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Desenvolupament d'un sistema de diagnòstic automatitzat per la malària i l'esquistosomiasi urogenital utilitzant eines d'intel·ligència artificial i un microscopi robotitzat universal de baix cost

Carles Rubio Maturana

Desenvolupament d'un sistema de diagnòstic automatitzat per la malària i l'esquistosomiasi urogenital utilitzant eines d'intel·ligència artificial i un microscopi robotitzat universal de baix cost

TESI DOCTORAL

Carles Rubio Maturana

DIRECTORS

Dr. Joan Joseph Munné i Dra. Elisa Sayrol Clols

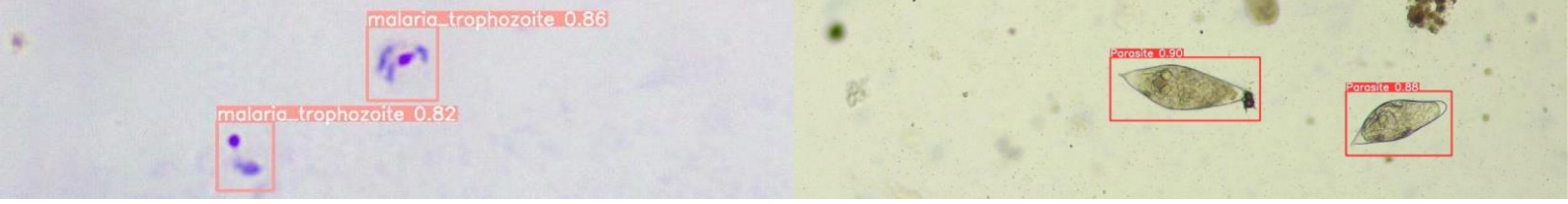
TUTORA

Dra. Elena Sulleiro Igual

Programa de doctorat en Microbiologia

Departament de Genètica i Microbiologia, Facultat de Biociències
Universitat Autònoma de Barcelona

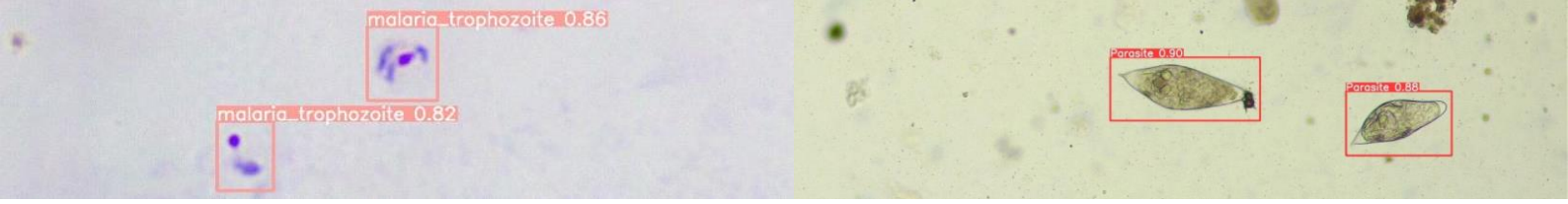
Barcelona, Maig de 2025



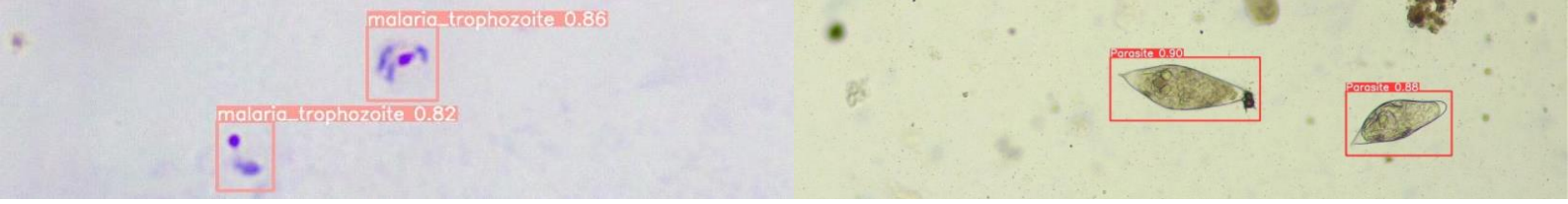
Desenvolupament d'un sistema de diagnòstic automatitzat per la malària i l'esquistosomiasi urogenital utilitzant eines d'intel·ligència artificial i un microscopi robotitzat universal de baix cost

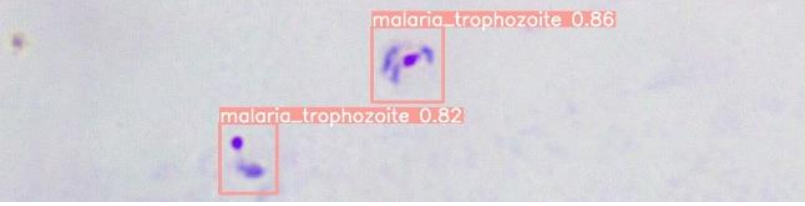
Tesi Doctoral presentada per en Carles Rubio Maturana per optar al grau de Doctor en Microbiologia, al programa de Doctorat en Microbiologia del Departament de Genètica i Microbiologia de la Facultat de Biociències de la Universitat Autònoma de Barcelona, sota la direcció del Dr. Joan Joseph Munné i la Dra. Elisa Sayrol Clols i la tutoria de la Dra. Elena Sulleiro Igual.

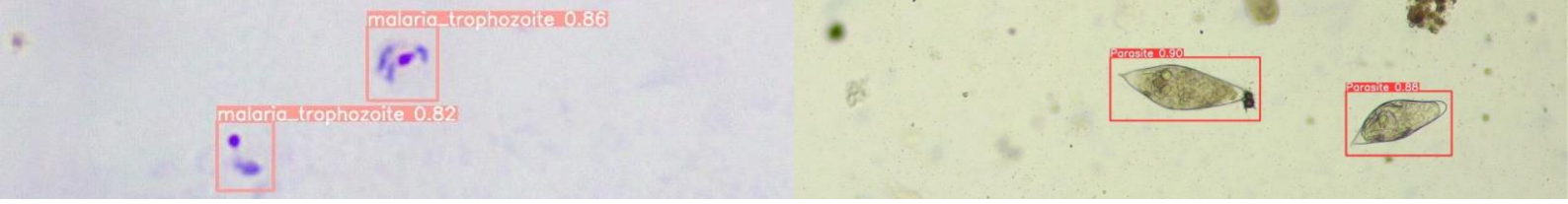
Maig de 2025, Barcelona



Índex







Certificat de dipòsit

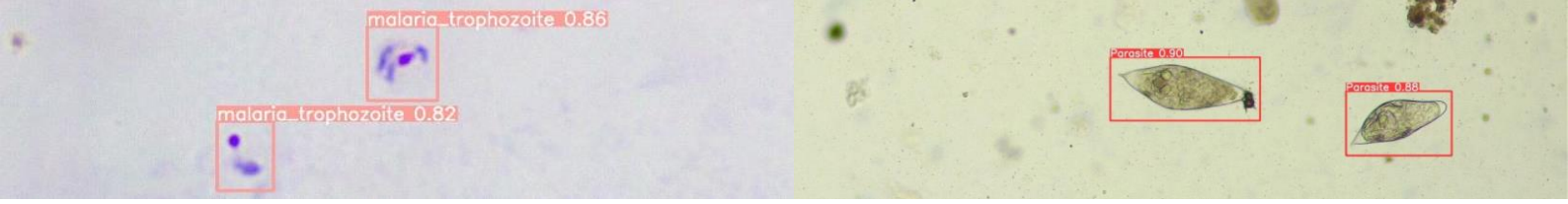
El **Dr. Joan Joseph Munné**, facultatiu especialista del Servei de Microbiologia del Hospital Universitari Vall d'Hebron, i investigador del Vall d'Hebron Institut de Recerca; i la **Dra. Elisa Sayrol Clols**, directora acadèmica del TecnoCampus Mataró (Universitat Pompeu Fabra); com a directors de la tesi doctoral d'en **Carles Rubio Maturana** certifiquen que el present treball titulat "***Desenvolupament d'un sistema de diagnòstic automatitzat per la malària i l'esquistosomiasi urogenital utilitzant eines d'intel·ligència artificial i un microscopi robotitzat universal de baix cost***" és apte pel seu dipòsit i presentació en el programa de doctorat de Microbiologia de la Universitat Autònoma de Barcelona (UAB).

La **Dra. Elena Sulleiro Igual**, Cap de Secció de Salut Internacional, facultativa especialista del Servei de Microbiologia de l'Hospital Universitari Vall d'Hebron i professora associada a al Universitat Autònoma de Barcelona, acredita, com els directors, la presentació de la tesi com a part de la universitat.

La tesi doctoral serà presentada per compendi de publicacions davant el tribunal que correspongui i compleix les condicions exigides per la Universitat Autònoma de Barcelona.

Directors de Tesi Doctoral: Tutora:

Dr. Joan Joseph Munné **Dra. Elisa Sayrol Clols** **Dra. Elena Sulleiro Igual**

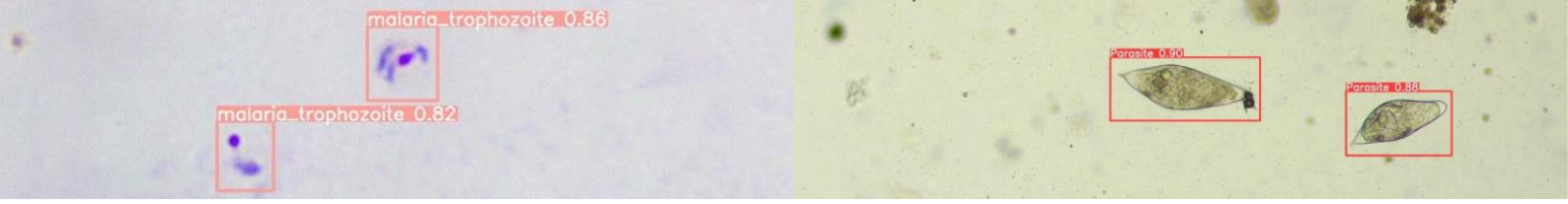


Agraïments

En primer lloc m'agradaria agrair als meus directors el Dr. Joan Joseph Munné i la Dra. Elisa Sayrol Clols. Joan, moltes gràcies per confiar en mi, per la feina feta durant el dia a dia, i per donar-me l'oportunitat de començar a treballar a l'Hospital Vall d'Hebron. Elisa, moltes gràcies pels consells i l'aprenentatge que m'has proporcionat en l'àmbit de la intel·ligència artificial i les seves aplicacions.

M'agradaria agrair i donar importància a tres institucions que han estat clau per poder realitzar la recerca presentada. En primer lloc al Centre de Malalties Transmissibles i Salut Internacional Drassanes Vall d'Hebron. El bon ambient del centre, i en concret del laboratori, han fet que em senti com a casa i que pugui treballar en un entorn envejable durant aquests anys. També em fa especial il·lusió que la nostra feina es relacioni directament amb Drassanes, donant-li la importància que es mereix degut al seu paper crucial per l'assistència, la docència i la recerca. En segon lloc m'agradaria agrair al Departament de Microbiologia de l'Hospital Universitari Vall d'Hebron, liderat pel Dr. Tomàs Pumarola i la Dra. Nieves Larrosa durant el temps transcorregut de la tesi doctoral. Moltes gràcies per oferir els mitjans i el suport necessari en tot moment, i permetre millorar dia a dia gràcies als professionals d'altíssim nivell dels que disposem. Per últim però no menys important, gràcies a la Universitat Politècnica de Catalunya i en especial al grup BIOCOMSC. Moltíssimes gràcies per ser generosos i confiar en mi en tot moment. M'heu acollit com un més del grup i m'heu ofert un munt d'oportunitats que penso no he deixat escapar.

Hi ha hagut 4 persones cabdals per que tot tirés endavant i que sense elles hagués estat impossible realitzar tota la feina feta durant la tesi. Allisson, moltes gràcies per venir des de Brasil amb la teva família, i per treballar mà a mà com a companys i amics. Ets una persona molt generosa i amb un caràcter alegre que permet treballar fàcilment. Francesc, has estat el meu mestre en tot moment. La teva passió per la feina és fascinant, però el més important és la teva qualitat humana. Sempre prudent i responsable, ajudant als demés sense demanar res a canvi. Trobarem a faltar els teus consells i coneixements, però has deixat un llegat que poca gent ha estat capaç d'aconseguir. Elena, una professional impecable i una líder com poques. Moltes gràcies per aportar els teus coneixements, oferir-me oportunitats i confiar en mi. La teva feina et situa com una referent en el teu àmbit, i ha estat un luxe poder tenir-te al costat. Dani, moltes gràcies per confiar en mi des de el primer moment. Gràcies per confiar en la gent jove, i deixar-nos créixer amb responsabilitats. Fer classes de Física mai se'm va passar pel cap, però m'has fet descobrir un món en el que em sento molt còmode.



Moltes gràcies especialment a l'Aroa, l'Àlex, la Patri, la Lidia, en Marc, la Neus, heu estat els meus companys i amics tant al Valle com a Drassanes. També a la Inés, la Núria, el Jesús, la Lupe, els companys de recerca, els residents... El vostre suport i companyia a l'hospital i fora d'ell m'han fet sentir com a casa. Aroa, moltíssimes gràcies pels consells i l'ajuda durant el dia a dia de la tesi, pels detalls dins i fora de la feina, i pel gran favor de revisar-te tota la tesi en un temps rècord!. Edurne, moltíssimes gràcies per tot. Aquells primers anys a Drassanes van ser fantàstics en gran part gràcies a tu. També a la Clara, l'Arnau, el Sergio, l'Enric, en Quim, la Qiao, l'Alberto, en Sergi, en Besim i moltes d'altres persones de la UPC. Aida, moltes gràcies pel suport, estic segur de que en uns anys seràs un científica de renom.

M'agradaria també agrair a la Fundació Probitas i als seus professionals, qui van finançar el projecte i la tesi durant els primers anys. Gràcies Marta, Anna, Mercè i Javi. Els vostres recursos han estat molt importants per poder dur a terme la feina feta.

Un agraïment especial per l'Hospital Nossa Senhora da Paz de Cubal, Angola. La meva estància allà va ser fantàstica. Especials agraïments a l'Arlette, una dona referent i empoderada que m'ha ensenyat a com es treballa en un laboratori del terreny. Sempre professional i amb ganes d'aprendre amb una humilitat incalculable. Gràcies també a en Joan Martínez, la Patri Álvarez, el Nano, la Rosa, l'Íñigo, el Miguel, la Mar, la Maria i tota aquella gent que va venir a Cubal. Vau fer que tot fos molt més fàcil i vam crear un equipàs. També agrair al Centre de Cooperació pel Desenvolupament de la UPC, qui va finançar l'estada i va donar facilitats perquè tot sortís correctament.

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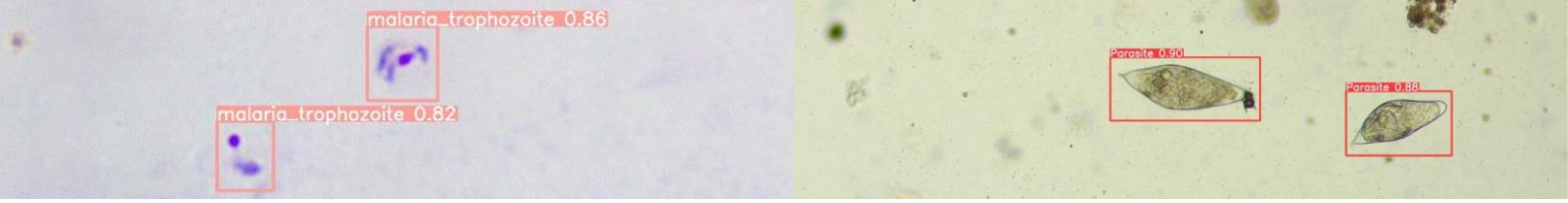
M'agradaria dedicar la tesi doctoral a la meva iaia Evi. Has sido un pilar fundamental en mi vida. Gracias por cuidarnos desde el primer día, por querernos tanto y por dárnoslo todo sin pedir nada a cambio. Nos has cuidado desde pequeños como una segunda madre hasta tus últimos días. Me alegra mucho que supieras que iba a ser doctor, he tenido la suerte de podértelo decir antes de partir. Por esto y mucho más, este trabajo va por ti.



Llistat d'acrònims

ADN: Àcid desoxiribonucleic

a.C.: abans de Crist

BIOCOM-SC: Biologia Computacional i Sistemes Complexos

CC: *Creative Commons*

CCD-UPC: Centre de Cooperació pel Desenvolupament de la Universitat Politècnica de Catalunya

Ct: *Cycle threshold*

ECLAT*: Equivalence Class clustering and bottom-up LAttice Traversal*

EI: esquistosomiasis intestinal

ELISA: Assaig immunoabsorbent lligat a enzim

EU: esquistosomiasis urogenital

FoV: Camp de visió, de l'anglès *Field of View*

G6PD: Glucosa 6 fosfat deshidrogenasa

HCRM: *Harris Corner Response Measure*

IEEE: Institut d'Enginyers Elèctrics i Electrònics

LAMP: Amplificació Isotèrmica per Bucles, de l'anglès *Loop-Mediated Isothermal Amplification*

mAP0.5: *mean average precision 0.5*

MTD: Malalties Tropicals Desateses

MVS: Màquines de Vector de Suport

NIAID: Institut Nacional d'Al·lergologia i Malalties Infeccioses

ODS: Objectius de Desenvolupament Sostenible

OMS: Organització Mundial de la Salut

PCR: Reacció en Cadena de la Polimerasa, de l'anglès *Polymerase Chain Reaction*

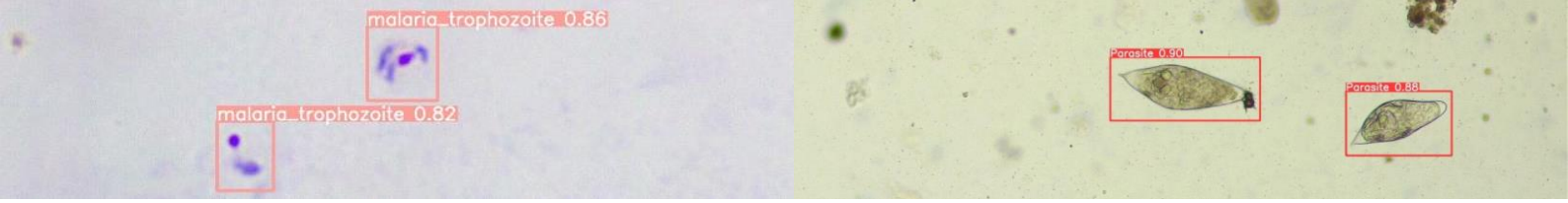
PDR: Proves de Diagnòstic Ràpid

pfprh2: Gen de la *Plasmodium falciparum* Proteïna Rica en Histidina II

pfprh3: Gen de la *Plasmodium falciparum* Proteïna Rica en Histidina III

p-LDH: lactat deshidrogenasa

PRH2: Proteïna Rica en Histidina II



Q1: Quartil 1

QBC: *Quantitative Buffy Coat*

LAMP: Amplificació isotèrmica per bucles, de l'anglès *Loop Mediated Isothermal Amplification*

R-CNN: *Region-based Convolutional Neural Network*

RMN: Ressonància Magnètica Nuclear

SMT: Subministració massiva de medicaments

SSD: *Single-Shot Detector*

TAI: Testing d'Anticòs d'Immunofluorescència

TAC: Tomografia Computada

UAB: Universitat Autònoma de Barcelona

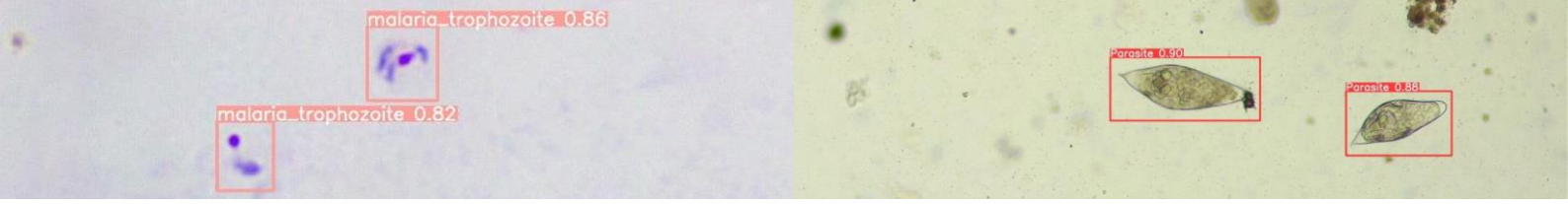
UK: Regne Unit

VFR: Visitar amics i familiars, de l'anglès *Visit Friends and Relatives*

VGG: *Visual Geometry Group*

VIH: Virus de la Immunodeficiència Humana

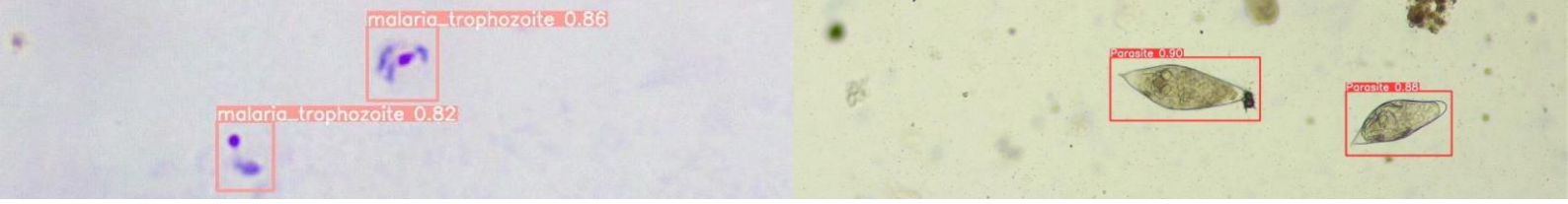
XNC: Xarxes Neuronals Convolucionals



Publicacions

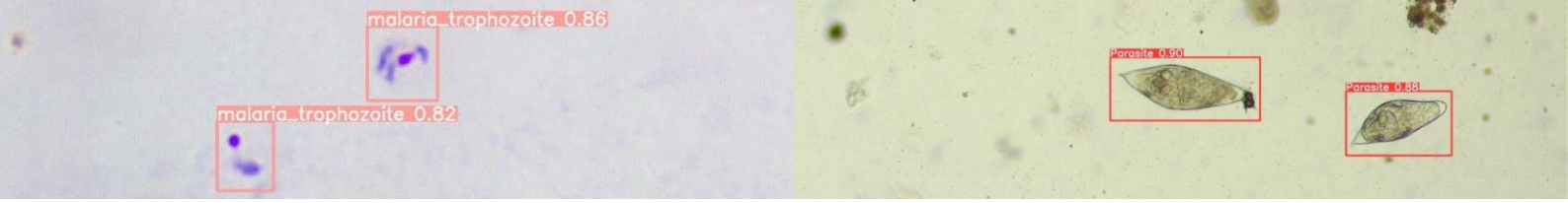
Articles científics en els que el doctorand ha participat

1. **Rubio Maturana, C**.; Dantas de Oliveira, A.; Nadal, S.; Bilalli, B.; Zarzuela Serrat, F.; Espasa, M.; Sulleiro, E.; Bosch, M.; Veiga, A.; Abello, A.; López-Codina, D.; Pumarola Suñé, T.; Sayrol Clols, E.; Joseph-Munné, Joan. Advances and challenges in automated malaria diagnosis using digital microscopy imaging with artificial intelligence tools: A review. *Front Microbiol* **2022** DOI: 10.3389/fmicb.2022.1006659
2. Perez-Ugarte, A.; **Rubio Maturana, C**.; Silgado, A.; Zarzuela Serrat, F.; Goterris, L.; Paredes-Carmona, F.; Sulleiro, E. Unexpected false-negative results in a traveller's malaria diagnosis. *J Trav Med* **2022** DOI: 10.1093/jtm/taac124
3. Treviño, B.; Zarzuela Serrat, F.; Oliveira-Souto, I.; **Rubio Maturana, C**.; Serre-Delcor, N.; Aznar, M.L.; Pou, D.; Goterris, L.; Salvador, F.; Bosch-Nicolau, P.; Rubio, J.M.; Ruiz, E.; Molina, I.; Sulleiro, E. Unexpected *Loa loa* finding in an asymptomatic patient from the Gambia: A case report. *Open Forum Infect Dis* **2023** DOI: 10.1093/ofid/ofad338
4. **Rubio Maturana, C**.; Dantas de Oliveira, A.; Nadal, S.; Zarzuela Serrat, F.; Sulleiro, E.; Ruiz, E.; Bilalli, B.; Veiga, A.; Abelló, A.; Pumarola Suñé, T.; López-Codina, D.; Sayrol Clols, E.; Joseph-Munné, J. iMAGING: a novel automated system for malaria diagnosis by using artificial intelligence tools and a universal low-cost robotized microscope. *Front Microbiol* **2023** DOI: 10.3389/fmicb.2023.1240936
5. Espiau, M.; Ajanovic, S.; Zarzuela Serrat, F.; **Rubio Maturana, C**.; Soler-Palacín, P.; Soriano-Arandes, A.; Sulleiro, E. Management of paediatric soil-transmitted helminthiasis in a non-endemic area: experience in a reference internatioal Health unit. *Parasitol Res*. **2024** DOI: 10.1007/s00436-024-08171-8
6. **Rubio Maturana, C**.; Guerrero, M.; Casas Claramunt, M.; Ayala-Cortés, S.N.; López, V.; Marínez-Vallejo, P.; Treviño, B.; Sulleiro, E.; Esperalba, J.; Rando Segura, A.; Aznar, M.L.; Bosch-Nicolau, P.; Salvador, F.; Oliveria-Souto, I.; Molina, I.; Serre-Delcor, N. Seroprevalence status of vaccine-preventable diseases in migrants livinf in shelter centres in Barcelona, Spain. *Enferm Infecc Microbiol Clin* **2024** DOI: 10.1016/j.eimc.2024.04.005
7. **Rubio Maturana, C**.; Dantas de Oliveira, A.; Gomez i Prat, J.; Sulleiro, E.; Zarzuela Serrat, F.; Joseph-Munné, J.; Prats, C.; López-Codina, D. Diagnóstico microscópico de la malaria



y la esquistosomiasis a través de intel·ligència artificial y robotización. *Enferm Emerg* **2024**

8. Dantas de Oliveira, A.; **Rubio Maturana, C**.; Zarzuela Serrat, F.; Motta Carvalho, B.; Sulleiro, E.; Prats, C.; Veiga, A.; Bosch, M.; Zulueta, J.; Abelló, A.; Sayrol Clols, E.; Joseph-Munné J.; López-Codina, D. Development of a low-cost robotized 3D-prototype for automated optical microscopy diagnosis: An open-source System. *Plos One* **2024** DOI: 10.1371/journal.pone.0304085
9. Pérez-Ugarte, A.; Oliveira-Souto, I.; Nadal-Baron, P.; Zarzuela Serrat, F.; Serre-Delcor N.; Treviño, B.; Aznar, M.L.; Pou, D.; Mediavilla, A.; **Rubio Maturana, C**.; Belsol-Alfonso, M.; Ruiz, E.; Goterris, L.; Sulleiro, E. High malaria PCR postivity in asymptomatic migrants from sub-Saharan Africa. *J Travel Med*. **2024** DOI: 10.1093/jtm/taae087
10. Mediavilla, A.; Silgado, A.; Sánchez-Marqués, R.; Bocanegra, C.; Nindia, A.; Salvador, F.; Pintar, Z.; Martínez-Vallejo, P.; **Rubio Maturana, C.**; Goterris, L.; Martínez-Campreciós, J.; Aixut, S.; Oliveira-Souto, I.; Aznar, M.L.; Espiau, M.; Molina, I.; Sulleiro, E. Usefulness of real-time PCR for urogenital schistosomiasis diagnosis in preschool children in a high-prevalence area in Angola. *Plos Negl Trop Dis*. **2024** DOI: 10.1371/journal.pntd.0012384
11. Mediavilla, A; Silgado, A; Febrer Sendra, B; Crego-Vicente, B; Martínez-Vallejo, P; **Rubio Maturana, C**; Goterris, L; Nindia, A; Martínez-Campreciós, J; Aixut, S; Aznar Ruiz de Alegria, ML; Fernández-Soto, P; Muro, A; Salvador, F; Molina, I; Berzosa, P; Oliveira-Souto, I; Sulleiro, E. Real-time PCR for malária diagnosis and identifiation of *Plasmodium* species in febrile patients in Cubal, Angola. *Parasit Vectors.* **2024** DOI: 10.1186/s13071-024-06467-3
12. **Rubio Maturana, C**; Dantas de Oliveira, A; Zarzuela, F; Ruiz, E; Sulleiro, E; Mediavilla, A; Martínez-Vallejo, P; Nadal, S; Pumarola, T; López-Codina, D; Abelló, A; Sayrol, E; Joseph-Munné, J. Development of an automated artificial intelligence-based system for urogenital schistosomiasis diagnosis using digital image analysis techniques and a robotized microscope. *PLoS Negl Trop Dis*. **2024** DOI: 10.1371/journal.pntd.0012614
13. **Rubio Maturana, C**; Martínez-Vallejo, P; Salvador, F; Beneitez-Pastor, D; Zarzuela Serrat, F; Sulleiro, E; Pou, D; Aznar, ML; Molina, I; Serre-Delcor, N; Oliveira-Souto, I. Primaquine treatment for *P. ovale* infection in migrants with G6PD deficiency. *J Travel Med*. **2024** DOI: https://doi.org/10.1093/jtm/taae150
14. **Rubio Maturana, C**; Dantas de Oliveira, A; Zarzuela, F; Mediavilla, A; Martínez-Vallejo, P; Silgado, A; Goterris, L; Muixí, M; Abelló, A; Veiga, A; López-Codina, D; Sulleiro, E;



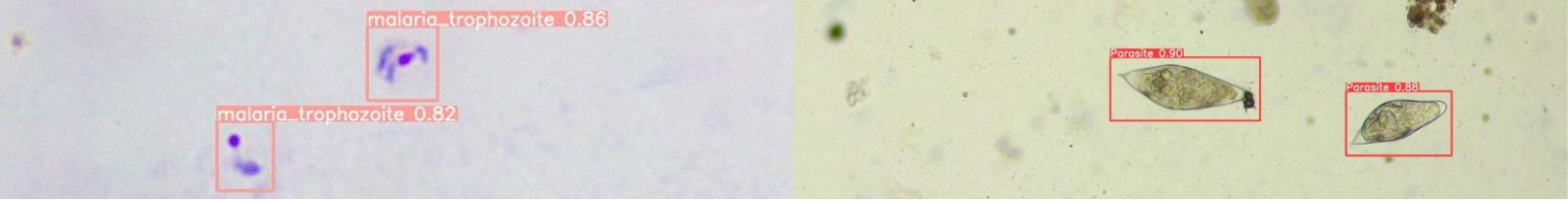
Sayrol, E; Joseph-Munné, J. Evaluation of an Artificial Intelligence-Based Tool and a Universal Low-Cost Robotized Microscope for the Automated Diagnosis of Malaria. *Int. J. Environ. Res. Public Health.* **2025** DOI: https://doi.org/10.3390/ijerph22010047

15. Mediavilla, A; Febrer-Sendra, B; Silgado, A; Martínez-Vallejo, P; Crego-Vicente, B; Nindia, A; **Rubio Maturana, C**; Goterris, L; Martínez-Campreciós, J; Aixut, S; Fernández-Soto, P; Aznar, ML; Muro, A; Oliveira-Souto, I; Molina, I; Sulleiro, E. Evaluation of dried blood spot sampling for real-time PCR malaria diagnostics in a rural setting in Angola. *Parasit Vectors.* **2025** DOI: 10.1186/s13071-025-06685-3

Comunicacions

Comunicacions orals que ha presentat, o en les que ha participat, el doctorand

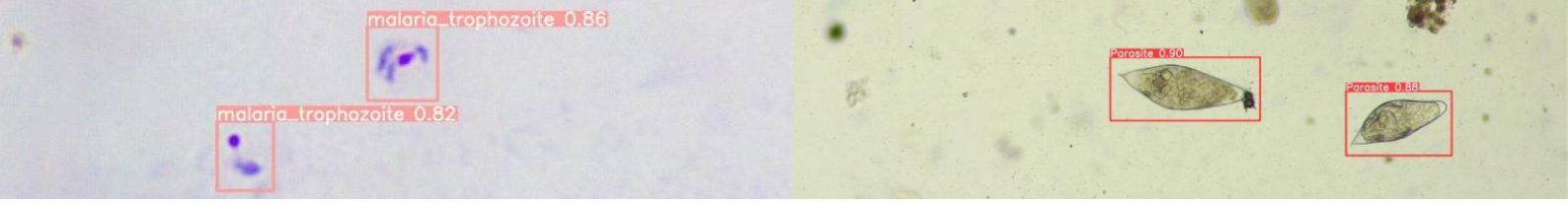
1. Comunicació oral Congrés SEMTSI 2022, Pamplona, 8-10 març 2022. *Desarrollo de una aplicación de dispositivo móvil para el diagnóstico automatizado de parásitos de malaria mediante el análisis de imagen digital con redes neuronales YOLOv5.*
2. Comunicació oral Congrés SEIMC 2022, Granada, 2-4 juny **Premi comunicació oral especial**: *Diagnóstico automatizado de malaria mediante el análisis de imágenes digitales microscópicas con redes neuronales e integración del sistema en una aplicación de dispositivo móvil.*
3. Ponència XIII Jornades Catalanes de Salut Internacional, Barcelona, 9 juny 2022: *Diagnòstic automàtic de mostres de malària amb tècniques d'IA i microscòpia robotitzada de baix cost.*
4. Ponència a les jornades de "Tecnologia per a la salut dels infants en contextos de pobresa" organitzada per la Societat Catalana de Pediatria i Centre de Cooperació UPC, Barcelona, 29 abril de 2022.
5. Coautoria Comunicació oral Congrés SEMTSI 2022, Pamplona, 8-10 març 2022: Automatización de bajo coste de microscopios ópticos básicos para facilitar el diagnóstico en laboratorios con pocos recursos.
6. Comunicació oral Congrés SEIMC, 1-3 juny 2023 Santiago de Compostela **Premi comunicació oral especial**: *Diagnóstico automatizado de la esquistosomiasis urogenital mediante técnicas de inteligencia artificial, un microscopio robotizado de bajo coste y una aplicación de dispositivo móvil.*



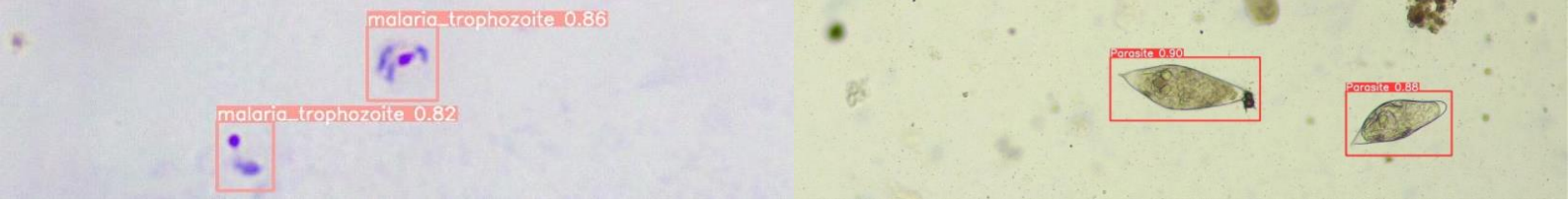
7. Ponència a l'Assemblea del projecte europeu AcToVax4NAM, 10-12 maig 2023, Barcelona.
8. Ponència a les Jornades de Recerca del grup de Biologia Computacional i Sistemes Complexos (BIOCOM-SC), 25 gener 2023, Castelldefels.
9. Mobile World Congress Barcelona 2024. Presentació de l'aplicació de dispositiu mòbil pel diagnòstic de la malària al stand del CIT-UPC durant els dies 26 al 29 de febrer de 2024.
10. Comunicació Oral al Congrès Europeu ESCMID 2024. "A novel automated smartphone application system for urogenital schistosomiasis diagnosis by using artificial intelligence tools and a universal low-cost robotised microscope" durant els dies 27 al 30 d'abril de 2024.
11. Jornada del Dia NTDs-IA al Instituto de Salud Carlos III, Madrid. Ponència "*Desarrollo de una herramienta de inteligencia artificial para el diagnóstico de la esquistosomiasis urogenital*" durant el dia 30 de Gener de 2024.
12. Sessió del Servei de Microbiologia de l'Hospital Clínic de Barcelona durant el dia 20 de Febrer de 2024.

Presentacions de pòster que ha presentat, o en les que ha participat, el doctorand

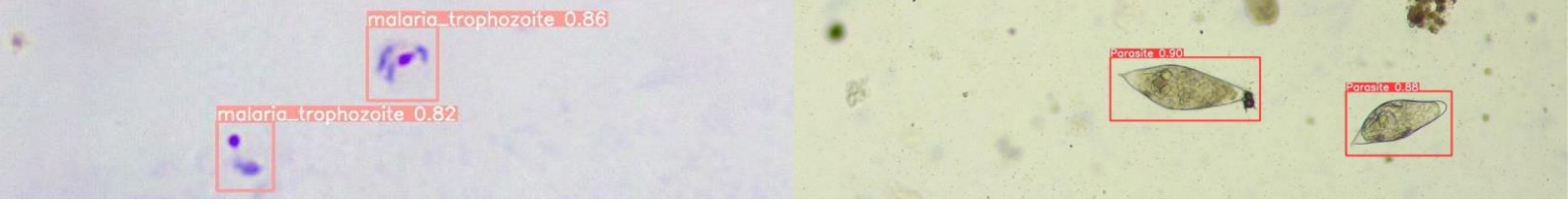
1. Jornades cooperació UPC organitzades pel Centre de Cooperació de la UPC (CCD-UPC). Presentació de pòster durant el dia 9 de Maig de 2024.
2. Presentació de e-pòster al Congrés SEIMC, Zaragoza 30 maig – 1 juny de 2024. "*Estado vacunal en migrantes recién llegados mediante el estudio de seroprevalencias en un centro de referencia en Barcelona, España*"



Índex de Figures



Índex de Taules

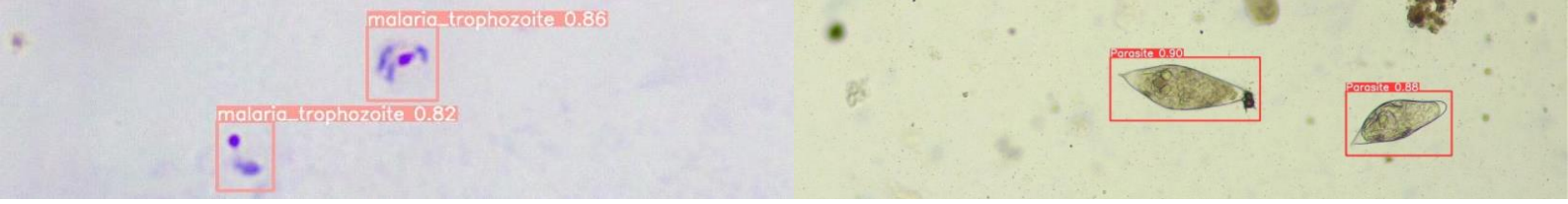


Resum (català)

La malària és una de les malalties infeccioses més prevalents a l'Àfrica subsahariana, amb 263 milions de casos reportats a tot el món durant el 2023 segons l'Organització Mundial de la Salut (OMS). L'esquistosomiasi és catalogada per l'OMS com a malaltia tropical desatesa, amb més de 253 milions de persones en risc d'infecció. La microscòpia continua sent la tècnica *gold standard* pel diagnòstic d'ambdues malalties. No obstant això, es tracta d'un mètode professional dependent amb un alt nivell d'implicació a la rutina diària dels laboratoris. Com a alternativa, s'estan desenvolupant noves tècniques de diagnòstic basades en l'anàlisi d'imatges amb eines d'Intel·ligència Artificial (IA).

La Intel·ligència Artificial és una de les tecnologies en desenvolupament més disruptives durant el segle present, que ha impulsat i millorat els mètodes tradicionals d'anàlisi d'imatges. L'aprenentatge profund i l'ús de xarxes neuronals convolucionals (XNC) per a la detecció d'objectes en imatges i vídeos podria ser una alternativa adequada al diagnòstic convencional per microscòpia. Els algoritmes d'IA utilitzen xarxes neuronals pre-entrenades per detectar formes biològiques en imatges de microscòpia. Pel seu desenvolupament i implementació és essencial disposar de conjunts de dades d'imatges digitals. Aquestes són comunament etiquetades pels experts mitjançant caixes contenidores que permeten delimitar les àrees de la imatge amb les formes biològiques d'interès. És determinant comptar amb un conjunt de dades d'imatges suficientment gran, per fer possible l'entrenament de XNC. Aquesta tecnologia confereix un diagnòstic ràpid, precís i automatitzat. D'altra banda, els telèfons intel·ligents són una solució òptima de baix cost per realitzar tot el diagnòstic mitjançant tècniques d'IA. La capacitat de capturar imatges de suficient qualitat amb una càmera pròpia, i la possibilitat d'integrar models de XNC per executar algoritmes d'IA i realitzar una detecció són, a dia d'avui, les principals característiques d'aquests dispositius per al diagnòstic.

Per abordar aquest problema, en el projecte presentat, hem desenvolupat un nou sistema automatitzat basat en tècniques d'anàlisi d'imatges per al diagnòstic de la malària i l'esquistosomiasi urogenital. El sistema es basa en una aplicació per a telèfons intel·ligents, i un microscopi robotitzat de baix cost. Per automatitzar tot el procés, es va dissenyar un prototip de peces impreses en 3D per a la robotització de la microscòpia òptica convencional, capaç d'autoenfocar i fer un escaneig de tota la mostra. L'objectiu principal de l'estudi és el desenvolupament d'un nou sistema automatitzat pel diagnòstic de la malària i l'esquistosomiasi

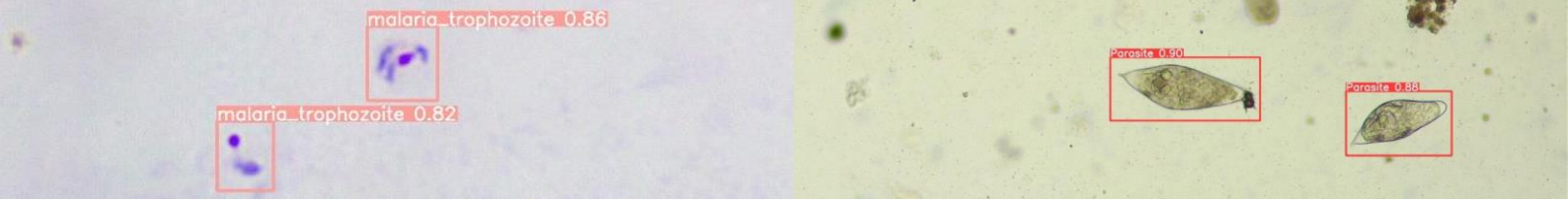


urogenital utilitzant eines d'intel·ligència artificial i un microscopi robotitzat universal de baix cost, dissenyat per la seva implementació en entorns amb pocs recursos on aquestes malalties són endèmiques.

Pel diagnòstic automatitzat de la malària es van utilitzar 148 mostres de gota gruixuda tenyides amb Giemsa per capturar imatges digitals microscòpiques. Es va crear un conjunt de dades de 2571 imatges etiquetades, i l'anàlisi comparatiu de xarxes neuronals (YOLOv5, Faster R-CNN, SSD i RetinaNet) va demostrar un rendiment òptim del model *You Only Look Once* versió 5x (YOLOv5x). Els resultats demostren, utilitzant mètriques d'aprenentatge automàtic per avaluar el rendiment, una precisió del 92.10%, un *recall* de 93.50% i un valor F de 92.79% per a la detecció de trofozoïts de *Plasmodium* immadurs, madurs i leucòcits. En el cas de l'esquistosomiasi urogenital, un total de 1017 imatges digitals etiquetades de 24 mostres de sediment urinari van ser emprades per l'entrenament dels algoritmes. Els ous de *S. haematobium*, tant ous viables com calcificats, van ser etiquetats manualment en imatges digitals per professionals de laboratori i utilitzats per entrenar els models YOLOv5 i YOLOv8. Les imatges de sediments urinaris també es van utilitzar per realitzar una classificació binària d'imatges per detectar la presència d'eritròcits/leucòcits en l'orina. L'entrenament de YOLOv5x va demostrar una precisió del 99.3%, un *recall* de 99.4% i un valor F de 99.3% per a la detecció d'ous de *S. haematobium*. El model de xarxa neuronal NasNetLarge va demostrar una precisió del 85.6% per a la detecció d'eritròcits/leucòcits en orina. Les imatges digitals de *Plasmodium spp.* i *S. haematobium* van ser capturades per la càmera de microscòpia digital integrada, i la càmera del telèfon intel·ligent al laboratori de Drassanes del Departament de Microbiologia de l'Hospital Vall d'Hebron.

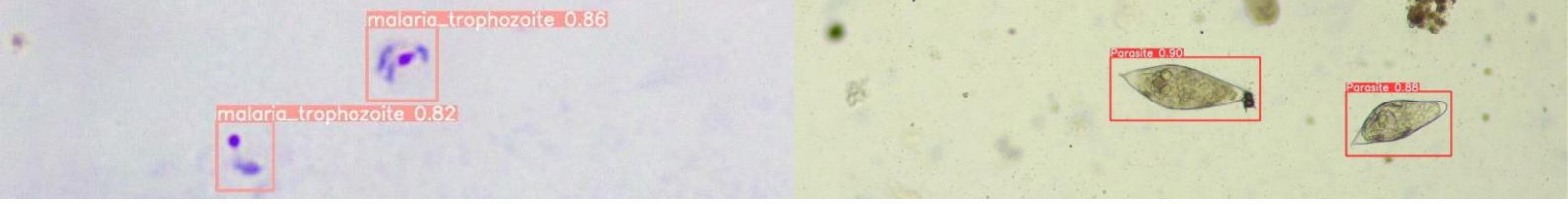
Els algoritmes de diagnòstic es van integrar en una aplicació per telèfons intel·ligents i un *software* de laboratori dissenyat pel seu maneig en ordinador. El sistema és capaç de realitzar un diagnòstic totalment automatitzat mitjançant: l'autoenfocament de la imatge i els moviments en els eixos X-Y del microscopi robotitzat, els models de XNC entrenats per a l'anàlisi d'imatges digitals i el telèfon intel·ligent. El nou prototip és capaç de determinar si (i) una mostra de gota gruixuda tenyida amb Giemsa és positiva/negativa per a la infecció de *Plasmodium*, i els seus nivells de parasitèmia; (ii) detectar la presencia d'ous de *S. haematobium* i d'eritròcits/leucòcits en imatges de sediments urinaris.

Es va realitzar una validació diagnòstica preliminar del sistema per comparar-la amb la tècnica *gold standard* de microscòpia convencional. Els resultats obtinguts a la validació del Centre



Drassanes Vall d'Hebron van demostrar una sensibilitat del 81.25% i una especificitat del 92.11% pel diagnòstic de la malària. D'altra banda, s'ha realitzat una prova de concepte a l'Hospital Nossa Senhora da Paz (Cubal, Angola) pel diagnòstic de la malària, demostrant resultats satisfactoris per a la implementació del sistema en laboratoris amb pocs recursos.

El projecte té un enfocament holístic amb un impacte transversal en la salut global. Altres aplicacions de la tecnologia podrien ser: (i) garantia de control de qualitat analítica i diagnòstica, (ii) generació de bases de dades d'imatges i digitalització de mostres biològiques, (iii) programes digitals educatius per a la formació de microscopistes i/o professionals de laboratori i (iv) notificació i vigilància per a la detecció de brots epidèmics. Finalment, la coalescència del sistema totalment automatitzat, la detecció autònoma de paràsits/cèl·lules en imatges digitals amb un programari per a telèfon intel·ligent i algoritmes d'IA, i altres aplicacions transversals confereixen al prototip les característiques òptimes per unir-se a l'esforç global per la lluita contra la malària i les malalties tropicals desateses.

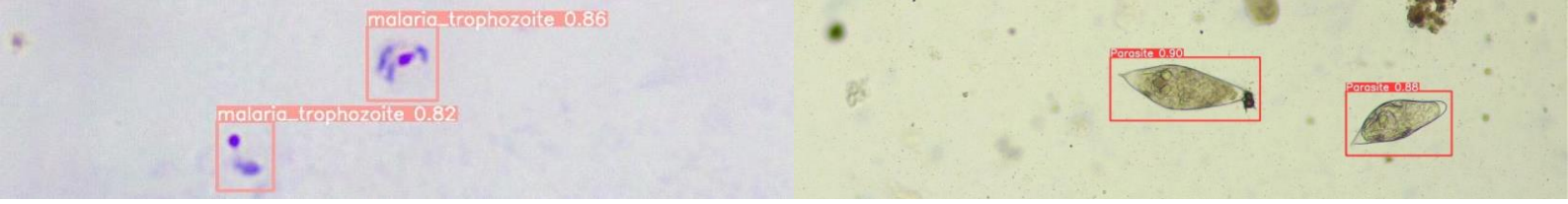


Resumen (castellano)

La malaria es una de las enfermedades infecciosas más prevalentes en África Subsahariana, con 263 millones de casos reportados en todo el mundo durante el 2023 según la Organización Mundial de la Salud (OMS). La esquistosomiasis es catalogada por la OMS como enfermedad tropical desatendida, con más de 253 millones de personas en riesgo de infección. La microscopia continúa siendo la técnica *gold standard* para el diagnóstico de ambas enfermedades. Sin embargo, se trata de un método profesional dependiente con un alto nivel de implicación en la rutina diaria de los laboratorios. Como alternativa, se están desarrollando nuevas técnicas de diagnóstico basadas en el análisis de imágenes con herramientas de Inteligencia Artificial (IA).

La Inteligencia Artificial es una de las tecnologías en desarrollo más disruptivas durante el siglo presente, que ha impulsado y mejorado los métodos tradicionales de análisis de imágenes. El aprendizaje profundo y el uso de redes neuronales convolucionales (RNC) para la detección de objetos en imágenes y videos podría ser una alternativa adecuada al diagnóstico convencional por microscopía. Los algoritmos de IA utilizan redes neuronales pre-entrenadas para detectar formas biológicas en imágenes de microscopía. Para su desarrollo e implementación es esencial disponer de conjuntos de datos de imágenes digitales. Estas son comúnmente etiquetadas por los expertos mediante cajas contenedoras que permiten delimitar las áreas de la imagen con las formas biológicas de interés. Es determinante disponer de un conjunto de datos de imágenes suficientemente grande, para realizar el entrenamiento de las RNC. Esta tecnología confiere un diagnóstico rápido, preciso y automatizado. Por otro lado, los teléfonos inteligentes son una solución óptima de bajo coste para realizar todo el diagnóstico mediante técnicas de IA. La capacidad de capturar imágenes de suficiente calidad con una cámara propia, y la posibilidad de integrar modelos de RNC para ejecutar los algoritmos y realizar una detección son las principales características de estos dispositivos para el diagnóstico.

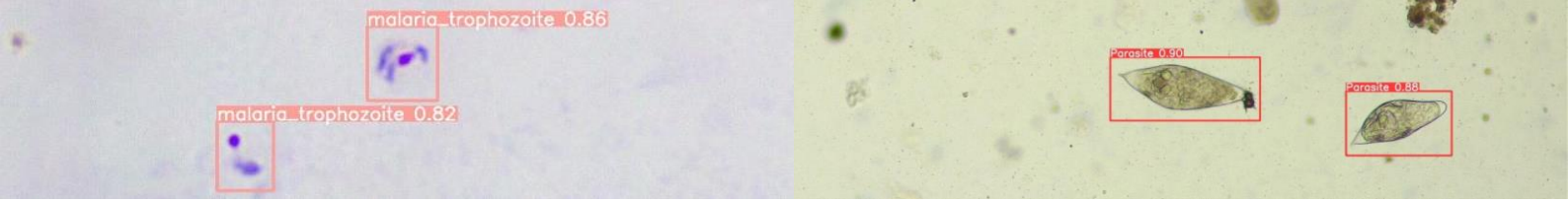
Para abordar este problema, en el proyecto presentado, hemos desarrollado un nuevo sistema automatizado basado en técnicas de análisis de imágenes para el diagnóstico de la malaria y la esquistosomiasis urogenital. El sistema se basa en una aplicación para teléfonos inteligentes, y un microscopio robotizado de bajo coste. Para automatizar todo el proceso, se diseñó un prototipo de piezas impresas en 3D para la robotización de la microscopia óptica convencional, capaz de auto-enfocar y hacer un escaneo de toda la muestra. El objetivo principal del estudio



es el desarrollo de un nuevo sistema automatizado para el diagnóstico de la malaria y la esquistosomiasis urogenital utilizando herramientas de inteligencia artificial y un microscopio robotizado universal de bajo coste, diseñado por su implementación en entornos con pocos recursos dónde estas enfermedades son endémicas.

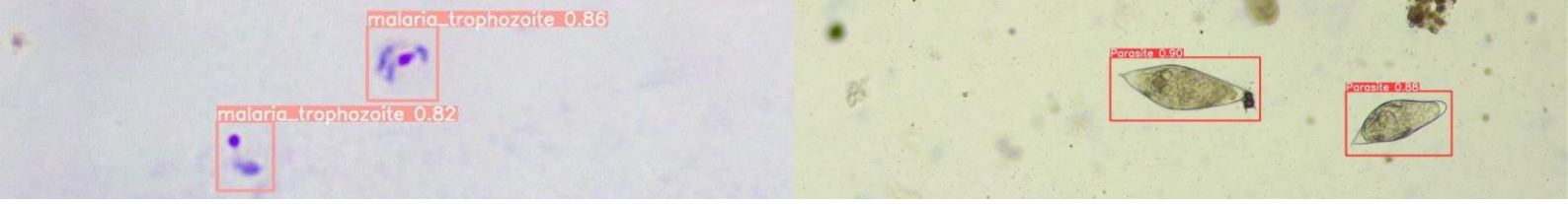
Por el diagnóstico automatizado de la malaria se utilizaron 148 muestras de gota gruesa teñidas con Giemsa para capturar imágenes digitales microscópicas. Se creó un conjunto de datos de 2571 imágenes etiquetadas, y el análisis comparativo de las redes neuronales (YOLOv5, Faster R-CNN, SSD y RetinaNet) demostró un rendimiento óptimo del modelo *You Only Look Once* versión 5x (YOLOv5x). Los resultados demuestran, utilizando métricas de aprendizaje automático para evaluar el rendimiento, una precisión del 92.10%, un *recall* de 93.50% y un valor F de 92.79% para la detección de trofozoítos inmaduros y maduros de *Plasmodium*, y leucocitos. En el caso de la esquistosomiasis urogenital, un total de 1017 imágenes digitales etiquetadas de 24 muestras de sedimento urinario fueron empleadas por el entrenamiento de los algoritmos. Los huevos de *S. haematobium*, tanto huevos viables como calcificados, fueron etiquetados manualmente en imágenes digitales por profesionales de laboratorio y utilizados para entrenar los modelos YOLOv5 y YOLOv8. Las imágenes de sedimentos urinarios también se utilizaron para realizar una clasificación binaria de imágenes para detectar la presencia de eritrocitos/leucocitos en la orina. El entrenamiento de YOLOv5x demostró una precisión del 99.3%, un *recall* de 99.4% y un valor F de 99.3% para la detección de huevos de *S. haematobium*. El modelo de red neuronal NasNetLarge demostró una precisión del 85.6% para la detección de eritrocitos/leucocitos en orina. Las imágenes digitales de *Plasmodium spp.* y *Schistosoma haematobium* fueron capturadas por la cámara de microscopía digital integrada, y la cámara del teléfono inteligente en el laboratorio de Drassanes del Departamento de Microbiología del Hospital Vall de Hebrón.

Los algoritmos de diagnóstico se integraron en una aplicación para teléfonos inteligentes y un *software* de laboratorio diseñado para su manejo por ordenador. El sistema es capaz de realizar un diagnóstico totalmente automatizado mediante: el auto-enfoque de la imagen y los movimientos en los ejes X-Y del microscopio robotizado, los modelos de RNC entrenados para el análisis de imágenes digitales y el teléfono inteligente. El nuevo prototipo es capaz de determinar si (i) una muestra de gota gruesa teñida con Giemsa es positiva/negativa para la infección por *Plasmodium*, y sus niveles de parasitemia; (ii) detectar la presencia de huevos de *S. haematobium* y de eritrocitos/leucocitos en imágenes de sedimentos urinarios.



Se realizó una validación diagnóstica preliminar del sistema para compararla con la técnica *gold standard* de microscopía convencional. Los resultados obtenidos en la validación del Centro Drassanes Vall de Hebrón demostraron una sensibilidad del 81.25% y una especificidad del 92.11% para el diagnóstico de la malaria. Por otro lado, se ha realizado una prueba de concepto en el Hospital Nossa Senhora da Paz (Cubal, Angola) para el diagnóstico de la malaria, demostrando resultados satisfactorios para la implementación del sistema en laboratorios con pocos recursos.

El proyecto tiene un enfoque holístico con un impacto transversal en la salud global. Otras aplicaciones de la tecnología descrita son: (i) garantía de control de calidad analítica y diagnóstica, (ii) generación de bases de datos de imágenes y digitalización de muestras biológicas, (iii) programas digitales para la formación de microscopistas y/o profesionales de laboratorio y (iv) notificación y vigilancia epidemiológica. Finalmente, la coalescencia del sistema totalmente automatizado, la detección autónoma de parásitos/células en imágenes digitales con un *software* para teléfonos inteligentes y algoritmos de IA, y otras aplicaciones transversales, confieren al prototipo las características óptimas para unirse al esfuerzo global por la lucha contra la malaria y las enfermedades tropicales desatendidas.

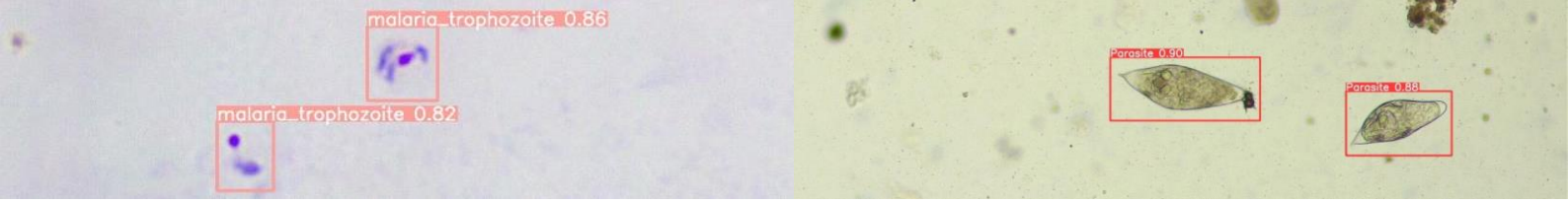


Abstract (english)

Malaria is one of the most prevalent infectious diseases in sub-Saharan Africa, with 263 million cases reported worldwide in 2023 according to the World Health Organization (WHO). Schistosomiasis is classified by the WHO as a neglected tropical disease, with more than 253 million people at risk of infection. Microscopy remains the *gold standard* technique for diagnosing both diseases. However, it is an expert dependent technique and results are difficult to reproduce. As an alternative, new diagnostic techniques are being developed based on image analysis with Artificial Intelligence (AI) tools.

Artificial Intelligence is one of the most disruptive developing technologies during the present century, which has promoted and improved traditional methods of image analysis. Deep learning and Convolutional Neural Networks (CNN) for object detection in images and videos could be an appropriate alternative to conventional microscopy diagnosis. AI algorithms are able to detect biological forms in microscopy images by using pre-trained neural networks with microbiological data. Image datasets are crucial to developing and implementing AI algorithms for diagnosis. Digital images are commonly labeled by experts to determine by bounding boxes the areas of an image with biological forms of interest. It is decisive to have a set of image data sufficiently large, to perform CNN training properly. This technology provides a fast, accurate and automated diagnosis. On the other hand, smartphones are an optimal low-cost solution to perform all the diagnosis using AI techniques. The ability to capture images of sufficient quality with their own camera, and the possibility of integrating CNN models to execute AI algorithms and perform a detection are nowadays the main characteristics of these devices for diagnosis.

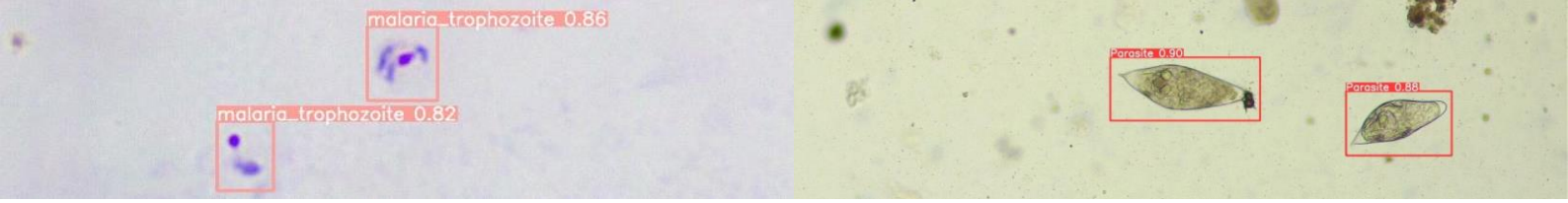
To address this problem, in the presented project, we have developed a new automated system based on image analysis techniques for the diagnosis of malaria and urogenital schistosomiasis. The system is based on a smartphone application, and a low-cost robotized microscope. To automate the entire process, a prototype of 3D printed pieces was designed for the robotization of conventional optical microscopy, capable of auto-focusing the sample and scanning the entire slide. The main objective of the study is the development of a new automated system for the diagnosis of malaria and urogenital schistosomiasis using artificial intelligence tools and a low-cost universal robotized microscope, designed for its implementation in resource-poor settings where these diseases are endemic.



For the automated diagnosis of malaria, 148 samples of Giemsa stained thick blood smears were employed to capture microscopic digital images. A dataset of 2571 labelled images was created, and the comparative analysis of neural networks (YOLOv5, Faster R-CNN, SSD and RetinaNet) demonstrated optimal performance of the *You Only Look Once* model 5x version (YOLOv5x). The results demonstrate, using machine learning metrics to evaluate performance, a precision of 92.10%, a recall of 93.50% and an F-score of 92.79% for the detection of early and mature *Plasmodium* trophozoite*s*, and leukocytes. In the case of urogenital schistosomiasis, a total of 1017 digital labelled images from 24 urinary sediment samples were employed for algorithm training. The eggs of *S. haematobium*, both viable and calcified eggs, were manually labeled in digital images by laboratory professionals, and employed to train the YOLOv5 and YOLOv8 models. Images of urinary sediments were also used to perform a binary classification, to detect the presence of erythrocytes/leucocytes in urine. YOLOv5x training demonstrated a 99.3% precision, a 99.4% recall and a 99.3% F-score for the detection of *S. haematobium* eggs in digital images. The NasNetLarge neural network model demonstrated an accuracy of 85.6% for the detection of erythrocytes/leucocytes in urine. The digital images of *Plasmodium spp.* and *Schistosoma haematobium* were captured through an integrated digital microscopy camera, and a smartphone camera in the Laboratory of the Drassanes-Vall d'Hebron Intenational Health and Infectious Diseases Centre.

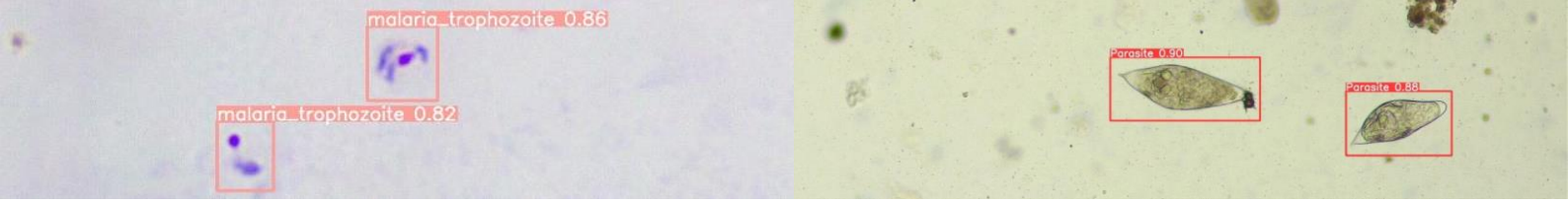
Diagnostic algorithms were integrated into a smartphone application and a laboratory software designed for computer handling. The system is able to perform a fully automated diagnosis through: (i) the auto-focusing of the sample and the slide scanning through the movements in the X-Y axes of the robotic microscope, (ii) the CNN models trained for the analysis of digital images and the smartphone. The new prototype is capable of determining whether a thick blood smear sample is positive/negative for *Plasmodium* infection, and its parasite levels; (ii) detecting the presence of *S. haematobium* eggs and erythrocytes/leucocytes in images of urinary sediments.

A preliminary diagnostic validation of the system was performed to compare it with the conventional gold standard microscopy technique. The results obtained at the validation of the Vall d'Hebron Drassanes Center demonstrated a diagnostic sensitivity of 81.25% and a diagnostic specificity of 92.11% for the diagnosis of malaria. On the other hand, a proof of concept has been carried out at the Nossa Senhora da Paz Hospital (Cubal, Angola) for the diagnosis of



malaria, demonstrating satisfactory results for the implementation of the system in laboratories with low resources.

The project has a holistic approach with a transversal impact on global health. Other applications of the technology could be: (i) guarantee of analytical and diagnostic quality control, (ii) generation of image databases and digitalization of biological samples, (iii) e-learning programs for microscopists and/or laboratory professionals and (iv) notification and surveillance for the detection of epidemic outbreaks. Finally, the coalescence of the fully automated system, the autonomous detection of parasites/cells in digital images with smartphone software and AI algorithms, and other transversal applications give the prototype the optimal characteristics to join the global effort to fight against malaria and neglected tropical diseases.



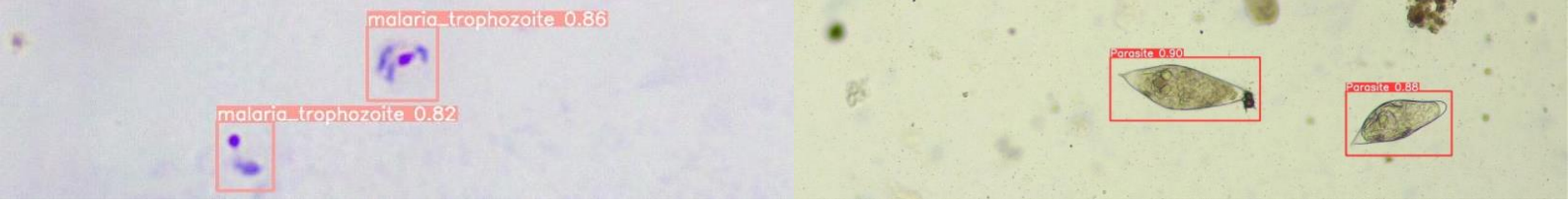
1. Introducció

1.1. La malària

La malària o paludisme és una malaltia tropical produïda per paràsits del gènere *Plasmodium*. El paràsit és un protozou unicel·lular intracel·lular de mida variable. La malaltia es transmet per la picada de mosquits femella infectats del gènere *Anopheles*, amb una gran prevalença a àrees tropicals i subtropicals a nivell global [1]. Principalment, les cinc espècies del paràsit que infecten als humans són *Plasmodium (P.) falciparum*, *P. vivax*, *P. ovale*, *P. malariae* i *P. knowlesi*. De totes elles, *P. falciparum* és la que produeix un major nombre de casos de malària i morts associades, amb especial rellevància a regions desfavorides i amb pocs recursos de l'Àfrica subsahariana [1].

El cicle biològic del paràsit *Plasmodium* és complex, i requereix el mosquit, que actua com a vector (**Figura 1)**. Els esporozoïts entren al corrent sanguini de l'ésser humà mitjançant una picada a nivell cutani d'una femella de mosquit *Anopheles* infectada. Les formes parasitàries són transportades pel corrent sanguini fins als hepatòcits, que són envaïts i infectats pels esporozoïts. Passats entre 5 i 15 dies després de la picada, els esquizonts desenvolupats al fetge alliberaran merozoïts mitjançant el trencament de les cèl·lules hepàtiques. El temps d'incubació dependrà de l'espècie de *Plasmodium*, sent *P. malariae* la més tardana. Els merozoïts entraran al corrent sanguini, iniciant així la fase intraeritrocitària del cicle biològic [2].

El desenvolupament del paràsit es pot donar per via asexual o sexual, donant a formes morfològicament diverses. La via asexual té lloc durant les properes 24-72 hores depenent de l'espècie de *Plasmodium*. En primer lloc, els merozoïts evolucionen a trofozoïts joves o trofozoïts en forma d'anell, seguits de trofozoïts madurs i esquizonts. Un cop els esquizonts (esquizogònia) són suficientment madurs, alliberen merozoïts per tornar a infectar nous eritròcits i repetir el cicle asexual. És conegut que *P. falciparum* infecta eritròcits de totes les edats, *P. malariae* té tendència pels més vells i *P. ovale* únicament envaeix els reticulòcits que expressen la proteïna CD71 (receptor de transferrina). Aquests factors tindran conseqüències directes en la parasitèmia, conjuntament amb molts d'altres com l'estat d'immunològic del pacient, l'edat i la desnutrició. En el cas concret de la infecció per *P. vivax* i/o *P. ovale* es desenvolupen al fetge unes formes parasitàries anomenades hipnozoits, responsables de reactivar la malaltia, setmanes, mesos o anys després de la infecció [3,4]. A aquest fenomen se'l coneix com a recidiva o recaiguda. Per altra banda, la via sexual s'acostuma a produir a la medul·la òssia, on els trofozoïts es desenvolupen fins a gametòcits. Els gametòcits maduren en un període d'entre 2-



12 dies, sent de 9-12 per *P. falciparum* i de 2-3 per *P. vivax*. Un cop al corrent sanguini, els gametòcits madurs són ingerits per la picada del mosquit on, a l'intestí de l'insecte, es produirà la reproducció sexual. Els gametòcits mascle i femella es diferencien donant lloc a microgametòcits i macrogametòcits, respectivament. Els microgametòcits fertilitzen els macrogametòcits per produir el zigot [5]. El zigot es transformarà en ooquinets, que seguidament formaran els ooquists. Els ooquists contenen centenars d'esporozoits, que migraran fins a les glàndules salivals del mosquit per infectar de nou a l'humà mitjançant una picada. El cicle biològic de la malària està representat i simplificat a la **Figura 1**.

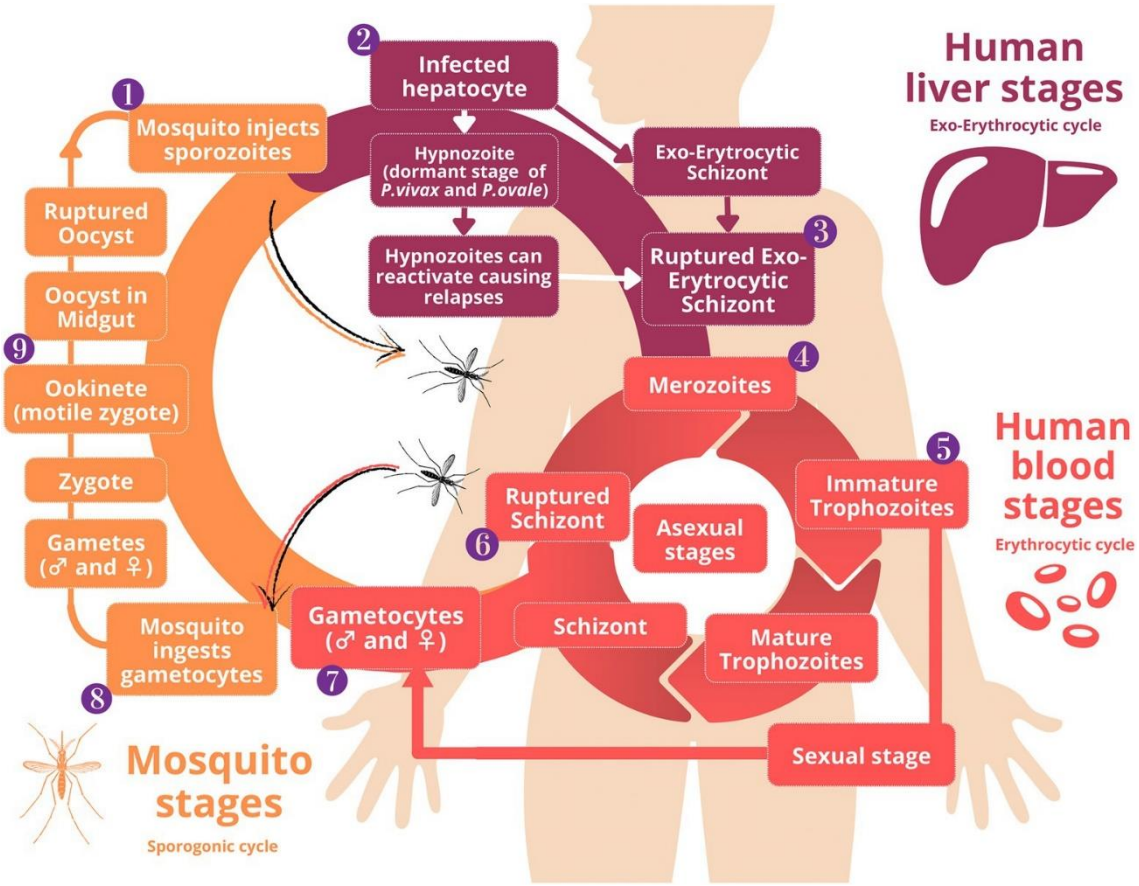
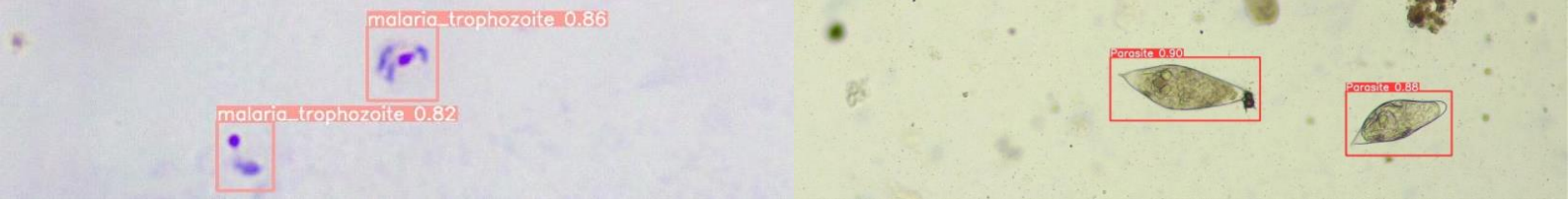


Figura 1 *Representació esquemàtica del cicle biològic del paràsit Plasmodium spp.* [6].

Actualment, l'estratègia de l'Organització Mundial de la Salut (OMS) per combatre la malària a nivell global està basada en diverses estratègies: prevenció, millora del diagnòstic i tractament [1]. La prevenció de la malària ha estat un repte global des de fa dècades. L'eliminació de zones amb aigües estancades i l'ús de mosquiteres són clau per evitar la proliferació del vector, i per tant la transmissió de la malaltia. S'utilitzen insecticides que redueixen la proliferació dels mosquits, la seva reproducció i inclús la seva infectivitat [7]. Encara així, molts mosquits estan

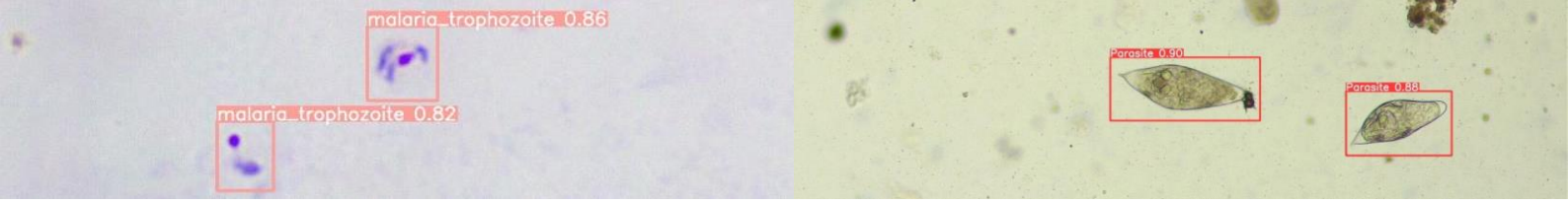


desenvolupant resistències als insecticides, que dificulten les tasques de control vectorial [8,9]. D'altres estratègies com la vacunació i la quimioprevenció també s'estan utilitzant. En alguns països de l'Àfrica subsahariana s'estan implementant programes de quimioprevenció massius per a infants en escoles, i per embarassades durant les èpoques d'altra transmissió de la malària [10,11]. D'altra banda, la vacunació del paludisme continua sent un repte global. Les vacunes de la malària les podem dividir segons el moment on pretenem que actuïn: (i) vacunes basades en les fases eritrocitàries, (ii) vacunes basades en les fases preeritrocitàries, (iii) vacunes basades en la malària placentària, i (iv) vacunes basades en el control de la transmissió, responsables d'eliminar les fases sexuals del cicle biològic del paràsit i les fases pròpies del mosquit. Algunes vacunes com la RTS,S/AS01E han estat recomanades per l'OMS, amb l'objectiu de vacunar infants en àrees amb alta transmissió de la malaltia [12]. Encara així, els avenços en la vacuna de malària s'han d'optimitzar per obtenir immunitat a escala global.

1.1.1. Ressenya històrica

La malària i les seves conseqüències ocupen un lloc únic en els annals de la història. Al llarg del temps ha tingut víctimes conegudes; des d'habitants neolítics, xinesos i grecs primitius, fins a personatges històrics com el faraó Tutankamon, l'explorador i metge britànic David Livingston o el navegant i explorador portuguès Vasco de Gama. Només durant el segle XX la malària va produir entre 150 i 300 milions de morts a nivell mundial, el que representava entre el 2-5% de tota la població del planeta [13].

Determinar l'inici i descobriment de la malària en humans és una controvèrsia. La prova inequívoca més antiga del paràsit de *Plasmodium* ha estat la detecció de l'àcid desoxiribonucleic (ADN) del paràsit en mosquits atrapats a pedres d'ambre de fa 30 milions d'anys [14]. Alguns estudis determinen que s'han trobat escriptures, manuscrits i taules d'argila de Mesopotàmia on s'esmenen febres mortals periòdiques que podrien referir-se a la malària [15]. Estudis moleculars, han detectat antigen de *Plasmodium* a restes egípcies datades de l'any 3200 a.C. [16], i a l'antiga Índia anomenaven el paludisme com "el rei de totes les malalties" (entre els anys 1500 i 800 a.C.) [13]. Hipòcrates al seu llibre "*Epidèmies*" de l'any 460-370 a.C. va descriure les primeres febres periòdiques, sense referir-se com a tal a la malària [17]. A l'antic l'Imperi Xinés anomenat *Nei Chin*, la medicina tradicional de la regió durant el 270 a.C., es descrivien febres amb periodicitat cada 3 o 4 dies, augment de la mida de la melsa, mals de cap i tremolors; tots ells símptomes típics de la malària [13]. També, al poema d'Homer "*La Ilíada*" es menciona la malaltia [18]. Totes aquestes proves escrites demostren que el paludisme ha tingut una



rellevància social gran durant segles, en cultures diverses i en persones de qualsevol estatus social.

Els primers tractaments descrits contra la malaltia daten del 168 a.C., quan es va començar a utilitzar a la Xina la planta medicinal *Artemisina annua*, també coneguda com a *Qinghao* en xinès, que conté l'alcaloide artemisinina, actualment utilitzat per la mateixa funció [19].

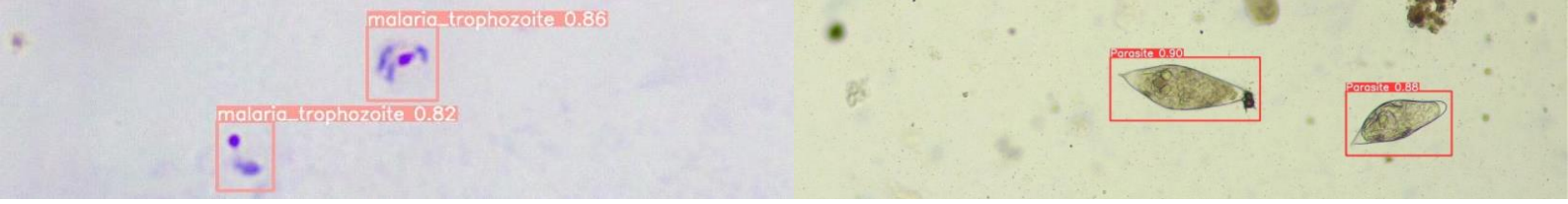
El científic Francesco Torti (1658-1741) és considerat la persona que va posar el nom de "*malària*" a la malaltia [23]. També va postular que es transmetia a través de l'aire que sortia dels pantans i llacunes estancades, sense mencionar la transmissió vectorial a través dels mosquits. El descobridor de l'agent causal de la malària va ser Charles Louis Alphonse Laveran, un cirurgià francès de l'exèrcit que estava de servei a Algèria, qui va rebre el premi Nobel per aquest descobriment l'any 1907. Durant l'observació al microscopi de la sang d'un pacient va percebre gametòcits de *P. falciparum.* També, va suggerir que podia haver-hi una relació directa entre la malària i els mosquits. Encara així, no va ser fins a la dècada de 1870, quan Patrick Manson va relacionar per primera vegada una malaltia amb el mosquit com a vector. Es tractava de la filariasis, una de les Malalties Tropicals Desateses (MTD) actuals, transmesa per mosquits i tàbacs. Finalment, els descobriments de Manson, Ronald Ross, Giovanni Battista i Amico Bigmani van postular que la picada dels mosquits era la responsable de la transmissió de la malària als humans. L'any 1902, Ronald Ross va guanyar el Premi Nobel de Medicina gràcies a aquest descobriment [20].

Actualment, es coneix amb detall el cicle de vida, la transmissió del paràsit, i les manifestacions clíniques de la malaltia. Encara així, la malària continua sent un problema de salut global, amb una àmplia distribució al llarg de tot el planeta.

1.1.2. Epidemiologia

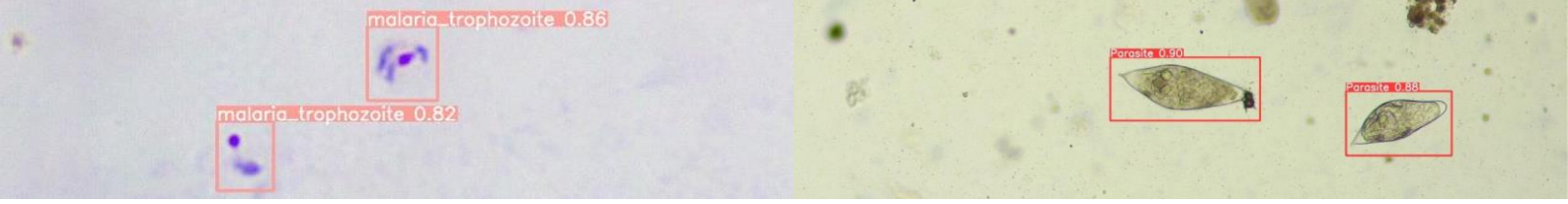
Actualment, les principals zones afectades per la malaltia són àrees amb pocs recursos de l'Àfrica subsahariana, el Sud-est Asiàtic, les conques de l'Amazones i d'altres regions tropicals. Encara així, actualment, aproximadament el 40% de la població mundial viu en llocs amb risc de contraure la malaltia, sent *P. falciparum* l'espècie que produeix la majoria de morts per malària a nivell global [1].

Les dades més recents sobre l'epidemiologia global de la malària són les descrites al World Malaria Report 2024 [21]. A nivell mundial durant el 2023, es van estimar uns 263 milions de casos de malària en 83 països endèmics. Respecte a l'any anterior s'ha observat un augment



d'11 milions de casos. Entre el 2000 i el 2019, el nombre de casos anuals de malària es va mantenir estable, variant entre 227 i 248 milions en els 108 països afectats. Des del 2020, el nombre de casos de malària estimats ha augmentat constantment, i la major part d'aquest augment s'ha produït en països de la Regió Africana (89,7%) i de la Regió de la Mediterrània Oriental (15,5%), ambdues regions delimitades per l'OMS. Els principals països que van contribuir a l'augment de casos entre el 2022 i el 2023 van ser Etiòpia (+4,5 milions), Madagascar (+2,7 milions), Pakistan (+1,6 milions), Nigèria (+1,4 milions) i la República Democràtica del Congo (+600.000). Les dades epidemiològiques sobre el nombre de casos i morts produïts per la malària des de l'any 2000 fins al 2023 estan representats a les **Figura 2 i Figura 3**.

La incidència de la malaltia va disminuir entre els anys 2000 i 2015, passant de 79.0 a 58.0 per cada 1000 persones en risc. En canvi, entre 2015 i 2022, la incidència es va mantenir estable. L'any 2023, la incidència de casos de malària va augmentar lleugerament en comparació amb el 2022, de 58.6 a 60.4 per cada 1000 persones en risc. Des de l'any 2000 les morts per malària van disminuir de 861.000, fins a 567.000 al 2019. Però, l'any 2020, es va produir un augment de 55.000 morts per malària, fins a augmentar el número a uns 622.000, com a resultat col·lateral de la pandèmia de la COVID-19. Entre el 2021 i el 2023, les morts es van reduir de nou a 597.000. El percentatge de morts totals per malària en nens menors de 5 anys va disminuir entre el 2000 i el 2023, passant del 86.7% al 73.7%. Al 2023, 29 dels 83 països (incloent-hi el territori de La Guaiana Francesa) endèmics de la malària representaven gairebé el 95% dels casos de la malaltia i el 96% de les morts a tot el món. Cinc països en concret: Nigèria (25.9%), la República Democràtica del Congo (12.6%), Uganda (4.8%), Etiòpia (3.6%) i Moçambic (3.5%); van representar poc més de la meitat de tots els casos globals. Tanmateix, quatre països van representar poc més de la meitat de totes les morts per malària a tot el món: Nigèria (30.9%), la República Democràtica del Congo (11.3%), el Níger (5.9%) i la República Unida de Tanzània (4.3%). Nigèria va representar el 39.3% de les morts globals de malària en menors de cinc anys, dada que s'ha de tenir en compte a causa de la gran població del país africà [21].



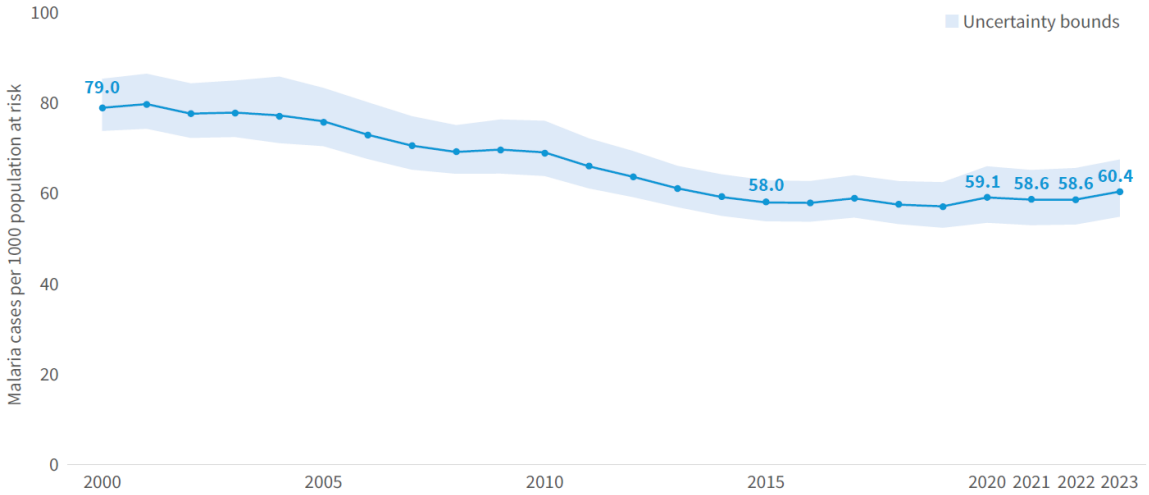


Figura 2 *Nombre de casos de malària per cada 1000 persones en risc entre els anys 2000 i 2023. Incidència de la malària a nivell mundial [21].*

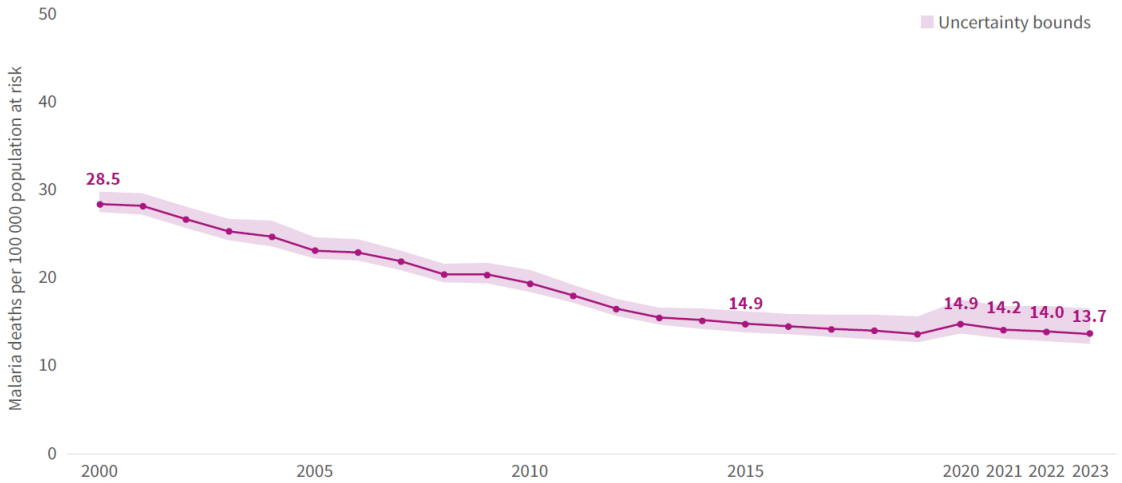
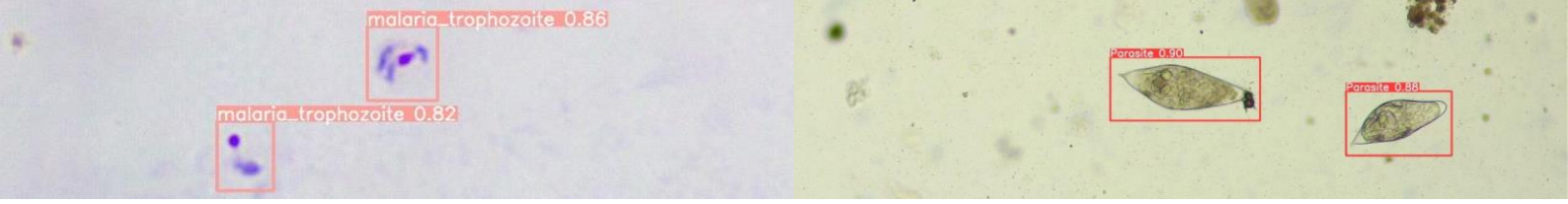


Figura 3 *Nombre de morts per malària per cada 100.000 persones en risc entre els anys 2000 i 2023. Mortalitat de la malària a nivell mundial [21].*

L'OMS posa a disposició estimacions, i no dades del tot confirmades, tenint en compte que en moltes ocasions, en països de mitjana i baixa renda, no es registren les dades epidemiològiques o no es disposa de registres a escala regional ni de districte.

En concret, durant el 2023 es van registrar 246 milions de casos i 569.000 morts a la regió africana de l'OMS. Centrant-nos en aquesta regió en els últims 5 anys, entre el 2019 i el 2023 els casos i les morts estimades per malària han augmentat en 23 milions i 24.000, respectivament, amb un pic de 598.000 morts estimades durant el 2020 vinculades a la COVID-19. Des de llavors, el nombre de morts estimades ha disminuït en 29.000. Aquesta regió ha representat al voltant



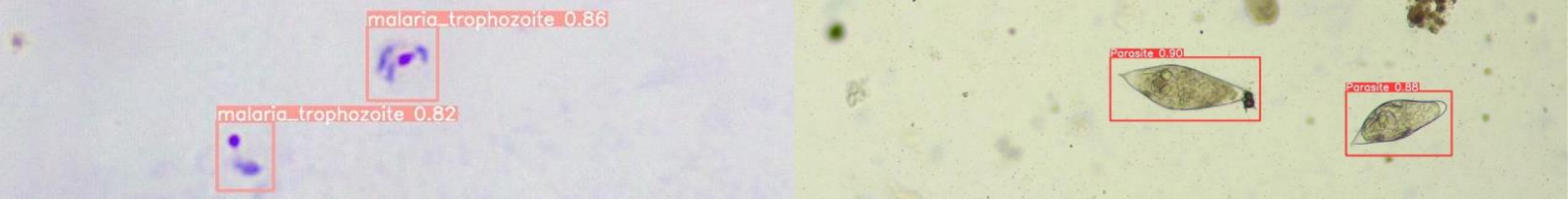
del 94% dels casos i el 95% de les morts a tot el món; el 76% de totes les morts en aquesta regió es van produir per infants menors de 5 anys al 2023, enfront del 91% a l'any 2000 [21].

1.1.3. Manifestacions clíniques i patogènia

La simptomatologia més comuna de la malària és la febre, encara que poden aparèixer altres símptomes com tremolors, mal de cap, miàlgies, nàusees, vòmits, diarrea, dolor abdominal i tos [22]. La malaltia es caracteritza per provocar símptomes clínics, dels quals es poden trobar períodes asimptomàtics i estones amb un gran nivell de malestar. Els períodes febrils s'associen a la ruptura dels esquizonts. En el cas de *P. vivax* i *P. ovale* els episodis de febres solen ser més espaiats en el temps, degut a la maduració dels esquizonts cada 48 h. Amb aquestes dues espècies, els símptomes poden aparèixer després de setmanes o fins i tot anys després de la infecció, si no són tractades correctament [23]. Per *P. malariae*, en canvi, els períodes febrils apareixen cada 72 h. En fases més avançades de la malaltia també es pot produir esplenomegàlia, hepatomegàlia, icterícia, hipertensió i major dolor a la palpació abdominal. En l'àmbit de laboratori, es poden observar multitud d'anormalitats que inclouen: anèmia normocròmica, normocítica, trombocitopènia, leucocitosi o leucopènia, hipoglucèmia, hiponatrèmia, proves elevades de funció hepàtica i renal, proteïnúria i coagulació intravascular disseminada. Al contrari que d'altres malalties parasitàries, la malària no produeix eosinofília.

En casos on el diagnòstic no està disponible i no es tracta al pacient, la malaltia pot derivar a fases anomenades complicades o malària greu [24]. En aquests casos els pacients acostumen a mostrar hemòlisi intravascular massiva amb hemoglobinèmia i hemoglobinúria. Principalment, si pateixen una infecció per *P. falciparum* es pot produir malària cerebral i anèmia severa. L'anomenada malària cerebral es defineix com a qualsevol estat d'anormalitat de l'estat mental degut a les afectacions neurològiques, en una persona que pateix malària. La malària cerebral presenta un percentatge de mortalitat d'entre el 15-50%. Altres alteracions clíniques i biològiques comunes de la malària severa són: hiperparasitèmia, hipoglucèmia greu, acidosi làctica, hipertèrmia prolongada, estat de xoc, disfunció pulmonar, cardíaca, hepàtica o renal, convulsions i sagnats espontanis. Les persones considerades d'alt risc de malaltia greu són les persones d'edat avançada (>65 anys), les dones embarassades, persones immunodeprimides o amb malalties cròniques, i els infants menors de cinc anys.

Els símptomes clínics són provocats principalment per les fases eritrocitàries de la malaltia. En canvi, no s'associa simptomatologia amb la presencia dels esporozoïts, les formes parasitàries



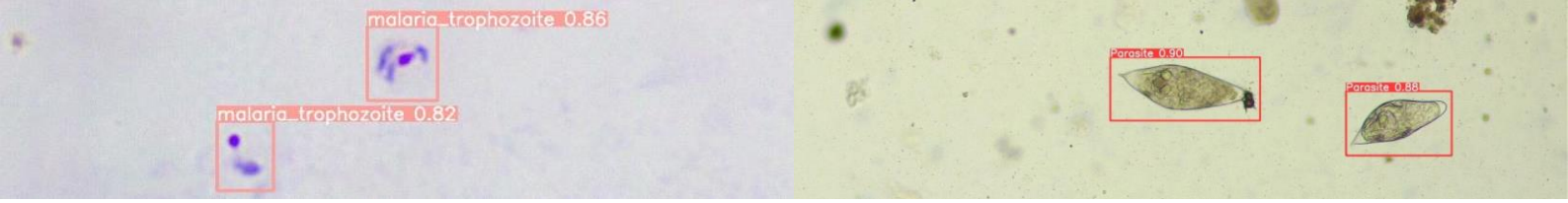
del fetge, els merozoïts o els gametòcits [22]. Els primers símptomes de la malària s'associen amb la ruptura continuada dels eritròcits a causa de la reproducció asexual dels paràsits. L'alliberació de paràsits al corrent sanguini provoca la reacció immunològica. Per tant, el nivell de parasitèmia que pateixi el pacient va directament associat a l'estat d'anèmia, i per tant la severitat de la malaltia. En el cas de la malària per *P. falciparum*, els eritròcits infectats s'adhereixen a l'endoteli dels capil·lars i les vènules post capil·lars, provocant l'obstrucció de la microcirculació i l'anòxia del teixit local. En el cervell aquest fet causa malària cerebral; en els ronyons pot causar necrosi tubular aguda i insuficiència renal; i en els intestins pot causar isquèmia i ulceració, donant lloc a hemorràgies gastrointestinals i a bacterièmia secundària per l'entrada de bacteris intestinals en la circulació sistèmica.

1.1.4. Diagnòstic

El diagnòstic de la malària és crucial per tractar i erradicar les infeccions del paràsit *Plasmodium*. La infecció podria produir malària en diverses formes i/o manifestacions: asimptomàtica, placentària, no complicada, i severa; depenent de la simptomatologia i fase de la infecció. Un diagnòstic precoç és determinant en la lluita efectiva contra la infecció. És per això, que el diagnòstic clínic i de laboratori s'utilitzen globalment per a la detecció de la malària. Hi ha diverses tècniques disponibles i utilitzades per detectar directament o indirectament la presència de paràsits de malària en sang. Encara així, el context i recursos són determinants a l'hora d'escollir o aplicar cadascuna de les tècniques [25].

1.1.4.1. Diagnòstic clínic

El diagnòstic clínic és el primer pas pel diagnòstic de la malària. Els antecedents epidemiològics, l'edat i la regió d'origen o regió visitada, en el cas dels viatgers o els *Visit Friends and Relatives* (VFR), són claus per correlacionar els símptomes amb la infecció. La simptomatologia no és específica i, per tant, és possible confondre-la amb altres infeccions o malalties amb manifestacions similars, com el dengue o d'altres arbovirosis [26]. Tanmateix, la simptomatologia clínica podria variar en funció de la fase de la malaltia i de l'espècie del paràsit de *Plasmodium* que produeix la infecció. Els principals símptomes de la malària són: les febres recurrents, calfreds, diaforesis i mals de cap [26]. El diagnòstic clínic s'ha de complementar amb tècniques de diagnòstic de laboratori, per confirmar la presència de paràsits a nivell microscòpic i/o molecular.



1.1.4.2. Diagnòstic de laboratori

El diagnòstic de laboratori de la malària engloba un conjunt de tècniques globalment emprades per la detecció del paràsit del gènere *Plasmodium*. Les mostres de sang s'utilitzen en la gran majoria de tècniques diagnòstiques. A continuació s'exposen les metodologies més utilitzades i acceptades als laboratoris d'arreu.

Exploració microscòpica de gota gruixuda i extensió fina de sang

La visualització microscòpica de mostres de gota gruixuda i extensió fina de sang per observar paràsits de la malària és la tècnica *gold standard* pel seu diagnòstic [27,28]. Abans de l'observació, la mostra es tenyeix principalment amb tinció de Giemsa o Leishman, per diferenciar els paràsits a través del microscopi. Les mostres de gota gruixuda de sang (**Figura 4**) són més eficients i proporcionen una major sensibilitat diagnòstica en comparació amb les mostres d'extensió fina [29]. Per altre banda, la visualització microscòpica de mostres d'extensió fina de sang permet identificar a l'espècie de *Plasmodium*. La combinació d'ambdós mètodes permet als experts determinar el tipus i la gravetat de la infecció amb un diagnòstic precís. És una tècnica senzilla i ràpida per visualitzar la forma activa intraeritrocitària dels paràsits en sang. El procediment operatiu estàndard de microscòpia de malària és el protocol recomanat per l'OMS, complementat amb manuals que permeten assegurar i avaluar la qualitat dels diagnòstics [30].

Conèixer el cicle de vida dels paràsits de *Plasmodium* (**Figura 1**) és important per realitzar una correcta identificació de les diferents etapes del desenvolupament dels paràsits i de l'espècie de *Plasmodium* **(Taula 1)**. *Plasmodium falciparum* normalment causa nivells de paràsits en sang alts o molt alts. Les taques de *Maurer*, els eritròcits poli-infectats i la característica forma de plàtan dels gametòcits són trets distintius de la infecció per *P. falciparum* [31]. En canvi, *P. vivax* i *P. ovale* són espècies que comparteixen algunes similituds en la morfologia i cicle de vida. Els hipnozoits, o formes dorments del fetge, són característiques d'aquests dos tipus de *Plasmodium*, ambdues espècies infecten els eritròcits joves, presenten el puntejat de *Schüffner*, tendeixen a no tenir múltiples anells per eritròcit infectat, i contenen pigment de malària [32,33].

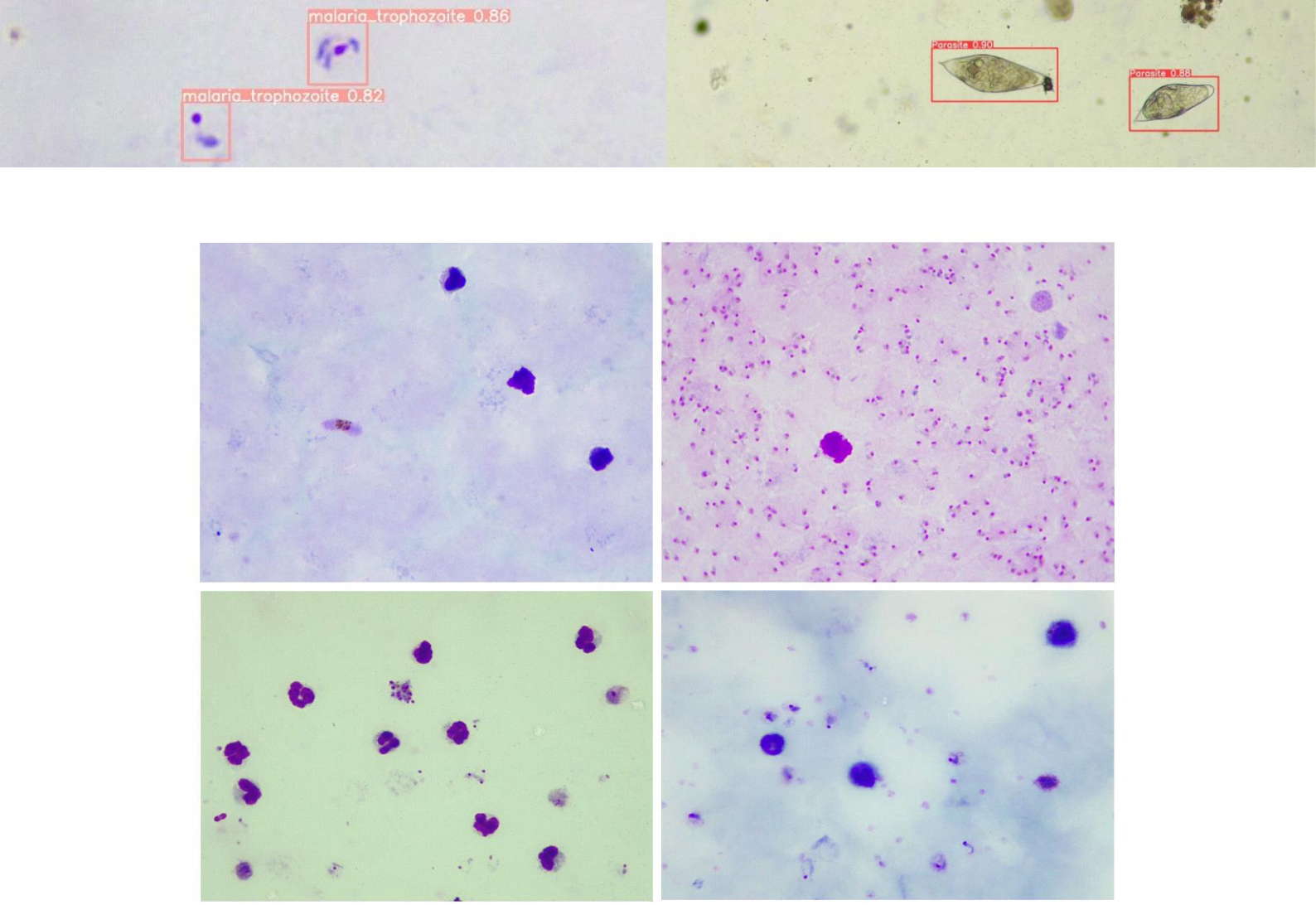
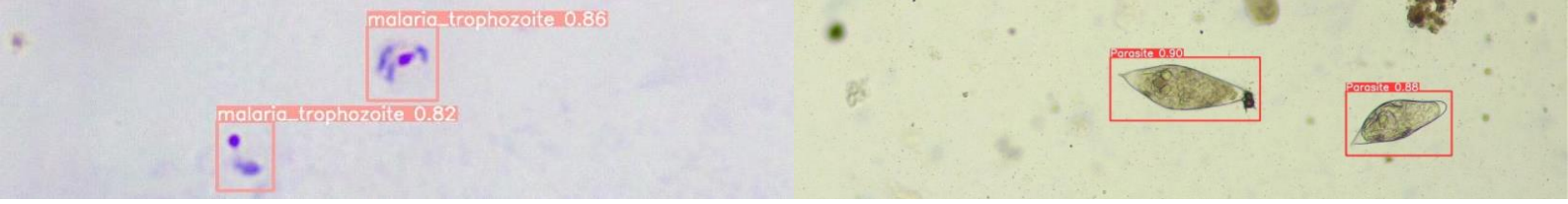
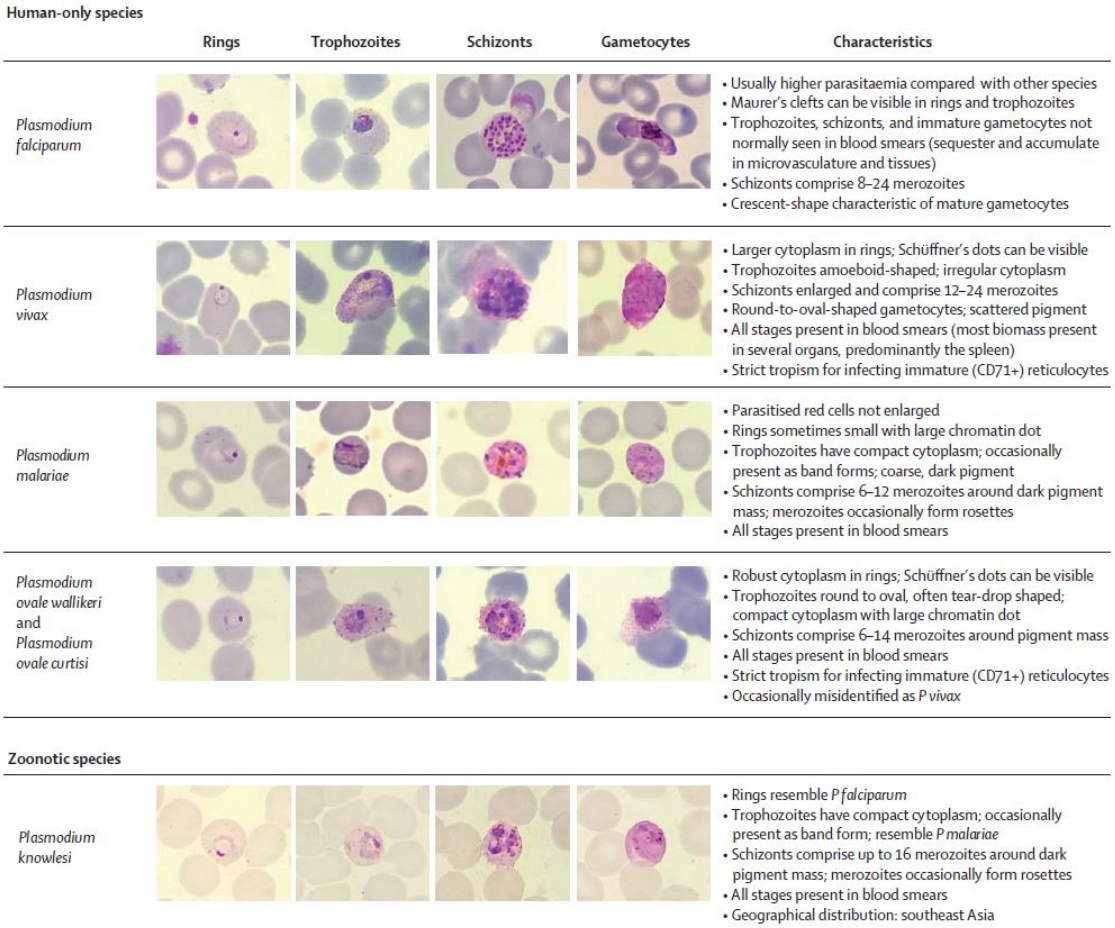


Figura 4 *Imatges microscòpiques de paràsits de Plasmodium en mostres de gota gruixuda de sang tenyides amb Giemsa. Imatges a 1000x augments. S'observa la presència trofozoïts immadurs, madurs, esquizonts i gametòcits. Imatges del Laboratori de Microbiologia del Centre de Malalties Transmissibles i Salut Internacional Drassanes-Vall d'Hebron.*

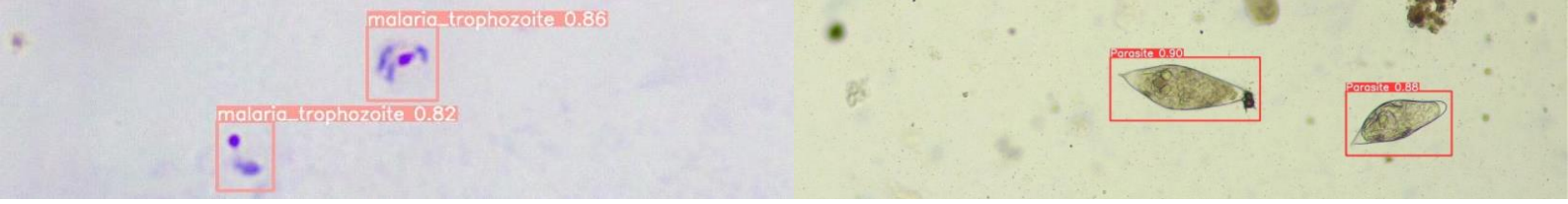
Per altra banda, *P. malariae* normalment causa nivells de paràsitemia més baixos, a causa del seu cicle de desenvolupament de 72h (24 h més llarg que en el cas de *P. falciparum* i *P. vivax*), genera una menor producció de merozoïts per cicle eritrocitari, i té predilecció per desenvolupar-se dins dels eritròcits més vells [34]. Finalment, *P. knowlesi* està present al Sud-est asiàtic i originalment era coneguda com la "*malària del simi"*. A causa del seu cicle asexual curt, de 24 hores de desenvolupament, la infecció per *P. knowlesi* pot progressar ràpidament i causar malària greu. Les formes de trofozoït jove de *P. knowlesi* s'assemblen a les de *P. falciparum*, i els trofozoïts i esquizonts madurs són similars a les formes de *P. malariae* [35,36].





Taula 1 *Taula resum de les espècies de Plasmodium que infecten als humans en mostres d'extensió fina. Es detallen les característiques morfològiques dels paràsits i de les seves fases del desenvolupament [2].*

Així doncs, la morfologia dels eritròcits i les característiques distintives en funció de l'espècie permeten determinar, amb els coneixements necessaris, quina espècie/s de *Plasmodium* trobem a la mostra biològica observada. En el cas de la gota gruixuda de sang els paràsits es visualitzen fora dels eritròcits, ja que s'hemolitzen durant el procés de fixació i tinció. L'absència dels eritròcits en les mostres de gota gruixuda dificulta la diferenciació de les espècies de *Plasmodium* en aquest tipus de mostres, encara així la visualització de gametòcits de *P. falciparum* o la presència d'esquizonts o trofozoïts madurs típics de *P. malariae*, permeten realitzar una orientació diagnòstica abans de la visualització de l'extensió fina de sang. Els càlculs de la densitat de paràsits en sang es realitzen manualment en ambdós tipus de mostres. L'observació per microscòpia òptica directa és una tècnica tediosa i que requereix experiència i entrenament. El procés d'observació de la preparació se sol realitzar mitjançant moviments



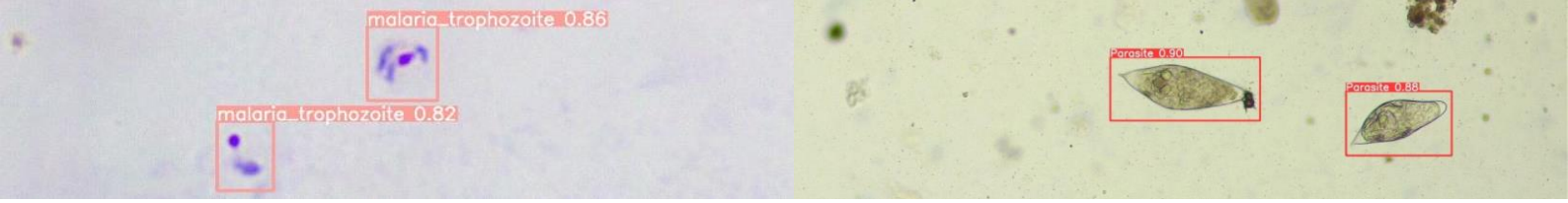
rectes horitzontals i verticals, per evitar observar els mateixos camps. La zona observada també és determinant, sobretot en les mostres d'extensió fina, sent la part distal la més òptima a causa de la no superposició dels eritròcits [37]. La visualització contínua de les mostres podria desencadenar errors diagnòstics a causa de la dificultat del procediment i la fatiga. La qualitat del microscopi i els reactius de tinció també són factors determinants, i limitants en cas de no disposar de bona qualitat. Els casos de falsos negatius condueixen a la progressió de la malària greu, derivant, en ocasions, en la mort del pacient. No obstant això, l'examen microscòpic s'utilitza habitualment en zones endèmiques i en entorns amb pocs recursos, a causa de la seva disponibilitat i fàcil maneig. La microscòpia també s'utilitza en països industrialitzats, conjuntament amb altres tècniques analítiques que poden complementar i millorar l'examen microscòpic.

Quantitative Buffy Coat (QBC)

El test *Quantitative Buffy Coat* (QBC) és un mètode de detecció qualitativa per detectar ràpidament la presència de paràsits de malària en sang centrifugada, tant capil·lar com venosa [38]. La sang és centrifugada en tubs QBC capil·lars, especialment recoberts, i es visualitza per microscòpia òptica de fluorescència. La tècnica es basa en un gradient de densitat que separa les cèl·lules sanguínies i permet la identificació de formes parasitàries mitjançant l'observació microscòpica fluorescent del tub capil·lar [39,40]. El tint que s'utilitza habitualment és taronja d'acridina, que permet la identificació de paràsits entre les àrees d'eritròcits i leucòcits. Els tubs QBC també tenen un anticoagulant per a la correcta visualització de la mostra i per evitar artefactes a causa de la coagulació de la sang. Presenta una sensibilitat diagnòstica més alta que la microscòpia convencional a causa de la concentració addicional de paràsits a la zona estreta dels tubs de sang. En canvi, té alguns inconvenients: requereix personal especialitzat, instrumentació específica, és més costosa que la microscòpia convencional, i és difícil determinar l'espècie i el nombre de paràsits. Encara així, és una tècnica de gran utilitat per realitzar diagnòstics ràpids, sempre que es disposi del material necessari.

Proves antigèniques de diagnòstic ràpid

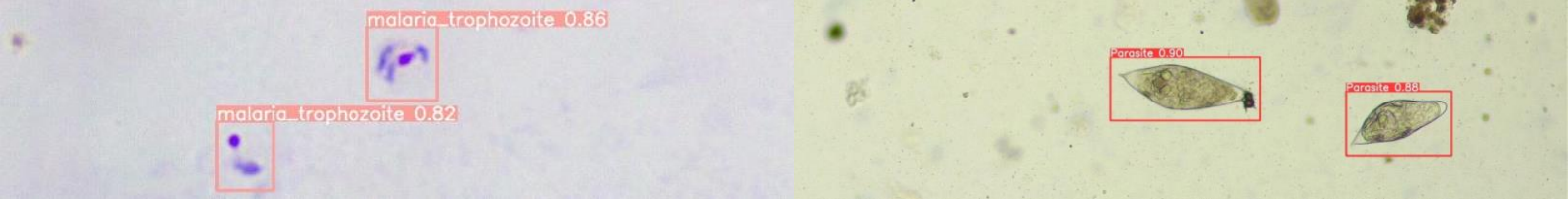
Les proves antigèniques de diagnòstic ràpid (PDR) són una opció adequada i complementària per detectar la infecció per *Plasmodium*. Les PDR són immunoassaigs de flux lateral que permeten la visualització d'interaccions específiques de reconeixement antigen-anticòs [41,42]. Atorguen un diagnòstic qualitatiu amb un temps de resposta ràpida, en la majoria dels casos de menys de 15-20 minuts. Les PDR funcionen envers l'observació d'una banda visible d'una tira de



nitrocel·lulosa produïda per la captura d'anticossos etiquetats amb marcadors colorimètrics. Una gota de sang perifèrica i una solució tampó s'utilitzen normalment per realitzar el diagnòstic en el dispositiu PDR mitjançant la detecció d'antígens específics de *Plasmodium*. La majoria de les PDR es basen en la detecció de la proteïna específica de *P. falciparum* anomenada Proteïna Rica en Histidina II (PRH2) [43]; o dianes d'antigen universals per a qualsevol espècie de *Plasmodium*, com ara la lactat deshidrogenasa (p-LDH) o l'aldolasa [44]. Diversos estudis han observat delecions genètiques en el gen codificant per la PRH2 (*pfhrp2*) i la seva homòloga, la PRH3 (*pfhrp3*) de *P. falciparum*, fet implicat en la ineficàcia de les PDR, produint resultats de falsos negatius [45–47]. Aquests resultats de falsos negatius a causa de la mutació del gen *pfhrp2/3* podrien desencadenar un problema de salut global. La baixa densitat del paràsit en sang, la interpretació incorrecta dels resultats o la dificultat per detectar les infeccions per *P. malariae* i *P. ovale* amb antígens panmalàrics també són causes de resultats falsos negatius. L'efecte prozona a causa de l'excés d'antigen podria provocar un diagnòstic incorrecte, tot i que no és un esdeveniment comú [48,48,49]. Els resultats de falsos positius són menys comuns, però plausibles. La reactivitat creuada a causa d'alts nivells d'antigen del paràsit o la presència d'altres antígens són les principals causes de resultats falsos positius [50]. Les PDR són una eina útil de suport al diagnòstic, però no haurien de substituir l'examen microscòpic.

Reacció en cadena de la polimerasa (PCR)

El diagnòstic molecular mitjançant la tècnica de la reacció en cadena de la polimerasa (PCR) és una alternativa complementària adequada a les tècniques convencionals. Es basa en l'amplificació de l'ADN de *Plasmodium*, i té una alta sensibilitat, especificitat i una complexitat metodològica relativament baixa [51]. És més sensible que la microscòpia, i permet identificar els paràsits de malària a nivell d'espècie quan els mètodes convencionals no són capaços de detectar-los. A més, la determinació d'espècies de *Plasmodium* mitjançant l'assaig PCR permet el diagnòstic inequívoc en la infecció mixta per vàries espècies o amb nivells baixos de parasitèmia, que són difícils de detectar mitjançant l'examen microscòpic. Alguns dels principals inconvenients del diagnòstic amb PCR són: la implementació d'una tècnica no rutinària en zones remotes, el temps necessari (2-3 h) pel diagnòstic, els requeriments de reactius i equipaments, i l'alt cost de la tecnologia. Actualment, la PCR s'està implementant com a tècnica diagnòstica per a la malària, tot i que no és el procediment *gold standard* i no s'utilitza majoritàriament en països endèmics. Les tècniques moleculars són útils per detectar infeccions en pacients asimptomàtics o amb nivells de parasitèmia molt baixos; el seu rendiment amb aquesta



casuística és considerablement millor al que tenen les altres tècniques diagnòstiques habitualment emprades. Aquesta tècnica de diagnòstic molecular s'usa majoritàriament en països o regions d'índex de desenvolupament alt i/o molt alt per realitzar estudis epidemiològics, per confirmació del diagnòstic i per cribratges en pacients asimptomàtics [52,53].

Amplificació isotèrmica per bucles (LAMP)

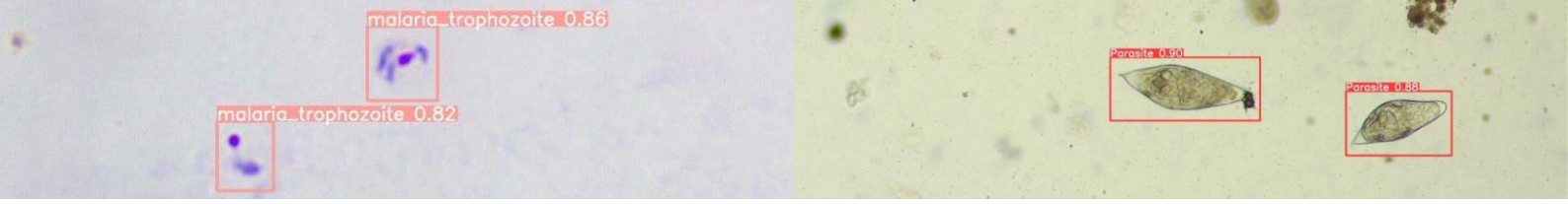
L'amplificació isotèrmica per bucles, més coneguda com a *Loop Mediated Isothermal Amplification* (*LAMP)*, és una tècnica molecular basada en l'amplificació d'àcids nucleics mitjançant l'ADN polimerasa de *Bacillus stearothermophilus* [54,55]. Alguns estudis determinen que la LAMP té una sensibilitat del 95.6% per a la detecció de paràsits de malària en comparació amb la microscòpia, i no requereix termocicladors [56]. Normalment, es necessita un espectrofotòmetre de fluorescència per llegir els resultats diagnòstics, la qual cosa dificulta l'aplicabilitat a les zones més rurals. No obstant això, els nous assajos amb LAMP estan dissenyats amb una unitat de lectura de fluorescència integrada per tal de detectar paràsits de *P. falciparum*, i en alguns casos permeten interpretar el resultat per canvis de terbolesa [57]. No està àmpliament implementat com a mètode diagnòstic, tot i que es postula com una alternativa interessant als mètodes moleculars convencionals, i podria ser progressivament implementat en entorns amb pocs recursos.

Tècniques serològiques

La serologia es basa en la detecció d'anticossos contra els paràsits de la malària. No s'utilitza habitualment per a un diagnòstic ràpid de la malaltia, sinó per realitzar estudis de seroprevalença. Com a exemple, el Testing d'Anticòs d'Immunofluorescència (TAI) utilitza antígens específics per a la quantificació d'anticossos IgG i IgM en mostres de sèrum [58]. Les estratègies combinades que utilitzen dades serològiques, de detecció d'antígens i d'ADN s'utilitzen per estimar la transmissió de la malària i realitzar estudis epidemiològics [59].

Citometria de flux

La citometria de flux és un mètode de comptatge de cèl·lules basat en un làser que permet la quantificació d'eritròcits infectats per paràsits de la malària. Ofereix els nivells de parasitèmia de manera automatitzada i té una sensibilitat generalment baixa. Nous avenços basats en la citometria de flux de fluorescència han demostrat que el dispositiu Sysmex XN-31 pot



determinar l'espècie de *Plasmodium* i quantificar els paràsits en sang [60]. No obstant això, pot generar falsos positius en cas de morfologia cel·lular anormal dels eritròcits.

Biomarcadors

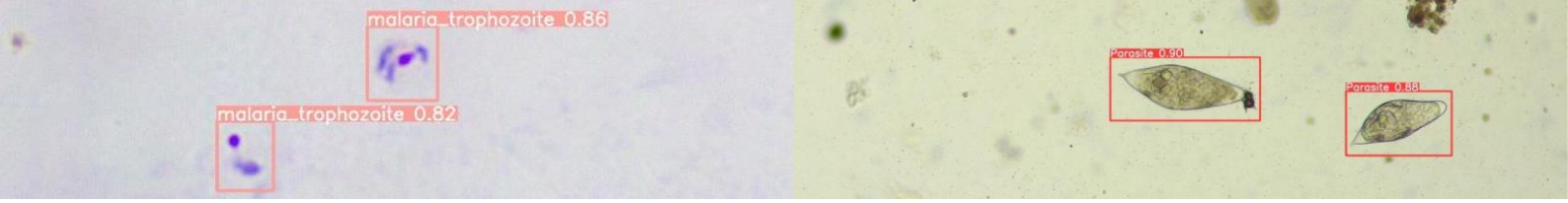
Els biomarcadors són alteracions cel·lulars, bioquímiques o moleculars que indiquen la presència de respostes biològiques, patogèniques o terapèutiques, amb un alt potencial diagnòstic [61]. El desenvolupament de la detecció de biomarcadors de malària, de biomarcadors múltiplex per a múltiples infeccions, i de biosensors són noves propostes a considerar com a eines de diagnòstic.

La **Taula 2** mostra un resum dels avantatges i inconvenients de cadascuna de les tècniques esmentades.

Diagnostic technique	Advantages	Disadvantages	References
Microscopic examination	(i) Availability (ii) Low-cost diagnosis (iii) Parasite level calculations (iv) Species identification	(i) Requires expert personnel (ii) Results are expert-dependent	Dowling and Shute (1966), Collins and Jeffery, (2007), Guintran et al. (2006), Wangai et al. (2011), Poostchi et al. (2018), Heide et al. (2019), *Malaria diagnosis and treatment* CDC, 2019
Quantitative Buffy Coat (QBC)	(i) Fast preparation and diagnosis results (ii) High sensitivity	(i) Requires expert personnel (ii) Requires fluorescent microscopy (iii) Specialized instrumentation	QBC Malaria Test, 2007, Tangpukdee et al. (2009), Siciliano and Alano (2015), *About Malaria* CDC, 2019, Shujatullah et al., 2006
Rapid Diagnostic Tests (RDTs)	(i) Fast preparation and diagnosis results (ii) Easy handling (iii) Low-cost diagnosis (iv) Species identification (usually *P. falciparum* from non-*P. falciparum* species)	(i) *pfHRP2/3* gene deletions (ii) Low sensitivity with low parasite levels (iii) Low sensitivity with *P. ovale* and *P. malariae* species. (iv) Cross-reactivity (v) Prozone effect	Wongsrichanalai et al. (2007), Gillet et al. (2009), Murray and Bennett (2009), Tangpukdee et al. (2009), Bejon et al. (2014), Nima et al. (2017), Orish et al. (2018), Cunningham et al. (2019), *Response plan to phrp2 gene deletions* (WHO, 2019), Ajakaye and Ibukunoluwa (2020), Kavanaugh et al. (2021), Kavanaugh et al. (2021)
PCR	(i) High sensitivity and specificity (ii) Species identification (iii) Reference tool for comparative studies	(i) Specialized instrumentation (ii) Difficult implementation in endemic areas (iii) Expensive diagnosis	Johnston et al. (2006), Li et al. (2014), Poostchi et al. (2018), Siwal et al. (2018), Haanshuus et al. (2019), Eshag et al. (2020), Leski et al. (2020), Feufack-Donfack et al. (2021), Mwenda et al. (2021)
LAMP	(i) High sensitivity and specificity (ii) Species identification (iii) No thermocyclers needed	(i) Specialized instrumentation (ii) Expensive diagnosis	Ocker et al. (2016), Selvarajah et al. (2020), Morris and Aydin-Schmidt (2021)
Serology	(i) Seroprevalence (ii) Malaria transmission	(i) Non-reliable diagnostic technique (ii) Not indicative of active infection	Tangpukdee et al. (2009), Oviedo et al. (2020)
Flow cytometry	(i) Quantification of infected erythrocytes (ii) Automated parasite level calculations	(i) Low sensitivity (ii) Specialized instrumentation (iii) Difficult implementation in endemic areas	Poostchi et al. (2018), Khartabil et al. (2022)
Biomarkers	(i) High diagnostic potential (ii) Easy handling	(i) Specialized instrumentation	Jain et al. (2014), Krampa et al. (2017))

QBC: Quantitative Buffy Coat, RDTs: Rapid Diagnostic Tests, PCR: Polymerase Chain Reaction, LAMP: Loop-Mediated Isothermal Amplification, Serology, Flow cytometry, and biomarkers.

***Taula 2** Avantatges i desavantatges de les tècniques de diagnòstic de la malària [6].*



1.1.5. Tractament

El tractament de la malària dependrà de l'espècie de *Plasmodium* que infecti al pacient, i de la fase i severitat de la malaltia. És per això que dividim el tractament segons malària no complicada o malària severa. A la **Taula 3** està representat un resum dels tractaments recomanats per la malària segons l'OMS.

***Panel 2*: Malaria treatment[81]**

Severe malaria

First line

- Intravenous artesunate: 2·4 mg/kg per dose at 0 h, 12 h, and 24 h, then every 24 h if necessary; if bodyweight is <20·0 kg, give 3·0 mg/kg per dose to ensure equivalent exposure to the drug

Alternatives if artesunate not available

- Intramuscular artemether: initial dose 3·2 mg/kg, then 1·6 mg/kg every 24 h
- Intravenous quinine diluted in 5% dextrose: loading dose of 20 mg/kg infused over 4 h, then 10 mg/kg every 8 h infused no faster than 5 mg/kg per h
- Pre-referral rectal artesunate: recommended in primary health-care settings in which parenteral drug administration is not possible[104]

Once eating and drinking

- Give full course of oral antimalarial treatment as per guidelines for uncomplicated malaria
- Avoid mefloquine-containing artemisinin-based combination therapies (ACT) in patients initially presenting with impaired consciousness because of increased risk of post-malaria neurological syndrome

Uncomplicated malaria

Uncomplicated Plasmodium falciparum, Plasmodium knowlesi, *or chloroquine-resistant* Plasmodium vivax *malaria*

- Artemether–lumefantrine: 0·83–4·00 mg/kg artemether and 4·83–24·00 mg/kg of lumefantrine given twice a day for 3 days with fatty food; the first two doses should be given 8 h apart
- Artesunate–mefloquine: 4 mg/kg per day artesunate (range 2–10 mg/kg) and 8·3 mg/kg per day mefloquine (7–11 mg/kg) given once a day for 3 days
- Dihydroartemisinin–piperaquine: 4 mg/kg per day dihydroartemisinin (range 2–10 mg/kg) and 18 mg/kg per day piperaquine (16–27 mg/kg) given once a day for 3 days for adults and children weighing ≥25 kg; children weighing <25 kg: at least 2·5 mg/kg per day dihydroartemisinin and 20 mg/kg per day piperaquine given once a day for 3 days
- Artesunate–amodiaquine (sensitive *P falciparum* only): 4 mg/kg per day artesunate (range 2–10 mg/kg) and 10 mg/kg per day amodiaquine (7·5–15 mg/kg) once a day for 3 days
- Artesunate–sulfadoxine–pyrimethamine (sensitive *P falciparum* only): 4 mg/kg per day artesunate (range 2–10 mg/kg) given once a day for 3 days and a single administration of at least 25 mg/kg sulfadoxine (25–70 mg/kg) and 1·25 mg/kg pyrimethamine (1·25–3·5 mg/kg) given as a single dose on day 1
- Infants weighing <5 kg with uncomplicated *P falciparum* malaria: treat with ACT at the same mg/kg dose as for children weighing 5 kg
- In the first trimester of pregnancy, use artemether–lumefantrine in preference to the other ACTs

Reducing risk of transmission of P falciparum *in endemic areas*

- Primaquine* single dose of 0·25 mg/kg without G6PD testing (maximum 15 mg base) given concomitantly with blood schizontocidal treatment

Uncomplicated chloroquine-sensitive P vivax, Plasmodium malariae, *and* Plasmodium ovale *malaria*

- Chloroquine: 10 mg base per kg day 1 and day 2, 5 mg base per kg on day 3; for children aged <5 years, total dose 30 mg/kg over 3 days

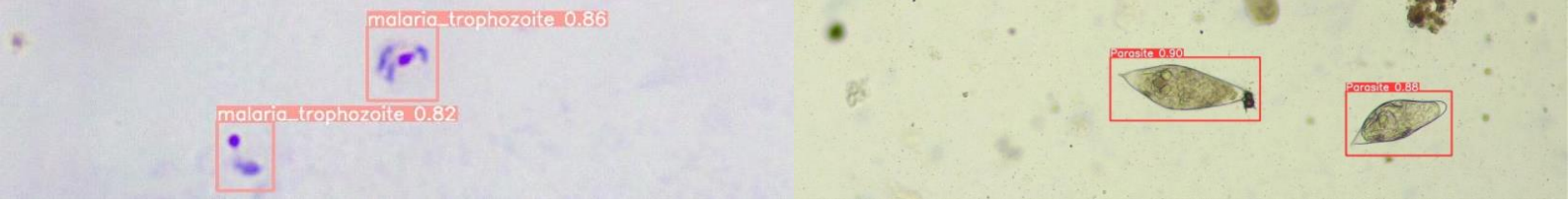
Antirelapse therapy for P vivax *or* P ovale *malaria*

- Primaquine*: 0·25–0·50 mg/kg per day for 14 days given concomitantly with blood schizontocidal therapy and after exclusion of G6PD deficiency; lower risk of relapse in areas of high relapse periodicity if a dose of 0·5 mg/kg per day is used

*Primaquine is contraindicated in pregnant women, infants aged <6 months, and women breastfeeding infants aged <6 months.

Taula 3 *Taula resum del tractament de la malària recomanat per l'Organització Mundial de la Salut [2].*

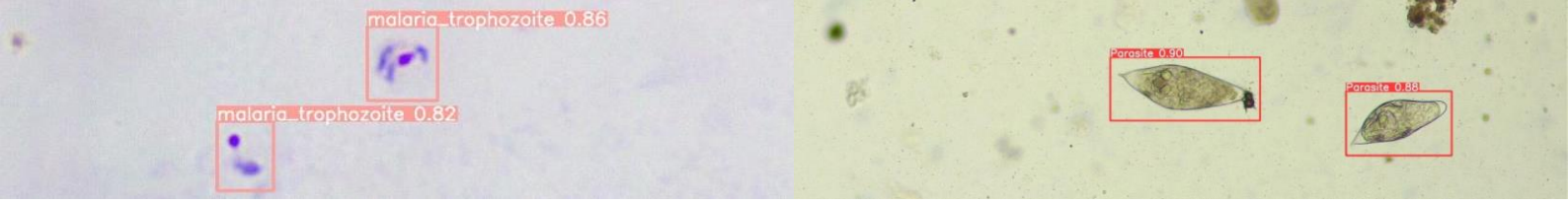
Per la malària no complicada, els pacients han de ser tractats amb combinats de les artemisines. Aquests tipus de tractaments eliminen el paràsit ràpidament de la sang, i progressivament van eliminant els romanents. Els tractaments combinats de les artemisines estan recomanats i



aprovats per l'OMS [28]. El més utilitzat a nivell global és el artemeter-lumefrantina (Coartem®) [62], encara que d'altres com el artesunat-pyronaridina han demostrat la mateixa eficàcia. La cloroquina també pot ser utilitzada per la infecció de totes les espècies de *Plasmodium*, encara que es va deixar d'utilitzar per *P. falciparum* degut a l'augment de resistències [63]. Actualment, l'OMS recomana el tractament amb combinats d'artemisina per les embarassades amb malària no complicada, a excepció del artesunat-sulfadoxina-pyrimethamina [28].

Els pacients amb malària severa han de ser tractats amb artesunat intravenós el més aviat possible, a causa de les disfuncions vitals que no els permeten prendre tractament oral [28]. En cas que l'artesunat no estigui disponible, es recomana injectar intramuscularment arthemeter, encara que és una mesura menys efectiva considerada de segona línia. La quinina intravenosa solament hauria de ser utilitzada en cas que no es disposi de tractament amb artemisines, o en regions on hi hagi resistències. Es recomana un mínim de 24 h amb tractament parenteral abans de passar a l'oral. Hi ha un seguit de tractaments de suport per la malària severa, que permeten mitigar i/o resoldre altres afectacions clíniques associades a la infecció [2]. En un 5-12% dels casos de malària severa per *P. falciparum* en infants s'observa la coinfecció per l'espècie del bacteri *Salmonella* [64]. La infecció per aquest bacteri pot produir sèpsia i/o meningitis, i és normalment tractada amb antibiòtics d'ampli espectre degut a les dificultats per la identificació bacteriana en àrees amb pocs recursos. Els nivells de parasitèmia dels pacients amb malària severa es recomana que es monitoritzin cada 12-24 hores, per així també comprovar l'eficàcia del tractament [65]. Altres mesures adjuntives, com el paracetamol, poden ajudar a reduir la simptomatologia del pacient.

En casos d'infecció amb les espècies *P. vivax* i *P. ovale* s'han d'administrar tractaments específics per eliminar les formes d'hipnozoits del fetge. L'opció més utilitzada és la 8-aminoquinolina primaquina (3.5-7.0 mg/kg), encara que d'altres tractaments com la tafenoquina també estan disponibles en algunes regions. Les dues drogues són incompatibles amb el dèficit de glucosa 6 fosfat deshidrogenasa (G6PD), i per tant és imprescindible estudiar l'enzim abans de prescriure el tractament [66]. Encara així, en molts països amb pocs recursos no es realitza la prova de la G6PD, amb el risc de poder induir hemòlisi en els pacients amb aquest dèficit. Alguns estudis demostren que el tractament amb primaquina en dosis més baixes (0.75 mg/kg) durant vuit setmanes, i amb els controls hematològics pertinents, poden ser una opció pels pacients amb dèficit de G6PD [67].



1.2. Les Malalties Tropicals Desateses

Les Malalties Tropicals Desateses (MTD) són un grup divers de malalties causades per multitud de patògens (virus, bacteris, paràsits, fongs i toxines) i associades a devastadores conseqüències sanitàries, socials i econòmiques. Les MTD són predominants entre les comunitats empobrides de les zones tropicals del Sud Global, encara que algunes tenen una distribució geogràfica molt més àmplia. S'estima, segons dades de l'OMS del 2024, que les MTD afecten més de 1.000 milions de persones, mentre que el nombre de persones que requereixen intervencions a causa de les MTD, tant preventives com terapèutiques, és d'uns 1.600 milions [68].

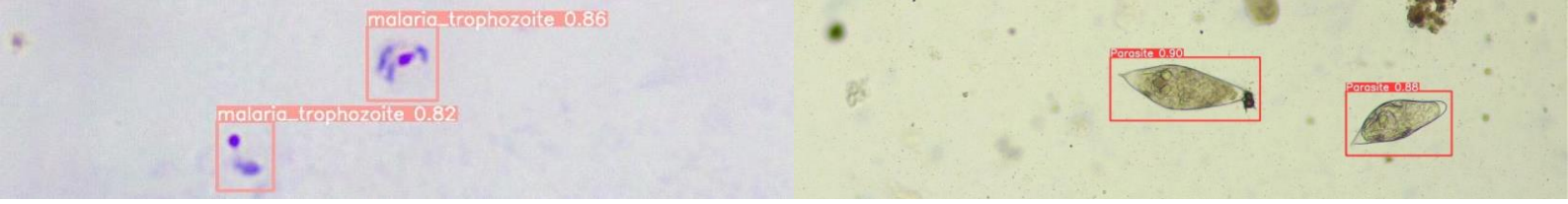
L'epidemiologia de les MTD és complexa i sovint relacionada amb les condicions ambientals. Moltes d'aquestes malalties són transmeses per vectors, tenen reservoris d'animals i estan associades a cicles de vida complexos. Tots aquests factors fan que el seu control a nivell de salut pública i global sigui un repte.

El llistat oficial de les MTD l'any 2024 segons l'OMS engloba les següents malalties: úlcera de Buruli; malaltia de Chagas; dengue i chikungunya; dracunculiasis; equinococcosi; trematodiasi alimentària; tripanosomosi humana africana (malaltia de la son); leishmaniosi; filariasi limfàtica; micetoma, cromoblastomicosi i d'altres micosis; sarna; oncocercosi; ràbia; esquistosomasi; helmintiasis transmeses pel sòl; teniasis/cisticercosi; tracoma; i malaltia del Pian [68].

1.3. L'esquistosomiasi

L'esquistosomiasi és una malaltia parasitària aguda i crònica causada per cucs del trematode del gènere *Schistosoma (S.)*, considerada per l'OMS com una MTD [69]. És la malaltia parasitària amb major morbimortalitat a nivell global, després de la malària [70]. Hi ha dues formes principals d'esquistosomiasi: l'esquistosomiasi intestinal (EI) i l'esquistosomiasi urogenital (EU) [71]. L'EI és causada per *S. mansoni*, *S. japonicum*, *S. mekongi*, *S. guineensis* i *S. intercalatum*. L'EU és causada per una única espècie anomenada *S. haematobium*. La distribució geogràfica de la malaltia està relacionada amb el tipus d'infecció i l'espècie. Es troba principalment a Àfrica, regions del Carib, Brasil, Xina, Indonèsia i Filipines.

Els símptomes de l'esquistosomiasi són causats per la reacció immunològica i física del cos als ous dels paràsits. El resultat d'aquesta infecció pot derivar en dolor abdominal, diarrea i sang a les femtes/orina. També pot produir engrandiment hepàtic i de la melsa, acumulació de fluid a la cavitat peritoneal i hipertensió dels vasos sanguinis abdominals. Altres manifestacions

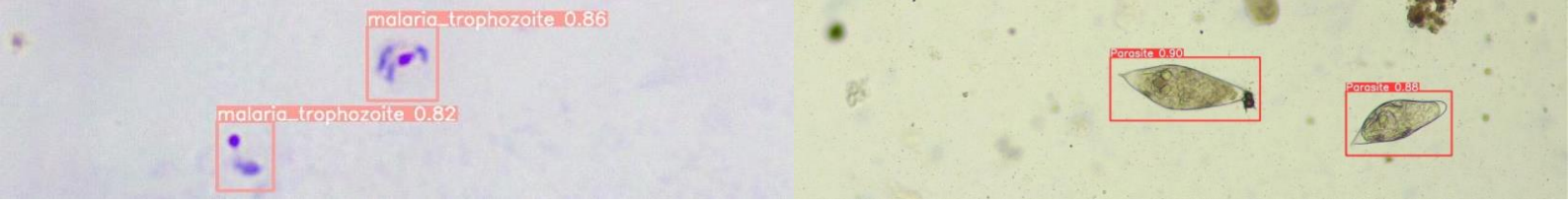


clíniques i biològiques de l'EU són: l'hematúria, la fibrosi de la bufeta i l'úter, i el dany renal. En fases cròniques greus pot produir càncer de bufeta, afectació de zones genitals, infertilitat, i insuficiència hepàtica i renal [72].

La tècnica *gold standard* pel diagnòstic de l'esquistosomiasi és la detecció d'ous dels paràsits en mostres de femta i/o orina. La visualització microscòpica amb concentració prèvia ens permet augmentar la sensibilitat diagnòstica. Els anticossos i la detecció d'antígens en mostres de sang o orina també són utilitzats com a indicadors d'infecció. Les tècniques moleculars PCR també s'estan implementant en països industrialitzats per la detecció del material genètic dels paràsits en mostres d'orina i femta.

El praziquantel és el tractament antiparasitari recomanat contra totes les formes d'esquistosomiasi. És eficaç, segur i de baix cost. La reinfecció pot ocórrer després del tractament, encara que el risc de desenvolupar una malaltia greu es redueix. També, el risc de reinfecció es reverteix quan el tractament s'inicia i es repeteix durant la infància.

En les persones, els ous d'esquistosoma s'eliminen en els excrements o en l'orina, segons l'espècie del paràsit. A la **Figura 5** es representa el cicle biològic simplificat de *Schistosoma* spp. En aigua, en condicions òptimes, els ous eclosionen i alliberen larves d'esquistosoma immadur anomenades miracidia o miracidis. Els miracidis neden i penetren en cargols del gènere *Biomphalaria* i *Bulinus*, entre d'altres, que actuen com a hostes intermediaris. Els miracidis evolucionen en esporocists dins del cargol. Els esporocists maduren a fase de cercaria, que són alliberades a l'aigua pel cargol i penetren a la pell dels humans. Les cercaries perden la cua i es converteixen en esquistosòmuls quan penetren a la pell. Les persones o hostes s'infecten quan les formes larvals del paràsit (cercaria) penetren a la pell durant el contacte amb l'aigua infestada. Seguidament, els esquistosòmuls es mouen al fetge per madurar fins a la forma adulta. Els mascles i les femelles es tornen corpulents i es desplacen a les vènules intestinals o blaves depenent de la seva espècie. *S. japonicum*, per exemple, es troba amb més freqüència en les venes mesentèriques superiors que drenen l'intestí prim, mentre que *S. mansoni* es troba amb més freqüència en les venes mesentèriques inferiors que drenen l'intestí gros. Les dues espècies, d'altra banda, poden viure en qualsevol lloc i moure's entre ambdues zones. *Schistosoma intercalatum* i *S. guineensis* es troben en el plexe mesentèric inferior, però es troben més baixos a l'intestí que *S. mansoni*. En el cas de *Schistosoma haematobium* es troba en el plexe venós vesicular i pelvià de la bufeta, així com en les vènules rectals. Allà, on romanen, les femelles comencen a pondre els ous. Els ous es mouen progressivament cap al lumen de



l'intestí (*S. mansoni, S. japonicum, S. mekongi, S. intercalatum/guineensis*) o de la bufeta i uretres (*S. haematobium*), i s'expulsen amb la femta o l'orina, respectivament.

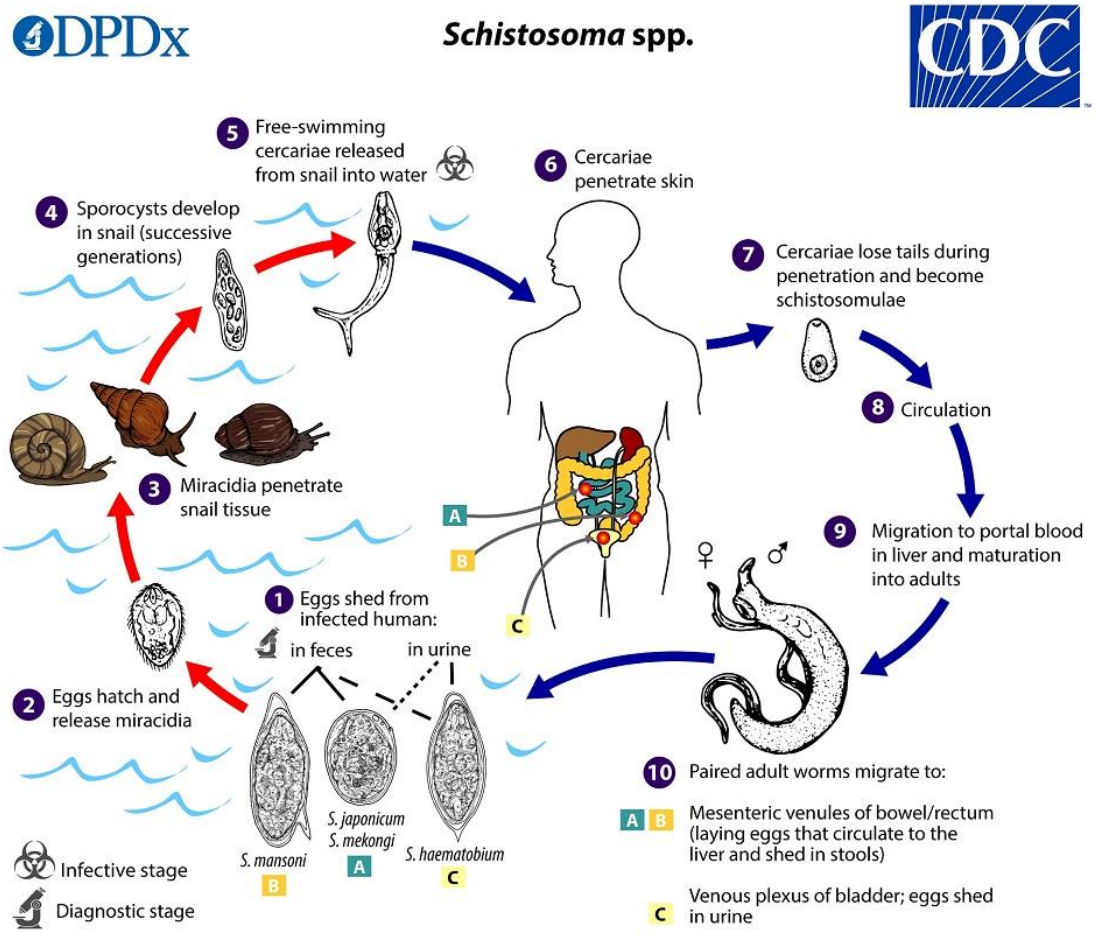
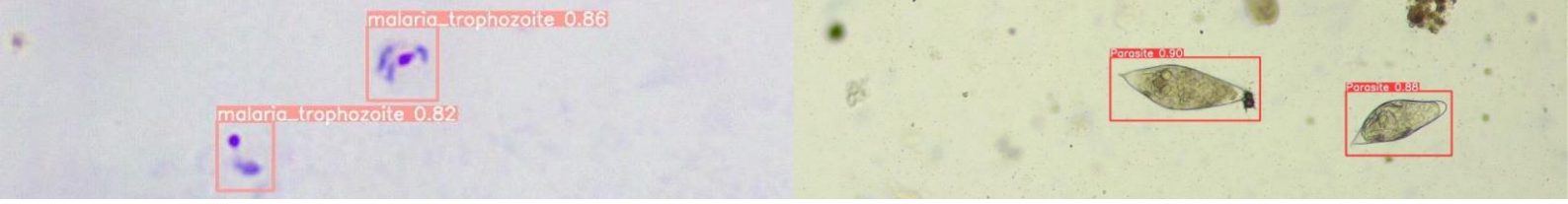


Figura 5 *Representació del Cicle biològic de Schistosoma spp. [27].*

1.3.1. Ressenya històrica

L'esquistosomiasi és una malaltia parasitària coneguda des de l'antiguitat. Fou descrita per primera vegada pel metge alemany Theodor Bilharz el 1851 al Caire, associant-la a l'hematúria, un problema comú entre els pagesos egipcis. La malaltia també és coneguda com a "*bilharziasis*", en honor al seu descobridor [70]. Durant els segles posteriors, es van identificar altres espècies com *Schistosoma japonicum* al Japó, l'any 1847, i *Schistosoma mansoni* a l'any 1915 [73]. Va ser Patrick Manson, el "pare de la medicina tropical", qui va identificar el paper dels cargols aquàtics com a hostes intermediaris en el cicle vital del paràsit. Finalment, Robert Thomson Leiper va completar la comprensió del cicle biològic i va diferenciar entre les espècies *S. haematobium* i *S. mansoni*.



L'esquistosomiasi es va fer rellevant militarment des del segle XVIII, afectant les tropes de Napoleó a Egipte i soldats britànics i australians durant la Guerra dels Bòers. Tot i ser més limitada a la Primera Guerra Mundial, es registraren números notables de casos fins al 1921 i en campanyes posteriors, com la invasió d'Etiòpia. A la Segona Guerra Mundial, destacà entre tropes alemanyes a l'Àfrica i britàniques a Nigèria, així com entre nord-americans al Pacífic, amb 144 casos documentats a Leyte el 1944.

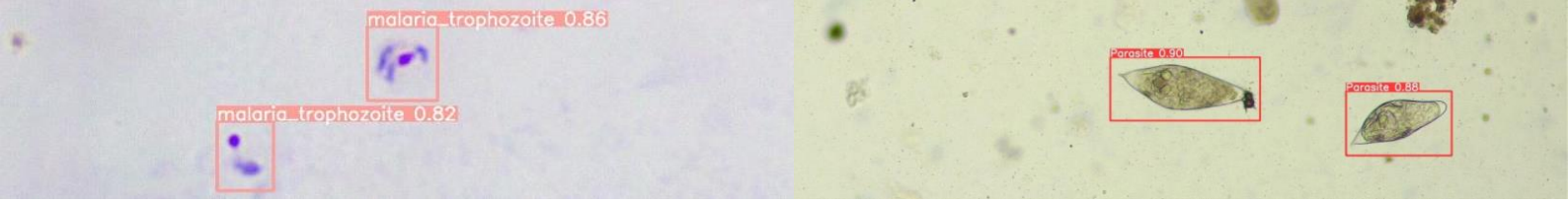
La transmissió del paràsit està directament vinculada a l'aigua contaminada, fet que connecta la malaltia amb l'evolució de la civilització. L'ús de tècniques de regadiu permanent durant el segle XIX, especialment a Egipte, va augmentar dramàticament la prevalença de la malaltia, afectant fins al 70% de la població masculina. També s'han trobat evidències de transmissió a través del comerç d'esclaus a Amèrica, introduint *S. mansoni* al Carib [74].

Mitjançant tècniques de biologia molecular, s'han descobert fragments d'ADN de paràsits en mòmies egípcies del 1250 a.C., confirmant la presència de la malaltia fa mil·lennis. Aquests estudis han permès traçar la relació contínua entre l'esquistosomiasi i la humanitat, destacant el seu impacte sanitari al llarg de la història. Tot i els avenços mèdics, la malaltia encara és un repte de salut global, amb més de 250 milions de persones afectades durant l'any 2021, i complicacions associades com la fibrosi hepàtica i el càncer de bufeta.

En conclusió, l'esquistosomiasi ha estat una amenaça recurrent en zones endèmiques, amb un impacte especialment significatiu durant conflictes bèl·lics del segle XX i durant l'actualitat en regions amb pocs recursos a nivell global.

1.3.2. Epidemiologia

L'esquistosomiasi és freqüent en zones tropicals i subtropicals, especialment en comunitats amb pocs recursos sense accés a aigua potable segura, i sanejament adequat. S'estima que almenys el 90% de les persones que requereixen tractament per a l'esquistosomiasi viuen al continent Africà. Les estimacions de la OMS mostren que almenys 251.4 milions de persones van requerir tractament preventiu per a l'esquistosomiasi durant l'any 2021, dels quals més de 75.3 milions van ser tractats [75]. L'esquistosomiasi afecta sobretot les comunitats pobres i rurals, especialment a les poblacions agrícoles i pesqueres. Les dones que fan tasques domèstiques en aigua infestada, com rentar roba, també estan en risc i poden desenvolupar esquistosomiasi genital femenina. La higiene inadequada i el contacte amb l'aigua infectada fan que els nens siguin especialment vulnerables a la infecció. També, la migració a les zones urbanes i els



moviments de població estan introduint la malaltia a noves regions. L'augment de la població i les necessitats creixents de l'accés a l'aigua, sovint, donen lloc a reestructuracions dels entorns, fet que facilita la transmissió de la malaltia. Amb l'augment de l'eco-turisme i els viatges a zones remotes, un nombre creixent de turistes estan contraient l'esquistosomiasi, que encara sent un problema menor, pot agreujar-se amb el pas dels anys, i introduir la malaltia a zones potencialment endèmiques.

La distribució geogràfica de la malaltia depèn de l'espècie del paràsit. L'espècie *S. mansoni* es troba a l'Àfrica, l'Orient Mitjà, la regió del Carib, Brasil, Veneçuela i Surinam. Tant *Schistosoma japonicum* com *S. mekongi* es troben en regions dels Sud-est Asiàtic i la Xina. Per altra banda, *S. guineensis* i *S. intercalatum* són propis d'àrees boscoses de l'Àfrica central. En el cas de *S. haematobium* el trobem distribuït a l'Àfrica, l'Orient Mitjà i s'han reportat alguns casos autòctons a l'illa de Còrsega (França) i a Almeria (Espanya) [76]. La distribució geogràfica de l'esquistosomiasi a nivell global està representada a la **Figura 6**.

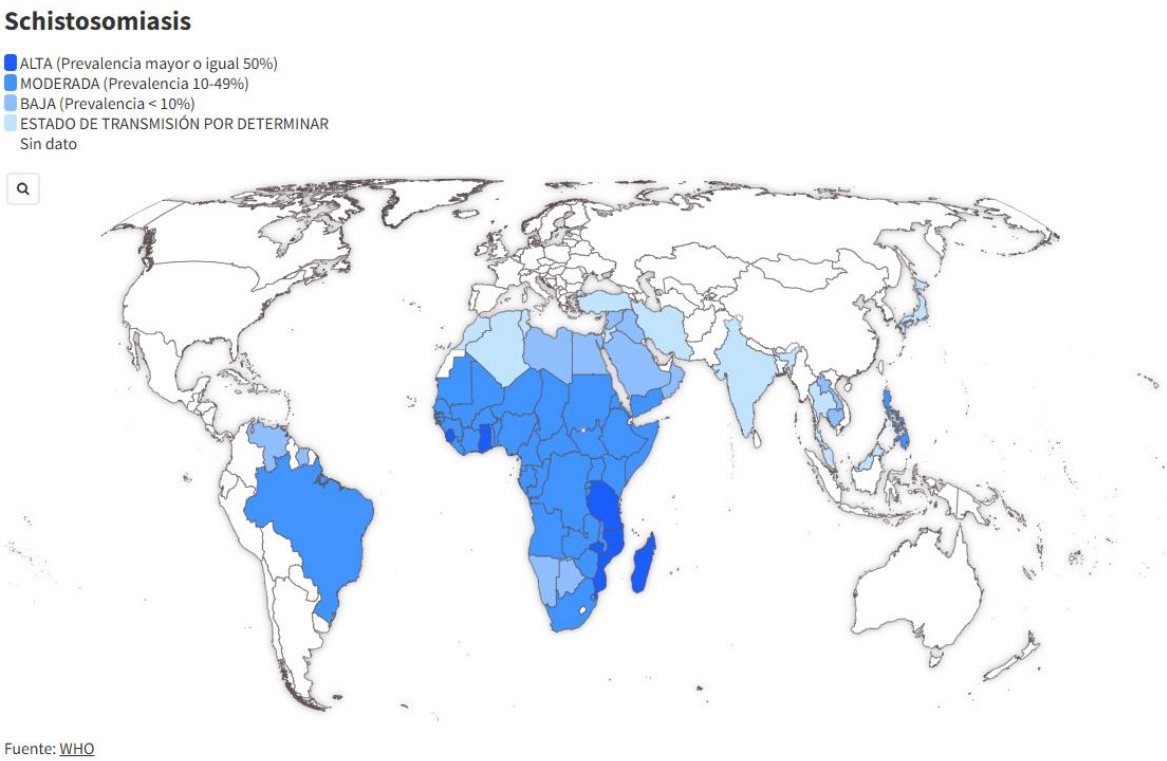
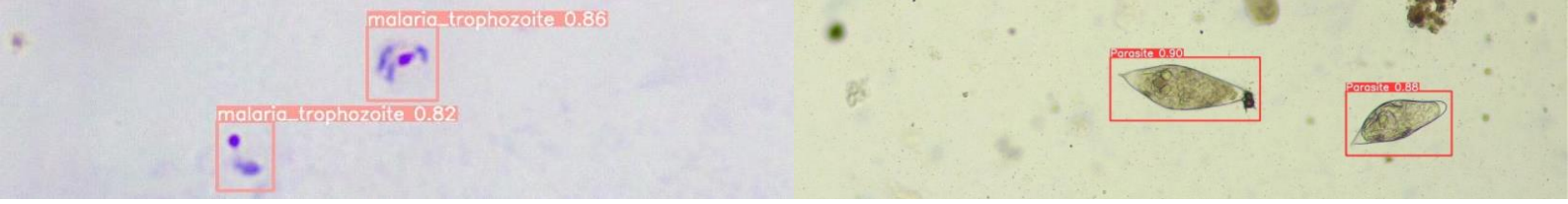


Figura 6 *Mapa interactiu de distribució geogràfica i prevalença de Schistosoma spp. [69].*



1.3.3. Manifestacions clíniques i patogènia

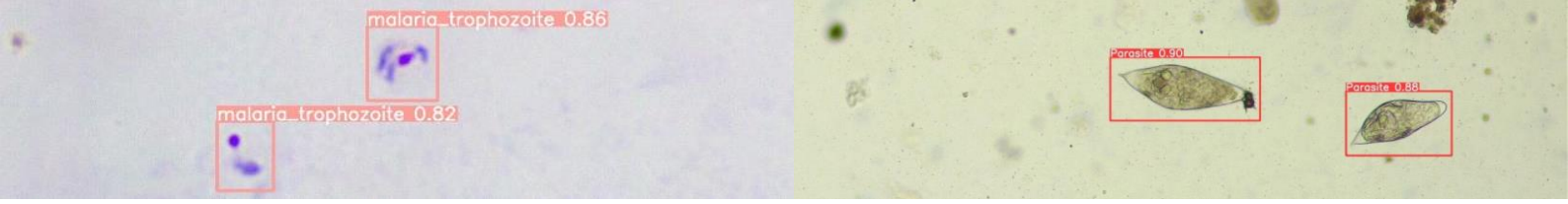
Els símptomes de l'esquistosomiasi humana són causats per la reacció del cos envers els ous dels paràsits, i no pels mateixos paràsits. Moltes infeccions són asimptomàtiques durant llargs períodes de temps. Després de la penetració de les cercaries a través de la pell, es poden produir reaccions cutànies d'hipersensibilitat, manifestant-se com a petites i picants nafres maculopapulars. La síndrome de Katayama és una reacció sistèmica d'hipersensibilitat causada per *S. japonicum* i *S. mansoni* que es pot produir setmanes després de la infecció inicial [77]. Els símptomes i signes clínics més comuns són: dolor estomacal, diarrea, febre, tos, eosinofília i hepatosplenomegàlia.

Les infeccions amb el paràsit d'esquistosoma poden causar lesions en el sistema nerviós central. Els òvuls ectòpics de *S. japonicum* en el cervell poden causar malaltia granulomatosa cerebral. També, les lesions granulomatoses al voltant dels òvuls ectòpics a la medul·la espinal poden ocórrer en infeccions per *S. mansoni* i *S. haematobium*. La infecció persistent pot donar lloc a respostes granulomatoses, i fibrosi en el fetge i la melsa. Diverses complicacions hepàtiques inflamatòries i les reaccions granulomatoses al cervell i la medul·la espinal s'associen amb *S. mansoni* i *S. japonicum*. L'hematúria, la calcificació de la bufeta urinària, el carcinoma de cèl·lules escamoses i els granulomes en el cervell i la medul·la espinal són tots símptomes, signes clínics i complicacions de l'esquistosomiasi produïda per *S. haematobium*.

1.3.4. Diagnòstic

1.3.4.1. Diagnòstic clínic

El diagnòstic clínic de l'esquistosomiasi, com el de moltes altres malalties tropicals, ve determinat per la regió d'origen del pacient, així com la clínica. L'epidemiologia de la malaltia ens pot determinar si és probable o no que el pacient estigui infectat per esquistosomiasi, i quina de les potencials espècies és la més probable. L'exploració física, l'historial mèdic del pacient (si se'n disposa), i els símptomes típics poden donar una orientació diagnòstica abans de realitzar el diagnòstic de laboratori. A l'exploració física, en ocasions, pot existir hepatosplenomegàlia dolorosa. Una estratègia emprada és el diagnòstic de l'esquistosomiasi mitjançant el cribratge del pacient asimptomàtic, ja que la majoria de la població (40-60%) infectada de regions endèmiques és asimptomàtica, i no presenta complicacions fins passats un mínim de 3 mesos des de la infecció [70]. Aquest tipus de pacients, en moltes ocasions, no presenten una clínica compatible, però els antecedents epidemiològics i la regió d'origen són determinants per a



detectar la malaltia. En els nens és freqüent trobar, com a única simptomatologia, una hematúria terminal indolora. És important preguntar de manera específica per aquest símptoma tant en els infants com en els adults en risc, ja que en moltes ocasions els pacients migrants no viuen l'hematúria com una malaltia a causa de la molt elevada prevalença que presenta a les seves regions d'origen [70].

1.3.4.2. Diagnòstic de laboratori

Visualització dels ous de Schistosoma spp. per microscòpia

La tècnica *gold standard* pel diagnòstic de l'esquistosomiasi, tant l'urogenital com la intestinal, és la visualització dels ous del paràsit mitjançant microscòpia òptica. En el cas de l'EU s'observen els ous de *S. haematobium* en mostres d'orina (**Figura 7**). Les tècniques de sedimentació, filtració amb filtres de nitrocel·lulosa i centrifugació són òptims per optimitzar l'eficiència en el diagnòstic de l'esquistosomiasi urogenital. És important destacar que d'altres paràmetres biològics com l'eosinofília i/o l'hematúria i leucocitúria són indicatius de la infecció. En el cas de l'EI s'observen mostres de femtes concentrades o en fresc per l'observació d'ous de *S. mansoni, S. japonicum, S. mekongi* i/o *S. intercalatum/guineensis*. Algunes de les tècniques més utilitzades per l'observació microscòpica dels ous en femtes són les basades en formol i sedimentació, i el Kato-Katz. Les infeccions no produeixen ous visibles en femtes/orina fins dos mesos després del contacte amb l'aigua infestada, fet que limita el diagnòstic primerenc en moltes ocasions [78].

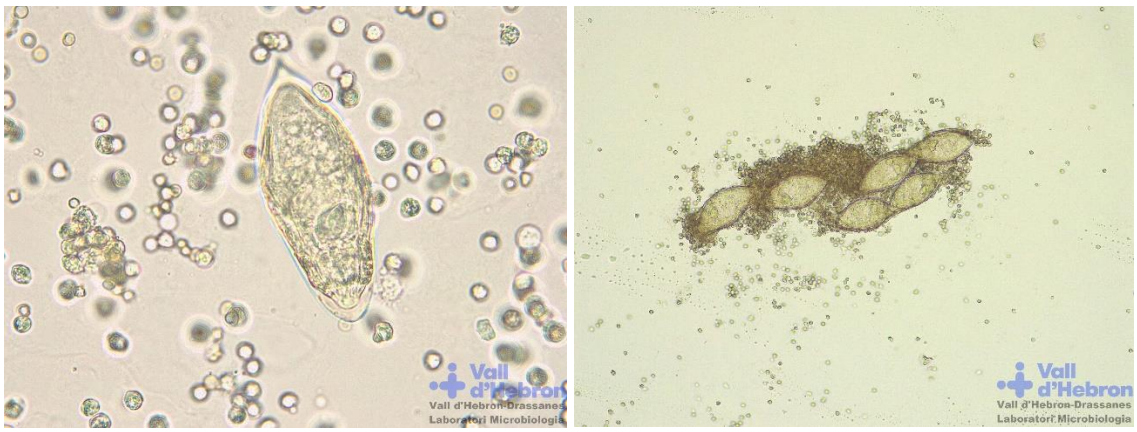
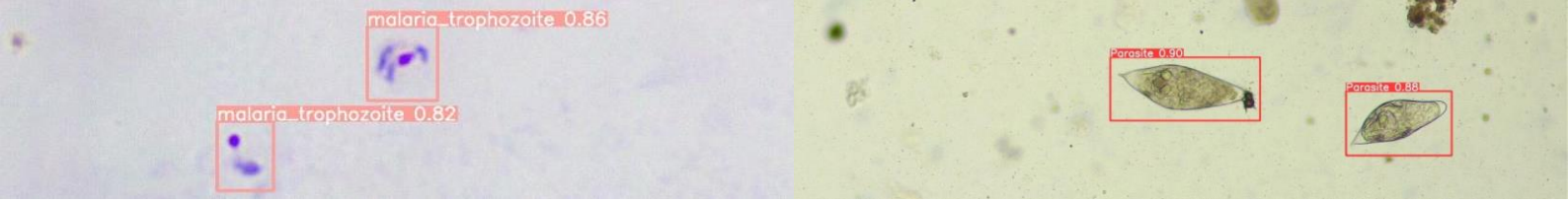


Figura 7 *Imatges microscòpiques d'ous de Schistosoma haematobium en mostres de sediment urinari. Imatge de l'esquerra 400x augments, dreta 100x augments. S'observa la presència de leucòcits i eritròcits a l'orina. Imatges del Laboratori de Microbiologia del Centre de Malalties Transmissibles i Salut Internacional Drassanes-Vall d'Hebron.*



Tècniques de biologia molecular

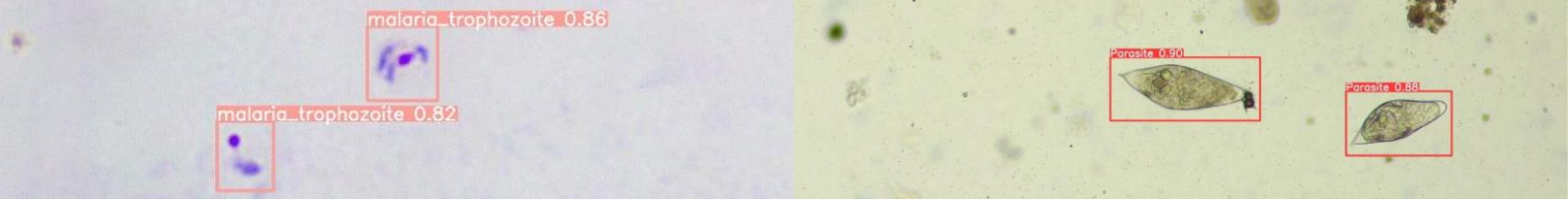
Les tècniques moleculars també són utilitzades per la detecció d'ADN del paràsit en mostres de femtes, sèrum i plasma. Aquest tipus de proves, com la PCR i LAMP, han demostrat resultats prometedors amb sensibilitats superiors al 90% [79]. La PCR és altament específica i sensible, però requereix equips de laboratori sofisticats i personal capacitat, la qual cosa limita el seu ús en àrees rurals o amb recursos limitats. D'altra banda, LAMP és una tècnica més accessible per a l'usuari i pot ser realitzada en condicions de camp amb un menor equipament. Actualment, també s'estan desenvolupant tècniques moleculars de PCR a temps real per la detecció de *S. haematobium* en mostres d'orina, amb resultats prometedors en infeccions de diferent intensitat [80]. Aquests tipus de tècniques permeten, en ocasions, detectar les fases més primerenques de la malaltia, així com la síndrome de Katayama.

Tècniques serològiques

Les tècniques indirectes per la detecció de l'esquistosomiasi es basen principalment en la serologia. És una tècnica àmpliament utilitzada, sobretot pel cribratge en pacients asimptomàtics i en pacients amb simptomatologia compatible, sobretot viatgers. Encara així, acostuma a donar un elevat nombre de falsos positius degut a les reaccions creuades, i no és considerada una tècnica confirmatòria. També s'utilitza per a la realització d'estudis epidemiològics de la malaltia, sobretot en àrees endèmiques o potencialment endèmiques. Els kits immunodiagnòstics disponibles són menys específics que els exàmens fecals múltiples a causa de la reactivitat creuada d'anticossos amb antígens d'altres helmints. La majoria de les tècniques detecten IgG, IgM o IgE contra l'antigen de l'helmint adult o l'antigen de l'ou, ambdós solubles; assaig immunoabsorbent lligat a enzim (ELISA); hemaglutinació indirecta; o immunofluorescència. La serologia no és una tècnica emprada per diferenciar una infecció activa o passada, i tampoc s'utilitza pel control de l'efectivitat del tractament [70].

Detecció d'antígens

Actualment, els antígens circulants anòdics i catòdics s'utilitzen pel diagnòstic d'infeccions actives per *Schistosoma*. Ambdós antígens són glico-conjugats procedents dels cucs adults, que poden ser detectats tant en sèrum com en orina [81]. Les tires reactives de l'antigen circulant catòdic, emprades per detectar les glicoproteïnes [82], poden ser d'utilitat pel cribratge de les espècies *S. haematobium* i *S. mansoni*, encara que no és considerada una tècnica definitiva pel diagnòstic [78].



Tècniques d'imatge radiològica

Les exploracions radiològiques tenen un paper important en el diagnòstic de la malaltia i les seves complicacions. La radiografia d'abdomen pot servir per mostrar principalment calcificacions en la paret de la bufeta. L'ecografia és l'exploració més rendible, que permet diagnosticar lesions en bufeta urinària com l'engruiximent de la mucosa, nòduls, o la fibrosi periportal. Altres exploracions com la Tomografia Computada (TAC) o la Ressonància Magnètica Nuclear (RMN) poden ajudar al diagnòstic, sobretot en casos de localitzacions ectòpiques com pot ser el pulmó o el sistema nerviós [70].

Biòpsia

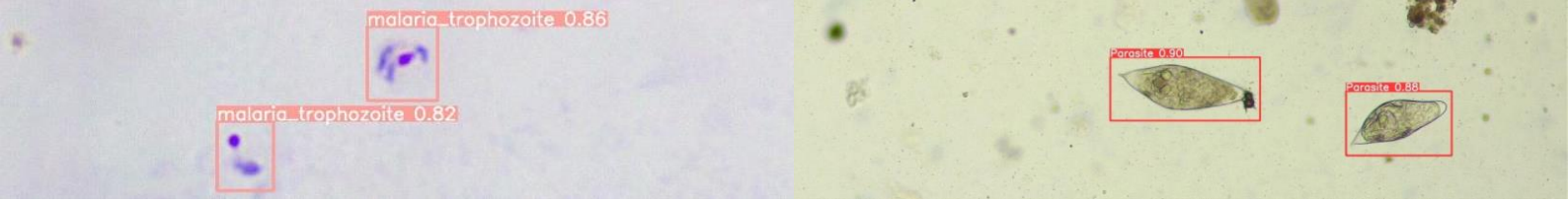
La biòpsia de bufeta i de la mucosa rectal són útils en pacients amb manifestacions clíniques típiques de la malaltia, però sense l'expulsió dels ous. Encara així són tècniques invasives i que requereixen intervencions quirúrgiques menors.

El resum de les principals tècniques diagnòstiques per l'esquistosomiasi, amb els seus avantatges i inconvenients, està representat a la **Taula 4**.

Técnica diagnóstica	Comentarios
Analítica general	- La eosinofilia es una constante en la fase aguda. En las formas crónicas su presencia es variable y cuando está presente suele ser leve - Otras alteraciones del hemograma: anemia (sobre todo en niños), trombocitopenia - Elevación de transaminasas, enzimas de colestasis y bilirrubina, prolongación tiempos de coagulación (fases avanzadas), ferropenia, elevación urea y creatinina (si uropatía obstructiva) - Tira reactiva de orina: hematuria, proteinuria, en ocasiones leucocituria
Detección de huevos en heces y orina	- Baja sensibilidad - Precisa de laboratorios con cierta experiencia - Precisa de técnicas de concentración o filtración - En la esquistosomiasis aguda tardan en detectarse los huevos de 30 a 50 días
Diagnóstico serológico	- Múltiples técnicas comercializadas (ELISA, radioinmunoanálisis, hemaglutinación indirecta, inmunofluorescencia indirecta, Western Blot y fijación del complemento) - Elevada sensibilidad pero muy variable entre las diferentes técnicas - Son las técnicas recomendadas para el cribado de la enfermedad en poblaciones de riesgo - No diferencian entre infección activa o pasada ni sirven para el control de la efectividad del tratamiento
Diagnóstico molecular y detección de antígenos	- Varias técnicas moleculares (PCR, LAMP) y de detección de antígenos (ACC) pero disponibles solo en centros de referencia
Radiología	- La radiología simple es útil para detectar calcificaciones vesicales - La ecografía es la técnica más rentable tanto para el diagnóstico de las lesiones a nivel urinario como hepatoesplénico

ACC: antígeno catiónico circulante; LAMP: *loop mediated isothermal amplification* (amplificación isotérmica mediada por bucles); PCR: reacción en cadena de la polimerasa.

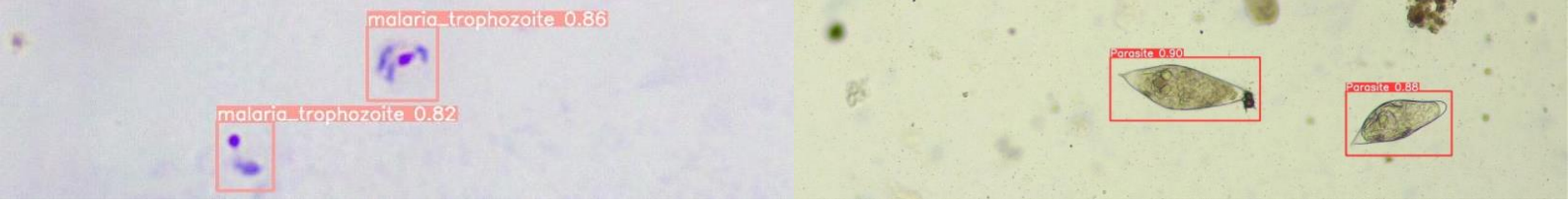
Taula 4 *Resum de les tècniques diagnòstiques emprades pel diagnòstic de l'esquistosomiasi [70].*



1.3.5. Tractament

El compost més eficaç i àmpliament utilitzat és el praziquantel, un derivat de la quinolina-pirazina acilat que ha estat el pilar del tractament de l'esquistosomiasi durant més de trenta anys. Els assajos clínics van demostrar que el tractament oral amb aquest antiparasitari és segur i eficaç contra els cucs adults de totes les espècies de *Schistosoma spp*. que afecten als humans, encara que el seu mecanisme precís d'acció està per establir. El praziquantel també és el tractament d'elecció dels programes globals basats en el control i prevenció de la malaltia, centrats en la subministració massiva del tractament (SMT), per reduir la morbiditat. El tractament és eficaç una hora després de ser ingerit; encara que ben absorbit, se sotmet a un extens primer pas hepàtic. Una fracció del fàrmac (80%) es metabolitza en el fetge i els metabòlits inactius són eliminats per via renal a través de l'orina. El praziquantel també és secretat en la seva forma activa en la llet materna, però no s'esperen esdeveniments o efectes adversos pels nadons, encara que es recomana que les dones no hagin de donar el pit el dia del tractament i durant les 72 hores posteriors. El praziquantel es pot utilitzar per tractar els infants menors de quatre anys i les dones embarassades després del primer trimestre [71].

En els programes de control de l'esquistosomiasi basats en el subministrament massiu de drogues, una sola dosi de praziquantel és recomanada per l'OMS, encara que aquest règim ha estat qüestionat i dades farmacodinàmiques i farmacocinètiques recents postulen una dosi més alta, especialment per a infants de sis anys o majors d'edat. Infeccions de *Schistosoma spp*. i els helmints transmesos pel sòl són molt comuns en zones on aquests cucs són co-endèmics, com a Àfrica i Àsia. En aquestes àrees, la teràpia preventiva conjunta de l'albendazole (un fàrmac antihelmíntic) i praziquantel és recomanada per l'OMS [83,84]. Els efectes adversos associats a la teràpia amb praziquantel, en general es consideren mínims, incloent-hi mareig, mal de cap, dolor abdominal, nàusees transitòries, prurit i erupció, i és probable que es deguin a les conseqüències de l'alliberament antigènic de cucs morts i no a al propi fàrmac. Les reaccions al·lèrgiques al praziquantel són també poc freqüents. En individus amb esquistosomiasi i cisticercosi concurrent, provocada per *Taenia solium*, el praziquantel pot induir convulsions i/o infart cerebral i lesions oculars permanents. És la conseqüència de les greus reaccions inflamatòries causades pels cucs de *T. solium* a l'aplicar-hi el tractament.



1.4. Nous mètodes de diagnòstic basats en la Intel·ligència Artificial

La Intel·ligència Artificial (IA) es considera un camp d'estudi molt ampli, que engloba totes aquelles tasques o funcions que es poden realitzar mitjançant sistemes computacionals. Segons la *Real Académia de la Lengua Española* la IA es defineix com "*Disciplina científica que se ocupa de crear programas informáticos que ejecutan operaciones comparables a las que realiza la mente humana, como el aprendizaje o el razonamiento lógico.*" [85]. Dintre de la IA trobem l'aprenentatge automàtic o *Machine learning*, que descriu tots aquells algoritmes automatitzats que permeten treballar amb dades per poder realitzar una tasca. Per últim, l'aprenentatge profund o *deep learning* és un conjunt de processos computacionals en el camp de la intel·ligència artificial, que permeten l'aprenentatge automatitzat i la generació d'algoritmes mitjançant una metodologia molt similar a la del cervell humà. Aquesta classificació està representada a la **Figura 8**.

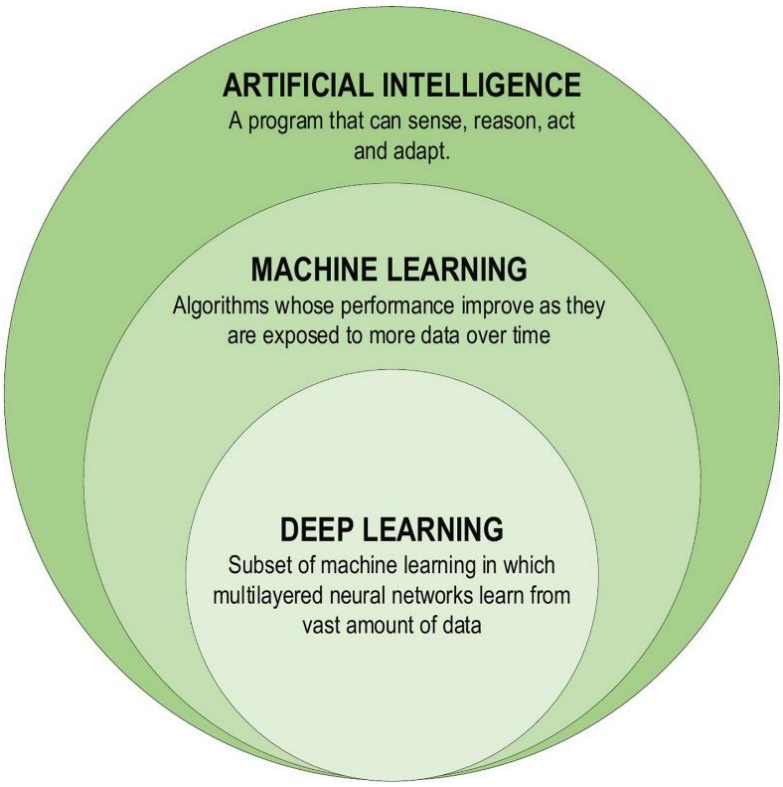
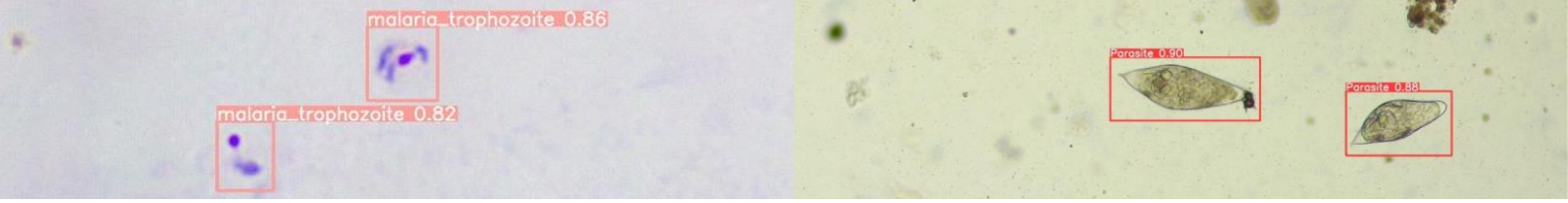
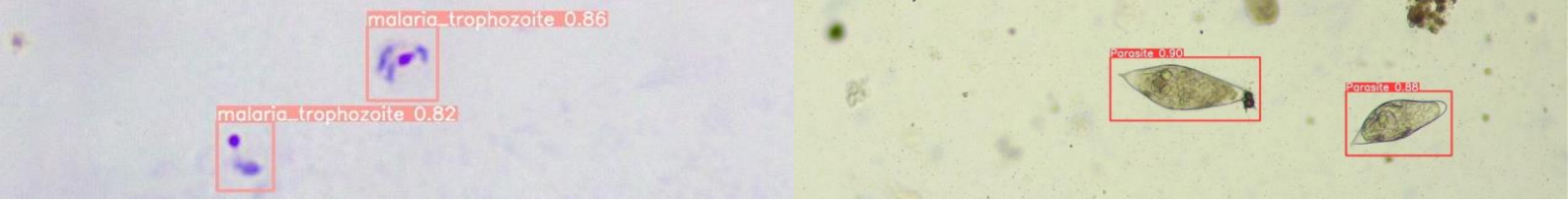


Figura 8 *Classificació jeràrquica dels conceptes "Intel·ligència Artificial", "Aprenentatge Automàtic" i "Aprenentatge Profund" [86].*



La base de l'aprenentatge és la informació de les bases de dades, que han de ser àmplies i completes. Els sistemes basats en *deep learning* consisteixen en xarxes neuronals artificials amb múltiples capes per entrenar i generar algorismes d'IA. L'aprenentatge profund ha desbancat els procediments de les tècniques tradicionals d'anàlisi d'imatge, molt menys automatitzades i que requerien gran coneixement per a la seva aplicació. Les Xarxes Neuronals Convolucionals (XNC) representen un ampli conjunt d'arquitectures de xarxes neuronals artificials. Es basen en la incorporació de filtres aplicats sobre els conjunts de dades, en una operació denominada convolució. Els models d'arquitectura de XNC s'entrenen per realitzar múltiples tasques, entre d'altres detectar objectes en imatges o vídeos digitals. Les XNC s'apliquen en molts camps científics, en concret destaquen com a eina d'atenció sanitària en el diagnòstic mèdic per analitzar i extreure característiques eficients de les imatges biològiques digitals.

Les tècniques més utilitzades són en l'àmbit de la radiologia i la dermatologia pel diagnòstic precoç [87,88]. *Kim* et al. 2024 va desenvolupar un model basat en IA mitjançant imatges de radiografia de tòrax i els valors de *Cycle threshold* (Ct) d'un sistema de PCR a temps real anomenat Xpert MTB/RIF, per predir els resultats del tractament de la tuberculosi [89]. Com a altre exemple, *Papachristou* et al. 2024, va avaluar una aplicació per a telèfons intel·ligents capaç de detectar, mitjançant l'anàlisi d'imatges demoscòpiques, lesions de la pell amb potencialitat per ser identificades com a melanomes [90]. Aquest tipus d'aplicacions, cada vegada s'utilitzen més per les malalties infeccioses i la microbiologia clínica [91,92]. L'anàlisi d'imatges digitals de microscopi utilitzant una *U-Net* (arquitectura de xarxa neuronal convolucional) per segmentar i detectar promastigots, amastigots i paràsits adherents de leishmaniosi és un estudi representatiu de la gran varietat de possibilitats de les XNC [93]. També, s'han desenvolupat sistemes d'IA pel diagnòstic de la malaltia d'Alzheimer. *Uysal* et al. 2020 va utilitzar imatges de ressonància magnètica de l'hipocamp per detectar possibles afectacions relacionades amb fases primerenques de la malaltia [94]. Un altre exemple és l'ús de la IA pel diagnòstic i prevenció de diversos tipus de càncer [95]. Algunes de les aplicacions desenvolupades es basen en el diagnòstic del càncer colorectal mitjançant la detecció de pòlips, biomarcadors o la predicció metastàtica de la malaltia amb models de xarxes neuronals [96]. En l'àmbit de laboratori, *Park* et al. 2021 van dissenyar i entrenar un model d'IA per determinar quines malalties patien una cohort de pacients, mitjançant l'anàlisi de les dades de les proves de laboratori [97]. L'alta capacitat de computació aconseguida en els últims anys i l'augment de les bases de dades han impulsat l'ús d'aquesta tecnologia per a aplicacions mèdiques i d'altres àmbits.



L'impacte en la salut global de la malària i l'esquistosomiasi ha accelerat el desenvolupament i la implementació de noves estratègies diagnòstiques per lluitar contra aquestes malalties. S'estan desenvolupant noves tècniques diagnòstiques basades en l'anàlisi d'imatges digitals mitjançant IA per a la detecció de paràsits. Aquest tipus de sistemes són una emulació de la visualització microscòpica, mitjançant la captura i processament d'imatges digitals, postulada com una alternativa ràpida i eficient a la realització tradicional del diagnòstic. En els últims anys, els mètodes computacionals basats en l'anàlisi d'imatges per a la detecció d'objectes han tingut cada cop més importància en estudis mèdics i biomèdics. S'estan desenvolupant diverses aplicacions de programari i eines per detectar paràsits de malària i/o d'altres patologies, en imatges de mostres biològiques utilitzant microscòpia d'òptica convencional [98–102].

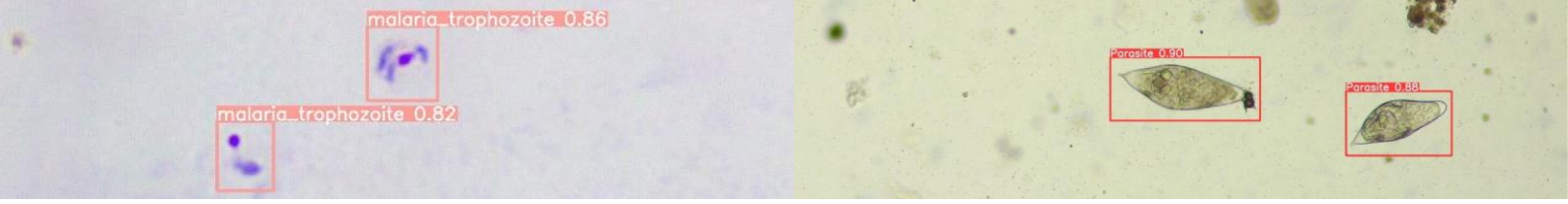
Els següents subapartats descriuen les etapes per a l'adquisició i processament automàtic d'imatges digitals, que inclouen les tècniques tradicionals i les tècniques actuals, que incorporen la intel·ligència artificial.

1.4.1. Adquisició d'imatges

La captura d'imatges és el primer pas per generar una base de dades d'imatges per a la seva anàlisi i identificació. L'adquisició depèn, principalment, de l'equipament i la infraestructura dels laboratoris. Les càmeres integrades al microscopi s'utilitzen sovint per adquirir imatges digitals amb microscòpia òptica convencional. No obstant això, les càmeres de telèfons intel·ligents o *smartphones* són una alternativa assequible per a aplicacions automatitzades de diagnòstic. Així, en països amb pocs recursos, les càmeres de telèfons intel·ligents poden ser una eina útil per a la captura d'imatges digitals i facilitar la substitució de les càmeres digitals integrades dels microscopis, que solen ser molt més costoses. La qualitat, resolució i morfologia de la imatge digital determinaran el processament i l'anàlisi de la imatge. L'adquisició d'imatges és el primer pas, tant per a les tècniques tradicionals de processament d'imatge com per als mètodes d'aprenentatge profund. Una bona captura d'imatges digitals marcarà la qualitat final de la base de dades, i conseqüentment la dels models d'IA.

1.4.2. Tècniques tradicionals de processament d'imatge

Les tècniques tradicionals del processament d'imatge, es basen en l'aplicació d'un seguit d'operacions per arribar a la detecció i anàlisi d'objectes en imatges digitals. A continuació s'inclou un breu resum que ajuda a interpretar les diferents passes a seguir.

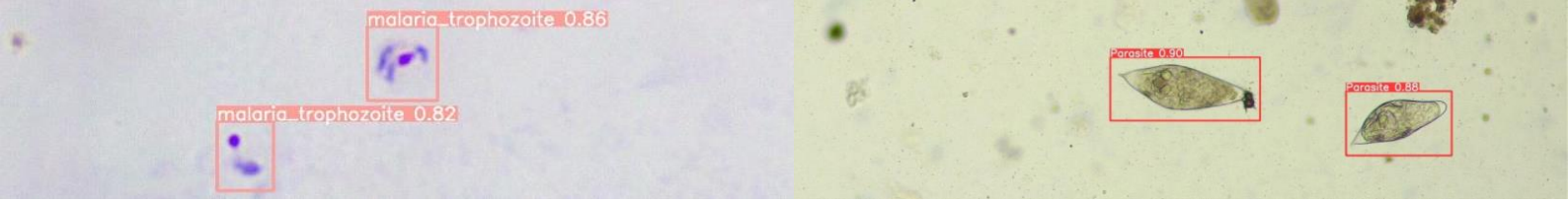


El preprocessament de la imatge s'utilitza en les primeres passes de les tècniques tradicionals de visió per computador. Aquestes modificacions faciliten els procediments posteriors d'identificació de característiques de manera automatitzada. La majoria de les estratègies estan basades en la reducció de soroll, millora del contrast d'imatge i redimensionament d'imatge [103,104]. Per exemple, els filtres mitjans gaussians o filtres de pas baix s'utilitzen per reduir el soroll de les imatges de microscòpia, per així detectar millor les formes d'interès abolint els possibles artefactes generats per la tinció de la mostra biològica. A més, la normalització i correcció del color per reduir els efectes de la il·luminació és una solució assequible per reduir els errors d'imatge associats a les càmeres digitals. La normalització del color també és un mètode de preprocessament per minimitzar problemes de tinció. En el cas de l'adquisició d'imatges amb telèfon intel·ligent, mètodes com la normalització del color i l'eliminació del fons són també eines útils per preparar imatges pel diagnòstic automatitzat [105].

La segmentació de la imatge és molt sovint necessària per extreure característiques rellevants, i consisteix a classificar cada píxel com a part dels objectes de la imatge original. Les operacions morfològiques, la transformació de *Hough*, l'agrupament de K-mitjanes, l'algorisme de conques hidrogràfiques, els algorismes de segmentació basats en vores, la segmentació basada en regles, la coincidència de plantilles i la conca controlada per marcadors són tècniques de segmentació habituals [106]. També s'ha utilitzat en malària per a imatges d'extensió i gota gruixuda [107]. Moltes d'elles es complementen amb tècniques de llindar (*threshold*) com a pas final per extreure i definir les diferents regions segmentades.

L'extracció de característiques o *feature extraction* és el següent procediment. Aquest procediment facilita els següents passos d'aprenentatge i classificació proporcionant informació quantitativa sobre determinats paràmetres d'imatge. Les característiques de les imatges, com la composició de colors, la textura i la morfologia són acuradament seleccionades. Per exemple, en el cas de la malària en mostres d'extensió fina de sang, *Nugroho* et al. 2015 va utilitzar una característica que es basa en un histograma de paràmetres de textura per detectar paràsits en forma de trofozoït, esquizont i gametòcit de *P. falciparum* [108]. Com a altre exemple per malària mitjançant un *software*, els càlculs de les característiques dels eritròcits en imatges d'extensió fina es realitzen mitjançant plataformes de codi obert com *PyRadiomics Documentation* (Harvard) [109].

L'aprenentatge automàtic pel reconeixement de patrons és el pas final del procediment abans de la identificació, i no solament s'utilitza en el camp de l'anàlisi d'imatges. Hi ha tècniques



d'aprenentatge automàtic, entre les quals destaquen: els arbres de decisions, els algoritmes de regles d'associació (Eclat, Frequent Pattern, entre d'altres), els algoritmes genèrics, les xarxes neuronals artificials, les màquines de vectors de suport (MVS), els algoritmes d'agrupament i les xarxes bayesianes [110]. Com a exemple de models d'aprenentatge automàtic, *Moslehi* et al. 2022 van utilitzar un sistema basat en arbres de decisions per classificar els factors que afectaven la mortalitat per la COVID-19 [111]. Per altra banda, *Kumari* et al. 2024 va emprar el model d'aprenentatge automàtic *Equivalence Class clustering and bottom-up Lattice Traversal* (ECLAT) per predir la severitat de la diabetis [112].

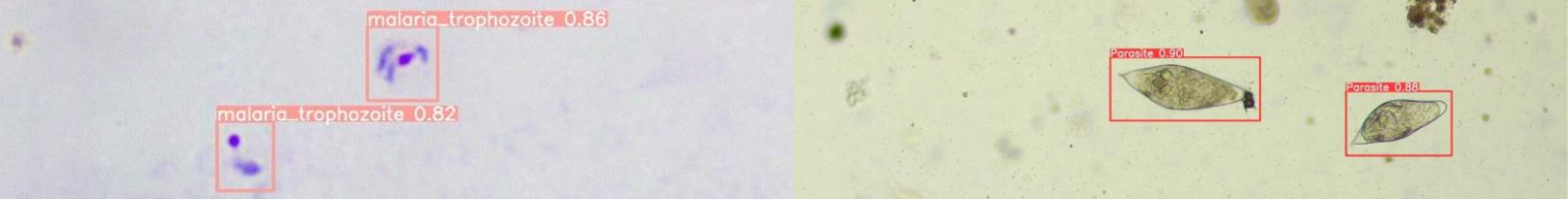
En el cas de la malària, els mètodes de classificació s'utilitzen per a la identificació de paràsits i leucòcits en mostres d'extensió fina i gota gruixuda. Com a exemple, la identificació d'espècies de paràsits i l'etapa de desenvolupament es poden detectar utilitzant tècniques tradicionals de reconeixement de patrons que inclouen, per exemple, la MVS [113] o classificadors de regressió logística [114]. La complexitat augmenta el temps de resposta, tot i que un balanç entre complexitat i temps és crucial per realitzar una identificació correcta i ràpida [6].

Els sistemes d'anàlisi d'imatges tradicionals han evolucionat cap a mètodes cada cop més automatitzats, que faciliten el procediment manual [115]. Les xarxes neuronals profundes van irrompre superant àmpliament els resultats de les tècniques tradicionals [116].

1.4.3. Aprenentatge profund

Les tècniques convencionals d'aprenentatge automàtic eren limitades en la seva capacitat de processar dades. Durant dècades, s'han desenvolupat sistemes de reconeixement de patrons o d'aprenentatge automàtic requerint una considerable experiència i domini de l'àmbit. L'aprenentatge profund ha millorat exponencialment el rendiment dels sistemes basats en l'aprenentatge automàtic.

Els mètodes d'aprenentatge profund són mètodes d'aprenentatge amb múltiples nivells de representació, obtinguts mitjançant la composició de mòduls simples però no lineals que eleven la representació a un nivell (dades d'entrada o *input*), en una representació a un nivell superior. Amb la composició de suficients transformacions de nivell, es poden aprendre funcions molt complexes. Per a les tasques de classificació, les capes més altes de representació amplien aspectes de l'entrada (*input*) que són importants per a la discriminació, i suprimeixen les variacions irrellevants. Una imatge, per exemple, ve en forma d'una matriu de valors de píxels, i les característiques apreses en la primera capa de representació representen típicament la

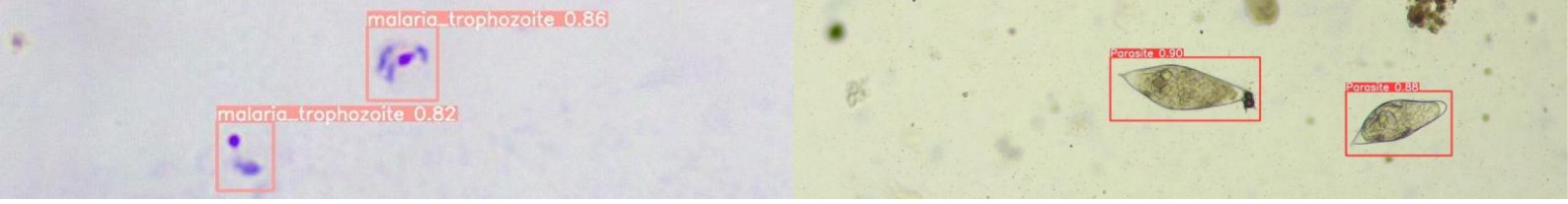


presència o absència d'arestes en orientacions i ubicacions particulars de la imatge. L'aspecte clau de l'aprenentatge profund és que aquestes capes de característiques no estan parametritzades pels humans, sinó que s'aprenen de dades utilitzant un procediment d'aprenentatge de propòsit general automàtic. Aquest canvi de paradigma és el responsable de l'optimització de l'aprenentatge automàtic [117].

Els models d'aprenentatge profund de detecció d'objectes són capaços d'identificar i localitzar objectes d'una determinada classe en imatges i vídeos digitals. Durant els últims anys, s'han millorat els models de detecció d'objectes i la majoria dels detectors d'última generació utilitzen xarxes d'aprenentatge profund. Normalment, com a molt les imatges en brut necessiten un preprocessament simple per redimensionar-les i proporcionar-les a la xarxa. El mateix model decideix i calcula les característiques apropiades, i proporciona una sortida que condueix a la identificació i localització d'objectes [118].

Les XNC són sistemes computacionals inspirats en neurones biològiques dissenyats per processar dades (**Figura 9**). L'entrada d'imatge s'analitza per reconèixer patrons visuals i completar la futura identificació d'objectes com a sortida. Els filtres convolucionals estan dissenyats per detectar patrons o característiques específiques en les dades d'entrada. En l'aprenentatge profund, els pesos d'aquests filtres s'aprenen automàticament a través de l'entrenament amb grans conjunts de dades. Les neurones en xarxes profundes (*deep neural networks*) estan controlades per una funció d'activació, que és l'encarregada de controlar la sortida (*output*). Operacions com els regularitzadors, amb normes L1, L2, la normalització per lots o l'abandonament són elements clau perquè els models predictius aprenguin millor i més ràpid.

Un fet important a tenir en compte a l'hora d'entrenar XNC és disposar de dades representatives suficients. En l'aprenentatge automàtic, així com en l'aprenentatge profund, les dades es distribueixen comunament en tres conjunts: entrenament, validació i testatge o *testing*. El model de predicció aprèn dels múltiples exemples del conjunt de dades i les mateixes dades d'entrenament s'introdueixen repetidament a la XNC en un procediment iteratiu. Durant l'entrenament, el conjunt de dades de validació permet la sintonització hiperparamètrica i l'avaluació de models mitjançant una optimització contínua. Finalment, s'utilitza un conjunt de dades de prova o *testing* per avaluar el model després de completar el procés d'entrenament, amb dades no vistes o inèdites per la xarxa [119]. Si la suma ponderada està per sobre d'un llindar, l'entrada es classifica com pertanyent a una categoria determinada [117].



Un dels principals problemes de les xarxes neuronals a causa d'una memorització de dades en detriment d'aprendre podria interferir en el resultat final de l'entrenament i l'obtenció de prediccions finals prou robustes. Aquest concepte és l'anomenat *overfitting*, molt comú quan les bases de dades no són suficientment grans o robustes, i present en tots els processos d'aprenentatge automàtic [120].

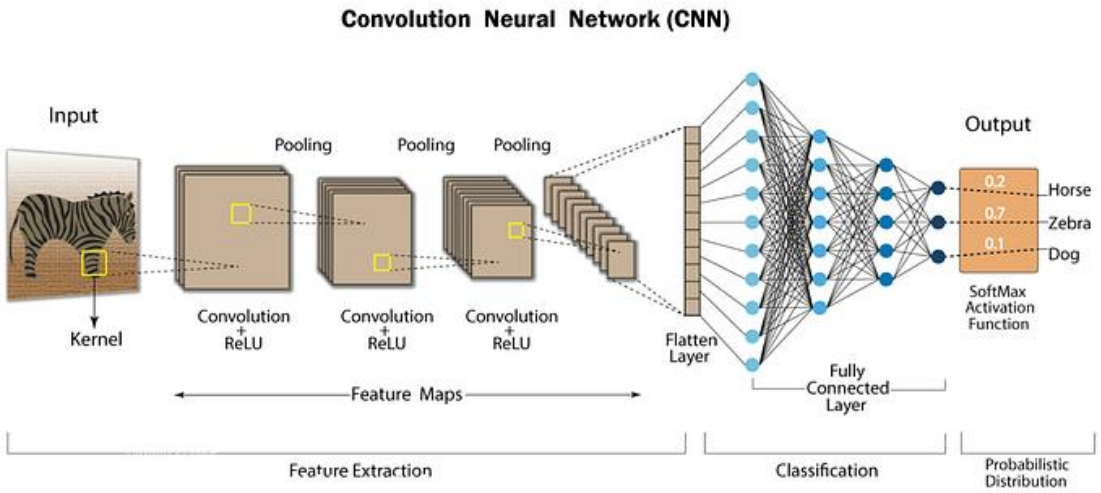
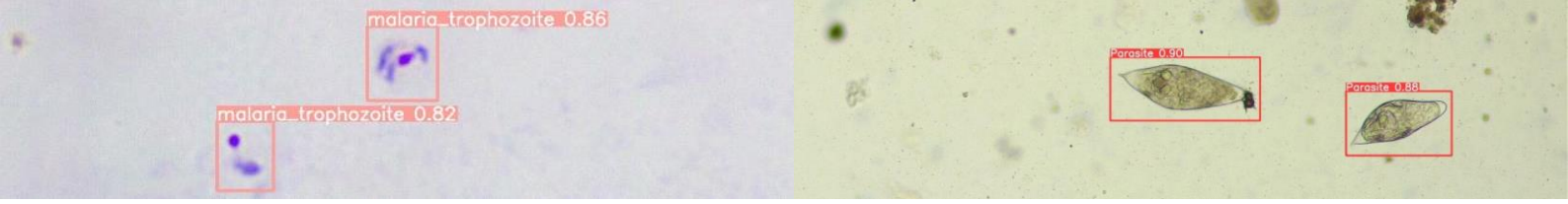


Figura 9 *Representació de l'arquitectura d'una xarxa neuronal convolucional* [121].

1.4.4. Detecció d'objectes amb xarxes neuronals convolucionals

Els detectors d'objectes basats en XNC es classifiquen segons funcionen en una o dues etapes. Els detectors de dues etapes tenen una alta precisió de localització i reconeixement d'objectes, mentre que els detectors d'una etapa aconsegueixen una alta velocitat d'inferència. El detector de dues etapes més representatiu és el Faster R-CNN i els detectors d'una etapa són *You Only Look Once* (YOLO) i *Single-Shot Detector* (SSD). La xarxa neuronal YOLO ha demostrat ser una alternativa òptima per la detecció d'objectes en imatges digitals, amb un alt rendiment i millora al llarg de totes les seves versions [122–131].

En la majoria dels casos es requereixen dades d'imatges etiquetades manualment per realitzar tots els processos esmentats. L'entrenament no supervisat o semisupervisat són una alternativa, encara que la majoria dels estudis d'imatge mèdica es realitzen amb dades d'entrenament supervisat. L'aprenentatge supervisat basat en l'anotació d'imatges és divers i s'han descrit multitud d'estratègies. La classificació d'imatge completa és l'anotació de tota la imatge com a tipus o classe. Els objectes no discernibles es detecten a la imatge, de manera que tota la imatge es classifica com un tipus. La detecció d'objectes utilitzant caixetes o *bounding boxes* és una altra



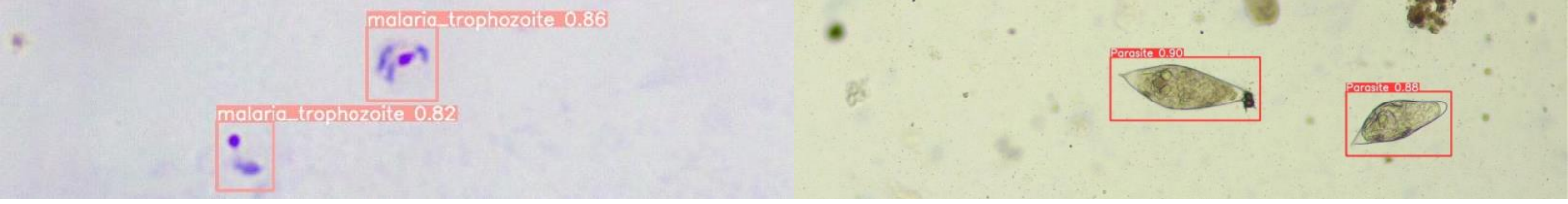
estratègia basada en l'anotació de diversos objectes dins de cada imatge. Requereix un procediment d'anotació supervisada de les coordenades dels diferents objectes de la imatge. Les XNC utilitzen el conjunt de dades i identifiquen cada quadre contenidor com una classe d'objecte. En el cas de la segmentació d'imatges, la identificació d'objectes es basa en una classificació a nivell del píxel. Cada píxel es classifica com un objecte de classe amb el seu propi valor, i les anotacions s'afegeixen manualment a les imatges. No obstant això, és una tasca que requereix encara més temps per a generar grans bases de dades anotades, per tant, s'estan desenvolupant procediments d'anotació automàtics com els *domain adaptations* [132].

1.4.5. Bases de Dades d'Imatges

La necessitat d'obtenir grans bases de dades pot arribar a ser un problema a l'hora de generar algoritmes eficients. *ImageNet* és un dels majors conjunts de dades disponibles d'imatges universals per a investigadors i amb un ús no comercial [133]. En el cas particular de la malària, es necessita un conjunt de dades prou gran d'imatges anotades per entrenar models de XNC i realitzar una identificació automatitzada dels paràsits. *DataPort* és un conjunt de dades d'imatge de codi obert [134]. Malauradament, no hi ha molts conjunts de dades públiques i de codi obert disponibles d'imatges de gota gruixuda i extensió, ni tampoc en el cas de l'esquistosomiasi. Les tècniques d'augment de dades, per ampliar artificialment els conjunts de dades d'imatge i obtenir millors rendiments, s'utilitzen actualment amb resultats prometedors [135]. El nombre d'etiquetes que es requereixen per obtenir un bon rendiment de les xarxes neuronals és una incògnita en molts casos, degut a la complexitat de cada model i a la tasca que han de realitzar dependent de la morfologia i tipus d'imatges. Encara així, alguns estudis, com *Mancebo-Martín* et al. 2024 van comprovar que un mínim de 100 etiquetes per cada categoria de classificació eren suficients per obtenir valors de valor F (*F-score*) superiors a 0.8, en mostres de sang per la identificació de múltiples espècies de paràsits [136].

1.4.6. La Intel·ligència Artificial pel diagnòstic de la malària i l'esquistosomiasi

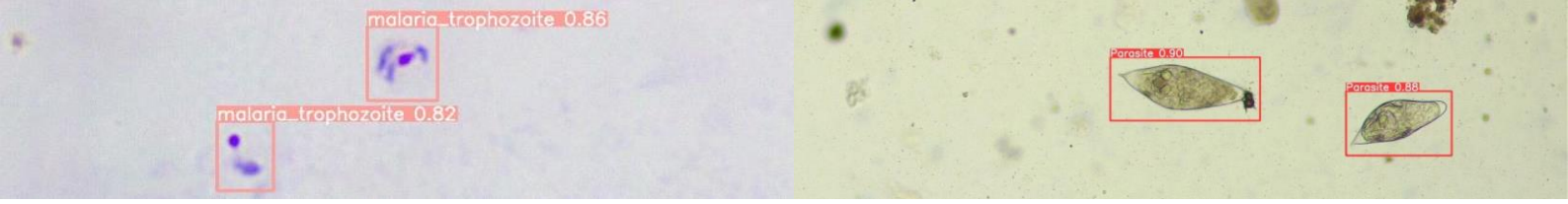
S'ha demostrat que les XNC tenen un rendiment òptim amb aplicacions de diagnòstic per la imatge mitjançant computadors en camps d'estudi específics, i es poden generalitzar per a altres tasques d'imatge mèdica. Els models de detecció d'objectes, com YOLOv3, YOLOv4, YOLOv5, YOLOv8 i YOLOv11 s'utilitzen per a aquest tipus d'aplicacions. A més, Faster R-CNN [100,137] i SPPnet són xarxes neuronals optimitzades que s'utilitzen per accelerar i millorar el temps d'identificació d'objectes. Estudis recents demostren el potencial de models anomenats



transformadors (*transformers*). Es tracta d'una tècnica basada en xarxes neuronals profundes, però no convolucionals, que utilitza mecanismes d'atenció i que està assolint resultats de gran qualitat, sobretot quan es disposa de grans bases de dades [138,139].

L'anàlisi automatitzada d'imatges de microscòpia podria ser una alternativa a l'examen de microscòpia convencional per al diagnòstic de la malària i l'esquistosomiasi. La preparació i el tipus de mostra són fets importants a tenir en compte per realitzar la correcta identificació de les característiques biològiques dels paràsits. La visualització de la gota gruixuda és crucial per a un diagnòstic correcte de la malària, fet que permet la consegüent visualització de l'extensió fina per a la identificació d'espècies de *Plasmodium*. La visualització microscòpica de la gota gruixuda té una sensibilitat analítica més elevada per a la detecció del *Plasmodium,* i sobretot té especial rellevància en casos de baixes parasitèmies. No obstant això, la freqüència d'artefactes observats en aquest tipus de mostres és més alta en comparació amb extensions. Segons la bibliografia actual, les XNC per a la detecció de paràsits de malària en gotes gruixudes són menys utilitzades i/o desenvolupades en comparació amb les d'extensió fina [6]. Altres factors importants a considerar en el cas de la visualització de gotes gruixudes són l'hemòlisi dels eritròcits, la ruptura del citoplasma dels leucòcits, i les formes variables dels trofozoïts en la fase intra-eritrocitària; fets que poden explicar la major dificultat per generar algoritmes d'IA per aquest tipus de mostres.

La majoria dels mètodes publicats per a la identificació del paràsit de la malària es basen en procediments supervisats, que requereixen un procediment d'etiquetatge manual previ de les imatges microscòpiques [140]. En el cas de l'esquistosomiasi urogenital, el procediment és molt similar, sent en la majoria dels casos XNC entrenades amb bases de dades d'imatges, en aquest cas de sediments urinaris. Les imatges digitals han de ser etiquetades per crear un conjunt de dades suficientment ampli per permetre la generació d'un model de detecció òptim. Quan es tracta de la detecció, aquest procés requereix definir manualment l'espai a la imatge on es troba cada paràsit, per entrenar el model de xarxa neuronal. Encara així, nous mètodes com els "*domain adaptations*" es comencen a postular com a alternatives a l'etiquetatge convencional, i permetre etiquetar automàticament les imatges mitjançant un petit grup d'imatges manualment etiquetades [141].



Càlculs automàtics de la densitat parasitària mitjançant mètodes d'aprenentatge profund

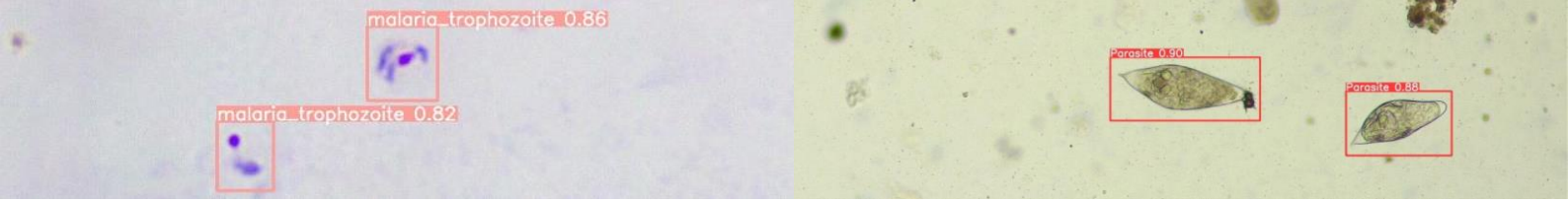
El recompte automàtic de parasitèmia mitjançant l'anàlisi d'imatges és una eina útil que podria superar i donar suport a la quantificació manual del nivell de paràsits, sobretot en el cas de la malària. Els càlculs convencionals de la parasitèmia de les mostres de malària no són precisos i en ocasions difícils de reproduir. Normalment, es realitza una estimació manual del nombre de paràsits en funció dels eritròcits parasitats i/o el nombre de leucòcits. També, quan la concentració de paràsits en sang és elevada, és una tècnica tediosa i que requereix temps. La quantificació parasitària de la gota gruixuda es realitza de manera rutinària en els laboratoris d'arreu, comptant el nombre de paràsits i leucòcits, i utilitzant la mesura de referència de 8000 leucòcits/µL de sang (**Equació 1**).

$$\frac{paràsits}{\mu L\ de\ sang} = \frac{nombre\ de\ paràsits\ comptats\ x\ 8000}{nombre\ de\ leucòcits\ comptats}$$

Equació 1: Càlcul del nombre de paràsits de *Plasmodium* per microlitre (µL) de sang en mostres de gota gruixuda.

En canvi, la quantificació de l'extensió fina de sang es basa en el recompte d'eritròcits infectats en cada camp microscòpic, suposant que tenim aproximadament 100 hematies/camp i donant el resultat en percentatge (%).

Com a alternativa, la quantificació dels nivells de paràsit en sang mitjançant tècniques d'anàlisi d'imatge digital requeririen períodes de temps més curts. Per acomplir aquestes tasques és, per tant, necessari no solament detectar els paràsits, sinó també els leucòcits i els eritròcits depenent del tipus de mostra que analitzem. Durant els inicis de la dècada del 2010 es va començar a desenvolupar un programari d'anàlisi d'imatges per a realitzar aquesta funció automàticament amb imatges de gota gruixuda [142]. Per als càlculs automatitzats de parasitèmia amb mostres d'extensió fina hi ha eines d'anàlisi d'imatges disponibles per millorar el recompte manual convencional, moltes provinents d'escàners utilitzats en hematologia. Com a exemple, *Plasmodium AutoCount* és una eina digital d'anàlisi d'imatges per realitzar un recompte automàtic de paràsits en mostres d'extensió fina de sang tenyides amb Giemsa [143]. Altres eines de processament d'imatges basades en llibreries de programari OpenCV també van demostrar la seva eficàcia en mostres també d'extensió. Els mètodes anteriorment esmentats utilitzaven tècniques de processament d'imatges, com ara la reducció del soroll amb filtres i transformacions binàries, per determinar la presència de paràsits de malària dins dels eritròcits

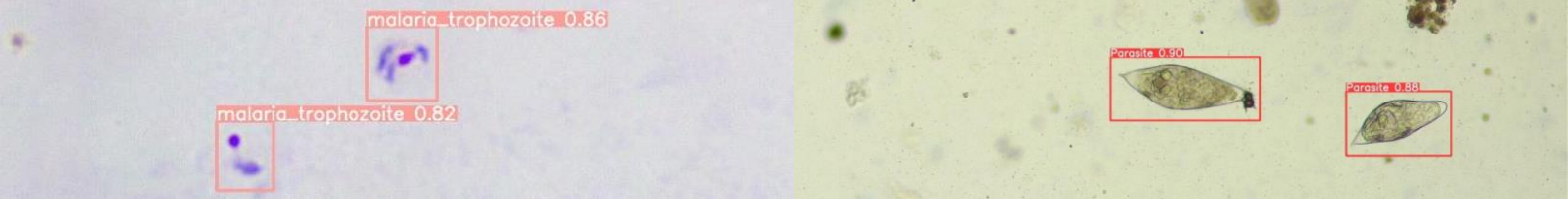


i realitzar un càlcul final del nivell del paràsit. Actualment, també existeixen tècniques basades en la IA per aquest tipus de tasques, que permeten quantificar amb precisió el nombre de paràsits en mostres tant de gota gruixuda [144] com d'extensió fina [145].

Aplicacions de telefonia mòbil per a la detecció de paràsits

S'estan desenvolupant aplicacions de telefonia mòbil per a la detecció automàtica de paràsits de la malària i l'esquistosomiasi. La captura d'imatges mitjançant telèfons intel·ligents és una alternativa adequada i fàcil per a l'adquisició d'imatges a través de la lent del microscopi. Només es necessita un microscopi òptic, un adaptador pel telèfon mòbil i un telèfon intel·ligent convencional per realitzar un diagnòstic per la imatge. Les càmeres de telefonia mòbil podrien substituir les càmeres de microscòpia integrades o externes, i realitzar un diagnòstic òptim mitjançant l'anàlisi d'imatges. A la **Figura 10** està representat un exemple d'aplicació per a telèfons intel·ligents pel diagnòstic de l'EU.

La integració de models predictius de XNC en una aplicació de programari per a telèfons intel·ligents és possible actualment. Els models de XNC adaptats realitzen tot el diagnòstic en un únic dispositiu. La coalescència entre el diagnòstic automatitzat i el programari per a telèfons intel·ligents és una fita i un repte per a la seva futura implementació en laboratoris de tot el món. L'anàlisi d'imatges i els procediments d'aprenentatge profund permeten que els telèfons intel·ligents siguin una de les millors alternatives per a la implementació de la detecció automatitzada de paràsits de malària i esquistosomiasi. Fins i tot en entorns amb pocs recursos, els telèfons intel·ligents són una opció disponible i relativament barata. Un dels principals problemes de les càmeres de telèfons intel·ligents és la qualitat de la captació de la imatge i l'adaptació a les lents del microscopi, fet que limita la seva implementació. Actualment, les càmeres de telèfon intel·ligent proporcionen una alta qualitat d'imatge, encara que l'adaptació al microscopi pot ser un problema. Les imatges podrien ser pertorbades per problemes en l'adaptació de la lent, o degradacions de la qualitat de la imatge relacionades amb l'enfocament. L'enfocament automàtic de la imatge del microscopi també és un problema a resoldre. Encara es requereix tecnologia per automatitzar completament tot el procediment d'adquisició i enfocament de la imatge per la identificació de paràsits en un únic dispositiu.



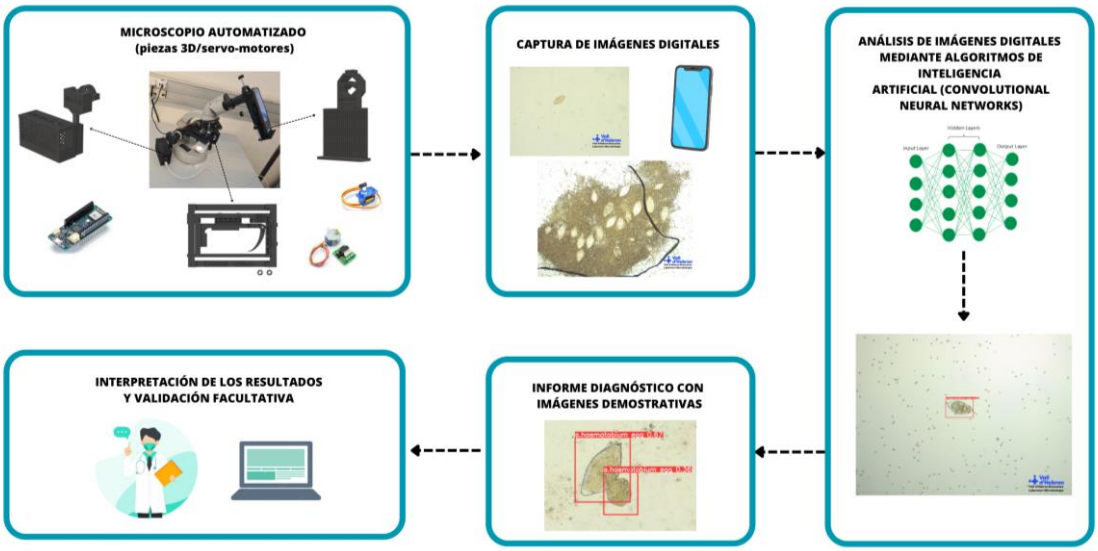


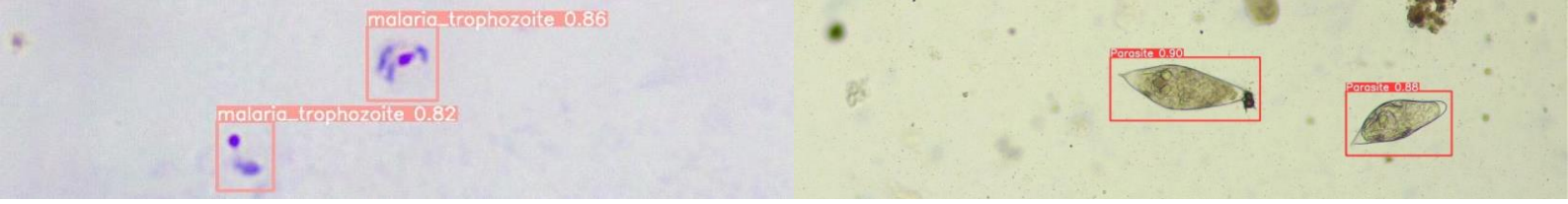
Figura 10 *Esquema del funcionament i circuit de l'aplicació per telèfon intel·ligent presentada a la tesi doctoral. L'esquema representa la detecció d'ous de Schistosoma haematobium mitjançant un microscopi robotitzat de baix cost i xarxes neuronals convolucionals* [146].

La *gamificació* o ludificació de la tecnologia per a la identificació de paràsits de malària en imatges digitals també és una aplicació innovadora. Com a exemple, es va desenvolupar un videojoc, on els jugadors voluntaris en línia analitzen imatges de sang per detectar paràsits de malària. Aquesta aplicació anomenada *Malaria Spot* va permetre la creació d'una base de dades d'imatges anotades privada per usos comercials [147].

En resum, les aplicacions per a telèfons intel·ligents podrien ser el futur per a l'adquisició i l'anàlisi d'imatges de manera automatitzada per part de les tecnologies d'IA, i una alternativa adequada per al diagnòstic de la malària i les malalties tropicals desateses. La possibilitat d'integrar models predictius i l'adquisició d'imatges en un únic dispositiu confereix una àmplia gamma d'aplicacions en el camp de l'anàlisi d'imatges per al diagnòstic.

1.4.7. Automatització del hardware per a microscòpia òptica

La robotització del microscopi per escanejar i autoenfocar les mostres biològiques és una opció per automatitzar la visualització al microscopi. L'automatització ajuda a resoldre les limitacions relacionades amb el procediment de diagnòstic. Els mètodes de processament d'imatges permeten l'automatització del diagnòstic, encara que, en moltes ocasions, es necessita personal tècnic per moure l'eix X-Y i enfocar la mostra. Actualment, es disposa de múltiples cases comercials que ofereixen dispositius automatitzats per realitzar funcions de microscòpia, però

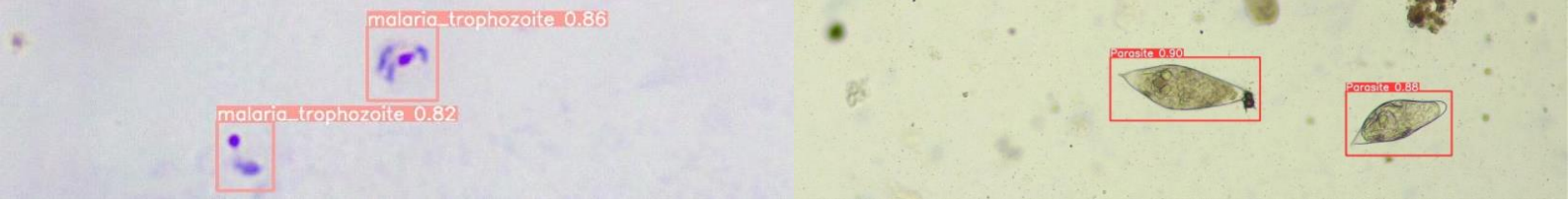


el cost sol ser molt elevat i no solen estar pensats per la seva implementació en països de mitjana o baixa renda. L'adaptació de sistemes de baix cost a les tècniques de microscòpia tradicionals és crucial per implementar plenament la tecnologia esmentada en pràctiques clíniques i diagnòstiques reals de qualsevol laboratori. Per això, l'optimització de maquinària o *hardware* de baix cost amb models d'impressió 3D per fabricar peces específiques del microscopi seria una opció adequada en entorns amb pocs recursos. Alguns estudis han presentat la possibilitat de desenvolupar un dispositiu òptic que emuli o substitueixi un microscopi òptic [148]. No existeix bibliografia descrivint l'adaptació de peces 3D a microscopis òptics convencionals per la seva automatització pel diagnòstic.

1.4.8. Implementació de tecnologies d'Intel·ligència Artificial en entorns amb pocs recursos

Els entorns amb pocs recursos són considerats aquelles zones o regions amb índex de desenvolupament humà baix o molt baix. En moltes ocasions són zones altament afectades per les malalties infeccioses associades a la pobresa, especialment per les MTD. D'altra banda, els pocs recursos dels quals es disposa en aquests entorns pot limitar la qualitat dels diagnòstics, i per contrapart la qualitat de vida de les persones. Noves tècniques accessibles basades en la IA podrien donar suport a aquest tipus d'entorns a nivell global, reforçant els plans nacionals de la lluita contra aquestes malalties.

Els telèfons intel·ligents ja són actualment dispositius accessibles a nivell global, i són una alternativa portàtil i adequada pel diagnòstic mitjançant tècniques d'anàlisi d'imatges digitals [149], que es podrien implementar en entorns amb pocs recursos i zones endèmiques remotes. Els models de XNC poden integrar-se dins del programari per a telèfons intel·ligents i no seria necessària una connexió a Internet. La provisió de centres sanitaris amb dispositius mòbils per part d'organitzacions governamentals i programes nacionals contra la malària seria un factor determinant per a la correcta implementació d'aquesta nova tecnologia per al diagnòstic mitjançant la visualització microscòpica i utilitzant la IA. No obstant això, a causa de les restriccions específiques de moltes zones endèmiques, aquest pot ser un problema important que s'abordarà en els pròxims anys per voluntat política. Els beneficis dels telèfons intel·ligents per al diagnòstic poden ser de gran valor, no només per a la malària, sinó també pel diagnòstic de moltes altres malalties tropicals o MTDs. Per tant, la implantació en hospitals regionals o petits centres sanitaris suposaria un repte per a futurs estudis. Els nous models de detecció



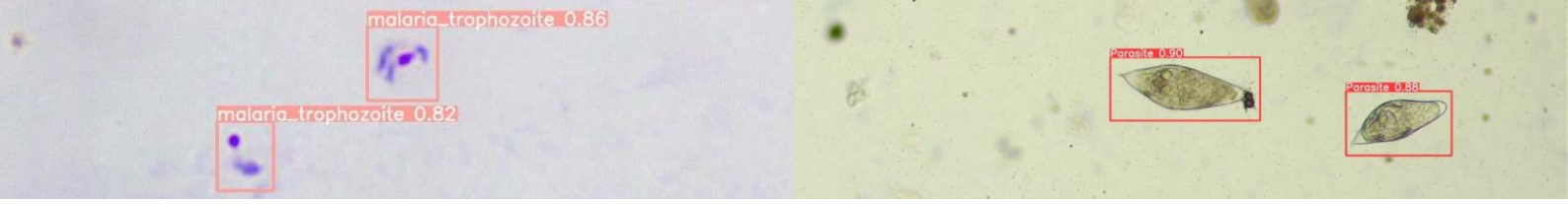
d'objectes entrenats amb imatges digitals per a telèfons intel·ligents són adequats pel desplegament del diagnòstic de la malària en entorns amb pocs recursos.

Els estudis de rendiment per validar la tecnologia són un requisit indispensable per a la futura implementació d'una eina diagnòstica. L'avaluació del sistema s'ha de realitzar en condicions ideals, i també amb recursos limitats, per determinar la seva eficàcia en diferents entorns. Hi ha moltes barreres a superar per a passar d'un producte o tecnologia en desenvolupament a la introducció i implementació com a producte comercial. Algunes de les principals barreres són l'ajust als sistemes de salut i laboratori necessaris per garantir l'adaptació, la posada en pràctica efectiva, la demostració del valor de la tecnologia, l'avaluació de la viabilitat operativa, les polítiques i els requisits reguladors de les organitzacions governamentals, la investigació operativa per avaluar l'efecte net de la tecnologia en el camp, la distribució, el servei de manteniment i reparació, i el control de qualitat [150].

La implementació de noves tècniques diagnòstiques en entorns de laboratori ha de ser regulada i controlada pels protocols a nivell mundial de l'Administració d'Aliments i Medicaments, i de l'OMS. La tecnologia ha de ser validada i acceptada per les autoritats internacionals i nacionals tal com es descriu a les regulacions [151,152]. La majoria dels esforços per implementar noves eines diagnòstiques en entorns pobres en recursos es centren en malalties infeccioses com el VIH, la tuberculosi i la malària. La comprensió profunda i la coordinació dels actors implicats en el desenvolupament i la implementació del diagnòstic són fites per a l'èxit de les intervencions diagnòstiques en àrees amb pocs recursos.

1.4.9. Aspectes ètics de la IA pel diagnòstic biològic

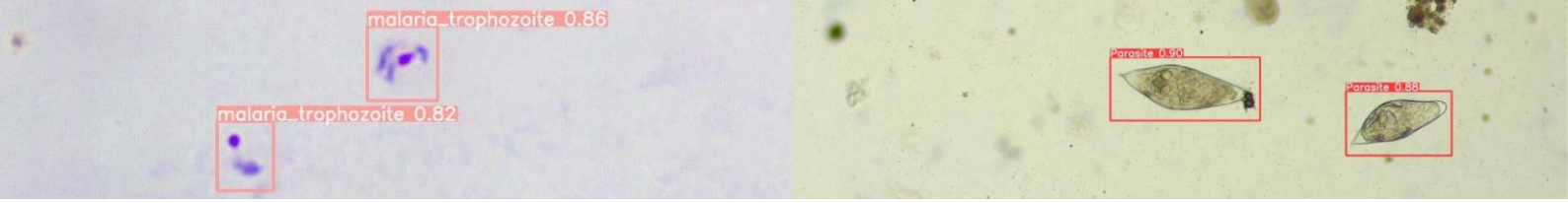
L'ús de la IA en el diagnòstic biològic planteja importants qüestions ètiques que han de ser abordades per garantir una aplicació segura i justa. Un dels primers aspectes a considerar és la privacitat i seguretat de les dades, ja que aquests sistemes requereixen grans quantitats d'informació mèdica confidencial per entrenar-se i millorar la seva precisió. La protecció d'aquestes dades és essencial per evitar usos indeguts i complir amb normatives en l'àmbit Europeu i global, mitjançant tècniques com l'anonimització i l'encriptació de les dades [153]. D'altra banda, la responsabilitat en la presa de decisions és un repte crucial. La IA ha de ser una eina de suport i no un substitut del professional de laboratori, assegurant sempre la supervisió humana. La qüestió de l'equitat i el biaix algorítmic s'ha de tenir en compte a l'hora de desenvolupar sistemes basats en IA, ja que poden reflectir i amplificar desigualtats presents en



les dades amb què han estat entrenats. Això podria portar a diferències en la qualitat del diagnòstic segons el gènere, l'ètnia o la situació socioeconòmica del pacient. Per evitar aquestes discriminacions, és necessari treballar amb conjunts de dades representatius i aplicar mecanismes per detectar i corregir possibles biaixos.

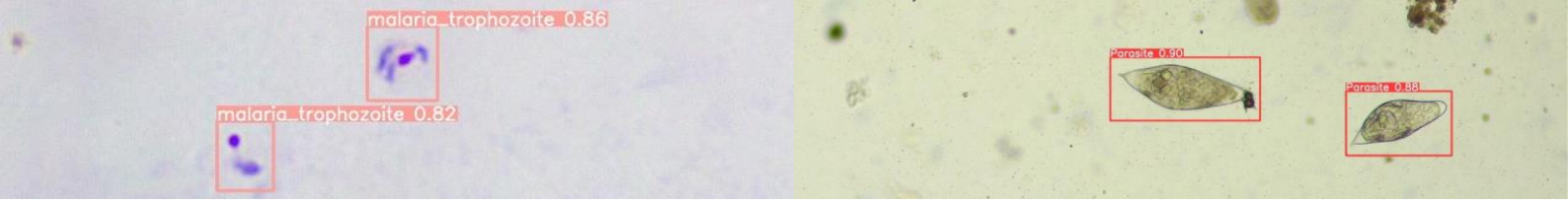
1.5. Cooperació internacional pel desenvolupament

Actualment, la cooperació internacional pel desenvolupament és un mecanisme global per mitigar les diferències entre regions, i aportar recursos i/o coneixements a les zones més desfavorides. Moltes de les accions que es duen a terme en aquest aspecte tenen com a objectiu millorar la qualitat de vida de les persones. En contextos amb pocs recursos, les malalties infeccioses són molt prevalents i tenen un gran impacte a la societat, agreujat pels dèficits en recursos humans i tècnics, i la poca disponibilitat de proves diagnòstiques i de medicaments. És per això que les accions han de ser viables i sostenibles a llarg termini, fins a la seva transferència als centres de salut on s'hagi implementat, i anar orientades a obtenir autonomia per part de les regions intervingudes. També és crucial la implicació de les organitzacions de base comunitària tant en la preparació del projecte com en la seva implementació, i sempre respectant la cultura, les creences i costums del lloc on s'actua, encara que puguin semblar contradictòries amb la ciència. Identificar els problemes i les necessitats de la contrapart local és molt important per la implementació de qualsevol projecte de col·laboració. Tanmateix, la cooperació entre les entitats governamentals i internacionals poden ajudar a implementar definitivament aquest tipus d'estratègies.



2. Justificació

Durant l'any 2023, es van produir 263 milions de casos de malària i 597.000 morts per la malaltia a nivell mundial. Sobre els casos d'esquistosomiasi globalment, no es disposa de dades tan fiables com en el cas de la malària. S'estima que 251.4 milions de persones van requerir tractament profilàctic l'any 2021 contra l'esquistosomiasi, per tant, que estaven en alt risc de contraure la malaltia; mentre que només van rebre el tractament 75.3 milions d'individus. El diagnòstic *gold standard* de la malària i l'esquistosomiasi urogenital, segons l'OMS, és la visualització dels paràsits mitjançant microscòpia òptica convencional. Per contrapart, són tècniques professional dependents i que requereixen un alt nivell de coneixement i formació per realitzar-les correctament. Tanmateix, quan el volum de mostres rebudes al laboratori és molt elevat, es poden cometre errors a l'hora d'observar les preparacions. Els sistemes automatitzats fiables, accessibles, sostenibles i pensats per la seva implementació en entorns amb pocs recursos podrien ser una eina de suport al diagnòstic d'aquestes malalties. És per això que hem dissenyat i desenvolupat un nou sistema automatitzat pel diagnòstic de la malària i l'esquistosomiasi urogenital, accessible i de baix cost. El sistema està basat en una aplicació de telèfon intel·ligent pel diagnòstic mitjançant l'anàlisi d'imatges digitals utilitzant eines d'IA. El sistema funciona amb algoritmes d'intel·ligència artificial, i un conjunt de peces 3D i servo motors per emular els moviments X-Y de la platina del microscopi i d'autoenfocament de la mostra. A causa de la problemàtica del diagnòstic a nivell global, i especialment en regions endèmiques on es disposa de pocs recursos, creiem que el sistema pot donar suport a les tècniques convencionals de diagnòstic de laboratori. També, pot ser una eina útil per a la formació de microscopistes, la digitalització de les mostres biològiques mitjançant bases de dades d'imatges, com a sistema de control de qualitat i/o per a la vigilància epidemiològica d'aquestes i d'altres malalties.



3. Objectius

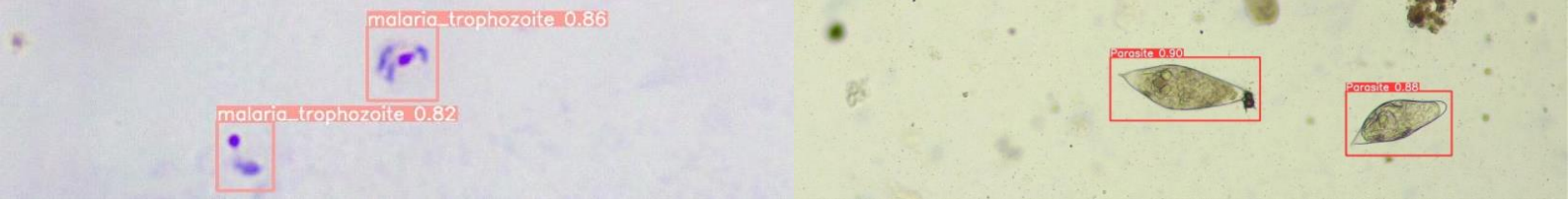
A continuació esmenem els objectius generals i específics de la tesi doctoral.

3.1. Objectiu general

Desenvolupament d'un nou sistema automatitzat pel diagnòstic de la malària i l'esquistosomiasi urogenital utilitzant eines d'intel·ligència artificial i un microscopi robotitzat universal de baix cost.

3.2. Objectius específics

- Captura i etiquetatge d'imatges digitals de mostres clíniques de malària i esquistosomiasi urogenital per a la creació d'una base de dades d'imatges.
- Entrenament de models basats en intel·ligència artificial per la creació d'algoritmes capaços de detectar automàticament els paràsits/cèl·lules en imatges digitals.
- Creació i disseny de peces 3D per la robotització universal de baix cost de microscopis òptics convencionals.
- Creació d'una aplicació per a telèfons intel·ligents capaç de guiar els moviments de la platina del microscopi i l'autoenfocament de la mostra, i integrar els algoritmes diagnòstics per la malària i l'esquistosomiasi urogenital.
- Validació del desenvolupament del sistema pel diagnòstic de malària i esquistosomiasi urogenital als laboratoris del Departament de Microbiologia de l'Hospital Vall d'Hebron i al Centre de Malalties Transmissibles i Salut Internacional Drassanes-Vall d'Hebron.
- Validació diagnòstica del sistema automatitzat pel diagnòstic de malària als laboratoris del Departament de Microbiologia de l'Hospital Vall d'Hebron i al Centre de Malalties Transmissibles i Salut Internacional Drassanes-Vall d'Hebron, i al laboratori de l'Hospital Nossa Senhora da Paz (Cubal, Angola).



4. Resultats

4.1. Articles publicats

4.1.1. *iMAGING*: a novel automated system for malaria diagnosis by using artificial intelligence tools and a universal low-cost robotized microscope

Revista: Frontiers in Microbiology

Impacte (any de publicació): Factor d'impacte 5.2 ; Quartil 1 (Q1) Microbiologia

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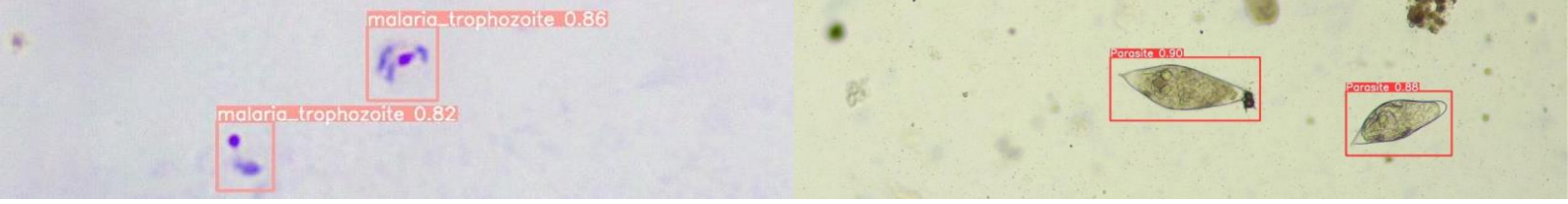
DOI: 10.3389/fmicb.2023.1240936



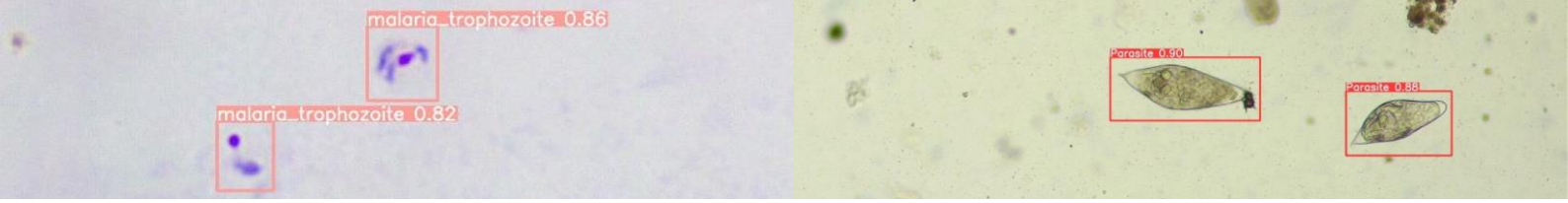
Resum del treball:

La malària és una de les malalties infeccioses més prevalents a l'Àfrica subsahariana, amb 247 milions de casos reportats a tot el món durant el 2021 segons l'Organització Mundial de la Salut. La microscòpia òptica continua sent la tècnica *gold standard* pel diagnòstic de la malària, però requereix experiència, temps i és difícil d'estandarditzar. Com a alternativa, les noves tècniques de diagnòstic basades en l'anàlisi digital d'imatges mitjançant eines d'intel·ligència artificial poden millorar el diagnòstic i ajudar a automatitzar-lo.

En aquest estudi, es va crear un conjunt de dades de 2571 imatges etiquetades de gota gruixuda de sang. Les xarxes neuronals YOLOv5x, Faster R-CNN, SSD i RetinaNet van ser entrenades per a la detecció d'objectes en el mateix conjunt de dades, i es va avaluar el seu rendiment en la detecció de paràsits de *Plasmodium*. Es van aplicar mòduls d'atenció i es van comparar amb els resultats de YOLOv5x. Per automatitzar tot el procés de diagnòstic, es va dissenyar un prototip de peces impreses en 3D per a la robotització de la microscòpia òptica convencional, capaç d'autoenfocar la mostra i fer l'escaneig de tota la preparació.

L'anàlisi comparativa va demostrar un rendiment per a la xarxa YOLOv5x de 92.10% de precisió, 93.50% de *recall*, 92.79% de valor F i 94.40% *mAP0.5* per a la detecció general de trofozoïts de *Plasmodium* primerencs, madurs i leucòcits. Els valors F corresponents a cada categoria van ser del 99.0% per als leucòcits, del 88.6% per als trofozoïts primerencs i del 87.3% per a la detecció de trofozoïts madurs. El rendiment dels mòduls d'atenció va demostrar diferències estadístiques no significatives en comparació amb el model original entrenat de YOLOv5x. Els models predictius es van integrar en una aplicació per a telèfons intel·ligents per al diagnòstic basat en l'anàlisi d'imatges. El sistema és capaç de realitzar un diagnòstic totalment automatitzat: mitjançant els moviments d'enfocament automàtic, d'escaneig en els eixos X-Y del microscopi robotitzat, els models de XNC entrenats per a l'anàlisi d'imatges digitals i el telèfon intel·ligent. El nou prototip és capaç de determinar si una gota gruixada de sang tenyida amb Giemsa és positiva/negativa per a la infecció de *Plasmodium* i els seus nivells de parasitèmia. Tot el sistema es va integrar a l'aplicació per a telèfons intel·ligents anomenada *iMAGING*.

La coalescència del sistema totalment automatitzat a través de moviments de rastreig i d'enfocament automàtic, i la detecció autònoma de paràsits de *Plasmodium* en imatges digitals amb un programari per a telèfons intel·ligents i algoritmes d'IA confereix al prototip les característiques òptimes per unir-se a l'esforç global per la lluita contra la malària, les malalties tropicals desateses i altres malalties infeccioses.



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iMAGING: a novel automated system for malaria diagnosis by using artificial intelligence tools and a universal low-cost robotized microscope

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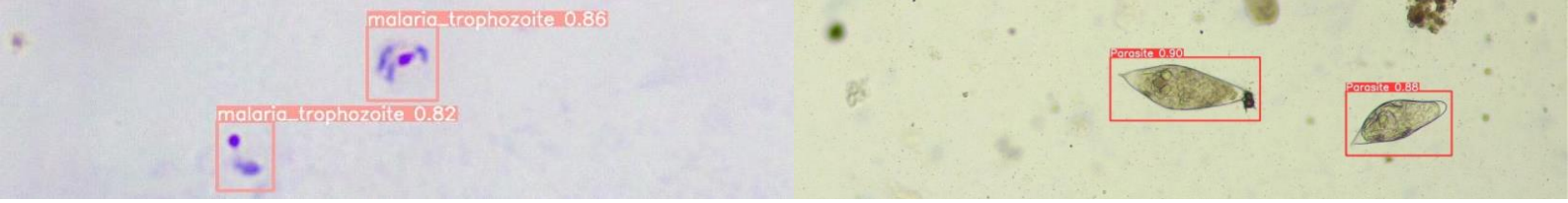
Introduction: Malaria is one of the most prevalent infectious diseases in sub-Saharan Africa, with 247 million cases reported worldwide in 2021 according to the World Health Organization. Optical microscopy remains the gold standard technique for malaria diagnosis, however, it requires expertise, is time-consuming and difficult to reproduce. Therefore, new diagnostic techniques based on digital image analysis using artificial intelligence tools can improve diagnosis and help automate it.

Methods: In this study, a dataset of 2571 labeled thick blood smear images were created. YOLOv5x, Faster R-CNN, SSD, and RetinaNet object detection neural networks were trained on the same dataset to evaluate their performance in *Plasmodium* parasite detection. Attention modules were applied and compared with YOLOv5x results. To automate the entire diagnostic process, a prototype of 3D-printed pieces was designed for the robotization of conventional optical microscopy, capable of auto-focusing the sample and tracking the entire slide.

Results: Comparative analysis yielded a performance for YOLOv5x on a test set of 92.10% precision, 93.50% recall, 92.79% F-score, and 94.40% mAP0.5 for leukocyte, early and mature *Plasmodium* trophozoites overall detection. F-score values of each category were 99.0% for leukocytes, 88.6% for early trophozoites and 87.3% for mature trophozoites detection. Attention modules performance show non-significant statistical differences when compared to YOLOv5x original trained model. The predictive models were integrated into a smartphone-computer application for the purpose of image-based diagnostics in the laboratory. The system can perform a fully automated diagnosis by the auto-focus and X-Y movements of the robotized microscope, the CNN models trained for digital image analysis, and the smartphone device. The new prototype would determine whether a Giemsa-stained thick blood smear sample is positive/



negative for *Plasmodium* infection and its parasite levels. The whole system was integrated into the *iMAGING* smartphone application.

Conclusion: The coalescence of the fully-automated system via auto-focus and slide movements and the autonomous detection of *Plasmodium* parasites in digital images with a smartphone software and AI algorithms confers the prototype the optimal features to join the global effort against malaria, neglected tropical diseases and other infectious diseases.

KEYWORDS

malaria, malaria diagnosis, convolutional neural networks, artificial intelligence, robotized microscope, smartphone application, YOLOv5, thick blood smears

1 Background

Malaria is a vector-borne disease caused by parasites of the genus *Plasmodium* (1). It is transmitted to humans by the bite of an infected female mosquito of the species *Anopheles* and has a high prevalence in tropical regions worldwide (Talapko et al., 2019). According to World Health Organization (WHO) estimates, 247 million cases of malaria were reported globally in 2021, increasing from 245 million in 2020 (World Health Organization, 2022). Malaria heavily affects the African Region with about 95% of all cases and 96% of all deaths (World Health Organization, 2022). There are five species of *Plasmodium* parasites that can infect humans: *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi* (Talapko et al., 2019).

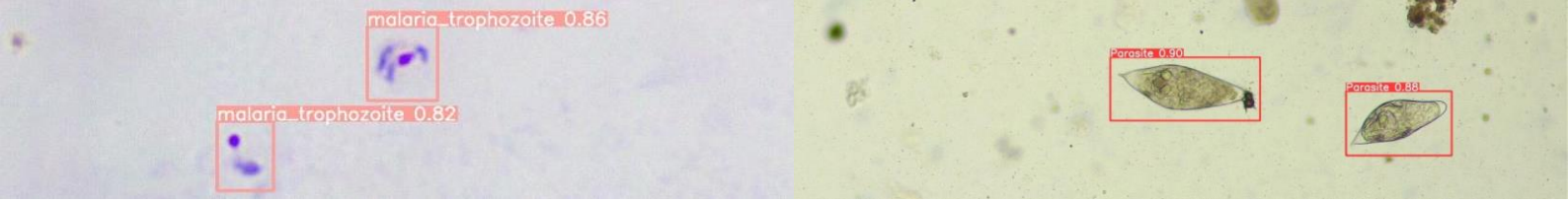
Microscopic visualization of blood smears is still the gold-standard method for malaria diagnosis (Calderaro et al., 2021). The examination of thick and thin blood smear samples by traditional optical microscopy allows the visualization of active parasitic forms in peripheral blood (WHO, 2022). Differentiation of parasite species is performed by the visualization of thin blood smears with Giemsa staining (WHO, 2016). Thick blood smear is a 20-40-fold more sensitive technique compared with thin smears and, therefore, can visualize lower parasite levels (Wangai et al., 2011). Microscopy is an inexpensive and efficient technique that allows the identification of *Plasmodium* parasites at the species level, the determination of different developmental stages of the parasites, and the quantification of parasite density. It is widely used in endemic and low-income areas from Sub-Saharan Africa and reference laboratories worldwide. However, it is an expert-dependent technique, can generate diagnostic mistakes due to the consecutive visualization of a high number of samples, and can lead to diagnostic inaccuracies due to fatigue and workload.

Rapid Diagnostic Tests (RDTs) are blood antigen detecting tests with an immunochromatographic lateral flow device that allows the diagnosis of malaria parasites (Cunningham et al., 2019). The limit of detection of malaria RDTs is about 100–200 parasites/μL of blood (Berzosa et al., 2018; Acquah et al., 2021). However, the emergence of gene deletions coding for the HRP2/3 proteins is causing an increase in false-negative results due to the lack of the detection antigen with consequences for the final diagnosis (Jejaw Zeleke et al., 2022). Polymerase Chain Reaction (PCR) for malaria diagnosis (i) is a highly sensitive technique (Leski et al., 2020); (ii) allows to distinguish between *Plasmodium* species (van Bergen et al., 2021); (iii) requires specific material, and (iv) is costly and relatively complex (Fitri et al., 2022). Quantitative Buffy Coat (QBC), Flow cytometry, or biomarker identification are used to complement traditional methods (Calderaro et al., 2021). Nevertheless, malaria diagnosis is still an issue in some regions, which could lead to misdiagnosis and generate several complications due to the difficulty of implementing these techniques in resource-poor settings (Boyce and O'Meara, 2017). The lack of resources and health care personnel in malaria-endemic areas are a limitation for accurate diagnosis (Wambani and Okoth, 2022). Moreover, traditional diagnostic techniques are time-consuming and require high-level trained professionals. Thus, the development of accessible, low-cost, automated diagnostic techniques is a major challenge for malaria parasite detection and would be a supportive complement to traditional techniques.

Nowadays, Artificial Intelligence (AI) is a disruptive technology with a high impact on health-related goals. Convolutional Neural Networks (CNNs) are artificial neural network models commonly used to analyze and classify images with deep learning tools; and they have improved traditional image-processing techniques through their faster and highly automated procedure. Novel diagnostic techniques based on AI are being developed and optimized for the detection of *Plasmodium* parasites in thick and thin blood smear digital images (Sankaran et al., 2017; Fatima and Farid, 2020; Yang et al., 2020; Abubakar et al., 2021; Islam et al., 2022). CNN-based malaria detection algorithms able to detect *P. falciparum* parasites in Giemsa stained thick blood smear slides were developed and demonstrate robustness with a wide variety of field-prepared samples (Mehanian et al., 2017a,b). As another example, DeepMCNN system was able to calculate parasitaemia estimations by counting parasites and leukocytes as recommended by the WHO (Manescu et al., 2020). Algorithm analysis is a crucial step to correctly evaluate and implement machine learning solutions for clinical usage with effective metrics (Delahunt et al., 2022).

Mobile software applications are used to integrate the technology and provide a fast and efficient diagnosis for *Plasmodium* detection (Rosado et al., 2016; Vasiman et al., 2019). However, smartphones have several limitations that should be addressed such as: image resolution required for malaria diagnosis, optical attachment and adaptation to the microscope, high number of fields-of-view (FoVs) for diagnosis, and the need for focused images and Z-stacks (Mehanian et al., 2017a,b; Delahunt et al., 2022).

Moreover, automation of the entire process, including autofocusing and slide tracking movements, would be of significant help to obtain an optimal diagnostic tool. Robotized microscopes that move the slide and capture images are usually expensive and not



designed for implementation in malaria-endemic countries. Observational studies to evaluate the performance of automated detection systems for malaria diagnosis were performed in in-field settings with promising results, such as EasyScan Go and Autoscope (Torres et al., 2018; Horning et al., 2021; Das et al., 2022). This type of device could be a useful diagnostic tool, not only for malaria diagnosis, but also for other infectious or Neglected Tropical Diseases (NTDs), such as schistosomiasis, trypanosomiasis, and filariasis. However, specific peculiarities in terms of optical train, number of FoVs for diagnosis, parasite sizes and sample preparation should be considered.

In this study, we trained multiple computational state-of-the-art deep learning models for malaria parasite detection in thick blood smear digital images. A malaria-labeled image database was created and employed to train the CNN models. We compared the performance of different neural networks with the same dataset, and applied attention modules. In addition, a low-cost robotized microscope was designed to automate the image auto-focus and slide movements. Arduino controllers, 3D-printed pieces, and servo motors were used to create a single prototype for the universal automation of optical microscopes. The system does not need internet connection and was power supplied by portable solar batteries. Finally, the diagnosis technology has been integrated into a smartphone application called *iMAGING*, which controls the microscope's slide movements and detects malaria parasites in digital images via trained CNNs on a computer. According to our knowledge, it is the first fully automated low-cost system for malaria diagnosis with artificial intelligence tools, and specially designed for low-income regions and malaria endemic regions. We consider that the development of this novel digital image diagnosis technology would contribute to erradicate malaria and other neglected tropical diseases, and would join the global effort to fight against infectious diseases of poverty.

2 Materials and methods

2.1 Convolutional neural network algorithms

To generate the malaria digital image database and train CNN algorithms, the following methodologies, summarized in Figure 1, were employed. The same dataset was used to train the different CNN models. This methodology follows quality standards and was the same for all experiments. This type of work allows us to standardize the methods thus obtaining reliable and comparable results between the neural network models employed.

2.1.1 Image acquisition

Giemsa-stained thick blood smear samples were used to capture microscopic digital images for further image labeling and identification. Thick blood smear biological samples were provided by the (i) Vall d'Hebron University Hospital (Barcelona, Spain), (ii) Drassanes-Vall d'Hebron International Health and Infectious Diseases Centre (Barcelona, Spain), (iii) Malaria collection samples of Drassanes-Vall d'Hebron, and (iv) Saint John of God Hospital (Lunsar, Sierra Leona). Parasites of *P. falciparum*, *P. vivax*, *P. ovale*, and *P. malariae* species were visualized in the samples used to correctly detect *Plasmodium* infection. Samples were validated by three expert clinical parasitology microscopists from Drassanes-Vall d'Hebron International Health and Infectious Diseases Centre Laboratory. All thick blood smear samples were positive for *Plasmodium* infection with parasite levels ranging from 80 to +10,000 parasites/μL. A Leica ICC50W integrated digital microscopy camera (5.0 MP) and the digital camera of a Samsung Galaxy S20 (64 MP, 0.8 μm, f/2.0, OIS) smartphone device were employed for image acquisition. Image pixel size (resolution) was 3,024 × 4,032 pixels for smartphone-captured images, and 2,992 × 1944 pixels for the Leica ICC50W digital camera. An adapter 3D bracket attached to the ocular lens of the microscope was used to standardize the image-capturing procedure with the smartphone device. Both, integrated camera and smartphone images were captured by the visualization of blood smear samples through a Leica DM750 microscope lens with 1,000x total magnification (10x ocular; 100x immersion oil objective).

2.1.2 Image pre-processing

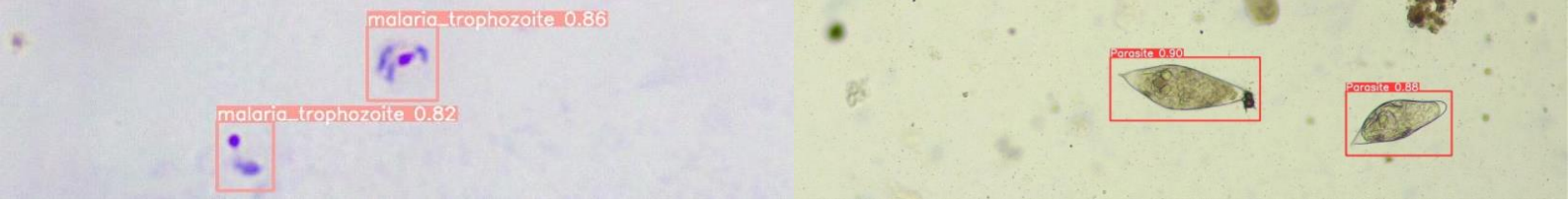
The images were cropped to highlight the area of interest and eliminate the black borders typical of acquisition with a smartphone device. Cropping was only performed in smartphone-acquired images to remove the outer edges without losing information. Original smartphone images were cropped automatically (Python script) to obtain a 4:3 image in the center, and subsequently rotated 90° for image reorientation. With this procedure, it is possible to crop an image regardless of its dimensions and number of pixels, as proportions were used to perform the cropping. The cropped images have the same 4:3 image proportion as those acquired with the microscope-integrated camera. Cropping confers a recomposition of the image that may positively affect the final results, providing a clearer image and removing elements irrelevant to the prediction and identification functions of the neural networks (Cheng et al., 2022). Pre-processed images were used for image annotation and CNN training.

2.1.3 Image annotation

Both, integrated digital camera and smartphone camera acquired images were labeled by experts of the Drassanes-Vall d'Hebron International Health and Infectious Diseases Centre. Parasite forms, leukocytes, and artifacts were labeled in malaria thick blood smear digital images. A personalized annotation software was developed with the Python programming language for digital image labeling (Python Software Foundation, 2019). For image annotation, the area of interest was selected by creating a bounding box with the object inside. Labels were considered single-object detections on digital database images, therefore an image contains multiple annotations. Parasite forms (malaria blood stage cycle) as early trophozoites and mature trophozoites were labeled. Leukocytes were annotated in digital malaria images for further parasite density calculations. Artifacts and confusing forms due to illumination issues, sample preparation, Giemsa staining reagents, or image capturing were also labeled. The annotation procedure is represented in Figure 1. Once labeling is finished, the Annotation App software creates a *json* file with annotations linked to the original image file, in which the coordinates of the labeled objects are specified.

2.1.4 Convolutional neural networks training

A comprehensive comparative study to evaluate the performance of several state-of-the-art object-detection CNN models was designed. The comparison of the convolutional neural networks has been



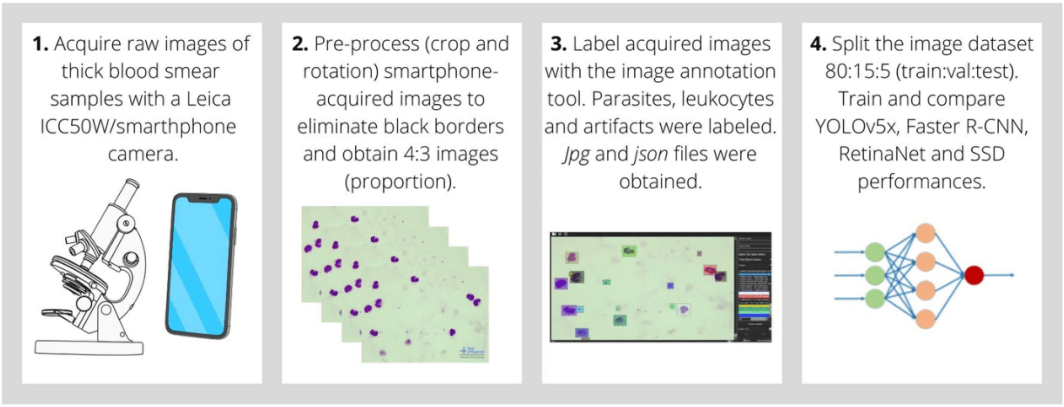


FIGURE 1
Sequential procedure of CNN algorithm generation for malaria parasite detection in thick blood smear digital images. **1.** Images were acquired through the microscope lens with an ICC50W integrated digital camera or a smartphone camera. **2.** Smartphone-acquired images were pre-processed to eliminate black borders from the surroundings of the original raw image by image cropping and rotation. New images have a 4:3 proportion, emulating integrated camera-acquired images. **3.** Image labeling by image annotation software. Parasites, leukocytes, and artifacts were labeled for further CNN training. JPG (image) and JSON (labels linked to image) files were generated. **4.** Split image dataset into 80% training, 15% validation, and 5% testing. Train and compare YOLOv5x, Faster R-CNN, RetinaNet, and SSD performances.

designed based on previous similar studies (Dong et al., 2017; Pattanaik et al., 2019). Pre-trained YOLOv5 (Redmon et al., 2016), Faster R-CNN (Ren et al., 2017), SSD (Liu et al., 2016), and RetinaNet (Pardede et al., 2020) models with the COCO dataset (Dataset, n.d.) were fine-tuned with our malaria thick blood smear positive *Plasmodium* infection samples dataset. CNNs were trained for multi-class classification with three categories: early trophozoites, mature trophozoites, and leukocytes. Their performances were evaluated by precision, recall, *F*-score, and mAP0.5 descriptive values with validation and test sets. The malaria dataset was split into the same proportions for each CNN model: 80% images for training, 15% for validation and 5% for testing. Images/samples for each subset were the same to standardize and compare performances under equal training conditions, preserving patient-level structure.

2.1.5 Attention modules

Preliminary tests to check attention modules perfomance in our malaria dataset with the same proportion split (80% train / 15% validation / 5% test) were carried out. In particular, we have trained the YOLOv5x with the Squeeze and Excitation (SE) attention module, as well as the Convolutional Block Attention Module (CBAM) as stated in Hu et al. (2018) and Woo et al. (2018) respectively. Their performances were evaluated by precision, recall, *F*-score, and mAP0.5 descriptive values with test set and compared with YOLOv5x results as demonstrated in other studies (Gong et al., 2022).

2.1.6 Statistical analysis

Statistical analyses to determine significant differences between validation and test subset performance for each CNN model was performed. Metric means were calculated individually for each CNN model and subset (validation/test) by t-test analysis ($p < 0.05$). To evaluate significant statistical differences between CNNs models and attention modules a paired *t*-test analyses ($p < 0.05$, *t*-value > 2/−2), mean (M) and standard deviation (SD) were employed. Statistical analyses were performed with IBM SPSS Statistics environment.

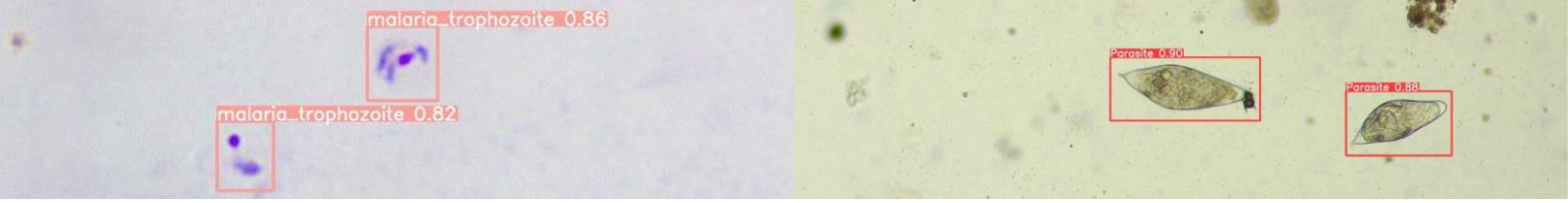
2.2 Microscope automation and smartphone application

2.2.1 Design of a 3D-printed prototype for microscopy automation

An Ender-3 Creality 3D printer was used to build Polylactic Acid (PLA) and Polyethylene terephthalate glycol (PETG) pieces for microscope focus automation. The entire prototype was designed with Tinkercad Open Source specialized software and Ultimaker Cura software (Ultimaker Cura: software de impresión 3D potente y fácil de usar | Ultimaker, n.d.). Autofocusing and two-dimensional *X*-*Y* track slide movements were performed by low-cost servo and step-by-step motors. Power requirements are: servo motor 9G / 5 V and 500 mA each; stepper motor Rohs 28BYJ-48 / 5 V and 240 mA; and Arduino MKR Wifi 1,010 / 5 V and 700 Ma. The whole system requirements are 5 V and 2A.

2.2.2 Auto-focus algorithm

In our mechanism we have employed the variance of Laplacian as a reference method for image auto-focusing. This method allows the calculation of a value for each image, which indicates the level of focusing of the acquired picture. Therefore, the analysis of variance of Laplacian values would determine which is the best focused image for each Field of View (FoV) (Salido et al., 2020). The variance calculation is performed in each FoV determined by *X*-*Y* movements of the robotized microscope. In a single FoV the smartphone camera observes different focused images by the continuous movement of the step-by-step motor on the fine adjustment wheel. The smartphone device by Bluetooth (*BLE*) connection with the controllers guides the step-by-step motor to move the wheel in both directions of rotation (30 position units of movement in each direction) in order to focus the biological sample. During the auto-focusing process a Laplacian variance value is computed to each of the images. The system visualizes the centroid of the original image by creating a new cropped image for



Laplacian analysis. This procedure allows the observation of only the center of the image, without the black borders produced by the ocular lens attachment. Once the image scanning in the two directions of rotation has been completed, the mechanism is able to return to the position of highest focus by analyzing the variance values. An image was captured in the focused position by the smartphone camera for further image analysis.

2.2.3 Integration of trained CNN models into computer software

A Lenovo ThinkPad intel Core i5 computer was employed to run CNN algorithms. A smartphone app was developed with the official open-access integrated development environment, Android Studio (Android Developers, 2021). The *iMAGING* smartphone app confers the possibility of integrating CNN and automated microscope technology into a single software. To increase computational power and speed up neural network detection, images captured from the smartphone device are sent via a *BLE* connection to the computer for further analysis by CNN models. This type of transmission does not depend on an internet connection.

3 Results

A novel automated diagnostic method for malaria parasite detection in thick blood smear digital images was developed and integrated into a smartphone app. The flowchart of the operational procedure is represented in Figure 2.

3.1 Convolutional neural network

3.1.1 Malaria thick blood smear image database analysis

Once all images were captured and labeled, a digital image database of malaria thick blood smears was generated; a total of 148 thick blood smear samples were used for image database generation. Between 10 and 20 images of different microscopy fields were captured for each sample employed. A total of 2,571 labeled images were imported into the database for further CNN training and diagnosis algorithm generation. Annotation numbers for each label class were: 24437 leukocytes, 37,820 early trophozoites and 1,641 mature trophozoites were labeled taking into account all database images. A total of 2,238 images were captured with the integrated microscope camera LEICA ICC50W and 333 images were acquired with the Samsung Galaxy S20 smartphone camera. Malaria thick blood smear image database summary information is represented in Table 1. The total number of images and labels employed for CNN training was decided considering other similar studies (Mehanian et al., 2017a,b; Torres et al., 2018; Yang et al., 2020), and were conditioned by the number of *Plasmodium* infection samples available in the laboratory and CNN algorithms proven performance results.

3.1.2 Convolutional neural network comparison

Object-detection state-of-the-art CNNs were trained and compared to evaluate their performance. Table 2 shows the most relevant metrics to evaluate the performance of YOLOv5x, Faster R-CNN, SDD, and RetinaNet with validation and test data image subsets. Results of the t-test analysis indicated that there was a non-significant statistical difference between neural network performance with validation and test data subsets, as expected (Table 2).

Precision test values ranged between 0.8913–0.9562, setting considerably optimized results with all trained CNN models. Precision parameter analysis indicates that all CNNs have an optimized identification algorithm, with low rates of failures when parasite and leukocyte detections were performed. Analysis of recall (sensitivity) test values generated a wide range of values: 0.4789–0.9331. These remarkable differences between CNNs indicate that YOLOv5x and Faster R-CNN, with recall values of 0.9350 and 0.9638, respectively, were the optimal CNNs for these type of detections. Low recall values, as for SSD, indicate that the algorithm could not detect all objects of interest and, therefore, high levels of false-negative results were obtained.

The *F*-score is the harmonic mean of precision and recall (Equation 1); therefore, *F*-score analysis of the different trained CNNs was relevant to determine the model with the best performance. The highest *F*-score value was 0.9279, corresponding to the YOLOv5x neural network. The Faster R-CNN *F*-score value was just lower than that of YOLOv5x, with a final result of 0.9261. Both neural networks were optimal in terms of accuracy and, therefore, with high precision and recall values. In Supplementary Figure 1, the precision-recall graph of the YOLOv5x algorithm demonstrates the aforementioned results.

$$F\ score = 2 \cdot \frac{precision \cdot recall}{precision + recall} \quad (1)$$

Equation (1): *F*-score value calculation.

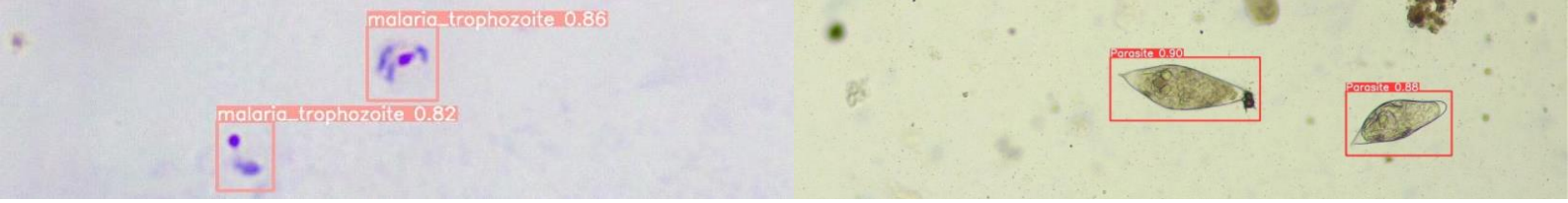
Finally, mAP values were between 0.9133–0.9489, indicating a high accuracy value for the trained object-detection models.

Once all descriptive parameters were analyzed, we concluded that YOLOv5x and Faster R-CNN were the best CNNs based on object detection for our image database. Results of the paired t-test indicated that there was a non-significant difference between YOLOv5x ($M = 0.9$; $SD = 0.02$) and Faster R-CNN ($M = 0.9$; $SD = 0.03$), $t(7) = 0.8$, $p = 0.429$. The low recall values for the SSD model indicate a non-reliable algorithm for parasite detection. The RetinaNet model had acceptable results, although not as positive as YOLOv5x and Faster R-CNN. As a result, we present several digital labeled images of a test data subset in Figure 3, and predictions performed by the YOLOv5x trained model.

Finally, in order to evaluate the multi-class model for each category, results of YOLOv5x were shown in Table 3. Descriptive parameters of each class are important to evaluate the model for each category. Leukocytes have higher predictive values than early and mature trophozoites for all descriptive metrics.

3.1.3 Attention modules comparison

Attention modules results of SE and CBAM training with YOLOv5x algorithm are shown in Supplementary Table 1. Precision values of CBAM (0.9350) are slightly better when compared with original YOLOv5x (0.9210) and SE (0.9040). In the particular case of recall values, SE showed the best performance (0.9380) just



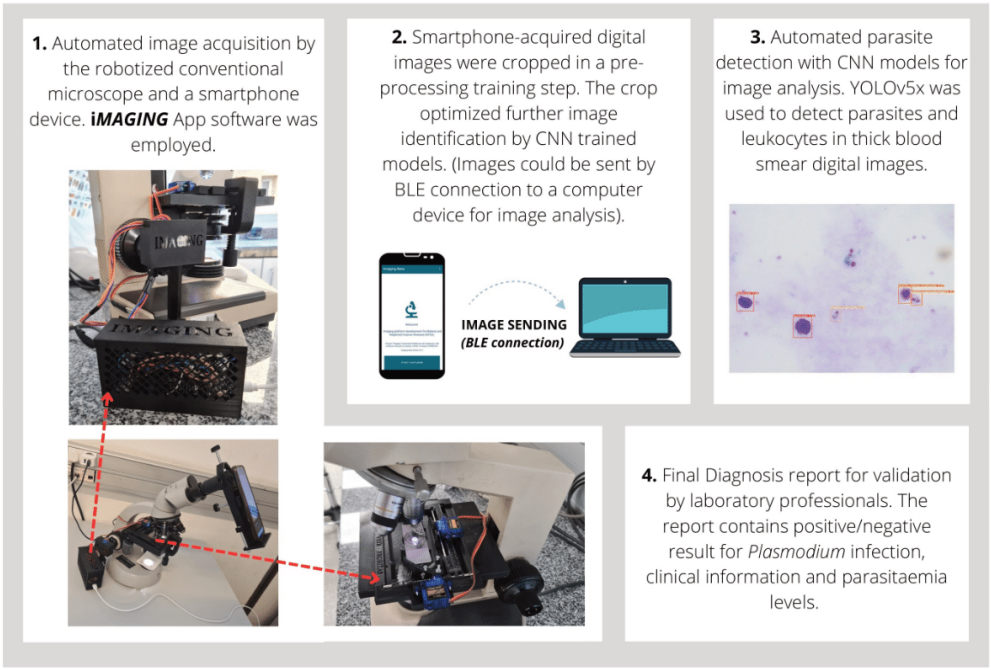


FIGURE 2
Flowchart of the malaria diagnostics procedure by *iMAGING* technology. The robotized microscope and smartphone were used as an emulation of traditional microscopic examination. **1.** The smartphone captures images via its camera using the *iMAGING* app. Images of the system prototype were represented. **2.** Images are cropped to eliminate areas of no interest for imaging diagnosis. Images are sent by a Bluetooth (*BLE*) connection to a computer for further analysis. **3.** Images are analyzed by computational technology and Convolutional Neural Network models for parasite detection. **4.** Finally a malaria diagnosis report containing clinical information is generated.

higher than that of YOLOv5x (0.9350). Therefore, *F*-score and mAP0.5 values of attention modules were similar to the original YOLOv5x trained model. Results of the paired t-test analysis indicated that there is a non-significant difference between YOLOv5x ($M = 0.9$, $SD = 0.01$) and CBAM ($M = 0.9$, $SD = 0.01$), $t(3) = 0.3$, $p = 0.779$; and that there is a non-significant difference between YOLO ($M = 0.9$, $SD = 0.01$) and SE ($M = 0.9$, $SD = 0.02$), $t(3) = 1.1$, $p = 0.348$. These results lead us to conclude that the application of attention modules in our database could help to obtain comparable results, although they did not improve on the performance of the original YOLOv5x CNN.

3.1.4 YOLOv5x negative sample validation

Analysis and validation tests were performed to evaluate the reliability of YOLOv5x model with confirmed negative samples. Thick blood smear samples from asymptomatic individuals (healthy control) from malaria endemic areas were (n = 5) provided by the Drassanes-Vall d'Hebron International Health and Infectious Diseases Centre with a negative RT-PCR result for *Plasmodium* infection (RealStar® Malaria Screen & Type PCR kit 1.0, Altona, 22,767 Hamburg, Germany). A total of 166 images were acquired from thick blood smear samples and analyzed by the trained YOLOv5x model (confidence threshold 0.5). The employed images were not used for CNN algorithms training. A minimum number of 200 leukocytes were detected in each sample to emulate microscopy examination. After analysis, 161/166 digital images (96.98%) were negative for *Plasmodium* infection, and five false-positive results were reported (five artifacts detected as early trophozoites). A total of 1,008 leukocytes were detected in all digital images. Validation tests were crucial to evaluate the reliability of the system and illustrate the importance of clinical validation by professionals to report a final diagnosis. Inspection by laboratory professionals is desirable to conclude that the sample was negative and discard false-positive results.

3.1.5 YOLOv5x Low-parasite density validation

Analysis and validation tests were performed to evaluate the reliability of YOLOv5x model with confirmed low parasite density *Plasmodium* infected samples. Thick blood smear samples ($n = 5$) provided by the Drassanes-Vall d'Hebron International Health and Infectious Diseases Centre with a positive microscopy examination result (<800 parasites/μL) for *Plasmodium* infection were analyzed (confidence threshold 0.5). A total number of 50 *Plasmodium* positive images (10 images/sample) were acquired. Low-parasite images with a single *Plasmodium* parasite were analyzed to detect false-negative results. 47/50 images (94%) were correctly analyzed,

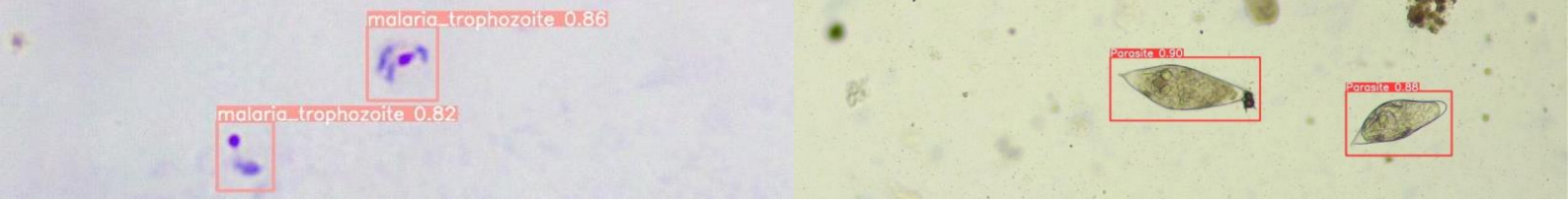


TABLE 1 Summary of Malaria thick blood smear sample/image database.

Category		Sub-Total	Total
Sample source	Drassanes-Vall d'Hebron International Health and Infectious Diseases Centre (Barcelona, Spain)	55 (samples/patients)	148 (samples/patients)
	Malaria collection samples of Drassanes-Vall d'Hebron (Barcelona, Spain)	82 (samples/patients)	
	Saint John of God Hospital (Lunsar, Sierra Leona)	13 (samples/patients)	
Plasmodium species	*P. falciparum*	47 (samples)	80 (samples)
	P. vivax/P. ovale	24 (samples)	
	P. malariae	7 (samples)	
	Mixed infection	2 (samples)	
Parasite density	Low (<1,000 p/μL)	57 (samples)	148 (samples)
	Medium (10,000–1,000 p/μL)	57 (samples)	
	High (+10,000 p/μL)	34 (samples)	
Image acquisition type	Integrated camera (ICC50W Leica)	2,238 (annotated images)	2,571 (annotated images)
	Smartphone (Samsung Galaxy s20)	333 (annotated images)	
Annotations category	Early trophozoites	37,820 (labels)	63,898 (labels)
	Mature trophozoites	1641 (labels)	
	Leukocytes	24,437 (labels)	

p, parasites; μL, microliter. Organizational database scale: Patient > Sample > Annotated Image > Annotation.

TABLE 2 Comparative table of object-detection CNN models' performance.

CNN model	Validation dataset				Test dataset				
	Precision	Recall	*F*-score	mAP 0.5	Precision	Recall	*F*-score	mAP0.5	*p*-value
YOLOv5x	0.8975	0.9197	**0.9085**	**0.9490**	0.9210	0.9350	**0.9279**	0.9440	0.157
Faster R-CNN	0.8753	**0.9331**	0.9033	0.9194	0.8913	**0.9638**	0.9261	0.9412	0.144
SSD	**0.9501**	0.4789	0.6368	0.8491	**0.9562**	0.5599	0.7063	0.9133	0.354
RetinaNet	0.9369	0.8155	0.8720	0.9180	0.9407	0.8719	0.9050	**0.9489**	0.187

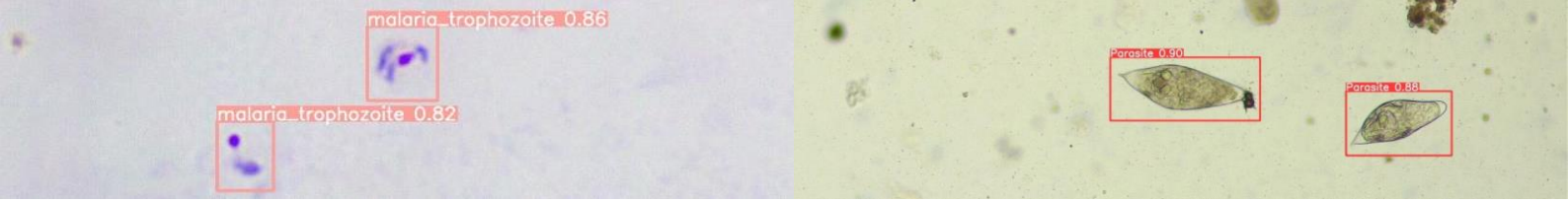
Descriptive parameter values of Precision, Recall, *F*-score, and Mean Average Precision (mAP0.5) are represented for Validation and Test datasets. YOLOv5x: You Only Look Once version 5 model x, Faster R-CNN: Faster R-Convolutional Neural Network, SSD: Single Shot Detector. Statistical analysis (*t*-test) to compare the performance of CNN models with validation and test data subsets ($p < 0.05$). Bold values represent the higher values of each parameter, in validation and test datasets.

with a total of three false-negative results (undetected mature trophozoites in a single sample). Final validation by laboratory professionals is desirable to conclude that the sample was positive and discard false-negative results.

3.1.6 Automated parasite density calculations

The parasite level calculation procedure was based on the Centers for Disease Control and Prevention (CDC) recommendations for parasite density calculation in thick blood smear samples (CDC – DPDx – Diagnostic Procedures – Blood Specimens, n.d.). The system can acquire images and detect the number of parasites and leukocytes in each microscopic field. Once the system has quantified 200 leukocytes, a parasite density result is obtained. The number of *Plasmodium* parasites and leukocytes was simultaneously counted to obtain a quantitative result. The CDC estimates that 1 μL of blood contains 8,000 leukocytes (CDC – DPDx – Diagnostic Procedures – Blood Specimens, n.d.), therefore, an automatic calculation of parasite number per microliter could be performed. In cases where less than 200 leukocytes were counted, an approximate value would be obtained with the same calculation. Equation (2) represents the algorithm for automatic parasite density calculation by image analysis.

$$Parasite\ density = \frac{\left(NDP : number\ of\ detected\ parasites\right) * \left(8,000\ leukocytes\right)}{\left(NDL : number\ of\ detected\ leukocytes \geq 200\right)} = parasites\ /\ \mu L \quad (2)$$



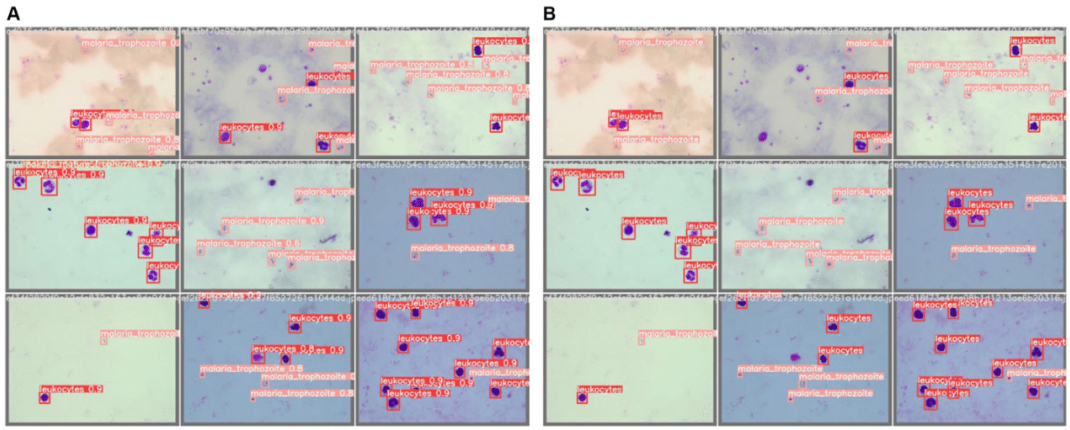


FIGURE 3
(A) Labeled test images by Vall d'Hebron-Drassanes professionals. **(B)** YOLOv5x trained model predictions of a test image subset with confidence values.

TABLE 3 YOLOv5x model parameters for each label classification category.

Category	Precision	Recall	*F*-score	mAP0.5
Leukocytes	0.989	0.993	0.990	0.994
Early *Plasmodium* trophozoites	0.908	0.866	0.886	0.938
Mature *Plasmodium* trophozoites	0.838	0.912	0.873	0.905

Equation 2: Parasite density calculation in thick blood smear samples by image analysis. The number of *Plasmodium* parasites and leukocytes detected in several digital images would determine the number of parasites per microliter of blood. A total number of 8,000 leukocytes were assumed in 1 μL of peripheral blood.

3.2 Microscopy automation and smartphone application

3.2.1 Universal microscopy automation prototype

A low-cost prototype was designed to automatically capture and detect malaria parasites in thick blood smear images on a conventional optical microscope. The main aim of microscope automation was to move the *X-Y* axis of the microscope stage and the auto-focus of the sample.

For the *X-Y* axis, an adapter was constructed to fit onto the microscope stage and hold the sample easily. Two servo-motors, one for each axis, capable of horizontal and vertical movements by means of toothed rotors, were attached to the same part. These pieces allow the movements across the sample, thus emulating the tracking of conventional microscopy. The *X-Y* control of the slide movement allows microscopic images to be captured for malaria diagnosis without overlapping each other. Each digital image corresponds to a microscopic field. The servo motors are controlled by Arduino controllers and connected via Bluetooth (version 5.0) to the smartphone device.

A step-by-step motor with an adapter arm was used for sample auto-focus. This was also monitored by Arduino controllers and connected via *BLE* to the smartphone device. For sample autofocusing the Variance of Laplacian has been employed (Salido et al., 2020). Additionally, an external cage was designed to store Arduino controllers, cables, and electronic parts. All 3D parts were designed as generic adapters by using measurements from various conventional optical microscopes commonly employed in laboratories (see Figure 2).

The motors used were finally controlled by a smartphone device, which captures microscopic images of different microscopy fields to detect biological forms in digital images. Image-capturing optimization was crucial for correct image acquisition. A smartphone adapter was designed to correctly attach the mobile phone lens to the ocular lens.

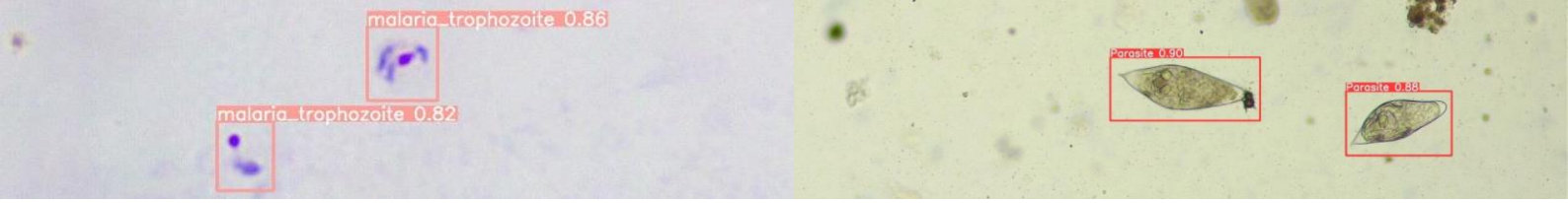
The whole 3D prototype was able to move the sample on the microscope stage, auto-focus the preparation, and capture digital images by means of a smartphone device. This mechanism allows automation of malaria diagnosis by the observation of thick blood smears and detection of parasite forms, thus emulating conventional microscopy. The smartphone device, step-by-step and servo motors, and controllers are powered by portable solar batteries.

3.2.2 Autofocus evaluation

An auto-focus experiment was performed to calculate the time of focus and prediction time of YOLOv5x CNN. A total number of 50 FoV of thick blood smear samples were auto-focused and analyzed by *iMAGING* prototype system. Results show an average time of focus of 8,144 ± 56 ms/FoV; and an average analysis time by YOLOv5x CNN and a Lenovo ThinkPad intel Core i5 of 2,126 ± 179 ms/image. Analyzed images show 6.2 ± 2.3 leukocytes/image and 7.1 ± 2.7 trophozoites/image (ring stange and mature), with a total of 13.3 ± 3.9 objects/image.

3.2.3 Imaging app software for malaria diagnosis

The imaging app software for smartphone devices was developed using the Android Studio development environment (Android Developers, 2021). The application allows the integration of all the technology developed for autonomous diagnosis. The smartphone, via the



application, could control and guide the movements of the microscope slide via a *BLE v5.0* connection and capture digital images in each microscopic field. Therefore, the mobile device acts as the key element of the process and is responsible for capturing the images that will subsequently be analyzed by the trained CNN models on the computer. Mobile phone devices are relatively low-cost and available worldwide; for this reason, they are ideal for imaging diagnostics and might be a suitable option for implementation in areas with resource-poor settings.

4 Discussion

CNNs have been employed for the detection of *Plasmodium* parasites in thick and thin blood smear microscopic images. However, whole procedure automation, huge labeled image datasets, system implementation in clinical laboratories and effectiveness are some of the major issues of these technologies.

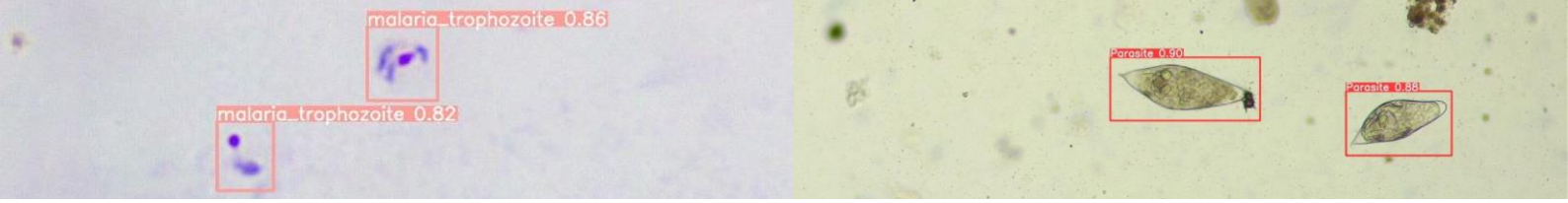
To address some of the main limitations, we have developed a fully automated diagnostic system for the detection of *Plasmodium* trophozoites and leukocytes in thick blood smear digital images by using AI tools and a low-cost robotized microscope. Training results showed optimal performance for early trophozoite, mature trophozoite, and leukocyte detection in a test dataset with the YOLOv5x and Faster R-CNN algorithms. Moreover, the adaptor 3D pieces confer the microscope the possibility to auto-focus the sample, scan the entire slide, and capture digital images with a smartphone camera for further image analysis and diagnosis. However, the system still has some limitations: (i) the need of trained personnel to prepare the Giemsa stain; (ii) it is able to detect *Plasmodium* spp. infection for malaria diagnosis, although it is not able to differentiate between *Plasmodium* species; and (iii) the difficulties that might appear in the field for its routinely implementation.

In our study, thick blood smears were used as reference samples for *Plasmodium* parasite detection. Thick blood smears should be the first step in microscopic visualization for malaria parasite detection, resulting in a positive/negative result for the sample analyzed (Maturana et al., 2022). Its observation is crucial to perform a malaria diagnosis and is widely employed in resource-poor settings due to its accessibility, conferring a valuable feature to the system. The detection of *Plasmodium* parasites and leukocytes by CNN algorithms provides a fast and efficient diagnosis. Moreover, species identification should be complemented with thin blood smear visualization for a complete diagnosis. Several studies demonstrate the possibility of detecting malaria parasites in thick (Kaewkamnerd et al., 2012; Rosado et al., 2016; Yang et al., 2020) and thin blood smear samples (Mushabe et al., 2013; Fatima and Farid, 2020), although few distinguish between species. The major limitation for *Plasmodium* species differentiation is the morphological similarities between them such as *P. ovale* and *P. vivax* (CDC, 2013), and the large image database required for the proper development of algorithms capable of differentiating between species (Krishnadas et al., 2022). Differentiation between *P. falciparum* and *P. vivax* could be the first step toward the generation of new models capable of detecting malaria parasites at the species level (Penas et al., 2017).

Moreover, the creation of a large database of thick blood smear labeled images (a total number of 63,898 labels) by professionals from an international health reference center confers an additional value to the diagnosis system. The employment of clinical biological samples is of vital importance for the acquisition and labeling of digital images as they more accurately emulate the practice of a microbiological diagnostic laboratory. In this study, there was an imbalance in label proportions between parasite stage forms due to biological reasons and the type of samples used. When thick blood smears were observed for *Plasmodium* parasite detection, the most common forms were early trophozoites or immature trophozoites (Phillips et al., 2017). In addition, most samples employed for database generation contain *P. falciparum* parasites, in which the majority of parasitic forms in peripheral blood are early trophozoites. Therefore, YOLOv5x, Faster R-CNN, SDD, and RetinaNet CNN models were trained with leukocyte, early trophozoite, and mature trophozoite image data labels. Generated models can detect the most common parasitic forms in thick blood smear samples and count leukocyte numbers to calculate parasite levels. Thus, trained CNNs could determine whether a sample is positive or negative for *Plasmodium* infection. *F*-score values are an optimal descriptive metric to evaluate or determine the best CNN model in cases of unbalanced data (Lopez-Nava et al., 2020). In cases of low-parasite levels a laboratory expert would be required to determine if the sample is positive/negative for *Plasmodium* infection. As demonstrated, with samples under 800 parasites/μL the system could trigger false-negative results (6%). Considering WHO guidelines in microscopy diagnosis, a parastitaemia of 80–200 parasites/μL are defined as difficult detection samples for certified WHO microscopists. Therefore, our system performance would be acceptable, although it should be evaluated following WHO microscopy diagnosis guidelines in clinical validation studies. RDTs might have a higher sensitivity and could also complement the final diagnosis (Slater et al., 2022).

Parasite density estimations by thick blood smear samples are performed following CDC recommendations to obtain autonomous calculations (CDC – DPDx – Diagnostic Procedures – Blood Specimens, n.d.). Parasite levels are crucial in *Plasmodium* infection and could determine the severity of malaria disease; therefore, leukocyte labeling is important and could provide valuable descriptive diagnostic information.

Descriptive metrics (precision, recall, *F*-score) can be compared with other predictive models based on CNNs for malaria parasite detection. When thick blood smear algorithms were compared with our predictive models, in most cases, *F*-score values (0.92–0.93) were very similar to the state-of-the-art reported in the literature (Yang et al., 2020; Kassim et al., 2021). In addition, a remarkable characteristic of our database is the heterogeneity of samples/images from different laboratories, preparation procedures, staining, smartphone/integrated camera acquisition, and *Plasmodium* species. Most studies used samples/images from a single laboratory or a single *Plasmodium* specie, commonly *P. falciparum*. In contrast, the addition of different images in terms of visual differences would affect the final descriptive parameters of the algorithm, although it would confer robustness to detect diverse preparations (Maron et al., 2021). Thin blood smear algorithms for parasite detection usually have higher values of precision, recall and, consequently, *F*-score, when compared with thick blood smears (Loddo et al., 2022; Magotra and Rohil, 2022). In addition, the customization of CNNs to improve detection results is generating optimal algorithms, such as the REONet method (modified ResNet-18) to classify malaria parasites on thin blood smears with 96.68% specificity, 94.79% sensitivity, and a 95.69% *F*-score (Zhu et al., 2022). This fact would explain the higher values of descriptive parameters in that type of study. Another important aspect was the comparative study of different object-detection CNNs. As demonstrated, the neural network model is crucial to obtain a reliable diagnosis, and the different structures and processing of each one would determine the final results. The same dataset was evaluated with different object detection CNNs, demonstrating optimal results with the YOLOv5x and Faster R-CNN models. The most efficient



neural network is YOLOv5, however, the recall values of the Faster R-CNN model are slightly higher, and may perform better for the detection of samples with low parasite concentrations. In our study, YOLOv5x has the highest *F*-score value (92.79%) in comparison with other evaluated CNNs and attention modules, and is the most balanced neural network in terms of descriptive metrics. In addition, it is the model that best fits with our technology, as the processing and analysis of the images are fast (Li et al., 2022), and allows it to be integrated into the software of smartphone devices (Liu et al., 2022). By analyzing the metrics, speed and applicability of the YOLOv5x neural network, we could determine that it is the most suitable option for our system. However, the Faster R-CNN model could be a similar alternative with comparable results, at the expense of the RetinaNet and the SSD models. In the medical field, the minimization of false positive and negative results is an added value for the diagnosis. The neural network with the best sensitivity and recall, and consequently *F*-score, is the one that will generate the lowest errors. This factor reaffirms the choice of the YOLOv5x model for our system. Recent studies demonstrated that modification by increasing the feature scale and adding detection layers to the YOLOv3 and YOLOv4 algorithms could be an optimal solution to improve their performance in thick blood smear images (Abdurahman et al., 2021). Modified YOLOv4 obtains a mAP value of 96.32% for the detection of early trophozoites in thick blood smear digital images. When compared with our database results, their performance is slightly better, although mature trophozoites were not included for training and detection. Finally, single category results demonstrate that the detection of leukocytes is superior (*F*-score 0.990) when compared with early and mature trophozoites (*F*-scores of 0.886 and 0.873 respectively). YOLOv5x is demonstrated to be better with the detection of larger objects in digital images, as our results confirmed and in concordance with other studies (Liu et al., 2022). SE and CBAM attention modules were applied to YOLOv5x model, although they did not improve its final performance as demonstrated in statistical analysis. However, precision values of CBAM and recall values of SE were slightly better when compared to YOLOv5x, leading to a lowest ratio of false-positive and false-negative results, respectively. As an alternative to CNNs, there are transformer-based methods such as Detection Transformer (DETR) which are a transformer encoder-decoder architecture and a set-based global loss that forces unique predictions via bipartite matching for object detection (Carion et al., 2020).

It is important to note that the descriptive metrics of the neural networks evaluate the models generated and determine the final development validation of the whole prototype, however, they do not provide information on the sensitivity and specificity of the entire diagnostic system. Image auto-focusing issues, illumination changes, staining artifacts, or microscope model could affect the quality of the acquired images, therefore CNN prediction values would be negatively affected. In order to reliably evaluate the complete diagnostic tool, clinical validation tests should be pursued in reference laboratories and resource-poor settings. However, an earlier development phase, such as the one presented in this study, is crucial to provide the basis for future validation and implementation studies.

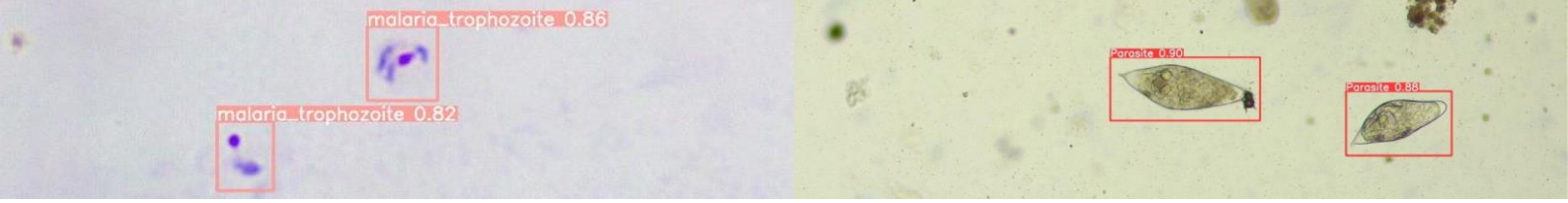
In addition, one of the key added values of the project is the universal microscope automation by means of the movement of the slide and the auto-focusing of the sample. The low-cost mechanism allows us to automate the process from beginning to end. A recent study demonstrated that the automation of a microscopic system for malaria detection could provide valuable results (Yoon et al., 2021). Yoon et al. (2021), developed a system with 100% sensitivity and specificity for the detection of *P. falciparum* cultures and *P. vivax* samples (Yoon et al., 2021). Lower-resolution images or microscope models could explain our different results, however, our novel diagnostic system is affordable, easy to use, universally adaptable, cheap, and specifically designed for any type of laboratory and infrastructure. Portable solar batteries provide an alternative to relying on electrical power to operate our system. Other design proposals for robotization and implementation based on a 3D-portable mobile microscope are of significant value to this area of study, and help improve and advance the development of portable and automated diagnostic systems (García-Villena et al., 2021).

Moreover, several studies demonstrated the utility of smartphone devices for the automatic diagnosis of malaria parasites via imaging techniques (Cesario et al., 2012; Poostchi et al., 2018; Yang et al., 2020; Yu et al., 2020; Zhao et al., 2020). Its powerful analog, digital, and telecommunication functions, combined with cloud data processing, confer smartphones with a wide range of diagnostic possibilities (Merazzo et al., 2021). Yu et al., 2023 evaluated the performance of a smartphone-based malaria diagnostic application in thick blood smear images with promising results, and could be considered as a milestone for further studies (Yu et al., 2023). However, parasite levels were not evaluated as distinct from our system.

The validation and implementation of the *iMAGING* prototype in resource-poor setting environments would be the next step. The continuous loss of microscopist experts is a major problem for clinical laboratories, although microscopy should remain a reference method of high relevance in microbiological diagnosis (Bradbury et al., 2022). Therefore, the system was designed to be a supportive tool for microscopists, in order to facilitate their routinely laboratory practice for malaria diagnosis and could be a suitable option for their training, by the continuous visualization of *Plasmodium* parasites in digital microscopy images. It is crucial to understand traditional microscopy to validate and implement novel diagnostic techniques based on AI. The direct visualization of parasites by microscopic observation of blood smears is an unequivocal sign of a positive diagnosis, one of the major strengths of this procedure compared with molecular or RDT techniques (Bradbury et al., 2022).

5 Conclusion

Automated malaria diagnosis is a major challenge to improve and support traditional microscopic techniques. Artificial intelligence diagnostic techniques would not only be useful for implementation in malaria-endemic countries but also for professional training, sample digitization, and diagnostic support for any laboratory, regardless of their resources. It is only a matter of time before novel diagnostic techniques based on AI and image digitalization erupt into medical environments to provide support for traditional microscopy. Microscopic visualization of thick blood smears can be tough and complex, therefore, assisted diagnostic methods based on AI, such as the one described herein, could be a suitable supportive tool of great potential. The automation of the entire process by the robotization of conventional optical microscopes provides added value to the diagnostic system. The possibility of completely emulating traditional microscopy with its *X*-*Y* slide movements and sample autofocus issues is a major challenge. The system has great potential, however, it needs to be refined and validated in different laboratories to evaluate its performance in clinical practice. Furthermore, comparison with other diagnostic techniques already established and regulated for malaria



diagnosis, such as conventional optical microscopy, RDTs, and PCR, would allow AI-based diagnostic techniques to be more accurately positioned within the currently available set of malaria diagnostic techniques. The diagnostic system described in this study, has significant value due to the automation of the process, the design of the prototype, the automated calculation of parasite density, and the support which it provides to conventional microscopy. However, the detection system would be optimized in terms of object detection, and the algorithms for differentiating the *Plasmodium* species should be implemented to provide a complete diagnosis in further studies.

In conclusion, we are ever closer to developing an AI-based diagnostic method for malaria parasite detection. Recent advances and improvements in convolutional neural network models confer a promising future for this type of methodology. The development of an effective automated diagnostic system with AI technology for malaria diagnosis is still a great challenge. Therefore, the coalescence of the fully-automated system via auto-focus and slide movements and the autonomous detection of *Plasmodium* parasites in digital images with a smartphone software and AI algorithms confers the prototype the optimal features to join the global effort against malaria, neglected tropical diseases and other infectious diseases of poverty.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary files, further inquiries can be directed to the corresponding authors.

Ethics statement

This study was conducted in accordance with the Declaration of Helsinki and approved by the Clinical Research Ethics Committee (CEIm) of the Vall d'Hebron University Hospital/Vall d'Hebron Research Institute with reference number PR(AG)40/2023.

Author contributions

CM, EC, and JJ-M: conceptualized and drafted the manuscript, wrote the manuscript, and designed the figures. AO provided advice about artificial intelligence topics and designed the figures. FS and ES provided continuous intellectual feedback on malaria diagnosis and the protocols used nowadays. All authors have revised the manuscript and provided valuable feedback. EC and JJ-M edited and revised the overall manuscript. All authors agreed to be accountable for the content of the work. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2023.1240936/full#supplementary-material

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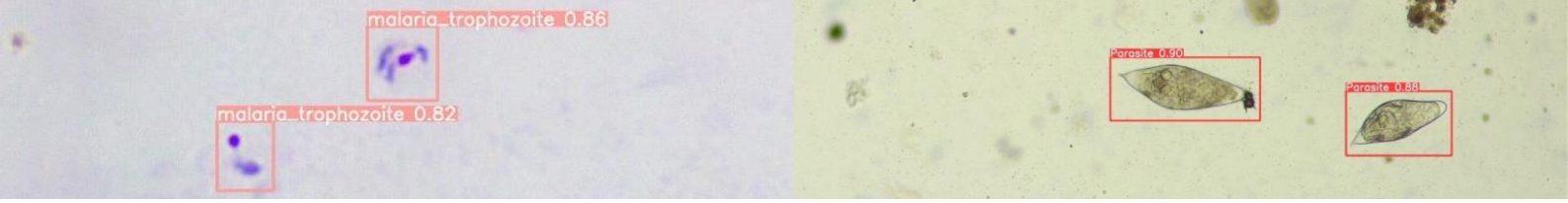

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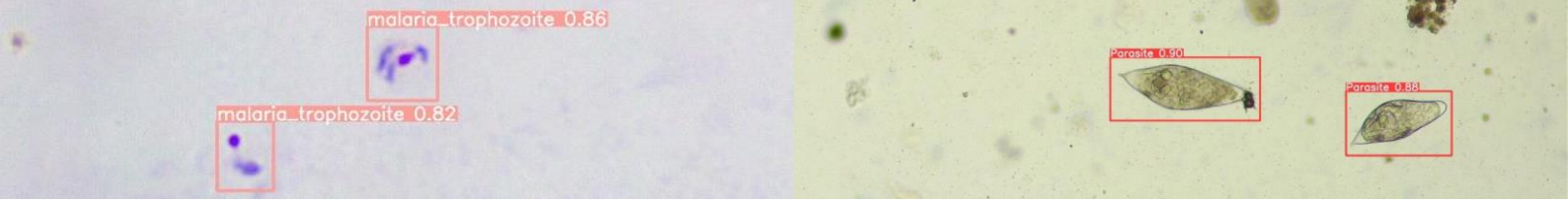

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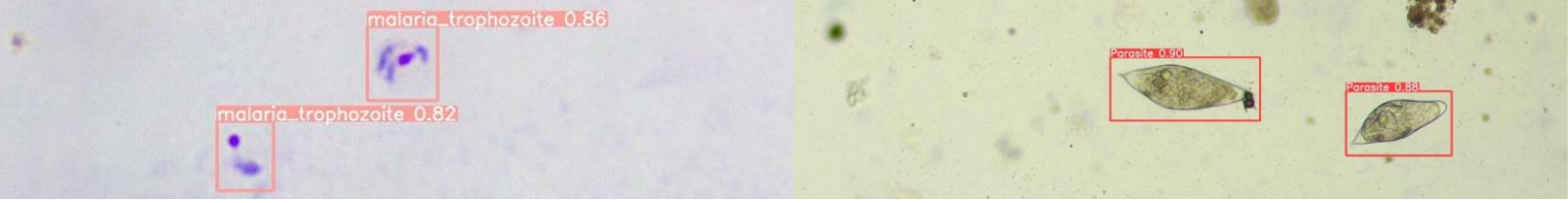

4.1.2. Development of a low-cost robotized 3D-prototype for automated optical microscopy diagnosis: An open-source system

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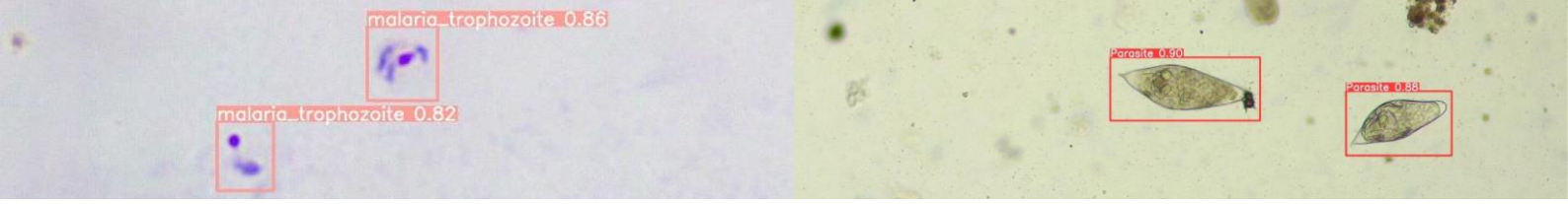
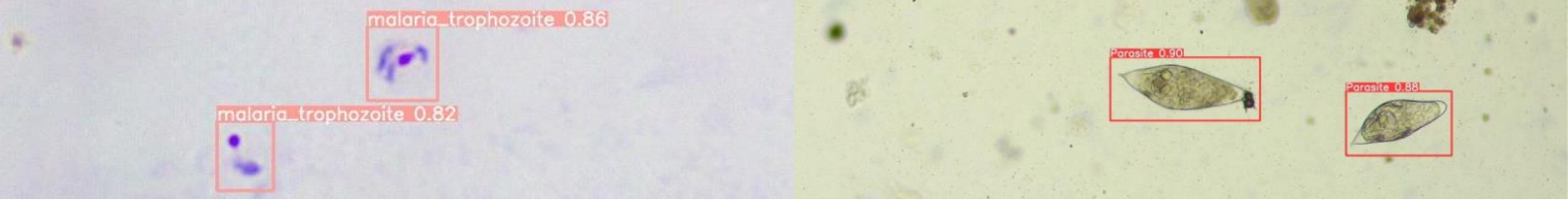


Resum del treball:

En un context clínic, la microscòpia òptica convencional s'utilitza habitualment per a la visualització de mostres biològiques per al diagnòstic. No obstant això, la disponibilitat de tècniques moleculars i proves de diagnòstic ràpid estan reduint l'ús de la microscòpia convencional als laboratoris, i en conseqüència el nombre de professionals experimentats comença a disminuir. A més, la visualització contínua durant llargs períodes de temps a través d'un microscopi òptic podria afectar els resultats del diagnòstic final a causa d'errors humans induïts per la fatiga. Per tant, l'automatització de la microscòpia és un repte a assolir, per abordar aquest problema. L'objectiu de l'estudi és desenvolupar un sistema automatitzat de baix cost per a la visualització de mostres microbiològiques/parasitològiques mitjançant un microscopi òptic convencional, i especialment dissenyat per a la seva implementació en laboratoris amb pocs recursos. Es va dissenyar un prototip 3D per automatitzar la majoria de microscopis òptics convencionals. Les peces van ser construïdes amb tecnologia d'impressió 3D i material biodegradable d'àcid polilàctic (PLA), i amb els programaris de treball *Tinkercad/Ultimaker Cura 5.1*. Els components del sistema es van dividir en tres subgrups: peces de microscòpia, peces d'emmagatzematge/autoenfocament, i peces per al telèfon intel·ligent. El prototip es basa en l'ús de servo motors controlats per la plataforma electrònica de codi obert *Arduino*, per emular els moviments X-Y i autoenfocament (Z) del microscopi. Es requereix un temps mitjà de 27.00±2.58 segons per a enfocar automàticament un sol FoV. L'avaluació de l'enfocament automàtic va demostrar un valor mitjà màxim de Variança Laplaciana d'11.83 amb les imatges testejades. Tot el procés d'automatització està controlat per un telèfon intel·ligent, que s'encarrega d'adquirir imatges per a realitzar el diagnòstic a través de xarxes neuronals convolucionals. El prototip està especialment dissenyat per a entorns amb pocs recursos, on el diagnòstic per microscòpia encara és un procés rutinari. La coalescència entre els models predictius de xarxes neuronals convolucionals i l'automatització dels moviments d'un microscopi òptic convencional confereixen al sistema una àmplia gamma d'aplicacions de diagnòstic basades en imatges. L'accessibilitat del sistema podria ajudar a millorar el diagnòstic i proporcionar noves eines als laboratoris de tot el món.



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RESEARCH ARTICLE

Development of a low-cost robotized 3D-prototype for automated optical microscopy diagnosis: An open-source system

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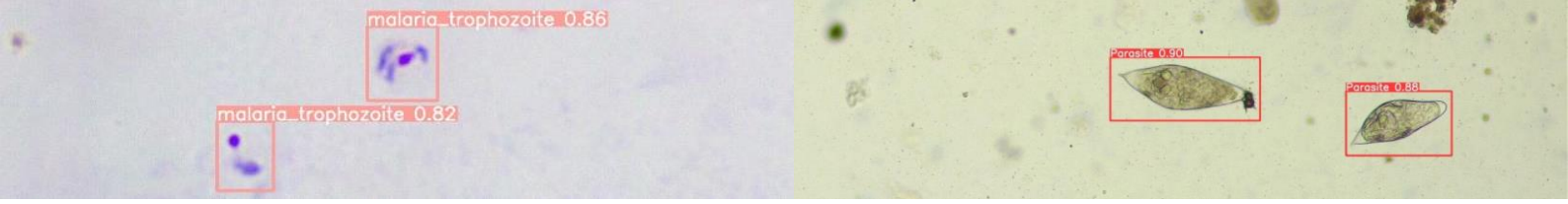
Abstract

In a clinical context, conventional optical microscopy is commonly used for the visualization of biological samples for diagnosis. However, the availability of molecular techniques and rapid diagnostic tests are reducing the use of conventional microscopy, and consequently the number of experienced professionals starts to decrease. Moreover, the continuous visualization during long periods of time through an optical microscope could affect the final diagnosis results due to induced human errors and fatigue. Therefore, microscopy automation is a challenge to be achieved and address this problem. The aim of the study is to develop a low-cost automated system for the visualization of microbiological/parasitological samples by using a conventional optical microscope, and specially designed for its implementation in resource-poor settings laboratories. A 3D-prototype to automate the majority of conventional optical microscopes was designed. Pieces were built with 3D-printing technology and polylactic acid biodegradable material with Tinkercad/Ultimaker Cura 5.1 slicing softwares. The system's components were divided into three subgroups: microscope stage pieces, storage/autofocus-pieces, and smartphone pieces. The prototype is based on servo motors, controlled by Arduino open-source electronic platform, to emulate the X-Y and auto-focus (Z) movements of the microscope. An average time of 27.00 ± 2.58 seconds is required to auto-focus a single FoV. Auto-focus evaluation demonstrates a mean average maximum Laplacian value of 11.83 with tested images. The whole automation process is controlled by a smartphone device, which is responsible for acquiring images for further diagnosis via convolutional neural networks. The prototype is specially designed for resource-poor settings, where microscopy diagnosis is still a routine process. The



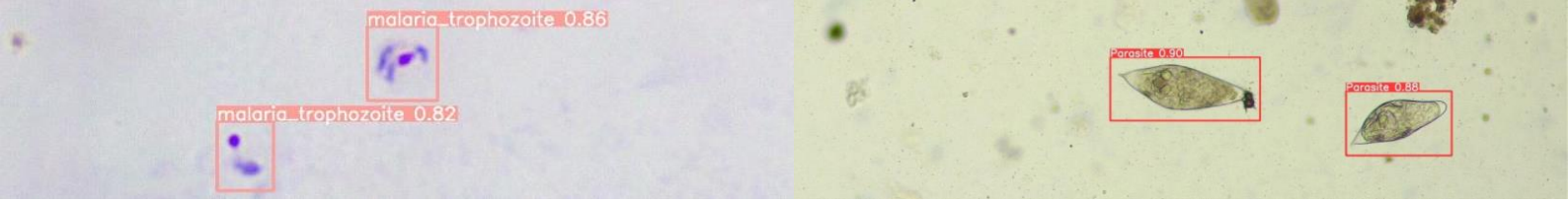
coalescence between convolutional neural network predictive models and the automation of the movements of a conventional optical microscope confer the system a wide range of image-based diagnosis applications. The accessibility of the system could help improve diagnostics and provide new tools to laboratories worldwide.

Introduction

Microscopy is a fundamental pillar in laboratories all over the world. The observation of the forms by magnifying the image through microscope lenses facilitated laboratory practice. One of the main functionalities of microscopy is diagnostics. Conventional microscopy is still used globally in clinical laboratories as a standardized diagnostic technique. Moreover, it is still the *gold standard* technique for the diagnosis of many diseases, such as malaria and some Neglected Tropical Diseases (NTD) [1]. In addition, microscopy remains a crucial tool in resource-poor settings, where the availability of novel and more expensive techniques is limited. The accessibility and easy-handling of microscopy allows its usage in laboratories worldwide. The direct observation of biological samples is expert dependent; needs high levels of expertise and knowledge for each type of observed sample; and could trigger on diagnostic errors due to fatigue or long periods of visualization time [2]. This casuistry is producing a decrease in the use of traditional microscopy as a reference diagnostic method, although it is still the mainstay of laboratories worldwide. In addition, because of the increasing failure of malaria rapid diagnostic tests (RDTs) due to *pfhrp2/3* gene deletions [3] and the low sensitivity in cases of low parasite density [4], microcopy remains the gold standard and reference technique for malaria diagnosis. Furthermore, its relevance in the diagnosis of parasitic infectious diseases is still important, and should be considered as a reference method regardless of the novel molecular techniques and the loss of microscopy professionals.

Last years, novel technologies based on the automation of the microscope movements and image acquisition and processing are being developed to solve human fatigue and errors due to the continuous observation through the lenses. On the one hand, the complete robotization of a conventional optical microscope should improve the traditional microscopy in terms of autonomous diagnostics. For this purpose, servo motors can be a proper alternative to emulate microscope stage movements through the X-Y and Z (focus) axis, with 3D-printed pieces providing the mechanical support for the device to carry out this type of movement. On the other hand, several studies based on automatized image-based diagnosis with artificial intelligence (AI) models have been published during the last years, making use of Convolutional Neural Networks (CNNs) to automatically detect bacteria, cells, parasites or microalgae in digital images, thus, becoming a new alternative for traditional microscopy [5–7]. Interestingly, the combination of an autonomous microscope for image acquisition and an autonomous diagnosis by CNN image analysis might be the best solution for supporting microscopy-based diagnoses. The coalescence of both technologies generates a completely automated diagnostic procedure, from the movements of the microscope to the final diagnosis by image analysis. Moreover, low-cost systems are crucial for the easy and accessible implementation of automated microscopes in any laboratory worldwide, especially in endemic NTD regions and resource-poor settings [8].

As for the automation of a microscope for image acquisition, stage movements and autofocus issue are the main strains. Ze-Jun 2012, developed an automatic movement stage with a parallelogram linkage mechanism in an optical microscope [9]. Sanz et al. 2021 reviewed state-



of-the-art autofocus procedures in order to propose a general applicable methodology [10]. Some studies demonstrate, with promising results, the possibility to create that type of autonomous devices for the detection of diatoms [11], such as Salido et al. 2020, that designed a fully operative low-cost automated microscope for diatom detection with *you only look once* (YOLO) deep learning library [11]. Other applications for the autonomous detection of *Plasmodium* parasites in blood samples [12], or *Schistosoma haematobium* eggs in urine samples [13], were also postulated as alternative methods for diagnosis. Moreover, Alexandrov et al. 2020 designed a super-resolution high-speed optical microscope for automated readout of metallic nanoparticles and nanostructures [14]. As reviewed, microscope automation has a wide range of applications, mainly for the detection and observation of microscopic objects by the emulation of traditional microscopy.

In this study, we address the automation of a conventional optical microscope up to the image acquisition, as a first step to prepare it for a further automated diagnosis. We designed a low-cost 3D-prototype that can be adapted and implemented to several conventional optical microscopes. The prototype pieces were designed and built with 3D-printing technology. Microscope stage and focus movements were performed by servo motors guided by an Arduino controller. The whole procedure was monitored by a smartphone device, which was responsible for the robotized microscope movements and the acquisition of digital images for further analysis. The automated prototype was able to move the sample on the stage of the microscope through the X-Y axis (horizontal and vertical movements), and on the Z axis by an auto-focus algorithm. Its characteristics make the prototype available for any laboratory regardless of their resources or limitations. Importantly, this automated diagnostic system was designed to be open-source and available.

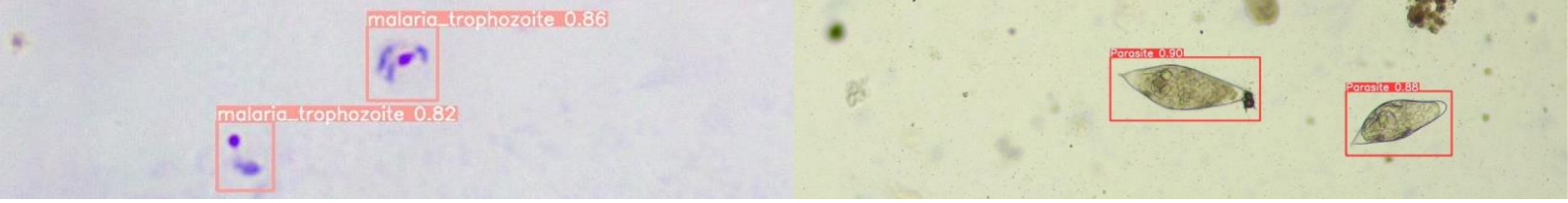
Materials and methods

The robotized prototype for conventional optical microscopes was designed following the methods explained in the subsequent section.

List of materials and devices

All material and devices specifications are listed in S1 Table. Below we present the list of materials and devices employed for the design, development and manufacturing of the robotized system.

- A conventional optical microscope with the standard characteristics to perform a diagnosis [15]. Binocular, with quadruple revolving objectives of 4x, 10x, 40x and 100x (immersion oil) magnification; and an ocular lens of 10x magnification. The microscope should consist of a fine and coarse adjustment wheels to focus the sample through the Z-axis, a microscope stage to deposit the sample with motion along X-Y axes, and light illumination.
- A 3D-printer Ender-3 Pro (Creality 3D). Ultimaker Cura 5.1 slicing software [16] and Tinkercad open-source software were used to design and print the 3D pieces. The filament employed for 3D pieces manufacturing was a Polylactic Acid (PLA), an easy-to-use, low-cost, biodegradable, and recyclable material.
- Three Micro Servo Motors 9G. The servo motors were employed to move the sample on the microscope stage through the X-Y-Z axes.
- Arduino MKR WiFi 1010 based on SAMD21 Cortex®-M0+ 32bit low power ARM® MCU microcontroller.
- Electronic components: cables, LED lights, and resistances (200 Ω).



- For the development of the autonomous microscope, three models of smartphones have been operated. A Samsung Galaxy S20, a Xiaomi Redmi 10C and a Samsung Galaxy A13.

Robotized system design

The system was designed to automatically replicate the movements of a conventional optical light microscope (Fig 1). It was designed as a universal prototype, which can be adapted to the majority of microscope models. Several representative microscopes were measured to define a range of measurements for building the pieces (Table 1). A 3D-prototype for the X-Y (microscope stage) and Z (focus issue) axis movements was designed. For X-Y axis, a rectangular adapter with 3 independent pieces was built. The adapter was deposited on the microscope stage, and 2 servo motors SG90 (5V, speed 0.5 seconds/120˚), one for each axis X and Y, were attached to the main 3D-piece to perform the microscope movements. Two metallic bars (86 mm *length*, 3mm ⌀) were positioned between the adapter pieces to easily allow the movements of the biological sample on the microscope stage. For the Z axis focusing movements, a third SG90 servo motor was employed. All motor movements were controlled by an Arduino MKR WiFi 1010, that is controlled and connected directly via *Bluetooth Low Energy* (BLE) protocol connection to a smartphone device. The electronic diagram and configuration of the entire system is represented in Fig 2.

Design and manufacturing of 3D pieces

3D printing technology was employed to build the pieces for microscope automation. Tinkercad and Ultimaker Cura 5.1 slicing software were used to design and print the 3D pieces of the prototype. The designed pieces were first visualized on a Tinkercad computer viewer to

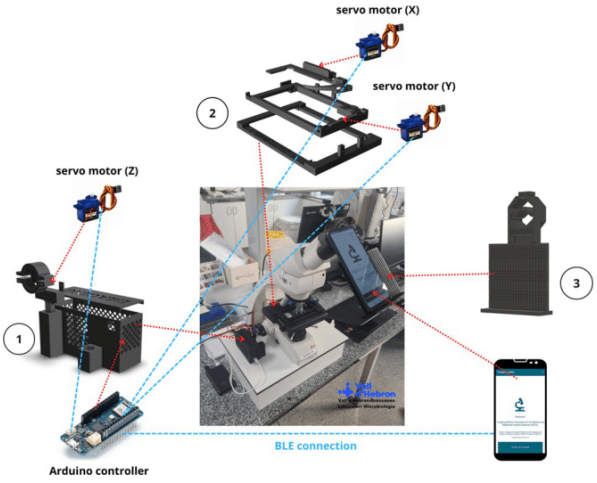


Fig 1. Robotized system representation. Robotized system representation on a Leica DM750 optical microscope from Microbiology Laboratory of Drassanes-Vall d'Hebron International Health Unit. Red discontinuous arrows represent space positions. Blue discontinuous lines represent connections. (1) Storage and focus 3D pieces. The Arduino controller is stored in the grid box. The servo (Z) motor is directly connected to the Arduino controller and subjected by a holder arm to change the Z position. (2) Microscope stage pieces. Three individual stage pieces were placed on the microscope stage. Two servo motors are attached to the stage 3D pieces to move the sample through the X-Y axis, and connected to the Arduino controller. (3) Mobile phone adapter pieces. The smartphone adapter is positioned on the ocular lens of the microscope. The smartphone is connected via *Bluetooth* (BLE) to the Arduino controller to guide the entire robotized procedure.

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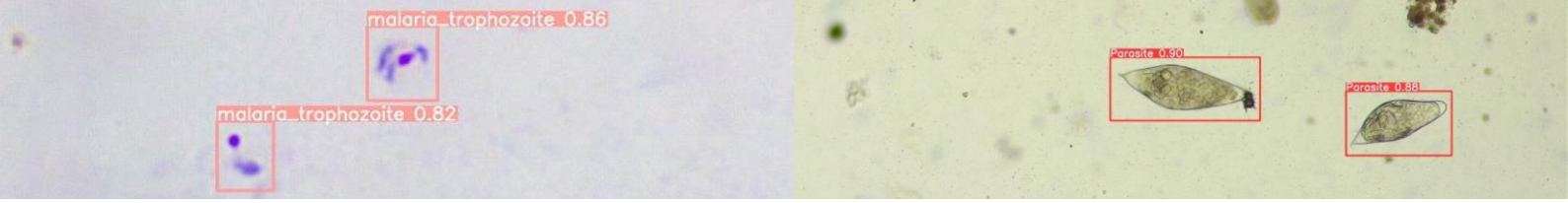

Table 1. Optical microscope measurements for the design of 3D universal adaptable pieces.

Microscope model	*Microscope stage dimensions (length x width x height)*	*Distance from the base to the micrometric wheel*	*Fine adjustment wheel*	*Ocular lens diameter*
Leica DM750	144mm x 185mm x 18mm	43mm	23mm (external diameter)	34.8 mm
Olympus CH2	152mm x 123mm x 10mm	110mm	24mm (external diameter)	31.7 mm
Nikon Eclipse E200	133mm x 220mm x 15mm	70mm	30mm (external diameter)	37.7 mm
Zeiss KF2	135mm x 125mm x 17mm	96 mm	22 mm (external diameter)	28 mm

Millimeters (mm).

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determine their measurements and lengths. The designed models were exported as a *stl* file for further building and exported for slicing to Ultimaker Cura 5.1, generating a *gcode* file. Finally, pieces were printed on an Ender-3 Pro printer with PLA material. The design of the 3D parts has been specially developed for adaptation to most conventional optical microscopes. Dimensions have been empirically collected from four different representative optical microscopes to establish a range of measurements for the design of the 3D prototype parts (Table 1). The measurements of the different microscope models confer the possibility to design 3D printed pieces that could be attached in any of the analyzed microscopes. Different microscope brands (Leica, Olympus, Nikon and Zeiss) were selected in order to obtain diverse designs and measurements with representative data.

A total number of 15 individual different pieces were designed and built to create 3 main systems or subsets of pieces. Each piece has different parts with specific functions. In Table 2, all pieces' designs are specified and classified in each of the subsets: microscope stage pieces (S4 Fig), auto-focus pieces/storage and controller parts (S5 Fig), and mobile phone adapter pieces (S6 Fig).

The pieces were constructed with a 3D-printer Ender-3 Pro (Creality 3D) employing PLA material.

Three subsets of pieces were designed and constructed for microscope automation. Auto-focus pieces and storage and controller parts are encompassed in the same subset, although their individual roles are different.

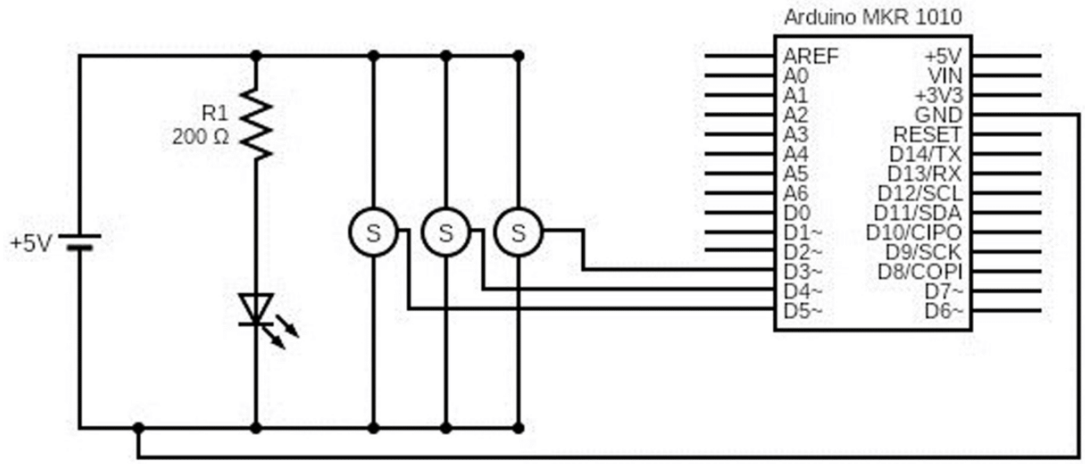

Fig 2. Representation of the electronic circuit and configuration. (S) Servo motor; (V) Volts; (R) Resistance; (Ω) Ohms; Ground (GND).

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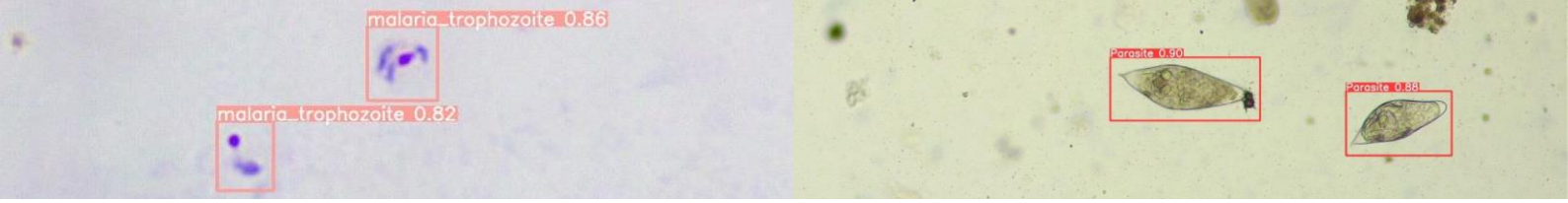

Table 2. Description of all 3D pieces employed for automated system building and assembly.

Subset pieces type	Pieces parts names	Design
Microscope stage pieces (5 independent pieces)	1. External stage holder 2. Screw hole for servo motor 3. Toothed rail for servo motor 4. Sample holder and internal stage holder 5. Medium stage holder 6. Sample clamp 7. Gears for toothed rail and servo motors	See S4 Fig
Auto-focus pieces / Storage and controller parts (5 independent pieces)	1. Storage box cover 2. Storage box 3. Auto-focus servo motor stick holder 4. Fine adjustment wheel clamps 5. Auto-focus system supports 6. Storage template for Arduino 7. USB hole 8. External cables hole	See S5 Fig
Mobile phone adapter pieces (5 independent pieces)	1. Smartphone holder 2. Adapter support 3. Ocular lens hitch 4. Wheels for regulating dimensions screws	See S6 Fig

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Microscope stage pieces. Specially designed to be attached on the microscope stage. Three sub-pieces (Fig 3A) confer a single structure to hold the biological sample and move it through the X-Y axes with the assistance of the servo motors. The original holding stage clip of the microscope should be removed to place the microscope stage 3D pieces. The three sub-pieces should be correctly assembled to avoid movement issues and inaccuracies due to gaps

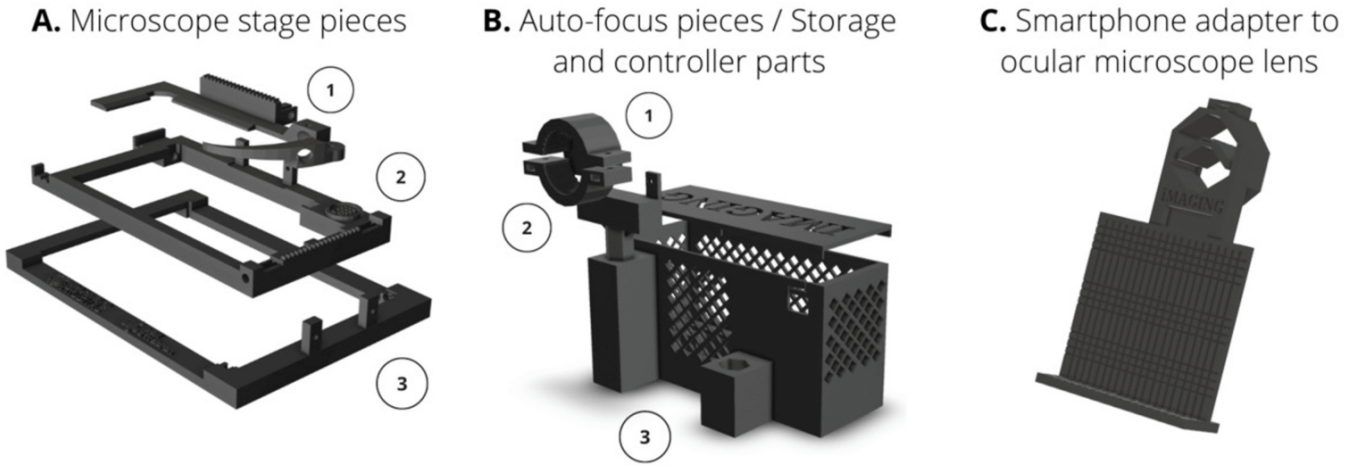

Fig 3. Microscope 3D pieces subsets. All pieces were designed with Ultimaker Cura 5.1 slicing and Tinkercad *softwares*. **(A)** Microscope stage pieces (1) Biological sample gripper to hold the slide. A toothed track allows for horizontal movement with the horizontal servo motor. (2) Stage holder. A toothed track allows for vertical movement with the vertical servo motor. (3) Main microscope stage piece to support the system. **(B)** Microscope auto-focus and storage pieces (1) Servo motor holder. This piece had an adjustable height to be attached on the fine adjustment wheel of the microscope. (2) Wheel holder with adjustable diameter. (3) Storage box for Arduino and board controllers. **(C)** Mobile phone adapter pieces for microscope ocular lens. An adjustable clamp allows the adapter to be attached to the eyepiece lens. The roughened PLA mount holds the smartphone device in order to capture images without moving.

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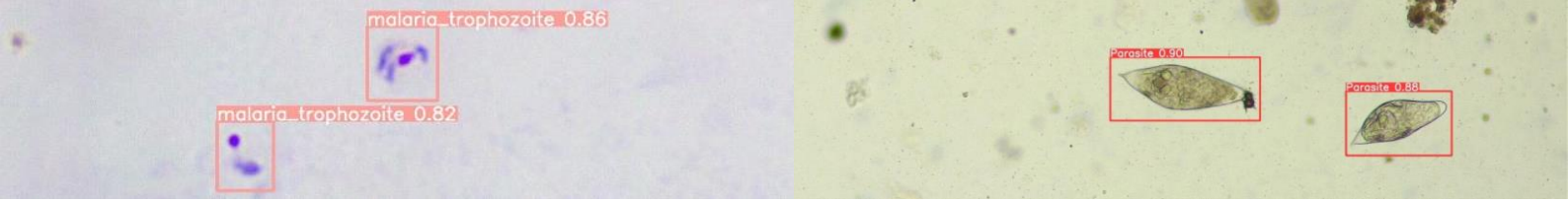


between items. Microscope 3D stage piece should be fastened on the original microscope stage with tweezers.

Auto-focus pieces. A holder on the fine adjustment wheel to emulate focusing movements with the motor (Fig 3B). The pieces have an adjustable diameter to be adapted to the fine adjustment microscope wheel and moving in both directions of rotation. Two metallic screws allow the subjection of the micrometric wheel, which would be responsible of the consequent auto-focus of the sample by the smartphone camera visualization. The sample must be correctly focused for further imaging detection of objects; such as bacteria, cells, parasites or other forms.

Storage and controller parts. A storage cage for Arduino and motor controllers (Fig 3B). The cage was specially designed to not disturb the adaptation to the microscope. An open port for USB 2.0 to provide Arduino controller with energy power was placed on the below lateral part of the box. The storage box has a mesh with small holes to prevent the system from overheating. Inside the controller box (Fig 3B, number 3) a template with the dimensions of the controllers allows them to be stored precisely and without any possibility of displacement.

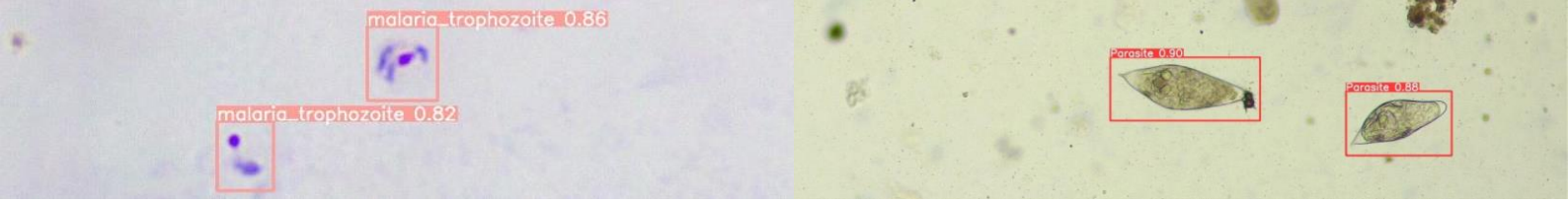
Mobile phone adapter. A smartphone adapter for ocular microscope lenses (Fig 3C). The adapter holds the smartphone device for the correct acquisition of microscopic images through its camera. The dimensions of the adapter were designed by the analysis of the different smartphone measurements. A PLA screw on the upper part of the holder allows to fit the adaptor to the microscope regardless of the diameter of the ocular lens.

Optical microscope stage movements emulation by servo motors

Once the 3D pieces were designed and constructed, a set of three Micro Servo Motor 9G were used to perform the focusing and shifting microscope movements. Two micro servo motors SG90 were employed to move the biological sample through the X-Y axes, fastened by microscope stage 3D pieces. The two motors were placed horizontally and vertically respectively, on the stage adapter and attached with two metallic screws. Two metallic bars allow the movement of the PLA pieces through the X-Y axes by the micro servo motor movements. Two gearwheels are positioned on the drive wheel of the servo motors, and by means of toothed rails allow movement in both X-Y directions. Both servo motors were controlled by the Arduino MKR WiFi 1010, connected by BLE connection to the smartphone device. One servo motor was employed to move the fine adjustment wheel of the microscope. The motor was subjected with a 3D PLA piece with a rounded hole fitting the form of the engine, and a hexagonal base stick (Fig 3B, number 1*)*. A circular screw with an adaptable dimension was attached to the servo motor. The screw (Fig 3B, number 2) holds the fine adjustment and moves it in both rotation directions. The auto-focus device was positioned on the storage box, conferring the system a more compact structure.

Auto-focus algorithm

An image auto-focusing method was designed to correctly acquire readable and focused images for further image analysis. The microscope must be in a focused position to capture an optimal image with the smartphone device. In our mechanism, we have employed the variance of Laplacian as a reference method for image auto-focusing. The variance of Laplacian allows the calculation of a value for each image, which indicates the level of focusing of the acquired picture. Focused images have higher values of variance of Laplacian than blurred images. Therefore, the analysis of variance of Laplacian values would determine which is the best focused image for each Field of View (FoV).



The calculation of the variance of Laplacian is specified in *Eq 1*; where Δ is the Laplacian operator (*Eq 2*) applied to an image $I(m,n)$ by convolving a 3 x 3 Laplacian kernel; and $\overline{\Delta I}$ is the average of the Laplacian (*Eq 3*) [11].

$$VAR\_LAP_{m,n} = \sum_{i}^{m}\sum_{j}^{n}[|\Delta I(i,j)| - \overline{\Delta I}]^2$$

Equation 1: The calculation of the variance of Laplacian (VAR_LAP).

$$\Delta(I) = I(m,n) * \begin{bmatrix} 0 & 1 & 0 \\ 1 & -4 & 1 \\ 0 & 1 & 0 \end{bmatrix}$$

Equation 2: The calculation of the Laplacian operator by convolving the image $I(m,n)$ to the 3 x 3 kernel.

$$\overline{\Delta I} = \frac{1}{m \times n}\sum_{i}^{m}\sum_{j}^{n}|\Delta I|$$

Equation 3: The calculation of the average of the Laplacian. The image (I) has "*m*" (width pixels) and "*n*" (height pixels) of dimensions.

The variance calculation is performed in each FoV determined by X-Y movements of the robotized microscope. In a single FoV, the smartphone camera observes different focused images (Z axis) by the continuous movement of the Z-servo motor on the fine adjustment wheel. The smartphone device by *BLE* connection with the controllers guides the Z-servo motor to move the fine adjustment wheel in both directions of rotation [50 position units of movement (u.m.) in each direction; 1 u.m. = 1˚] in order to focus the biological sample. Pseudo-code details are represented in Fig 4. During the auto-focusing process a Laplacian variance value is computed to each of the images/frames of a real-time video. In order to avoid unfocused positions, the system visualizes the centroid of the original image by creating a new cropped image with 50% width and 33% height image for Laplacian analysis. In addition, a 40 milliseconds delay was added to correctly acquire the images. This procedure allows the observation of only the center of the image, without the black borders produced by the ocular lens attachment and the blurred edges. Once the image scanning in the two directions of rotation has been completed, the mechanism is able to obtain the image with the highest Variance of Laplacian value for further image analysis.

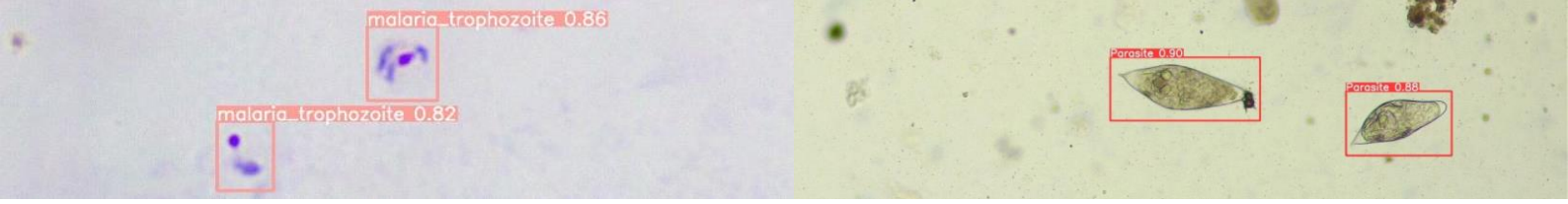
Ethics statement

This study was conducted in accordance with the Declaration of Helsinki and approved by the Clinical Research Ethics Committee (CEIm) of the Vall d'Hebron University Hospital/Vall d'Hebron Research Institute with reference number PR(AG)40/2023.

Results

Universal system adaptation

The developed system is specially designed to be adaptable to most conventional optical microscopes. The size measurements collected from optical microscopes of different brands and models allows the design of a universal adaptable system (Table 1). Each of the subgroups of 3D parts has been designed for a range of measurements within the dimensions of the microscopes analyzed. The microscope stage mounted pieces have 141 x 100 x 7.5 mm dimensions.



Algorithm 1 Android Application

```
Initialize Bluetooth connection with Arduino
BluetoothConnection.initialize()
Send command to Arduino to move microcretic motor
BluetoothConnection.sendCommand("MOVE_MOTOR")
Wait for acknowledgment from Arduino
if BluetoothConnection.receiveAcknowledge() == "ACKNOWLEDGED" then
    Motor movement started successfully
    Capture and process images while motor is moving
    ImageProcessing.startCapturingFrames()
    while ImageProcessing.getMalariaParasiteCount() < 5 and ImageProcessing.getCapturedImageCount() < 100 do
        Capture frame
        capturedFrame = ImageProcessing.captureFrame()
        Calculate Laplacian difference
        laplacianDifference = ImageProcessing.calculateLaplacianDifference(capturedFrame)
        Send image data to Arduino for display (optional)
        BluetoothConnection.sendImageData(capturedFrame)
        if BluetoothConnection.receiveCommand() == "STOP" then
            Break                                   ▷ Exit loop if stop command is received from Arduino
        end if
    end while
    Stop capturing frames
    ImageProcessing.stopCapturingFrames()
    Send command to Arduino to stop motor
    BluetoothConnection.sendCommand("STOP_MOTOR")
    Wait for acknowledgment from Arduino
    if BluetoothConnection.receiveAcknowledge() == "ACKNOWLEDGED" then
        Motor stopped successfully
        Process the image with the largest Laplacian difference using YOLOv5
        bestFocusedImage = ImageProcessing.getBestFocusedImage()
        detectionResult = YOLOv5Mobile.detectLeukocytesAndParasites(bestFocusedImage)
        Display or use detectionResult as needed
        Send acknowledgment to Arduino
        BluetoothConnection.sendAcknowledge("PROCESSING_COMPLETE")
    end if
else
    Motor movement failed, handle error
    handleError("Motor movement failed")
end if
Close Bluetooth connection
BluetoothConnection.close()
```

Algorithm 2 Arduino

```
Initialize Bluetooth connection
BluetoothConnection.initialize()
Wait for command from Android app
receivedCommand = BluetoothConnection.receiveCommand()
if receivedCommand == "MOVE_MOTOR" then
    Start the microcretic motor movement from 50 to 0 to 100 and back to 50
    MicrocreticMotor.startMovement()
    Send acknowledgment to Android app
    BluetoothConnection.sendAcknowledge("ACKNOWLEDGED")
    Wait for stop command from Android app
    receivedStopCommand = BluetoothConnection.receiveCommand()
    if receivedStopCommand == "STOP_MOTOR" then
        Stop the microcretic motor
        MicrocreticMotor.stopMovement()
        Send acknowledgment to Android app
        BluetoothConnection.sendAcknowledge("ACKNOWLEDGED")
    end if
else
    Handle unknown command or error
    BluetoothConnection.sendAcknowledge("ERROR_UNKNOWN_COMMAND")
end if
Close Bluetooth connection
BluetoothConnection.close()
```

Fig 4. Pseudo-code. Pseudo-code of the android application settings (algorithm 1) and arduino controller (algorithm 2).

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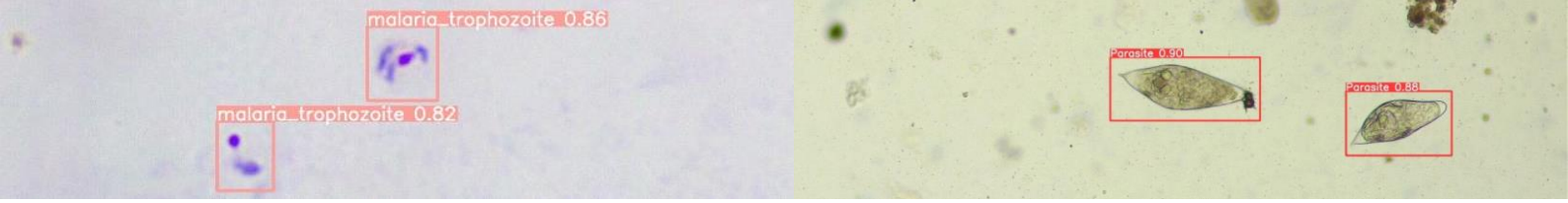
Final measures of length and width can be adjusted to any of the analyzed microscopes. The height dimension of 7.5mm was designed to be as small as possible in order to not interfere with the stirring of objective lenses of the microscope. The sample slide is in direct contact with the stage of the original microscope, in order to emulate a conventional microscopic visualization and do not modify the distances between the objective lenses and the biological



sample. Auto-focus pieces have a range of diameters of the coupling hitch with a micrometric wheel of 20–30 mm. In order to attach the auto-focus pieces from the base to the fine adjustment wheel, two vertical holder pieces were built with a range of measures of 38–48 mm length. Smartphone adapter pieces have a range of measures of 25–50 mm length, regulated by a metallic screw, on the ocular lens attachment. The specific dimensions of the prototype are summarized in S1–S3 Figs, and 3D pieces' models are open-source and publicly available in *supplementary material* section as *stl* files.

Robotized and automated slide scanning

A movement pattern has been developed which can be adapted to the needs of observation according to the type of biological sample. Horizontal and vertical movements through the X-Y axis of the sample slide are crucial to scan the maximum area in order to detect pathogens of interest or morphologies. The biological sample is placed on the glass slide and usually has a rectangular, circular or square shape, although sometimes the sample has an irregular form. It is crucial to only observe the area of interest, avoiding non-stained or empty spaces in the slide. The most efficient scanning procedure to observe the maximum surface of a rectangular or square shaped sample is a snake like movement from left to right and from the top to the bottom (Fig 5). To scan a single FoV with 100x objective magnification, servo motors perform the snake-like movement every 5 units of movement (u.m.) (Operating Speed 0.12 sec/60˚). The system stops in each FoV in order to acquire a new image with unseen information. The system allows the selection of the sample shape and the magnification of the observation in order to move the sample and capture images correctly.

Auto-focus scanning

The Arduino is connected via *BLE* to the smartphone and applies the variance of Laplacian algorithm to auto-focus the sample. It is necessary to help the system with the first focus in order to reduce diagnosis time by the observation of a blurred image on smartphone screen. Once the first focus was performed, the system initiated the auto-focus of the sample in each

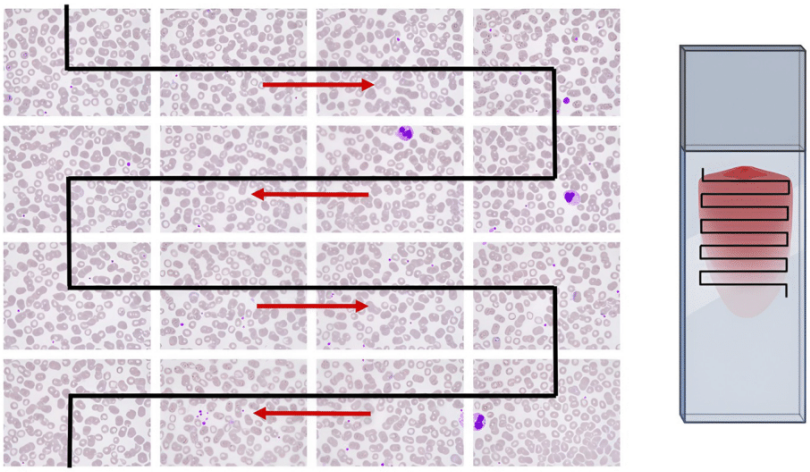


Fig 5. Sample scanning X-Y movement representation. Automated snake-like movement of the system to capture and scan the whole blood smear samples. Automated scanning strategies using imaging techniques for object detection through X-Y axes. Snake-like movement for image acquisition of a rectangular shape sample. Images from Microbiology Laboratory of Drassanes-Vall d'Hebron International Health Unit.

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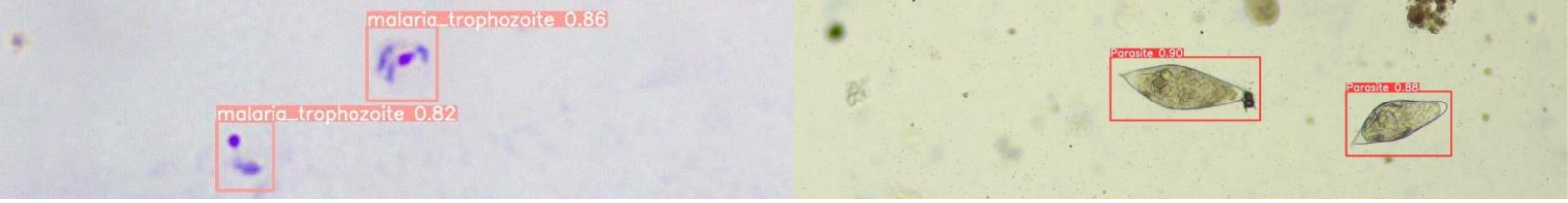

microscope FoV, by the observation of a real-time video. Biological samples are not perfectly plane and have a slight relief that might affect the focus of each FoV. Therefore, the first auto-focus is wider (50 u.m. of the servo motor) in comparison with the following auto-focus movements of the subsequent FoV (30 u.m. of the servo motor). This procedure allows the process to be faster, avoiding delays due to autofocusing of individual microscopic fields.

In order to evaluate the performance of the proposed auto-focus algorithm, an analysis experiment was designed. Auto-focus evaluation was described by the observation of different FoV of Giemsa stained thick blood smear samples (n = 6) at 1000x total magnification for malaria diagnosis, with a Zeiss KF2 microscope. A total number of 5 FoV images of each sample were acquired (30 FoVs in total). The observation was emulated with the digital camera of a Samsung Galaxy A13 smartphone device. Variance of Laplacian analysis was performed in each FoV while the servo motor moves the fine adjustment wheel of the microscope in order to find a focused image/frame of the real-time video. A total number of 60 micro-metric positions (30˚ on each side) were postulated in order to scan different focused images of the same FoV. The system determines the image/frame linked with the higher Variance of Laplacian value (most focused image), in order to capture it for further CNN analysis. Time of focus and Variance of Laplacian value were analyzed. Results were presented in Fig 6 and Table 3. An average time of 27.00 ± 2.58 seconds was required to auto-focus a single FoV with the described technology. Variance of Laplacian values of ≤ 6 are considered unfocused or empty FoVs. Images with non-biological material or stain (transparent) were not optimal for autofocusing and analysis. Results demonstrate a mean average maximum Laplacian value of 11.83, representing focused images for further CNN image analysis.

System features were represented in Table 4. Several state-of-the-art published systems were selected in order to be compared with our purpose in terms of: pieces materials, image acquisition, autofocus, cost, camera settings, point of care design and power requirements [13, 17–20].

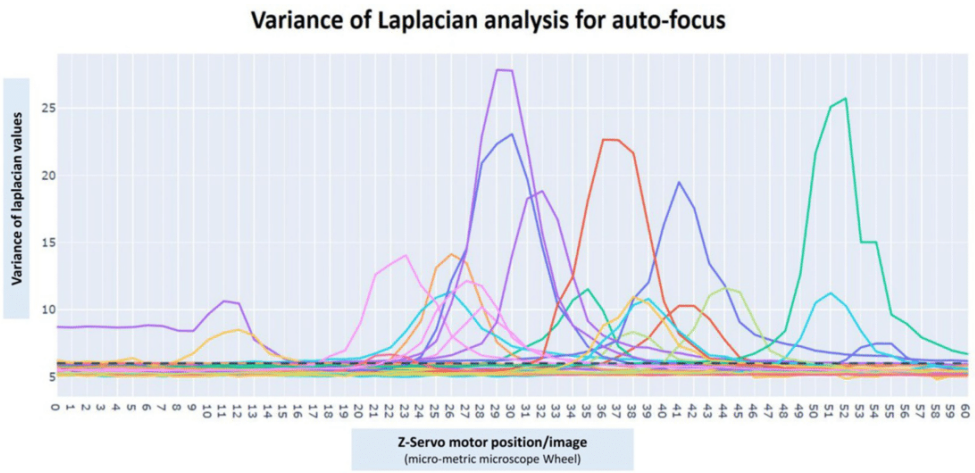

Fig 6. Variance of Laplacian values for auto-focus system analysis. A total number of 30 microscopic fields (images) of 6 different Giemsa stained thick blood smear samples (5 FoV/sample) for malaria diagnosis were analyzed. Each FoV has a range of Variance of Laplacian values depending on the position/image [60 total positions (30˚ on each side)] of the fine adjustment microscope wheel. Focused images were represented as the higher Variance of Laplacian values (peaks correspond to in-focus positions/images).

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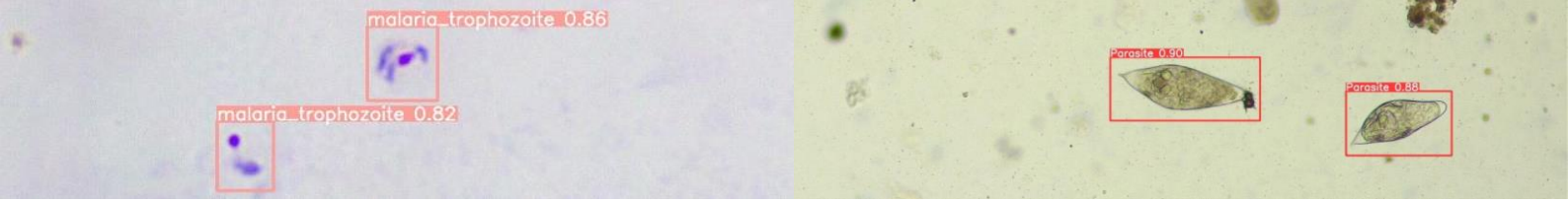

Table 3. Autofocus analysis of thick blood smear Giemsa stained.

Sample Number	Field Number	Time of Focus (milliseconds)	Laplacian Variance (maximum)	Position/Image (0–60)
P1	1	23.131	7,47	54
P1	2	25.116	10,28	41
P1	3	26.123	11,52	35
P1	4	29.878	10,63	11
P1	5	27.451	14,13	26
P25	1	27.584	11,33	26
P25	2	30.082	5,5	10
P25	3	25.657	8,33	38
P25	4	27.213	10,2	28
P25	5	29.767	8,5	12
P26	1	25.177	19,5	41
P26	2	28.167	6,1	22
P26	3	24.161	6,01	47
P26	4	26.584	18,83	32
P26	5	31.504	6,01	1
P19	1	23.428	11,24	51
P19	2	31.620	6,01	1
P19	3	24.454	11,61	44
P19	4	27.967	14,1	23
P19	5	30.837	6,39	5
N3	1	27.046	23,1	30
N3	2	26.003	22,66	36
N3	3	23.273	25,75	52
N3	4	26.959	27,85	29
N3	5	31.222	5,73	3
P40	1	25.405	10,8	39
P40	2	28.174	6,66	22
P40	3	22.830	5,41	55
P40	4	27.445	12,14	27
P40	5	25.721	10,96	38
MEAN		*26.999*	*11,83*	

Giemsa stained samples (n = 6). Time of Focus, Laplacian Variance and Position/Image values were represented.

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Discussion

According to our knowledge, it is the first fully low-cost adaptable automated system for infectious diseases diagnosis. These characteristics are one of the major strengths of the project, and facilitates the implementation of the system in any clinical laboratory environment. Out-of-measures microscopes could also be adaptable for our system, although new pieces should be designed for its correct implementation. In addition, it is also compatible with microscope systems containing an integrated camera. However, many of the published automated systems are based on a single design, and therefore make them difficult to implement in any laboratory due to their low adaptability. However, the acquisition of complete systems such as EasyScan Go could complement traditional laboratory tools with promising results [21]. Our system is low-cost ($220-$300) due to its manufacturing materials and servo motors; its power requirements (10W) are accessible; does not need internet connection; has an efficient auto-focus technology; and is specially designed for resource-poor settings implementation. However,

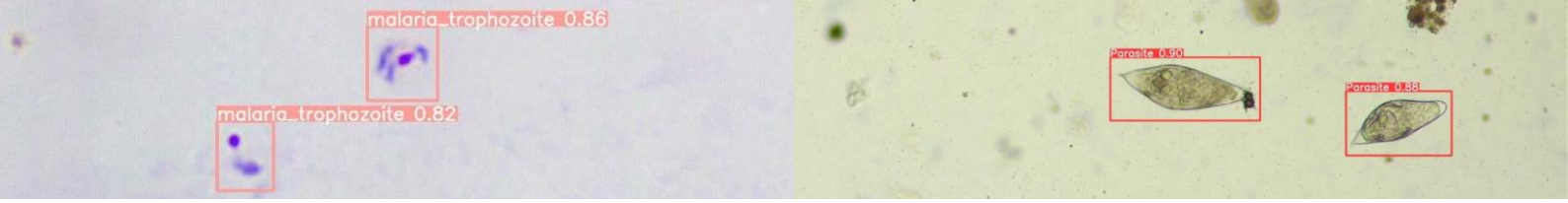


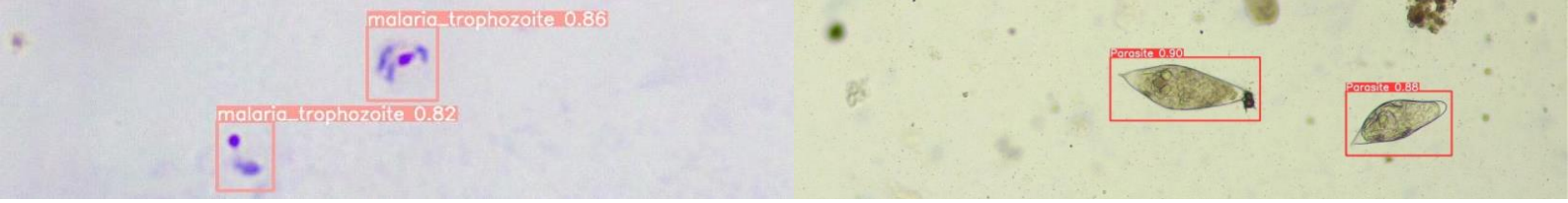
Table 4. Summary table comparison of the state-of-the-art automated microscope designs.

Design	Pieces materials	Automated image acquisition	Autofocus (focusing time)	Approximate cost	Camera	Point of care design	Power requirements / Internet connection	Additional requirements
Delahunt et al 2015 [17]	Aluminium	Yes	Yes. Brenner gradient algorithm (Z-stack)	1500-4000	MIGHTEX camera	Yes	15.8W / Non-specified	
Veerendra et al 2015 [20]	Non-specified	Yes	Non-required	Low-cost (non-specified)	Samsung Galaxy camera (EK-GC100)	Yes	Non-specified / Non-needed	Microfluidic-based system
García-Villena et al 2021 [18]	Polylactic acid (PLA) (3D printing)	Yes	Manual focus	Low-cost (non-specified)	Samsung S9 camera (minimum pixel resolution of 1440 × 1080)	Yes	Stepper drivers (12 V), LED light (3W) / Non-required (desirable)	Smartphone device
Oyibo et al 2022 [13]	Aluminium profiles (3D printing)	Yes	Yes. Greyscale 2D Laplacian filter.	Low-cost (non-specified)	Raspberry Pi High-Quality Camera Module V2.1, equipped with a Sony IMX477R	Yes	60W / Non-specified	
Gordon et al 2022 [19]	Aluminium and acrylonitrile butadine styrene (ABS) (3D printing)	Yes	Yes (algorithm non-specified)	1300$	1.6 MP monochromatic Blackfly S camera with a Sony IMX273 sensor	Yes	Non-specified / Non-required	Microfluidic-based system. Fluorescent stain.
de Oliveira et al 2024	Polylactic acid (PLA) (3D printing)	Yes	Yes. Laplacian variance (27.00 ± 2.58 seconds)	220–300 $ + (300 $ smartphone) = 520-600	Samsung Galaxy S20 camera (minimum 5MP)	Yes	10W / Non-needed	Conventional optical microscope + smartphone device

https://doi.org/10.1371/journal.pone.0304085.t004

some of the limitations of the system are the conventional microscope requirement for its operation, the non-automated change of magnification, and the need of an external camera (smartphone). Other designs described in Table 4, such as Oyibo et al 2022 do not need a conventional optical microscope for its functioning, although its power requirements are higher and therefore it is not an adaptable design [13]. García-Villena et al 2021 purposes a low-cost portable prototype specially design for its implementation in resource-poor settings, although it requires manual focusing [18]. Moreover, Gordon et al 2022 and Veerendra et al 2015 designed similar alternatives based on microfluidic technology for diagnosis and object detection.

Diagnosis procedures by microscopic visualization of biological forms are different depending on the type of observed sample and disease. For example, in the case of malaria disease, the observation of *Plasmodium* parasites in thick and thin blood smear samples with a total magnification of 1000x (100x immersion oil + 10x ocular lens) is crucial to perform a correct diagnosis [22]. Moreover, it is not necessary to observe the whole sample to perform a diagnosis, and consider a positive/negative result for *Plasmodium* infection. If a single parasite is observed it is considered as a positive diagnosis, although if the microscopist observes enough microscope fields (at least 100) without any parasite observation, the sample is considered as a negative result [23]. However, for the diagnosis of other parasitic diseases such as *Schistosoma haematobium* in urine sediment samples, the whole sample should be observed in order to detect the eggs at 100x and 400x total magnification (10x and 40x objective lens + 10x ocular lens) [13]. These differences in the observation methodologies and procedures are quite common in microscopy, and should be considered for the development of automated diagnostic methods.



With our system, the snake-like path of the X-Y servo motors allows its movements to be adaptable to most types of microscopic clinical specimens.

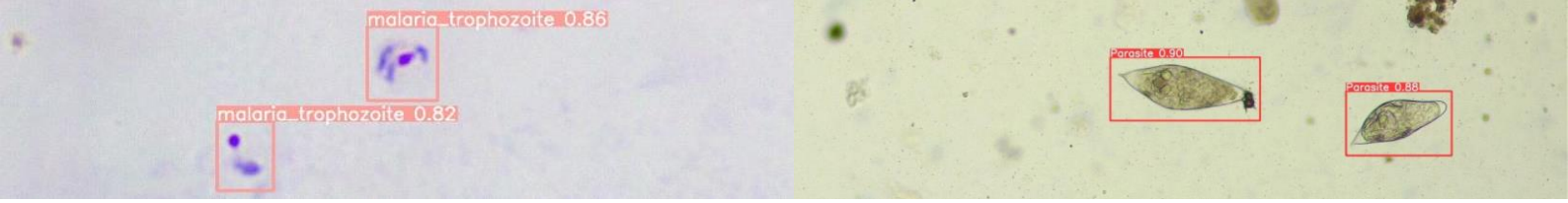
Autofocusing the sample for the correct acquisition of images is crucial to perform a correct image analysis and automated diagnosis. Variance of Laplacian technique is widely used as a reference method to evaluate the autofocus of digital images in other studies [24–26]. However, focus time is an issue when quickness and effectiveness for microscopy diagnosis is a requirement. As an alternative to our work, Bueno-Ibarra et al. proposed a fast auto-focus algorithm for automated microscopes by Fourier Transform and Pearson correlation [27]. The auto-focus algorithm requires time of analysis and the correction of the focus in each FoV slows down the acquire process. The morphology of biological samples should be correctly interpreted, assuming three dimensions of observation. The height or Z dimension of a sample on a microscope slide is variable and often irregular, since the disposition of the cells, bacteria or staining reagents create a relief on the slide that would consequently affect the autofocus of the sample in each FoV. In addition, preprocessing techniques such as noise reduction, background correction, contrast enhancement and image cropping could help to eliminate undesirable artifacts or effects that would affect image focusing [11]. However, these methodologies would increase even more the time of acquisition and analysis.

It is crucial to determine a balance between time and image quality in order not to obtain diagnoses that are too slow, or incorrectly captured images that hinder the object detection and classification performed by the CNN systems.

Diagnostic AI-based applications and perspectives

The developed automated system can be combined with artificial intelligence-based convolutional neural network models to perform fully autonomous diagnostics. Artificial Intelligence (AI) is one of the most outbreaking developing technologies during the current century. Improvements in deep learning techniques will allow the development of better and new AI applications in several research topics. European Parliament published recent studies related with AI in diplomacy, environmental impact green policies, open-source approaches, capital flows or data availability [28]. Artificial intelligence, in nowadays years, has played a crucial role in diagnostic support and biomedical research, and should become even more important in the near future. As some examples, COVID-19 computer-aided diagnosis by classification of CT images with deep learning models [29]; AI algorithms for the prognosis, diagnosis and treatment selection for precision oncology improvements [30]; machine learning and statistical techniques for differentiating tropical infectious diseases such as malaria, dengue and leishmaniasis [31]; or an automated microscopy for the diagnosis of *Schistosoma haematobium* eggs in resource-poor settings by AI techniques [13], are some of the main applications of this promising technology.

As discussed in the introduction, the development of novel diagnostic techniques to solve microscopy issues and improve resource-poor settings environments by its implementation will be a major challenge for the following years. Therefore, the low-cost automation of a standard microscope presented in this study in combination with image AI-based diagnosis tools would be an excellent alternative in these contexts. Evaluation of state-of-the-art CNN algorithms for malaria parasite detection in thin blood smear samples demonstrate a 97% of accuracy to distinguish between an infected and an uninfected erythrocyte [32]. Literature shows a wide variety of systems based on AI able to detect malaria parasites in digital images, in order to support and complement traditional microscopy [7, 22, 32–37]. Our research group trained multiple smartphone-based computational state-of-the-art deep learning models for malaria parasite detection in thick blood smear digital images and tested the robotized *iMAGING*



prototype with promising results [38]. This work was part of the same project presented in the manuscript, in which AI algorithms were a complement to the low-cost automation system and the smartphone device application. A dataset of 2571 annotated digital images of thick blood samples were employed. Comparative analysis yielded a performance for YOLOv5x on a test set of 92.10% precision, 93.50% recall, 92.79% F-score, and 94.40% mAP0.5 for leukocyte, ring stage and mature *Plasmodium* trophozoites overall detection. F-score values of each category were 99.0% for leukocytes, 88.6% for early trophozoites and 87.3% for mature trophozoites detection [38].

Moreover, schistosomiasis diagnosis is mainly based on the visualization of parasite eggs in stool (Kato-Katz technique) or urine sediment samples by microscopic examination [39]. As an alternative, Schistoscope system is an optical diagnostic device for the automated detection of *Schistosoma haematobium* eggs through the X-Y-Z-axis movements for sample scanning. A robust image dataset containing over 5000 FoV images of filtered spiked and clinical urine samples was employed for the generation of AI models for image analysis detection [13]. Our research group has assessed if our developed system is able to detect *Schistosoma haematobium* eggs in automatically acquired images with YOLOv8x neural network showing 95.3% precision, 89.9% recall, 92.5% F-score and 96.8% mAP0.5. Example images of thick blood smear (Fig 7) and urine sediment samples (Fig 8) analysis are represented.

As another example, Soil-transmitted helminthiasis (STH) is an NTD caused by intestinal parasitic worms transmitted through contaminated soil. Eggs of *Ascaris lumbricoides*, *Trichuris trichuria*, *Ancylostoma duodenale* and *Necator americanus* are passed in the feces of infected people, and could be observed by optical microscopy for diagnosis. Ward et al. 2022 prototyped an AI device to detect helminth eggs and *Schistosoma mansoni* eggs in stool. The system achieved an average precision of 94.9 ± 0.8% and recall of 96.1 ± 2.1% for helminth egg species detection [40]. In addition, american trypanosomiasis or Chagas disease is a NTD produced by the protozoan parasite *Trypanosoma cruzi*. During the acute phase of infection, Chagas disease diagnosis is performed by the direct microscopic observation of *Trypanosoma cruzi* parasite in blood smears. Morais et al. 2022 developed an automated system for the detection of *Trypanosoma cruzi* parasites in blood smears using machine learning algorithms applied to smartphone images. The final system demonstrates a final precision of 87.6%, and sensitivity of 90.5%, illustrating promising results for diagnosis [41]. Finally, Leishmaniasis is a disease caused by more than 20 species of *Leishmania* protozoan parasite. Microscopy diagnosis to detect amastigotes in giemsa-stained lesions of scrapping, biopsies, or impression smears are commonly employed as a reference diagnostic technique [42]. Zare et al. 2022 developed a machine learning-based system for detecting leishmaniasis parasites in microscopic images. The Viola-Jones algorithm was employed for parasite detection, with 50% precision and 65% recall for infected macrophages detection; and 71% precision and 52% recall for amastigotes outside macrophages detection [43].

Conclusions

A robotized system for automated diagnosis with a conventional microscope and a standard smartphone was fully developed for its low-cost implementation in resource-poor setting laboratories. Microscope robotization is a crucial step for automated diagnosis based on AI technology. Without the robotized movements of the microscope the entire process would not be completely autonomous. In addition, the design and implementation of a universally adaptable system should be affordable for any laboratory, regardless of their resources. Low-cost materials and a simple process make our system a valuable technology. Moreover, auto-focus movements are not commonly implemented in low-cost and conventional microscopes for AI

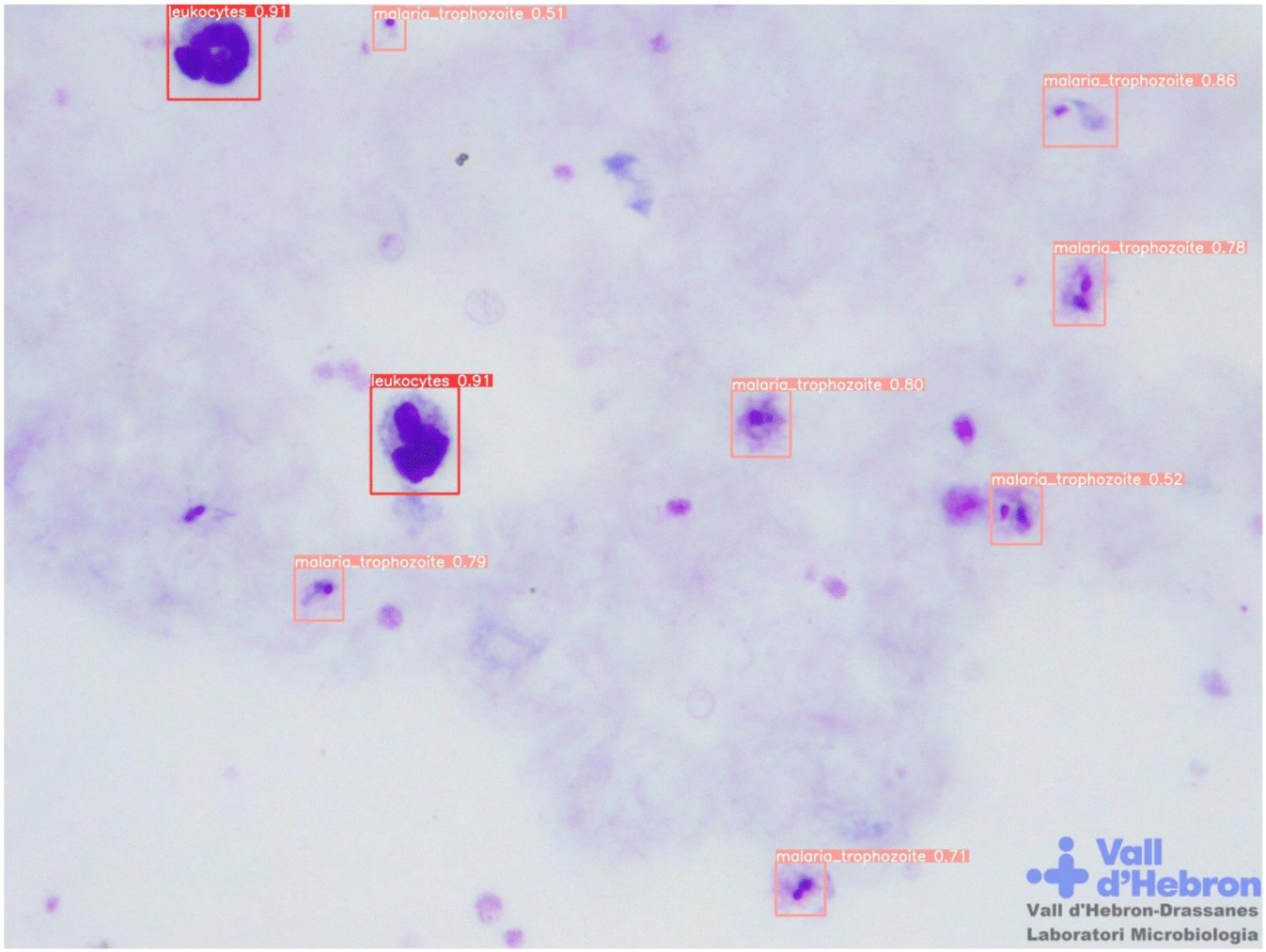


Fig 7. Microscopic images from Microbiology Laboratory of Drassanes-Vall d'Hebron International Health Unit. Giemsa stained thick blood smear sample with detection of leukocytes and malaria trophozites by YOLOv5x neural network performance. 1000x magnification.

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detections. Finally, Z-movements confer the system a fully automated analysis without the mechanical support of a human. The combination of hardware, low-cost materials and accessible technology, plus the adaptation of the 3D-pieces to a conventional optical microscope and the use of smartphone devices, gives the system the innovative nature required for its implementation in the field. Moreover, the freely available 3D-pieces designs provide the scientific community with open-source prototypes for its improvement and usability.

The digitalization of diagnosis would be the next step for the autonomous diagnosis worldwide. The standardization of automated diagnosis procedures should be addressed to provide reliable results and increase its efficiency [44]. Furthermore, the generation of data from microscopic images would help to generate databases for further AI algorithms training. The coalescence of autonomous movements and image analysis provides a milestone for the implementation of available automatic diagnosis with conventional optical microscopes [45].

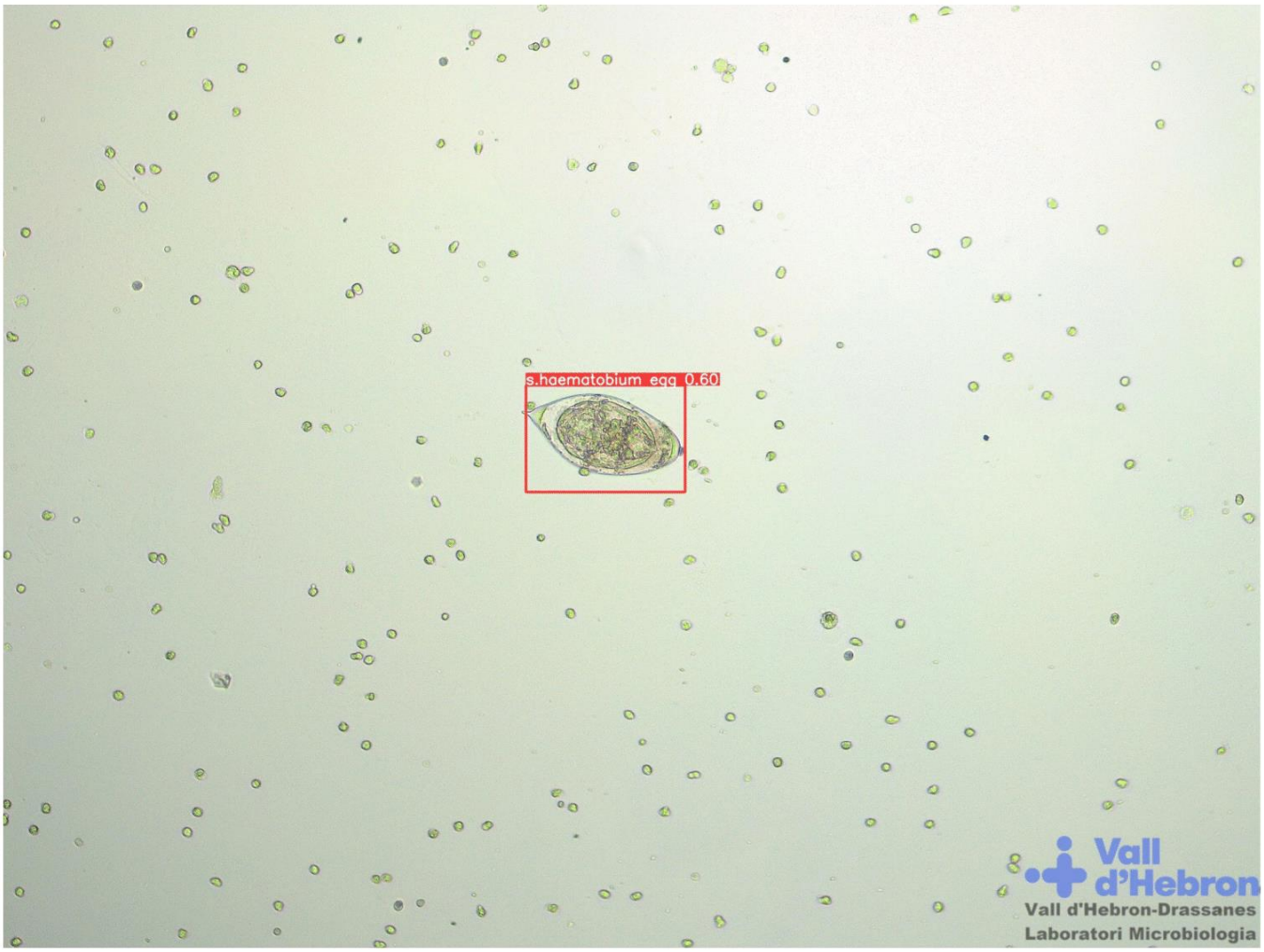


Fig 8. Microscopic images from Microbiology Laboratory of Drassanes-Vall d'Hebron International Health Unit. Urine sediment sample with detection of *Schistosoma haematobium* eggs by YOLOv8x neural network performance. 100x magnification.

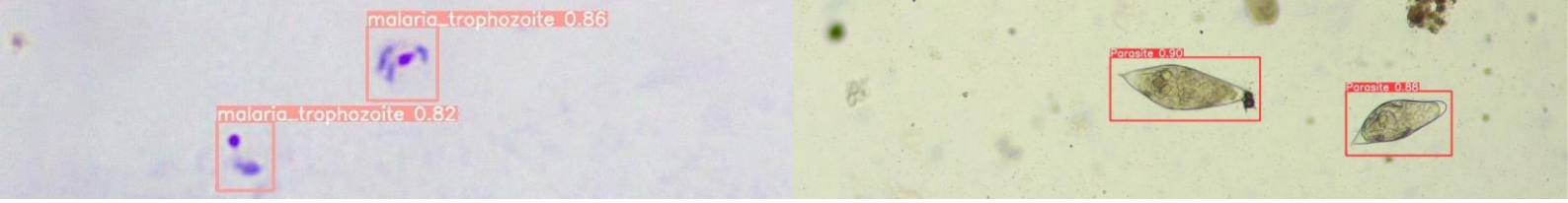
https://doi.org/10.1371/journal.pone.0304085.g008

In conclusion, we are ever closer to develop a fully automated system to perform autonomous microbiological diagnosis emulating traditional microscopy techniques. After successfully implementing the low-cost automation of a conventional optical microscope, the irruption of AI technology for the diagnosis during the near years allows us to postulate that convolutional neural networks for image analysis will be improved, regulated and optimized to be considered as a reference diagnosis technique for malaria and NTDs detection.

Supporting information

S1 Table. List of materials and devices employed for the development, design and manufacturing of the robotized conventional optical microscope system.
(DOCX)

S1 Fig. Microscope stage 3D pieces dimensions representation.
(TIFF)



S2 Fig. Mobile phone adapter 3D pieces dimensions representation.
(TIFF)

S3 Fig. Auto-focus 3d pieces / Storage and controller parts dimensions representation.
(TIFF)

S4 Fig. Microscope stage pieces (5 independent pieces). (1) External stage holder; (2) Screw hole for servo motor; (3) Toothed rail for servo motor; (4) Sample holder and internal stage holder; (5) Medium stage holder; (6) Sample clamp; (7) Gears for toothed rail and servo motors.
(TIFF)

S5 Fig. Auto-focus pieces / Storage and controller parts (5 independent pieces). (1) Storage box cover; (2) Storage box; (3) Auto-focus servo motor stick holder; (4) Fine adjustment wheel clamps; (5) Auto-focus system supports; (6) Storage template for Arduino; (7) USB hole; (8) External cables hole.
(TIFF)

S6 Fig. Mobile phone adapter pieces (5 independent pieces). (1) Smartphone holder; (2) Adapter support; (3) Ocular lens hitch; (4) Wheels for regulating dimension of the screws.
(TIFF)

S1 Data.
(ZIP)

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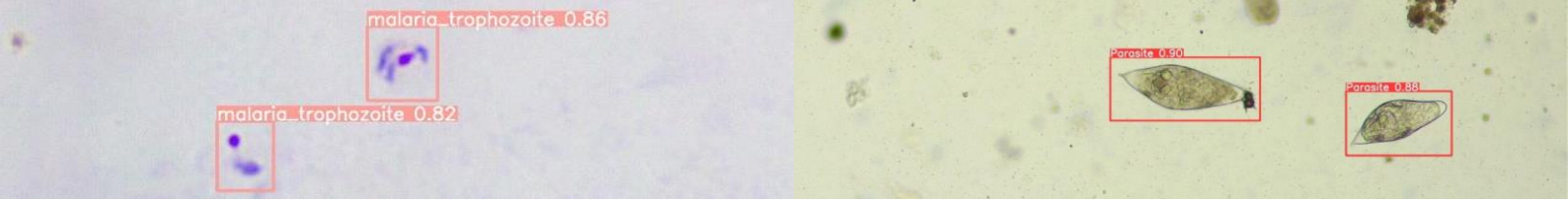
Methodology: Allisson Dantas de Oliveira, Carles Rubio Maturana, Elena Sulleiro, Daniel López-Codina.



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Writing – review & editing: Carles Rubio Maturana, Bruno Motta Carvalho, Elena Sulleiro, Clara Prats, Anna Veiga, Mercedes Bosch, Javier Zulueta, Alberto Abelló, Elisa Sayrol, Joan Joseph-Munné, Daniel López-Codina.

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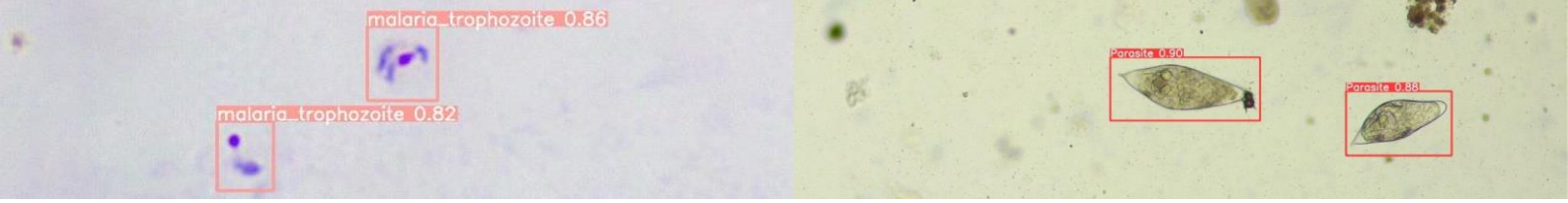

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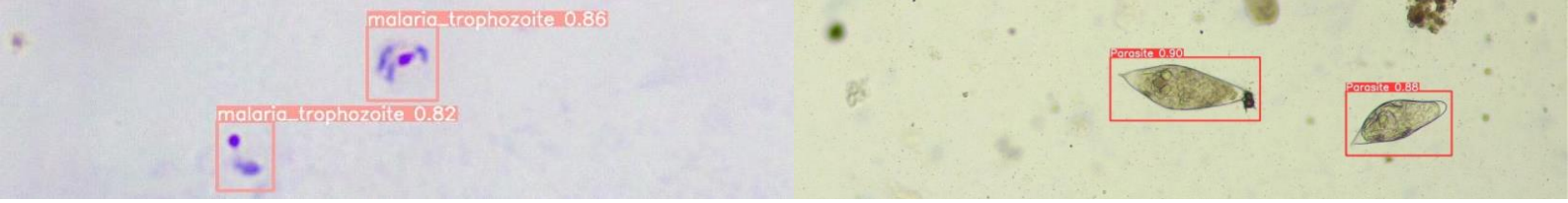

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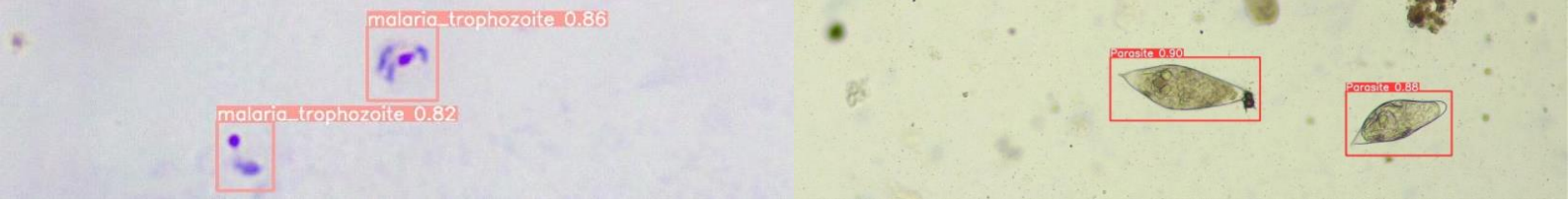

4.1.3. Development of an automated artificial intelligence-based system for urogenital schistosomiasis diagnosis using digital image analysis techniques and a robotized microscope

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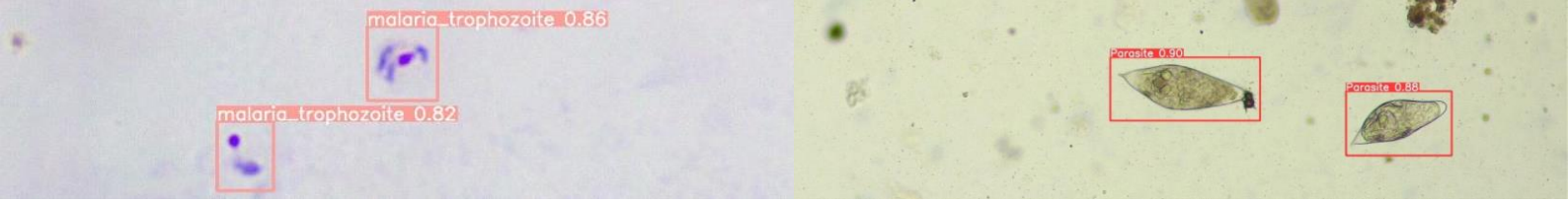


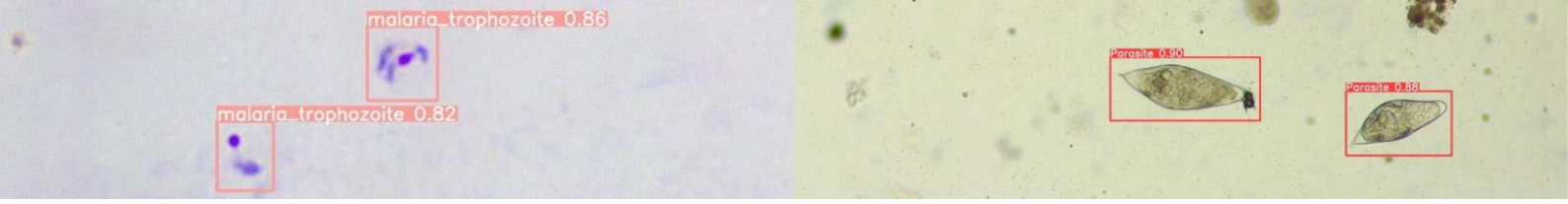
Resum del treball:

L'esquistosomiasi urogenital és considerada una malaltia tropical desatesa per l'Organització Mundial de la Salut. S'estima que afecta 150 milions de persones a tot el món, amb especial rellevància en els entorns amb pocs recursos del continent africà. El diagnòstic *gold standard* continua sent l'observació directa dels ous de *Schistosoma haematobium* en mostres d'orina per microscòpia òptica. Les noves tècniques de diagnòstic basades en l'anàlisi d'imatges digitals mitjançant eines d'Intel·ligència Artificial (IA) són una alternativa adequada per al diagnòstic de l'esquistosomiasi.

Es van adquirir imatges digitals de 24 mostres de sediments d'orina en àrea no endèmica. Els ous de *S. haematobium* van ser etiquetats manualment en imatges digitals per professionals de laboratori, i les imatges etiquetades es van emprar per entrenar els models de detecció automàtica YOLOv5 i YOLOv8. Les imatges de sediments d'orina també es van utilitzar per realitzar una classificació binària d'imatges per detectar eritròcits/leucòcits amb els models MobileNetv3Large, EfficientNetv2, i NasNetLarge. Es va utilitzar un sistema de microscopi robotitzat per moure automàticament la mostra a través dels eixos X-Y i per l'autoenfocament.

Un total de 1189 etiquetes van ser anotades en 1017 imatges digitals de mostres de sediments d'orina. L'entrenament de YOLOv5x va demostrar una precisió del 99.3%, un *recall* del 99.4%, un valor F de 99.3% i un 99.4% de *mAP0.5* per a la detecció de *S. haematobium*. NasNetLarge té una precisió del 85.6% per a la detecció d'eritròcits/leucòcits amb el conjunt de dades de prova. L'entrenament i comparació de xarxes neuronals convolucionals va demostrar que el model YOLOv5x per a la detecció d'ous, i el model NasNetLarge per a la classificació binària d'imatges per detectar eritròcits/leucòcits eren les millors opcions per a la nostra base de dades d'imatges digitals.

El desenvolupament de noves tècniques diagnòstiques de baix cost basades en la detecció i identificació d'ous de *S. haematobium* en orina mitjançant eines d'IA pot ser una alternativa adequada a la microscòpia convencional. Aquest estudi permet establir les bases per a la millora del sistema, i optimitzar la seva implementació en els laboratoris d'arreu.



PLOS NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

Development of an automated artificial intelligence-based system for urogenital schistosomiasis diagnosis using digital image analysis techniques and a robotized microscope

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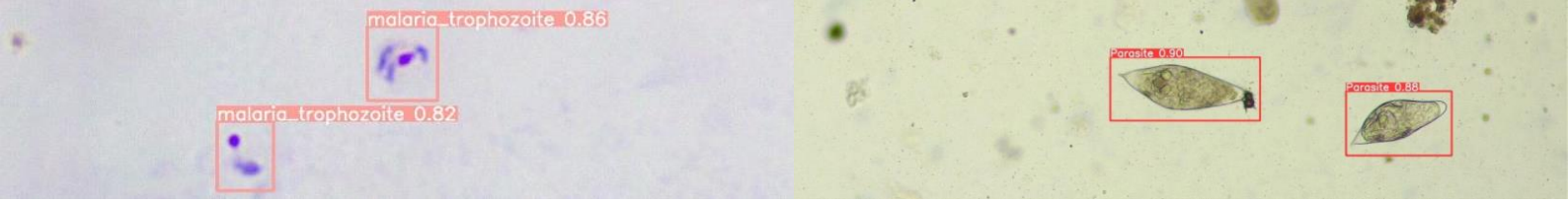
Abstract

Background

Urogenital schistosomiasis is considered a Neglected Tropical Disease (NTD) by the World Health Organization (WHO). It is estimated to affect 150 million people worldwide, with a high relevance in resource-poor settings of the African continent. The gold-standard diagnosis is still direct observation of *Schistosoma haematobium* eggs in urine samples by optical microscopy. Novel diagnostic techniques based on digital image analysis by Artificial Intelligence (AI) tools are a suitable alternative for schistosomiasis diagnosis.

Methodology

Digital images of 24 urine sediment samples were acquired in non-endemic settings. *S. haematobium* eggs were manually labeled in digital images by laboratory professionals and used for training YOLOv5 and YOLOv8 models, which would achieve automatic detection and localization of the eggs. Urine sediment images were also employed to perform binary classification of images to detect erythrocytes/leukocytes with the MobileNetv3Large, EfficientNetv2, and NasNetLarge models. A robotized microscope system was employed to automatically move the slide through the X-Y axis and to auto-focus the sample.



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Competing interests: The authors have declared that no competing interests exist.

Results

A total number of 1189 labels were annotated in 1017 digital images from urine sediment samples. YOLOv5x training demonstrated a 99.3% precision, 99.4% recall, 99.3% F-score, and 99.4% mAP0.5 for *S. haematobium* detection. NasNetLarge has an 85.6% accuracy for erythrocyte/leukocyte detection with the test dataset. Convolutional neural network training and comparison demonstrated that YOLOv5x for the detection of eggs and NasNetLarge for the binary image classification to detect erythrocytes/leukocytes were the best options for our digital image database.

Conclusions

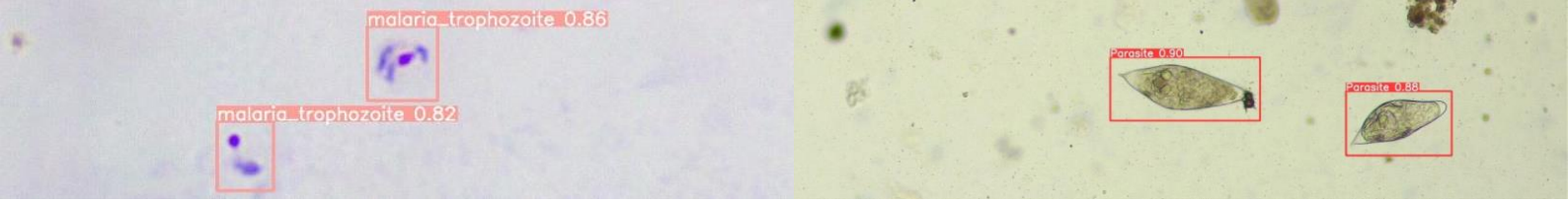
The development of low-cost novel diagnostic techniques based on the detection and identification of *S. haematobium* eggs in urine by AI tools would be a suitable alternative to conventional microscopy in non-endemic settings. This technical proof-of-principle study allows laying the basis for improving the system, and optimizing its implementation in the laboratories.

Author summary

Urogenital schistosomiasis, categorized as a Neglected Tropical Disease (NTD) by the World Health Organization (WHO), affects approximately 150 million individuals globally, predominantly in resource-limited regions of Africa. Gold standard diagnosis relies on visually identifying of *Schistosoma haematobium* eggs in urine samples using optical microscopy. However, novel diagnostic techniques based on digital image analysis by Artificial Intelligence (AI) tools are a suitable alternative for schistosomiasis diagnosis. In this technical proof-of-principle study, a small number (n = 24) of urine sediment samples were analyzed using AI models in non-endemic settings. The study involved manually labeling *S. haematobium* eggs in digital images, for training YOLOv5 and YOLOv8 models for automatic egg detection, and employing MobileNetv3Large, EfficientNetv2, and NasNetLarge models for binary classification of erythrocytes/leukocytes. A robotized microscope system facilitated automated sample movement and focusing. Results indicated high precision (99.3%) and recall (99.4%) for *S. haematobium* detection with YOLOv5x. NasNetLarge achieved 85.6% accuracy in erythrocyte/leukocyte detection. Overall, YOLOv5x for egg detection and NasNetLarge for cell classification proved most effective. The study suggests AI-based techniques offer a cost-effective alternative to conventional microscopy for diagnosing *S. haematobium* infections. The automated system's robustness and simplicity could facilitate widespread adoption in laboratories worldwide.

1 Introduction

Schistosomiasis is a parasitic disease caused by *trematode* worms of the genus *Schistosoma* [1]. It affects more than 250 million people worldwide, with a high prevalence in tropical and subtropical areas [2]. Its transmission occurs by direct contact with contaminated water, in which *Schistosoma cercariae* can penetrate human skin thus initiating infection. Freshwater snails of the genera *Biomphalaria*, *Oncomelania*, and *Bulinus* act as intermediate hosts [3]. Schistosomiasis is considered a Neglected Tropical Disease (NTD) by the World Health Organization



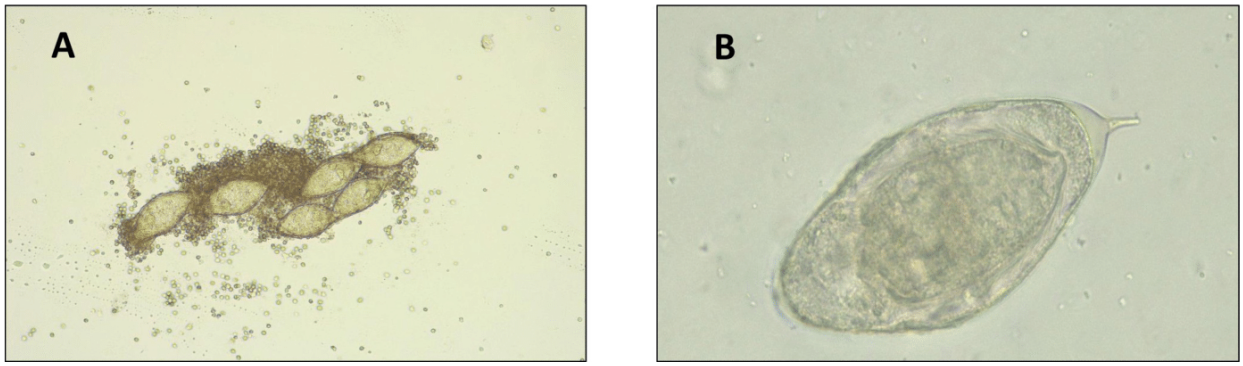


Fig 1. Panel A. *S. haematobium* eggs in a urine sediment sample (10x ocular; 10x objective lens). **Panel B.** *S. haematobium* egg in a urine sediment sample (10x ocular; 40x objective lens).

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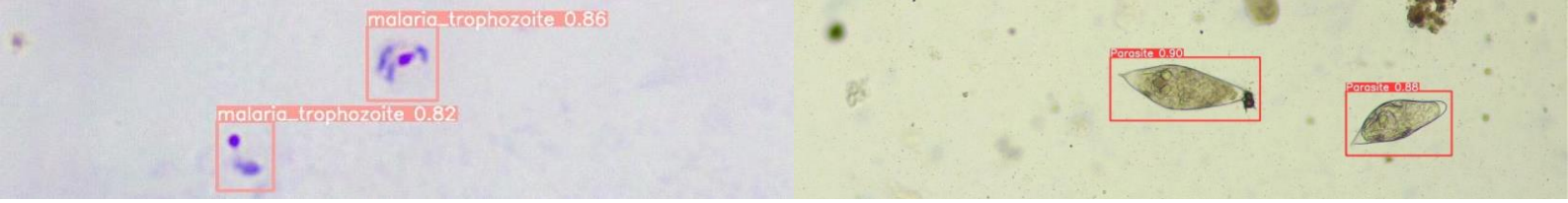
(WHO) due to its impact in resource-poor areas and its correlation with poverty [4]. The most predominant causative species are *Schistosoma haematobium* and *Schistosoma mansoni*, classified as urogenital and intestinal schistosomiasis, respectively [1]. More than 90% of schistosomiasis cases occur in the African continent, and 66% are caused by *S. haematobium* [5]. Some of the main countries with a high prevalence of urogenital schistosomiasis are Senegal, Nigeria, Angola, and Cameroon, among others [6–8]. Therefore, urogenital schistosomiasis has a major impact globally, with a significant incidence in the pediatric population of endemic areas [5]. Resource-poor areas near infested rivers are considered high-risk communities in which the prevalence of urogenital schistosomiasis is considerably high [9]. Digital images of *S. haematobium* eggs are represented in **Fig 1**. Most patients are asymptomatic, although if the disease is left untreated it can become chronic, cause hematuria and leukocyturia, and in some cases it can lead to bladder cancer [10].

According to the WHO, the gold standard diagnostic technique for schistosomiasis is the microscopic observation of parasite eggs in stool/urine samples [1]. Serological tests are also widely employed for the diagnosis of this disease [11], and molecular techniques such as real-time PCR are currently being developed to improve the sensitivity and specificity of traditional techniques [12]. However, in schistosomiasis-endemic areas, microscopy is still the most employed technique for diagnosis. Microscopic diagnosis is expert-dependent, time-consuming, and could trigger diagnostic errors due to observing large numbers of samples in short periods of time [13].

As an emulation of traditional microscopy, novel image analysis techniques based on Artificial Intelligence (AI) tools are being developed to automate the diagnostic procedure. As an example, a prototype based on AI image analysis was developed for the detection of soil-transmitted helminths and *S. mansoni* parasites in Kato-Katz stool thick smears [14]. Moreover, other studies demonstrate the utility of mobile phone-based microscopes for urogenital schistosomiasis diagnosis in Côte d'Ivoire [15]. Nowadays, Convolutional Neural Networks (CNNs) are the models most employed for object detection in digital image analysis [16]. Particularly, CNNs for poverty related diseases are postulated as a suitable supportive tool for the microscopic diagnosis of several diseases, such as malaria [17,18], tuberculosis [19], and NTDs [20]. Moreover, novel computational strategies, such as attention modules [21] and transformers [22], are improving the performance of traditional CNNs, opening up a new range of prospects for object detection algorithms in digital imaging. Several studies demonstrate that YOLO models have outperformed other state-of-the-art CNN models for object detection,



such as Faster R-CNN and RetinaNet [23,24]. YOLOv5 and YOLOv8 used similar backbones with changes in the CSPLayer/C2f module, and they were considered the best YOLO algorithms [25]. Moreover, each YOLO model has different versions depending on its architecture, considering YOLOv-s (small) and YOLOv-x (extra-large) as suitable versions for comparison, regarding the remarkable differences in performance and speed [25]. The YOLOv8 model is newer and considered an update of YOLOv5, nevertheless, the latter is still considered the best model for object detection tasks in terms of accuracy [26].

In this study, we fine-tuned object detection algorithms (YOLOv5s, YOLOv5x, YOLOv8s, and YOLOv8x) for the automated identification of *S. haematobium* eggs in urine samples. A small number of biological samples from non-endemic settings were employed for digital image acquisition and labeling of parasites by a smartphone/microscope camera. CNN training and metrics comparison were performed to determine the optimal algorithm for schistosomiasis diagnosis with our image database. Binary image classification algorithms (MobileNetv3Large, EfficientNetv2, and NasNetLarge) were also trained to detect digital images with the presence of erythrocytes and/or leukocytes in urine, as a suggestive, although not exclusive, clinical sign of urogenital *S. haematobium* infection.

An automated robotized low-cost microscope prototype was employed for sample analysis, with X-Y movements of the slide and autofocusing. A first proof-of-principle technical study in non-endemic settings of the development of the system has been conducted, allowing to evaluate the performance and to lay the basis to enhance it in further in-field studies.

According to the state-of-the-art there are several studies describing detection methods for the automated identification of *S. haematobium* eggs in urine sediment samples. However, our system could contribute novelties in terms of adaptability to conventional optical microscopes and its autofocus algorithm, the usage of a smartphone device as a key controller for image acquisition and AI-analysis, and the identification of erythrocytes and leukocytes in urine for *S. haematobium* diagnosis orientation. Many of the published systems involve the detection of *S. haematobium* eggs in digital images, although the combination of all system features provide new advances in low-cost automated detection.

Thus, our image analysis strategy would join the global effort to fight against NTDs, providing clinical laboratories with novel diagnostic tools able to complement state-of-the-art traditional technologies.

2 Materials and methods

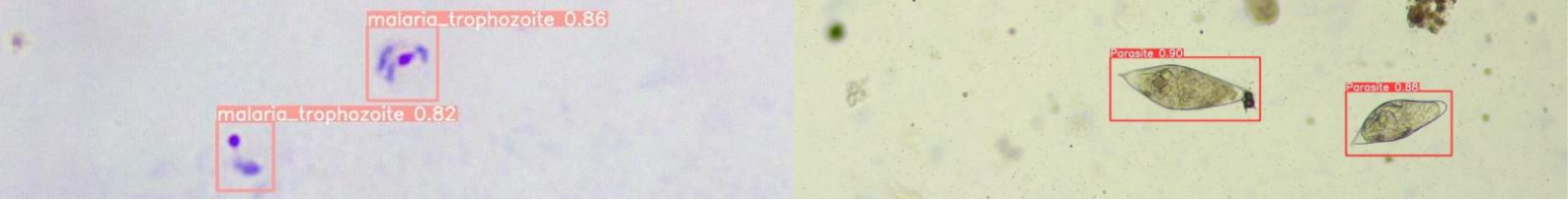
In this section, we describe the materials and methods used to develop the diagnostic system based on image analysis by image classification and object detection. The details of all procedures are sufficient to allow for experimentation by third parties.

2.1. Ethics statement

This study was conducted in accordance with the Declaration of Helsinki and approved by the Clinical Research Ethics Committee (CEIm) of the Vall d'Hebron University Hospital / Vall d'Hebron Research Institute, with reference number PR(AG)40/2023. The urine samples were not collected for the study, they were obtained from the regular clinical visits in our International Health Center and retrospectively analyzed. Formal consent was not obtained with ethics committee approval.

2.2. Sample preparation and observation

In this study, 24 *S. haematobium*-positive urine sediment samples and eight negative urine sediment samples from (i) the Drassanes-Vall d'Hebron International Health and Infectious



Diseases Centre (Barcelona, Spain), and (ii) the Microbiology Department of the Vall d'Hebron University Hospital (Barcelona, Spain) were employed. Urine sediment samples were collected directly in a 100 mL recipient. Samples are left to settle for 1 hour, and 10 mL were centrifuged for 5 minutes at 15,000 rpm. Parasite eggs were searched in urine sediment samples for microbiological diagnosis. Standard objective lenses, 10x and 40x, were employed to perform a large-scale observation and confirm the diagnosis, respectively. Between 1–20 images of different microscopy fields were acquired from each sample, considering parasite densities, presence/absence of erythrocytes and leukocytes in urine, and number of *S. haematobium* eggs per sample. This range of image numbers avoids an imbalance in the training of the neural networks by employing a stipulated number of data. Biological samples were obtained from symptomatic/asymptomatic patients from *S. haematobium*-endemic areas, mainly migrants, and Visit Friends and Relatives (VFR), following our reference center's protocols [27]. Most individuals came from Sub-Saharan Africa region, mainly from Gambia, Mali and Senegal. Regarding epidemiological studies, the prevalence of *S.haematobium* infection in these countries is between 9% to 10.2% [28–32]. Microscopic examination of clinical samples was performed following WHO sample observation statements for *S. haematobium* diagnosis [32]. Samples were observed at most 48h after extraction and discarded, following our International Health Laboratory protocols.

2.3. Image acquisition

Digital images of each microscopic Field of View (FoV) were acquired with an integrated Leica ICC50W camera attached to a Leica DM750 microscope (5.0MP / 2592 x 1944 pixels) and consecutively with the camera of a Samsung Galaxy S20 smartphone device (64MP, 0.8μm, f/2.0, OIS / 3024 x 4032 pixels). Smartphone-acquired images were captured using an ocular adapter for smartphone attachment to the microscope. A 3D adapter bracket attached to the ocular lens of the microscope was used to standardize the image-capturing procedure with the smartphone device. Both, the integrated camera and smartphone-acquired images were captured by the visualization of urine sediment samples with a Leica DM750 microscope lens with 100x and 400x total magnification (10x ocular; 10x and 40x objective lens). Image acquisition is represented in **Fig 2**.

2.4. Image pre-processing

Images acquired with a smartphone device were cropped to highlight the central area of interest of the image and to eliminate black borders due to ocular lens attachment. Cropping was performed to remove the outer edges without losing any information (**Fig 2**). Original smartphone images were cropped automatically (Python script) to obtain a 4:3 image in the center and subsequently rotated 90˚ for horizontal image reorientation. With this procedure, it is possible to crop an image regardless of its dimensions and the number of pixels as proportions were used to perform the cropping. The cropped images have the same 4:3 image proportion as those acquired with the microscope-integrated camera. Cropping confers a re-composition of the image that may positively affect the final results, providing a clearer image and removing elements irrelevant to the prediction and identification functions of the neural networks, as mentioned in the previous literature [33].

2.5. Image annotation and classification

Digital images were labeled by laboratory professionals from the Drassanes-Vall d'Hebron International Health and Infectious Diseases Centre. For image labeling, the area of interest was selected by creating a bounding box with the object inside (**Fig 2**). This bounding

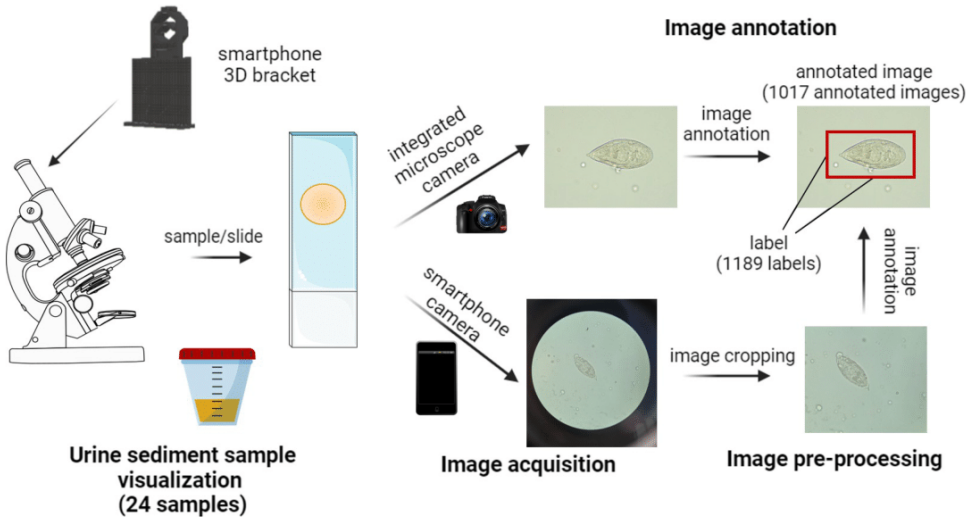


Fig 2. Representative scheme of sample visualization, image acquisition, image pre-processing, and image annotation procedures. Sample/slide, image, annotated image, and label are represented. *Schistosoma haematobium* digital images were acquired with an integrated camera/smartphone camera in the Microbiology Laboratory of the Vall d'Hebron Drassanes International Health and Infectious Diseases Centre. Illustrations were obtained from open source resources.

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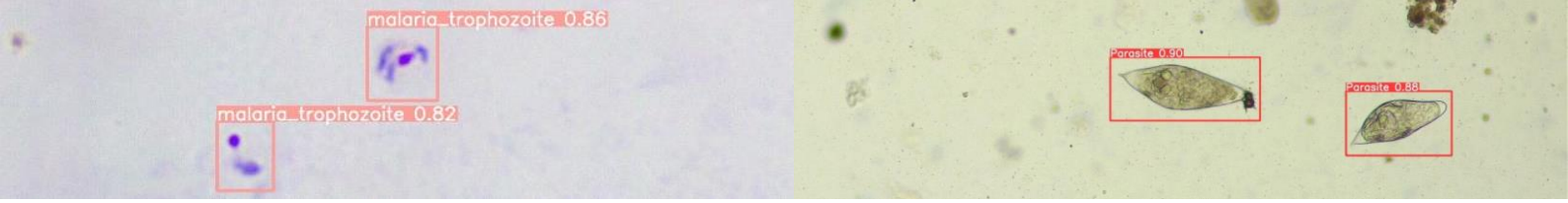
box defines the label. *S. haematobium* eggs (viable and calcified) were labeled in digital images using Annotation App software. Labeling eggs with bounding boxes was required to train the object detection algorithms based on CNNs. Once labeling was finished, the Annotation App software created a *json* type file with labels linked to the original image file, in which the coordinates of the labeled objects are specified (annotated images). Whole-image classification was performed by creating two subgroups of images depending on whether erythrocytes/leukocytes were present or not in urine sediment samples.

2.6. Binary classification algorithms for hematuria and leukocyturia detection

Whole-image binary classification models (MobileNetv3Large [34], EfficientNetv2 [35], and NasNetLarge [36]) were employed to automatically classify digital images in two subgroups. Digital images with or without the presence of erythrocytes/leukocytes in urine samples, with 100x magnification (10x ocular; 10x objective) were manually classified by three clinical laboratory experts from the Vall d'Hebron International Health and Infectious Diseases Centre. The image database was divided, allocating 90% for training/validation and 10% for testing. Images were resized by default to 331x331 pixels for NasNetLarge, 224x224 pixels for MobileNetv3Large, and 300x300 pixels for EfficientNetv2, and trained for 30 epochs and batch size 20. Only 100x magnification images (10x ocular; 10x objective) were employed for binary classification algorithm training.

2.7. Object detection algorithm training and comparison analysis

CNN object detection models were fine-tuned with our *S. haematobium*-labeled digital image database. You Only Look Once (YOLO) [37] versions 5s, 5x, 8s, and 8x were trained. YOLO was considered an optimal algorithm for object detection tasks, with several studies in the area of diagnosis [25,38,39]. The YOLO versions were also crucial, considering YOLO "s" (small) as



a fast and efficient alternative and YOLO "x" (extra-large) as a more accurate algorithm [25,39]. The *Schistosoma* image database was divided, allocating 80% for training, 15% for validation, and 5% for testing. Images were resized by default to 640x640 pixels and CNNs were trained for 30 epochs and batch size 16. Images were organized randomly considering the proportions, and test subset images were unseen by the CNN model to avoid unreliable results and preserve patient-level structure [40]. Neural networks were pre-trained with the Common Objects in Context (COCO) dataset [41]. Object detection CNNs were compared using the analytical metrics of precision, recall, F-score, and mAP0.5. Two consecutive image datasets were employed for CNN training and evaluation. An initial image dataset containing 491 images was employed, and thereafter, a second dataset containing 1017 images, which also included the images of the initial database (491). The number of images employed for CNN training was determined considering supervised single-class classifiers performance and other similar studies, although the minimum amount of labels to obtain reliable results depends on the labels quality and classifier architecture [42,43].

2.8. Statistical analysis

Statistical analyses were performed to determine significant differences between validation and test subset performance for each CNN model. Metric means were calculated individually for each CNN model. To evaluate significant statistical differences between CNN models, a paired t-test analysis (p-value<0.05, t-value $>$ -2 or 2), mean (M), and standard deviation (SD) were employed. The same statistical analysis was employed for binary classification algorithms. The IBM SPSS software statistics environment was used.

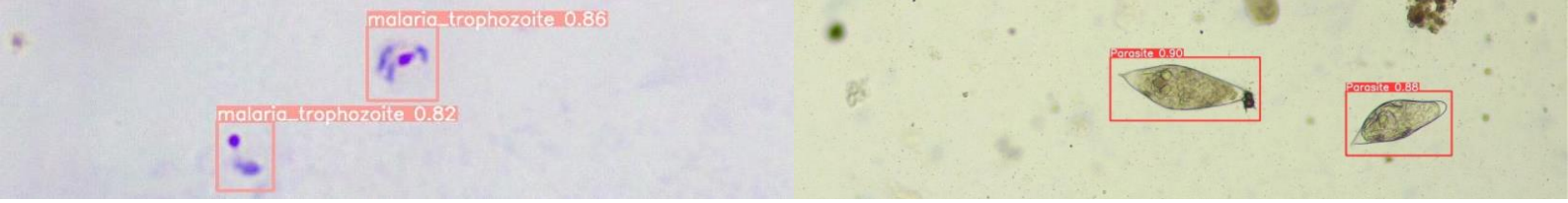
2.9. Microscopy automation system

The system [44] was employed to automate a conventional Leica DM750 optical microscope for malaria diagnosis. This technology can autofocus the image/FoV and guide the automated movements of the slide through the X-Y axis of the microscope. All the diagnostic technology is embedded into a smartphone/computer application, responsible for acquiring the images, automating microscope auto-focus and stage movements, and using CNN algorithms for parasite detection. The system is designed with 3D-printing technology and does not need an internet connection or an electrical power supply. Smartphone-based, adaptability and auto-focus results were previously published [44].

3 Results

3.1. *Schistosoma haematobium* urine sediment image database analysis

S. haematobium urine sediment samples, digital raw images, annotated images, and labels were analyzed. A total of 1017 annotated digital images were imported into the database for further CNN training and algorithm generation. A total of 1189 labels identifying *S. haematobium* eggs (calcified/non-calcified) were annotated in digital images. Of the 1017 digital images, 744 were acquired with the LEICA ICC50W integrated microscope camera, and 273 were acquired with the Samsung Galaxy S20 smartphone camera. For the detection of erythrocytes and/or leukocytes in urine, 762 images were acquired with the integrated microscope camera LEICA ICC50W and manually classified as urine samples with (493 images) or without (269 images) erythrocytes/leukocytes, respectively. A summary of the urine sediment sample image database information is shown in **Table 1**.



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3.1. *Schistosoma haematobium* urine sediment image database analysis

S. haematobium urine sediment samples, digital raw images, annotated images, and labels were analyzed. A total of 1017 annotated digital images were imported into the database for further CNN training and algorithm generation. A total of 1189 labels identifying *S. haematobium* eggs (calcified/non-calcified) were annotated in digital images. Of the 1017 digital images, 744 were acquired with the LEICA ICC50W integrated microscope camera, and 273 were acquired with the Samsung Galaxy S20 smartphone camera. For the detection of erythrocytes and/or leukocytes in urine, 762 images were acquired with the integrated microscope camera LEICA ICC50W and manually classified as urine samples with (493 images) or without (269 images) erythrocytes/leukocytes, respectively. A summary of the urine sediment sample image database information is shown in **Table 1**.

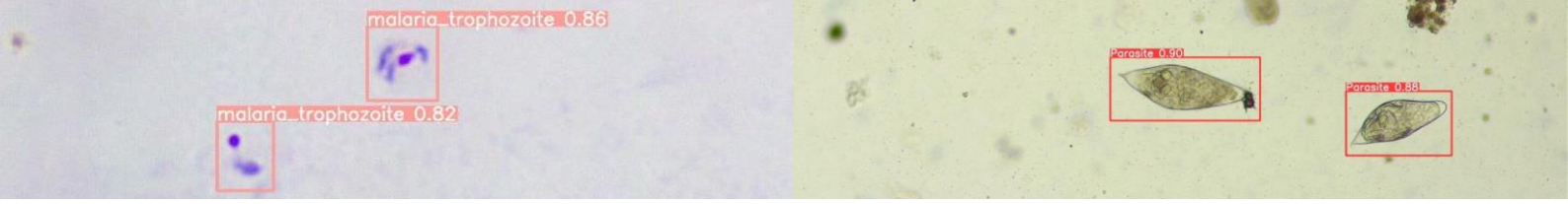

Table 1. Summary of the urine sediment sample image database.

Category		Sub-total	Total
Sample source	Drassanes-Vall d'Hebron International Health and Infectious Diseases Centre (Barcelona, Spain)	24 (samples/patients)	24 (samples/patients)
Image acquisition type	Microscope integrated camera (ICC50W Leica)	744 (annotated images)	1017 (annotated images)
	Smartphone camera (Samsung Galaxy S20)	273 (annotated images)	
Image magnification	10x ocular lens and 10x objective lens	500 (annotated images)	1017 (annotated images)
	10x ocular lens and 40x objective lens	517 (annotated images)	
Annotation category	*Schistosoma haematobium* eggs	1165 (labels)	1189 (labels)
	Schistosoma haematobium calcified eggs	24 (labels)	
Binary image classification	Presence of erythrocytes and/or leukocytes in urine	493 images	762 images
	Non-presence of erythrocytes and/or leukocytes in urine	269 images	
Negative sample validation	Non-presence of *Schistosoma haematobium* eggs in urine	8 (samples/patients)	400 images

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3.2. Convolutional Neural Network performance comparison for *S. haematobium* egg detection

CNN models were trained with our *S. haematobium* image database and compared to evaluate their performance in a test subset. Table 2 shows the most relevant metrics of the YOLOv5s, YOLOv5x, YOLOv5x-DA, YOLOv8s, and YOLOv8x CNNs trained with 491- and 1017-image databases. Overall analysis confirms that the 1017-image database provides higher metric results for CNN training than the 491-image database, as expected. Considering the 1017-image database training, precision analysis shows optimal values with the YOLOv5 model, with 99.3% for YOLOv5x and 97.1% for YOLOv5s. Recall analysis demonstrates a 99.4% rate for YOLOv5x and 97.2% for YOLOv5s. Consequently, F-score analysis demonstrated optimal values with the YOLOv5x (99.3%) and YOLOv5s (97.1%) models. Mean average precision (mAP0.5) analysis shows higher values for YOLOv5x, YOLOv5s, and YOLOv8s with 99.4%, 98.8%, and 98.7% respectively, all with the 1017-image database. Overall metric analyses in terms of precision, recall, F-score, and mAP0.5 indicated that the best CNN model for *S. haematobium* detection in urine sediment samples with our image database was YOLOv5x (1017).

Table 2. Summary of Convolutional Neural Network training and performance parameters with the test image dataset. DA: Data augmentation, mAP: mean average precision, YOLO: you only look once.

Neural Network model	Epochs	Precision (%)	Recall (%)	F-score (%)	mAP0.5 (%)	Images
YOLOv5x	30	92.3	73.3	81.7	81.7	491
YOLOv5x - DA	30	88.2	72.4	79.5	85.3	491
YOLOv8s	30	94.3	97.0	95.6	97.3	491
YOLOv8x	30	95.3	89.8	92.5	96.8	491
YOLOv5s	30	97.1	97.2	97.1	98.8	1017
YOLOv5x	30	**99.3**	**99.4**	**99.3**	**99.4**	1017
YOLOv8s	30	96.3	96.5	96.4	98.7	1017
YOLOv8x	30	96.3	95.1	95.7	96.6	1017

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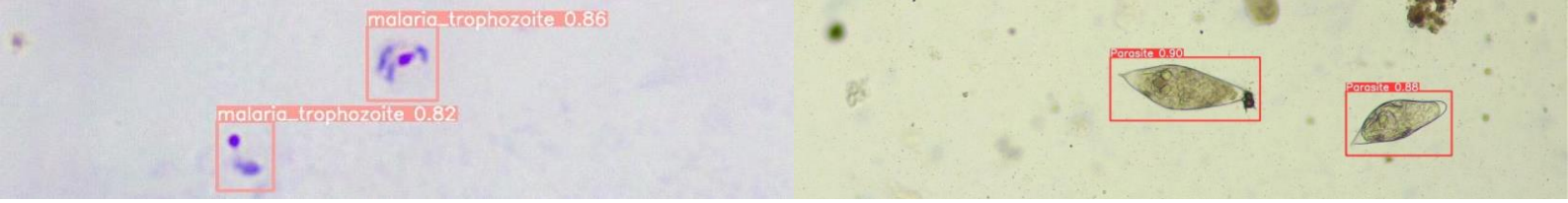

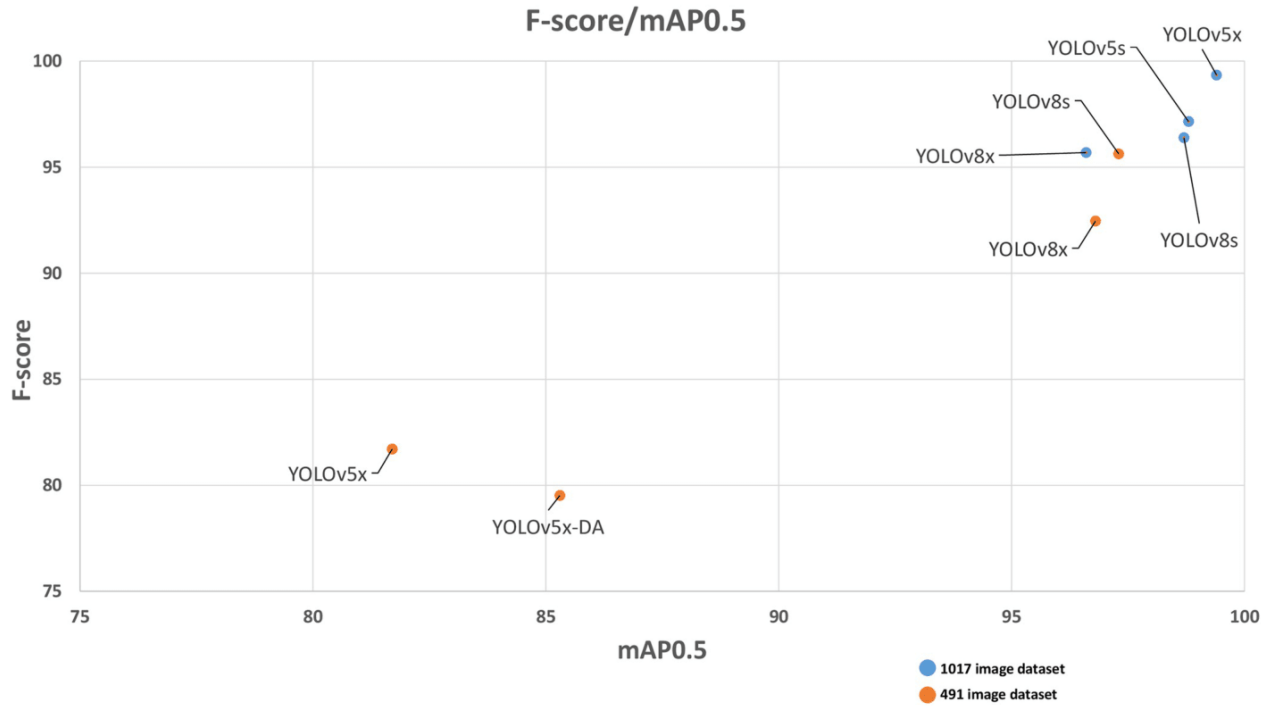

Fig 3. Graphical representation of F-score/mAP0.5 of the different CNNs trained on the test dataset. Orange dots represent the performance of CNNs trained with the 491-image dataset. Blue dots represent the performance of CNNs trained with the 1017-image dataset.

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To determine differences between neural network performances for *S.haematobium* egg detection in digital images, a statistical analysis was conducted considering the test data subset. As expected, models trained with a larger image dataset (1017) show considerably higher performance when compared with the previous smaller image dataset (491) ($p<0.05$). The impact of the size of the database was studied, even though it might seem obvious, to analyze whether results using more images were necessary. Results of the paired t-test indicated that there were significant statistical differences between YOLOv5x–DA-491 (M = 42.3, SD = 41.7) and YOLOv5x-1017 (M = 51.3, SD = 51), t = 2.4, $p<0.05$. Moreover, results of the paired t-test indicated that there were significant statistical differences between the YOLOv5-491 (M = 42.8, SD = 40.1) and YOLOv5-1017 trained versions (M = 50.7, SD = 49), t = 3, $p<0.05$). These data demonstrate the performance gain due to the higher number of data/images for training the YOLOv5x model. In contrast, there are non-statistically significant differences between models YOLOv8s-491 and YOLOv8s-1017 ($p>0.05$) and models YOLOv8x-491 and YOLOv8x-1017 ($p>0.05$). The performance gain due to the image dataset increase was not obtained with model YOLOv8s or YOLOv8x. The optimal results of YOLOv8 with a relatively small image database (491) demonstrate the efficiency of the neural network compared with YOLOv5. F-score values, which are the harmonic mean between precision and recall, and mAP0.5, provide valuable information to determine the best model for our image dataset. Fig 3 shows the correlation between the F-score and mAP0.5 values of the multiple trained neural networks. The dots represented in the top right part of the graph reflect optimal performance. Overall analysis and comparisons demonstrate that the YOLOv5x model is the most optimal CNN for our digital image dataset, although the YOLOv8 models are more efficient in terms of training and image database size. Differences between small "*s*" and large "*x*" CNN

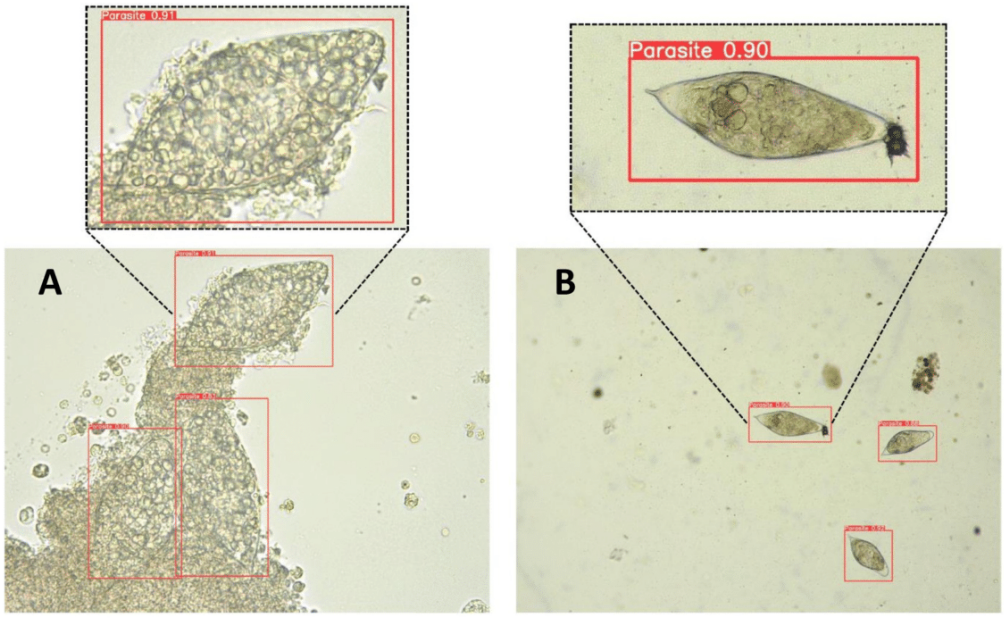

Fig 4. Panel A. Digital image (400x) of a urine sediment sample with hematuria and leukocyturia and three *Schistosoma haematobium* eggs detected with the YOLOv5x trained model. **Panel B**. Digital image (100x) of a urine sediment sample with three *S. haematobium* eggs detected with the YOLOv5x trained model.

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architectures were not statistically significant ($p>0.05$). Image identification was represented in Fig 4.

3.3. Binary image classification performance for erythrocyte/leukocyte detection in urine sediment images

Whole-image classification algorithms were trained with our *S. haematobium* image database and compared to evaluate their performance. Table 3 shows the most relevant metrics to evaluate the performance of the MobileNetv3Large, EfficientNetv2, and NasNetLarge models. There were no statistically significant differences between the performance of these three models ($p>0.05$), or between validation (M = 85.5, SD = 3.9) and test (M = 82.4, SD = 4) subsets in terms of accuracy results ($t = 1.8$, $p > 0.05$). However, NasNetLarge was considered the best option for the erythrocytes/leukocytes image classification task with an 85.6.0% accuracy, followed by the MobileNetv3-Large and EfficientNetv2 models, with 83.7% and 77.9% accuracy, respectively.

3.4. Negative sample validation

Analysis and validation tests were performed to evaluate the reliability of the YOLOv5x-1017 model trained with confirmed negative samples. Urine sediment samples (n = 8) with a

Table 3. Summary of binary image classifier training and performance parameters on validation and test image datasets.

Image classifier model	Epochs	Batch size	Validation Accuracy	Test Accuracy
MobileNetv3Large	30	20	83.3	83.7
EfficientNetv2	30	20	83.3	77.9
NasNetLarge	30	20	90.0	85.6

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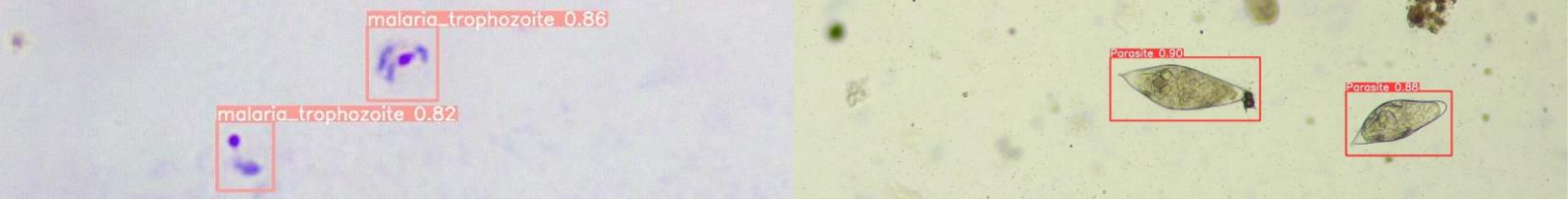


negative microscopic examination result for *S. haematobium* eggs were employed. Samples were observed and validated by professional microscopists with proven experience in parasitological diagnosis. Five microscope slides of each sample were prepared to observe the whole urine sediment. A total of 400 images were acquired from urine sediment samples and analyzed by the trained YOLOv5x model (confidence threshold value = 0.7). These negative sample images were not used for CNN algorithm training. After analysis, 394/400 (98.5%) were negative (true-negative) for *S.haematobium* infection, and six false-positive results were reported (epithelial cells, urine crystals, and artifacts). All false-positive images were from a single urine sediment sample.

3.5. Testing analysis with other image databases

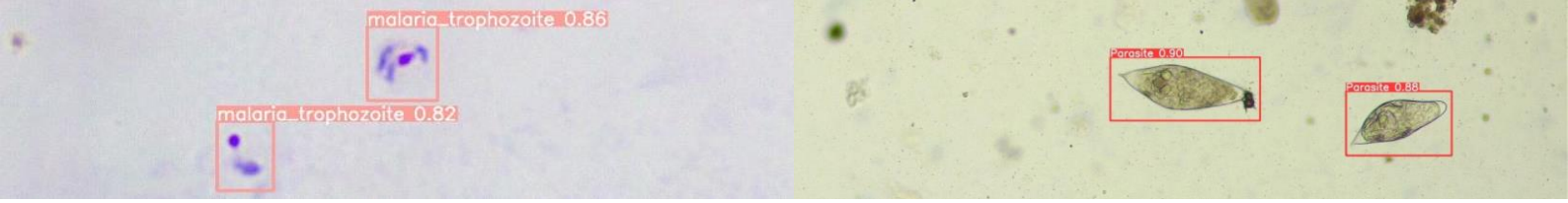
To determine the robustness of our trained models, we tested the considered optimal model, YOLOv5x, with digital images from other sources. However, there are no publicly available large state-of-the-art *S. haematobium* urine sediment databases to compare our results. As an alternative, publicly available single images were obtained from contrasted cited sources [23–26,45–47]. We selected and employed images with different input sizes (from 1600 x 1200 pixels to 400 x 300) and different image weights (from 895 KB to 25.9 KB), ensuring a representative sample (n = 19). Digital images with parasite eggs were empirically tested with our fine-tuned YOLOv5x model with positive results (27/27 eggs detected, average detection probability value = 0.79).

3.6. Automated microscope for diagnosis and smartphone software application

To automatically perform an autonomous detection of *S. haematobium* eggs in urine sediment samples, we designed a fully automated low-cost robotized microscope. Three-D polylactic acid pieces for microscope automation were built with an Ender 3-Pro printer. Automation allows the emulation of optical microscope movements via 9G servo motors and an Arduino MKR Wi-Fi 1010 controller. X-Y and Z (auto-focus) through the microscope slide permit the system to acquire images of different FoVs for further CNN detection. To auto-focus each FoV, a Variance of the Laplacian algorithm was employed [48]. Considering Schistosomiasis-endemic areas, which are usually resource-poor settings, the microscope was designed with low-cost materials and does not require continuous electric power supply. However, its implementation in resource-poor settings should be tested. Portable solar batteries grant the system energy autonomy if this is not available in the laboratory. In addition, the 3D pieces were designed with a range of measures as universal adapters for the vast majority of optical microscopes. A smartphone device controls the system's movements, via servo motors, through the X-Y-Z axis of the microscope. The Arduino controller is connected to the smartphone device via a Bluetooth low-energy connection, which additionally acquires the images and autofocuses the sample for further CNN object detection analysis. The whole diagnostic process is integrated into a smartphone-based application developed with Android Studio Programming environment v.2021 [49]. This automated system has already been employed by our research group for the automated detection of *Plasmodium* trophozoites and leukocytes in thick blood smear samples for malaria diagnosis [50].

4. Discussion

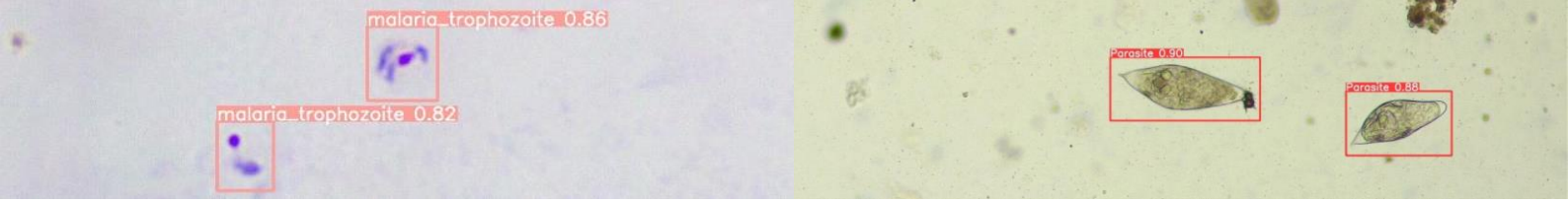
Schistosomiasis diagnosis by microscopic examination of urine and stool samples is still the gold standard technique and is widely used in resource-poor areas. However, the continuous reduction in microscopy parasitologist experts [13] requires the development and



implementation of novel diagnostic techniques for the diagnosis of schistosomiasis and other NTDs. In this study, we have developed a novel diagnostic technique based on the automatic detection of *S. haematobium* eggs in urine sediment samples using AI tools and a robotized low-cost 3D microscope system. Moreover, a first proof-of-principle study was performed to evaluate its detection potential and implementation in non-endemic settings.

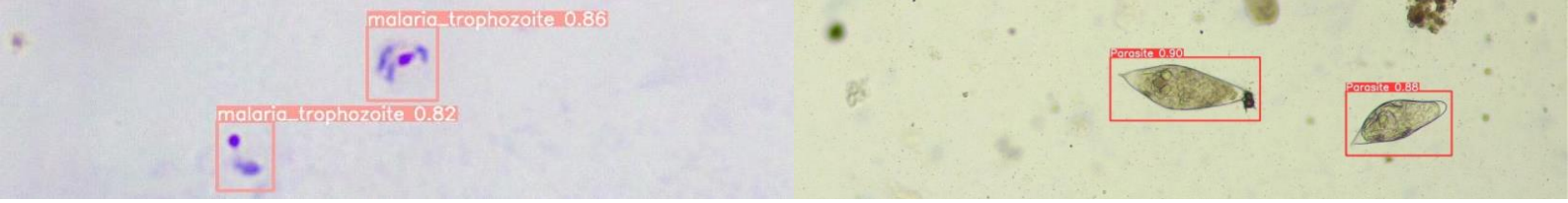
Following WHO guidelines for Schistosomiasis diagnosis, we have employed urine sediment samples and consequent image acquisition [32]. Clinical urine sediment samples are crucial to properly acquire digital images of *S. haematobium* eggs, train CNN models, and finally emulate a traditional microscopic diagnosis by AI image analysis techniques. *In vivo* and *in vitro* cultures of *S. haematobium* for laboratory-kept parasite lifecycle were described in the mid-1960s [51]; according to our knowledge, there are no publications describing their use for digital image acquisition and database generation for further CNN training. The conservation of fresh *S. haematobium* eggs can be difficult due to degradation. It would be optimal to capture images of *S.haematobium* eggs *in situ* at field laboratories, in order to obtain a more robust and representative database. As a limitation of the study, (i) images should be acquired at most 24/48 hours after sample collection, if not kept at 4˚C [52]. Moreover, (ii) system development was performed in non-endemic settings, resulting in sample collection difficulties due to the lower number of cases received in comparison with endemic regions. Sample size was small, therefore further investigation is needed to obtain more robust and conclusive results. Finally, (iii) a system validation should be pursued in *S. haematobium* endemic regions, to evaluate its performance in such environments. Although the system has been designed for its implementation in resource-poor settings, it has initially been evaluated in non-endemic areas. Therefore, it is crucial to conduct pilot tests in the field and further studies to assess diagnostic performance in such environments. Some of the system aspects that would allow its implementation in rural areas with few resources are: its adaptability to conventional optical microscopes, its portability, the low-cost of the system and the non-internet connection required.

As an attribute of the study, the detection of *S. haematobium* basal stage and calcified eggs was implemented to detect both egg forms. Calcified eggs are typically found in chronic bladder infections and, sometimes, basal-stage eggs were not present, making their detection essential for a proper diagnosis [53]. One of the main strengths of our study is the training and comparison of different YOLO neural network models. The continuous advancements in CNN development and improvement are generating more efficient models for object detection. The YOLOv5 and YOLOv8 models were considered optimal options in terms of accuracy and inference speed for object detection tasks [38]. Our results have shown that YOLOv5x was the best option for *S. haematobium* egg detection with our digital image database (**Table 2**). The improved YOLOv8 model shows a more efficient performance in terms of training in comparison with YOLOv5; YOLOv8 models trained with the 491-image dataset demonstrated optimal performances for all descriptive parameters. However, when the 1017-image dataset was employed, the YOLOv5 models demonstrated better performance compared with YOLOv8 (**Fig 3**). Other studies have compared the performance of the YOLOv5 and YOLOv8 models with very variable results [39,54]. Sary et al. 2023, compared the performance of both models for human detection in aerial images, showing better precision and F-score values for YOLOv8 and higher recall values for YOLOv5 [55]. Nevertheless, Sirisha et al. 2023, reported that the YOLOv5 model has a higher mAP0.5 value compared with other YOLO versions [38]. In addition, both YOLOv5 and YOLOv8 were employed for diagnostic tasks such as detecting developmental dysplasia of the hip in radiography images [56], and localization of dermoscopic structures [57], conferring the algorithms a contrasted efficacy for image-based diagnosis.



Another important aspect of this study was the identification of erythrocytes and leukocytes in the urine with the NasNetLarge model, allowing a diagnostic orientation due to the high correlation between the presence of erythrocytes in urine and *S. haematobium* eggs. It is important to note that haematuria may appear in multiple clinical situations; however, in schistosomiasis diagnostic protocols, a high correlation rate (78%) was observed, as shown in other studies [58]. We could not confirm that haematuria and leukocytes are specific to the infected individual, although if they were detected through images we could continue observing several sample replicas to find parasite eggs. Moreover, the NasNetLarge model had provided contrasted results for binary image classification with other similar diagnostic tasks, such as melanoma skin lesion detection and classifying diabetic retinopathy severity in digital images [59,60].

Other studies demonstrated the applicability of object detection algorithms for *Schistosoma* egg detection in stool and urine samples [61–64]. Before the irruption of CNNs, other strategies, such as the multi-class support vector machine (MCSVM) for parasite egg classification, were developed with an overall performance of 97.7% [62]. However, CNNs improved traditional image object detection in terms of computational potential, speed, and performance. Werd et al. 2022, developed an affordable AI-based system for the detection of soil-transmitted helminths and *S. mansoni* eggs in stool samples with the R-FCN ResNet101 COCO model [14]. They obtained an F-score of 88.9% for *Schistosoma* detection in stool samples, in comparison with the 99.3% value in urine samples of our fine-tuned YOLOv5x model. However, we must consider that the number of artifacts in stool samples is much higher than in urine samples. In addition, the number of images employed for CNN training was larger in our study. The difficulty in obtaining parasite egg digital images has led to several studies using data augmentation (DA) strategies [63]. Oliveira et al. 2022, used a DA strategy (66 original images) for *S. mansoni* egg detection in microscopy images with the Faster R-CNN model, showing a 76.5% precision [64]. Nevertheless, with our image dataset, we did not observe any statistically significant differences between DA and non-DA training, as shown in **Table 2**. As a breakthrough, Oyibo et al. 2022, developed an optical automated system based on AI for the detection of *S. haematobium* and *S. mansoni* eggs in urine and stool, respectively, for implementation in resource-poor settings [65]. Moreover, they mainly used non-clinical samples for UNET model training, which may interfere with its final performance. In addition, Schistoscope 5.0 shows an 80.1% sensitivity and 95.3% specificity for *S. haematobium* detection. However, it is a non-optimal system for transportation and needs to be suitable for the WHO target product profile for new diagnostics [66]. Its contribution is a milestone for automated schistosomiasis diagnosis and could be an alternative to conventional diagnosis in coalescence with other similar studies. Additionally, they have demonstrated that the employment of negative urines from endemic regions is crucial in validation studies, due to the presence of abundant artifacts that could be identified as parasite eggs [66]. Therefore, in our study, a negative sample validation should be pursued with a large amount of samples (>8) to evaluate the system performance in the field. Oyibo et al. 2023 also developed a detection framework for the diagnosis of urogenital schistosomiasis in microscopy images with from low-resource settings. The framework demonstrates a clinical sensitivity, specificity, and precision of 93.8%, 93.9%, and 93.8%, respectively, using results from an experienced microscopist as reference [67]. As another example, Coulibaly et al. 2023 performed a community based schistosomiasis screening program with a smartphone-based AI device showing 85.7% sensitivity and 93.3% specificity [15]. Its design is also 3D-printed, innovative and portable; although there are some differences with our system in terms of adaptability to other smartphone devices or microscopes. An alternative to using CNN-based models such as YOLO would be to use Vision Transformer models. However, they require larger model sizes, greater memory, and larger



databases [68]. Since our database is relatively small, we decided to use CNN-based models, which provide excellent results. AiDx multi-diagnostic microscope was tested in 17 communities in Abuja, Nigeria; showing 89% sensitivity and 99% specificity for the identification of *S. haematobium* eggs in urine with the fully automated AiDx Assist mode [69].

Our system has proven to be robust in our microbiology laboratory (non-endemic settings) and should be validated for its implementation in resource-poor settings for further evaluation. However, developmental stages, such as the ones presented in this manuscript, were crucial to evaluate the reliability of the system before a diagnostic validation, and were the basis for further in-field studies. Overall, we believe that this novel automated low-cost AI-based diagnostic system for parasite detection could join the global effort to fight NTDs and poverty-related diseases worldwide.

5. Conclusions

Automated schistosomiasis detection is a big challenge to support and optimize traditional microscopic diagnosis. Deep learning-based diagnostic techniques would help improve diagnostics and could be a suitable tool for the training and education of professionals. NTDs, such as schistosomiasis, are being significantly overlooked by national and international health organizations; therefore, novel solutions to improve the management of these diseases would be of significant benefit to the most vulnerable affected populations. Comparison of different YOLO object detection models allowed us to choose the best algorithm for our detection, ensuring that these results could be replicated in other similar studies. The automation of the entire process by robotization with 3D parts and servomotors for a conventional optical microscope allows the emulation of the X-Y slide movements and sample auto-focus. The integration of image analysis and microscope automation provides the system with attributes that render it accessible, affordable, and highly autonomous. Moreover, the low-cost and easy-to-handle technology was designed to be implemented in any laboratory, regardless of their resources. To this end, it is important to understand the needs related to the diagnosis of schistosomiasis and other NTDs in the field and to jointly develop solutions for the correct implementation of new AI-based technologies. In conclusion, we are getting closer to developing an automated diagnostic system for schistosomiasis diagnosis based on AI tools, to fight NTDs and other poverty-related diseases.

Supporting information

S1 Dataset. Training dataset configuration.
(YAML)

S1 Table. Fig 3 values.
(XLSX)

S2 Table. Negative sample validation values.
(XLSX)

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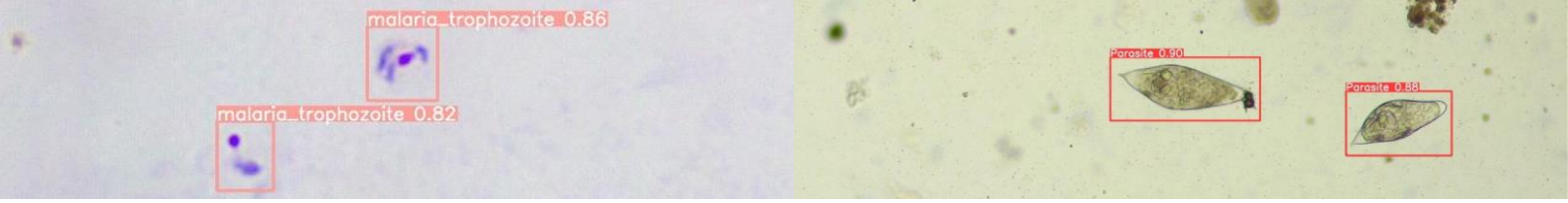

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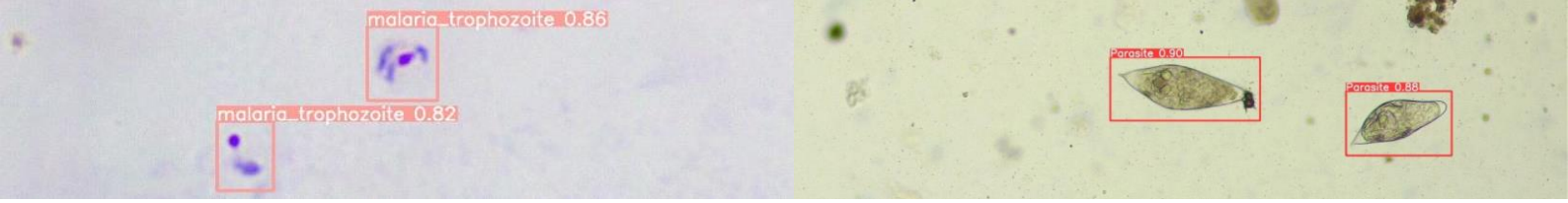

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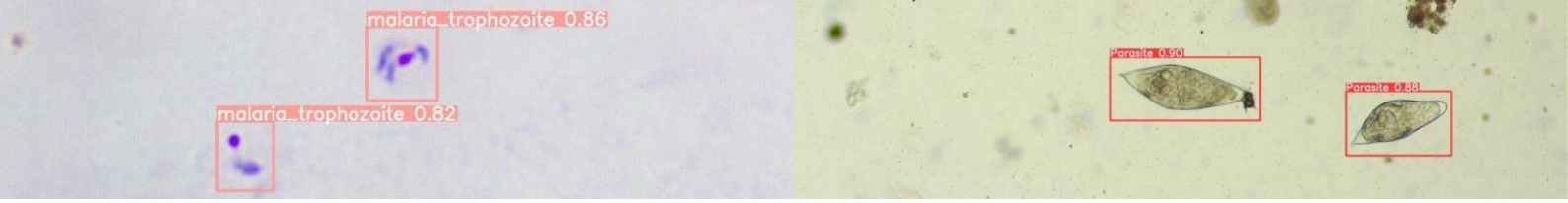

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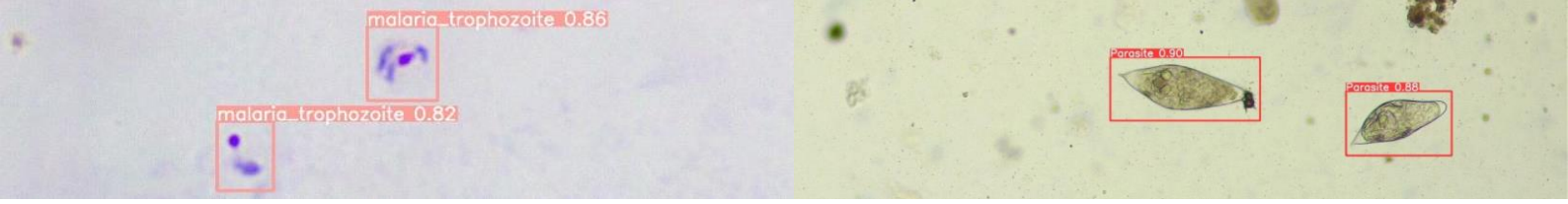

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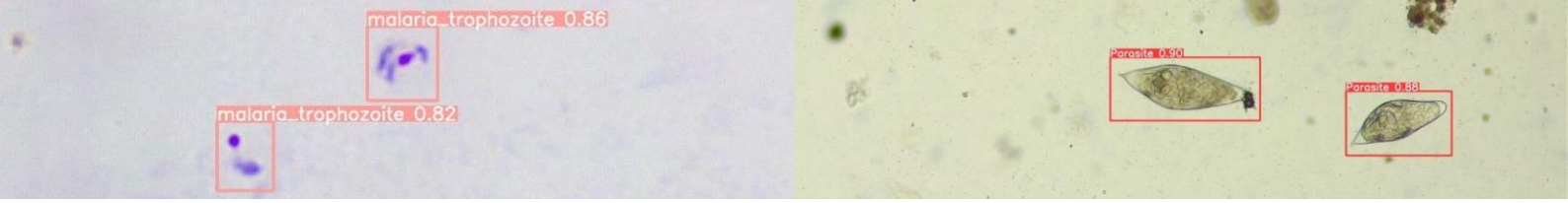

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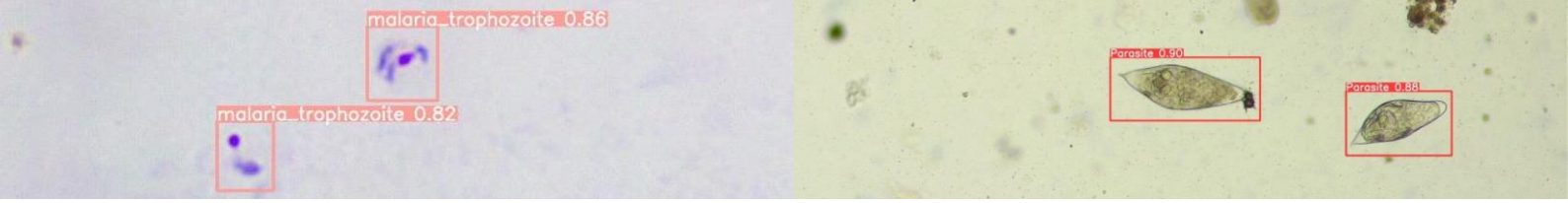

4.1.4. Evaluation of an Artificial Intelligence-Based Tool and a Universal Low-Cost Robotized Microscope for the Automated Diagnosis of Malaria

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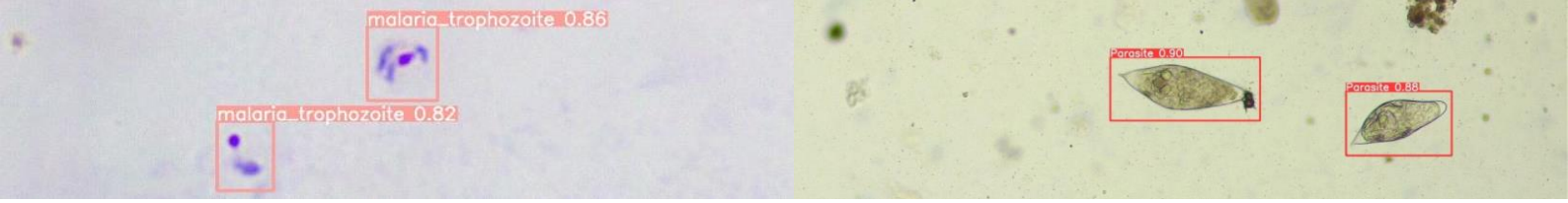
Resum del treball:

El diagnòstic *gold standard* de la malària és la visualització microscòpica de mostres de sang per identificar els paràsits de *Plasmodium*. És una tècnica professional depenent i podria desencadenar en errors de diagnòstic. Les eines d'Intel·ligència Artificial (IA) basades en l'anàlisi d'imatges digitals es van postular com una alternativa de suport adequada per al diagnòstic automatitzat de la malària. Al laboratori de referència de la Unitat de Salut Internacional Drassanes-Vall d'Hebron de Barcelona, es va realitzar una avaluació diagnòstica del sistema basat en IA anomenat *iMAGING*. *iMAGING* és un dispositiu automatitzat pel diagnòstic de la malària mitjançant l'ús d'eines d'anàlisi d'imatges basades en intel·ligència artificial, i un microscopi robotitzat de baix cost. Es van emprar un total de 54 mostres de gota gruixuda tenyides amb Giemsa de viatgers i migrants procedents de zones endèmiques, i es van analitzar per determinar la presència/absència de paràsits de *Plasmodium*. Els resultats del diagnòstic de la IA es van comparar amb els resultats del mètode *gold standard*. El sistema d'IA mostra una sensibilitat del 81.25% i una especificitat del 92.11% en comparació amb el mètode convencional de microscòpia. En total, 48/54 (88.89%) mostres van ser correctament identificades [13/16 (81.25%) com a positives i 35/38 (92.11%) com a negatives]. El temps mitjà del sistema d'IA per determinar un diagnòstic positiu de malària va ser de 3 minuts i 48 segons, amb una mitjana de 7.38 FoV analitzats per mostra. L'anàlisi estadística va donar com a resultat en un índex Kappa de 0.721, demostrant una correlació satisfactòria entre el mètode de diagnòstic *gold standard* i els resultats d'*iMAGING*. El sistema d'IA va demostrar resultats fiables pel diagnòstic de la malària en un laboratori de referència a Barcelona. La validació en regions endèmiques de malària serà el següent pas per avaluar el seu potencial en entorns amb pocs recursos.



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Brief Report

Evaluation of an Artificial Intelligence-Based Tool and a Universal Low-Cost Robotized Microscope for the Automated Diagnosis of Malaria

Carles Rubio Maturana [1,2], Allisson Dantas de Oliveira [3], Francesc Zarzuela [1], Alejandro Mediavilla [1,2], Patricia Martínez-Vallejo [1,2], Aroa Silgado [1,2,4], Lidia Goterris [1,2], Marc Muixí [1], Alberto Abelló [5], Anna Veiga [6], Daniel López-Codina [3], Elena Sulleiro [1,2,4,*], Elisa Sayrol [7,*] and Joan Joseph-Munné [1,*]

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Abstract: The gold standard diagnosis for malaria is the microscopic visualization of blood smears to identify *Plasmodium* parasites, although it is an expert-dependent technique and could trigger diagnostic errors. Artificial intelligence (AI) tools based on digital image analysis were postulated as a suitable supportive alternative for automated malaria diagnosis. A diagnostic evaluation of the *iMAGING* AI-based system was conducted in the reference laboratory of the International Health Unit Drassanes-Vall d'Hebron in Barcelona, Spain. *iMAGING* is an automated device for the diagnosis of malaria by using artificial intelligence image analysis tools and a robotized microscope. A total of 54 Giemsa-stained thick blood smear samples from travelers and migrants coming from endemic areas were employed and analyzed to determine the presence/absence of *Plasmodium* parasites. AI diagnostic results were compared with expert light microscopy gold standard method results. The AI system shows 81.25% sensitivity and 92.11% specificity when compared with the conventional light microscopy gold standard method. Overall, 48/54 (88.89%) samples were correctly identified [13/16 (81.25%) as positives and 35/38 (92.11%) as negatives]. The mean time of the AI system to determine a positive malaria diagnosis was 3 min and 48 s, with an average of 7.38 FoV analyzed per sample. Statistical analyses showed the Kappa Index = 0.721, demonstrating a satisfactory correlation between the gold standard diagnostic method and *iMAGING* results. The AI system demonstrated reliable results for malaria diagnosis in a reference laboratory in Barcelona. Validation in malaria-endemic regions will be the next step to evaluate its potential in resource-poor settings.

Keywords: artificial intelligence; malaria; automated diagnosis; tropical medicine; *Plasmodium*; point-of-care; infectious diseases

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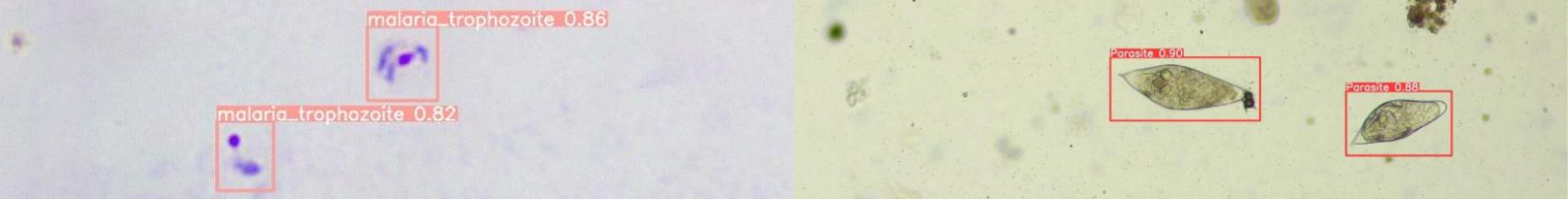


1. Introduction

Malaria is a parasitic disease caused by *Plasmodium* spp. with a high prevlence in tropical regions worldwide and transmitted through the bites of *Anopheles* female mosquitoes. The five *Plasmodium* species that cause malaria infection in humans are *Plasmodium falciparum*, *P. vivax*, *P. ovale*, *P. malariae,* and *P. knowlesi*. Regarding World Health Organization (WHO) data, globally, in 2022, there were 249 million malaria cases and 608,000 deaths [1]. Most of the deaths are caused by *P. falciparum*, mainly in children under five years of age from resource-poor setting areas of the Sub-Saharan Africa region. Therefore, malaria early diagnosis is crucial to decrease morbidity and mortality. The microscopic examination of thick and thin blood smear samples to determine the presence and species of *Plasmodium* parasites is the gold standard technique for malaria diagnosis. However, it is an expert-dependent methodology and could trigger diagnostic errors. As a supportive tool for microscopy examination, artificial intelligence (AI) methods are being postulated due to their easy handling. Novel image analysis techniques encompass Convolutional Neural Networks (CNNs), attention modules, and transformers, which all determine and identify objects of interest in digital images [2]. In addition, the robotization of the microscope stage and image autofocus would provide a fully automatic process. Low-cost robotized designs were proposed as suitable alternatives for conventional optical microscopy automation [3]. Moreover, several AI-based systems have been evaluated for the diagnosis of malaria and schistosomiasis in laboratories from endemic areas [4–6]. Most systems employ a smartphone device to acquire and analyze digital images or videos by CNN models in order to identify parasites or bacteria and perform a final diagnosis.

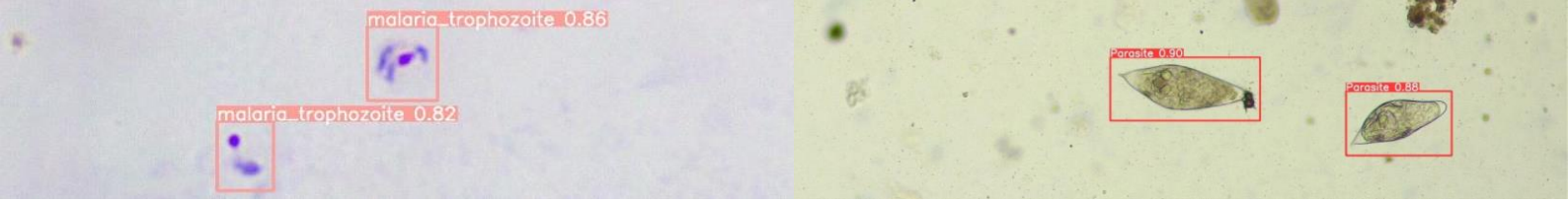
The *iMAGING* system is based on a robotized low-cost system consisting of 3D pieces and servo motors to automate a light optical microscope and a smartphone application responsible for acquiring the images and analyzing them by the You Only Look Once (YOLOv5) CNN pre-trained model. The development and design of the diagnostic system were already published in previous studies [7,8].

In this study, a preliminary diagnostic evaluation of the *iMAGING* system for the diagnosis of malaria in Giemsa-stained thick blood smear samples was performed. We evaluated the diagnostic sensitivity and specificity of the AI system in comparison with the gold standard methodology. Therefore, this study represents the initial step before the diagnostic validation in malaria-endemic settings.

2. Materials and Methods

2.1. Study Design

A diagnostic validation study was conducted using a collection of samples from travelers, Visit Friends and Relatives (VFR), and migrants coming from malaria-endemic areas (mainly Sub-Saharan Africa, South America, and Southeast Asia regions [9]) attending the International Health Unit Drassanes-Vall d'Hebron. The sample collection period was encompassed between 1 February 2023 and 30 November 2023. A total of 54 samples from 46 individuals were analyzed. Different samples from the same individual/patient were drawn at different times, before and after antimalarial drug treatment. Giemsa-stained thick blood smear samples were examined by conventional optical microscopy and were analyzed by the *iMAGING* system for malaria diagnosis. All *Plasmodium*-negative samples were also analyzed by RT-PCR (RealStar® Malaria, Altona, Hamburg, Germany) for diagnosis confirmation. Positive samples were classified according to parasite density (<800 parasites/µL; 801–10,000 parasites/µL; or >10,000 parasites/µL). *Plasmodium* species information was also collected. All samples were retrospectively revised by a laboratory technician to ensure sample quality.



2.2. Sample Size

A total of 54 thick blood smear samples were estimated to be statistically representative of our study. The sample size was calculated from epidemiological data of the International Health Unit Drassanes-Vall d'Hebron laboratory, following statistics guidelines for sample size calculation with proportions [10]. The sample size equation requires population size, probability of a condition (malaria positive), confidence level, and accepted error. Since malaria is not endemic in Spain, an approximation with real clinical data was employed. The population size was taken to be the number of blood smears analyzed from patients with suspected malaria (84 patients from 1 January 2020 to 31 December 2020), with a prevalence of 10.71% in our center. The calculations were performed using a value of 95% confidence and a 5% accepted error.

2.3. Microscope Automation

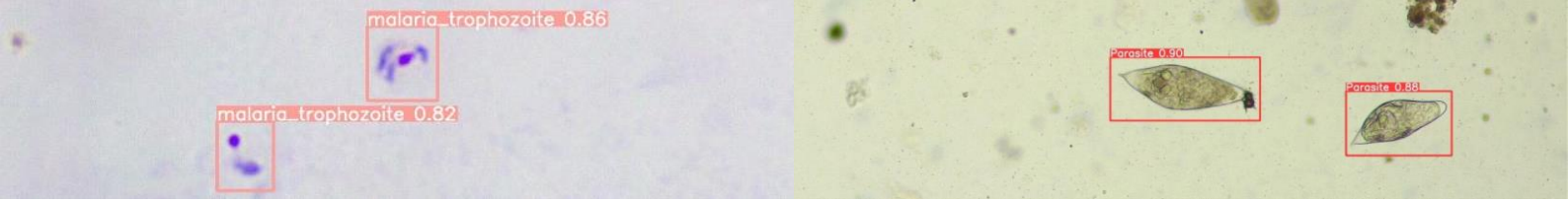
Samples were analyzed using the *iMAGING* software version 1.0. smartphone application for *Plasmodium* parasite detection in thick blood smear samples. Sample scanning was automated by the robotization of a conventional optical microscope (Olympus CH-2, Olympus, Feasterville-Trevose, PA, USA) with polylactic acid (PLA) 3D pieces. Microscope stage movements and autofocus issues were automated by three 9G low-cost servo motors, one for each axis, *X*-*Y* and *Z* (focus). All the system was controlled by an MKR Wifi 1010 Arduino controller (Arduino, Monza, Italy), connected by Bluetooth (BLE) to the smartphone device (Xiaomi Redmi 10, Xiaomi Corporation, Beijing, China). The robotized microscope system design has been published in open-access format [8]. Samples were analyzed through a snake-like movement in order to correctly visualize the thick blood smear sample. Autofocus analysis was performed via the Laplacian Variance algorithm in each Field of View (FoV).

2.4. Image Analysis and Diagnostic Algorithm

Digital images were acquired through the ocular lens of the robotized microscope with a Xiaomi Redmi 10C smartphone device (Camera: 720 × 1650 pixels resolution; Dual 50 MP, f/1.8, 26 mm (wide), PDAF 2 MP, f/2.4, (depth), HDR). A minimum of 30 MP resolution camera is required for image analysis. A two-step process was designed for the analysis of the images via the YOLOv5 CNN model to obtain more reliable results. YOLOv5 is a state-of-the-art CNN algorithm with high-performance results in multiple image analysis applications in terms of accuracy and speed. The detection of objects (parasites and cells) in digital images is a suitable task for that kind of model. Previous studies also demonstrate that the YOLOv5 model is the best option for the *iMAGING* diagnostic system for thick blood smear image analysis [7].

First, a live video of each FoV was recorded and analyzed using the Laplacian Variance algorithm. The captured video frame with the highest value of Laplacian Variance was analyzed by the neural network model YOLOv5s (smartphone) integrated into the *iMAGING* smartphone application. The YOLOv5s confidence threshold value was set to 0.25 for leukocyte and *Plasmodium* trophozoite detection via a smartphone device. This screening procedure was repeated until the identification of 15 possible parasites or the observation of 100 FoV, following data from previous studies.

Secondly, analyzed images were sent by BLE connection to ARIS software (computer device) for diagnosis confirmation. YOLOv5x (computer) confidence threshold value was set to 0.25 (iMAGING) and 0.50 (iMAGINGv2) for leukocyte and *Plasmodium* trophozoites detection via a computer device. The variation in the threshold value (0.25 or 0.50) allows us to evaluate the diagnostic results of the CNN according to the level of restriction for object detection. Leukocyte detection allows us to confirm that the sample is correctly stained and



serves as a quality control. If a single *Plasmodium* parasite was detected with the YOLOv5x model, the sample was considered positive. Conversely, if parasites were not detected in the analyzed images, the sample was considered negative for *Plasmodium* infection. The YOLOv5 metrics performance has already been published in previous studies by our research group [7]. A diagnostic flowchart is represented in Figure 1.

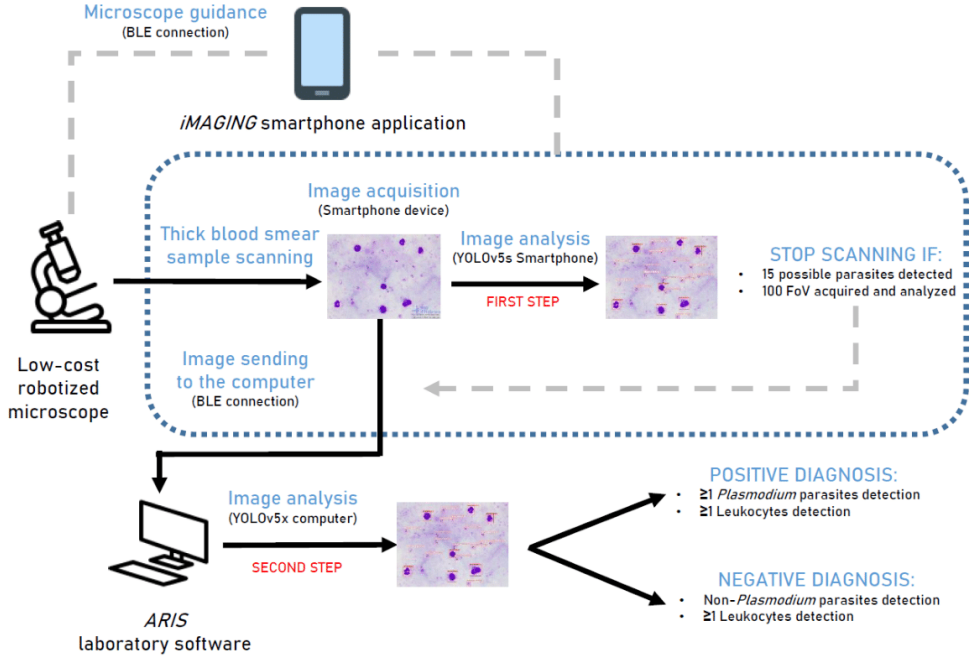


Figure 1. Diagnostic flowchart of the *iMAGING* smartphone application software and ARIS software for *Plasmodium* parasites detection in thick blood smear samples.

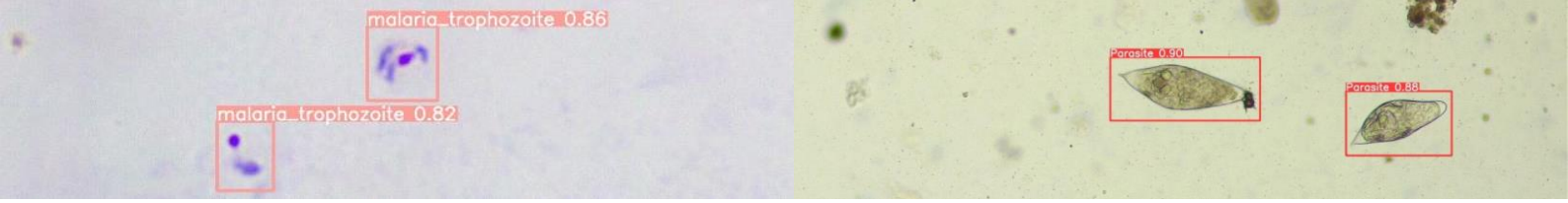
2.5. Statistical Analysis

iMAGING diagnostic results were compared with the reference gold standard diagnostic method (conventional optical microscopy). SPSS (version 27) and Excel 2016 were employed for statistical analysis. Diagnostic performance parameters for the *iMAGING* system were calculated: sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), parasitemia densities, and time of analysis.

3. Results

3.1. Diagnostic Accuracy of the iMAGING System Compared with Light Optical Microscopy

A total of 54 thick blood smear samples from 46 individuals were collected and evaluated by optical microscopy (gold standard) and *iMAGING* (AI system). Among the 46 participants of this study, 35/46 (76%) were male and 11/46 (24%) female. Their mean age was 32 years old (y.o.) [Interquartile range (IQR): 21–42 y.o.], and 24/46 (52.2%) were migrants, 11/46 (23.9%) VFR, and 11/46 (23.9%) travelers. Samples analyzed by optical microscopy show 16/54 (29.6%) positive and 38/54 (70.3%) negative for *Plasmodium* infection. The positive samples showed parasitemia ranges between 80 to >10,000 parasites/µL of blood [7/16 (43.75%) show <800 parasites/µL; 4/16 (25.0%) show 801–10,000 parasites/µL; and 5/16 (31.25%) show >10,000 parasites/µL]. Samples were predominantly 10/16 (62.5%) *P. falciparum* infections. A total of 4/16 (25%) samples were *P. vivax*/*P. ovale* infections, and 2/16 (12.5%) were *P. malariae* infections. All negative samples, 38/38 (100%), were screened by RT-PCR (RealStar® Malaria, Altona), showing negative results for *Plasmodium*



infection. When compared to light microscopy, the *iMAGING* system demonstrated an 81.25% sensitivity (CI 95%: 53.69, 95.03), 92.11% specificity (CI 95%: 77.52, 97.94), 81.25% PPV (CI 95%: 53.69, 95.03), and 92.11% NPV (CI 95%: 77.52, 97.94). Overall, the AI system demonstrates an 88.89% accuracy (48/54). The *iMAGING* system correctly identified 48/54 samples, with three false-positive (3/16) and three false-negative results (3/38). Results are represented in Table 1. The Kappa value (CI 95%) = 0.721 (0.511, 0.931) demonstrated a relatively strong correlation.

Table 1. Diagnostic evaluation of the *iMAGING* system versus light optical microscopy for the diagnosis of malaria in Giemsa-stained thick blood smear samples.

		Light Microscopy (Gold Standard)			Sensitivity (CI 95%)	Specificity (CI 95%)	PPV (CI 95%)	NPV (CI 95%)	Kappa Value (CI 95%)	*p*-Value	Mean Time of Analysis	
		(+)	(−)	Total							Positive (*Plasmodium*+)	Negative (*Plasmodium*−)
***iMAGING* (AI system)**	(+)	13	3 (FP)	16	81.25 (53.69, 95.03)	92.11 (77.52, 97.94)	81.25 (53.69, 95.03)	92.11 (77.52, 97.94)	0.721 (0.511, 0.931) Significant *	0.05	3 min 48 s/sample	5 min 5 s */sample
	(−)	3 (FN)	35	38								
	Total	16	38	54								

PPV: positive predictive value; NPV: negative predictive value; AI: artificial intelligence; CI: confidence interval; FP: false positive; FN: false negative. * In most occasions, less than 100 Fields of View (FoV) were observed, significantly reducing the meantime of analysis. * Kappa Cohen's value interpretation following Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics. 1977 criteria.

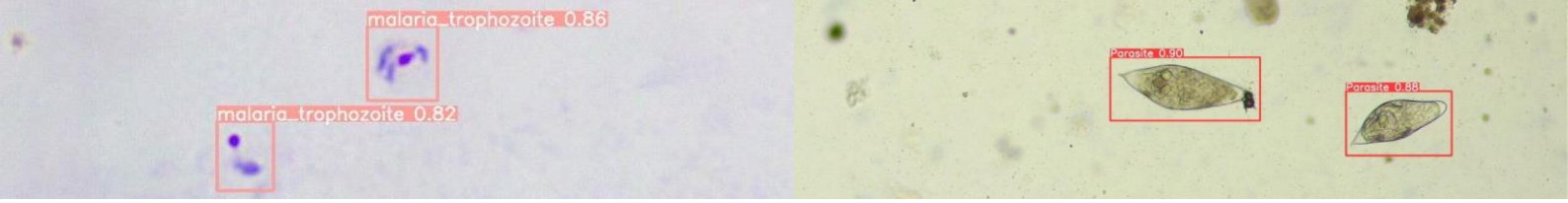
3.2. Parasite Density and Plasmodium Species

In detail, diagnostic results comparisons are represented in Table A1. Regarding positive samples (n = 16), 13/16 (81.25%) were correctly considered positive for *Plasmodium* infection by the *iMAGING* device. False-positive and false-negative results were analyzed in detail to avoid diagnostic errors. Parasite density is a crucial feature for malaria diagnosis, and discordant results were interpreted. Regarding positive samples, 5/5 (100%) with >10,000 parasites/µL, 4/4 (100%) with 801–10,000 parasites/µL, and 4/7 (57.14%) with <800 parasites/µL were correctly diagnosed by the *iMAGING* system. A high percentage of false-negative results (3/7, 42.85%) were reported in low-parasitized samples (<800 parasites/µL), representing 5.55% (3/54) of all analyzed samples. Regarding negative samples (n = 38), 35/38 (92.10%) were correctly considered negative for *Plasmodium* infection by the *iMAGING* device. Overall, there were 3/38 (7.89%) false-positive results, mainly due to the high presence of artifact samples because of the Giemsa staining procedure. Diagnostic evaluation results are summarized in Figure 2. In terms of *Plasmodium* species, the system is not able to distinguish them, although results were analyzed regarding diagnostic positivity/negativity. In summary, 8/10 (80%) of *P. falciparum* samples, 3/4 (75%) of *P. vivax/P. ovale*, and 2/2 (100%) of *P. malariae* were correctly considered positive *Plasmodium* infections.

3.3. iMAGING System Scanning Performance

The mean number of FoV observed was 7.38 FoV for positive samples and 21.86 FoV for negative samples. Considering parasite density, a mean number of 4.4 FoV was observed in samples with >10,000 parasites/µL, 10 FoV in samples with 801–10,000 parasites/µL, and 8.5 FoV in samples with <800 parasites/µL (Figure 2). The number of observed FoV was considerably higher in negative samples than in positive samples ($p < 0.01$) due to the stop scanning criteria (15 possible parasites detected or 100 FoV observed).

In terms of time of analysis, the *iMAGING* system demonstrates a mean time of scanning of 3 min and 48 s for positive samples (n = 16). Negative samples (n = 38) were scanned in a mean time of 5 min and 5 s, considering that in most occasions the stopping criteria of 100 FoV observed was not reached.



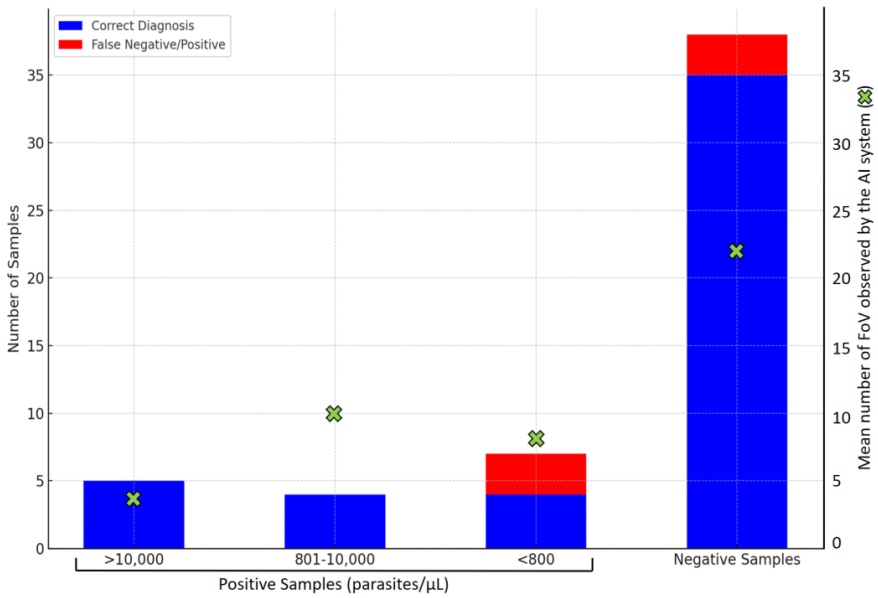
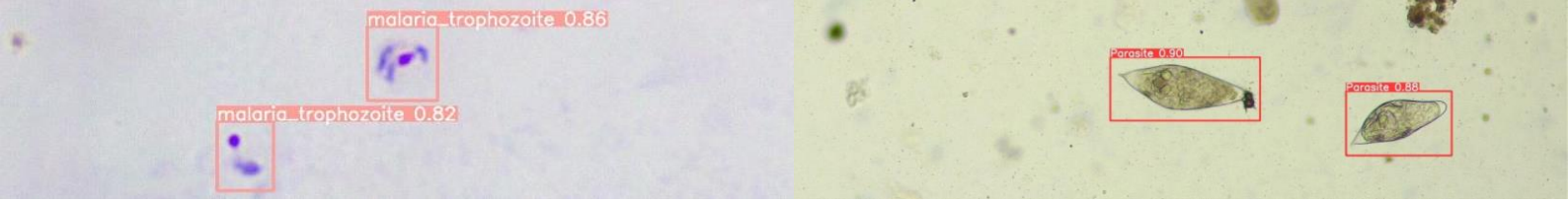


Figure 2. Diagnosis accuracy results of the *iMAGING* system. Positive samples were divided by parasite densities (>10,000 parasites/µL, 801–10,000 parasites/µL, and <800 parasites/µL). Green crosses represent the mean number of Fields of View (FoV) observed for each sample category.

4. Discussion

The *iMAGING* AI-based diagnostic system has demonstrated reliable results for malaria diagnosis in non-endemic settings. A fully automated procedure in a smartphone application and a low-cost robotized microscope confer the system the suitable features to complement traditional microscopy diagnosis. Diagnostic evaluation results of sensitivity and specificity by using the *iMAGING* AI system are in concordance with other similar studies [6,11,12]. A similar system, called EasyScan GO, based on AI malaria diagnosis with deep neural networks, demonstrated a range of 88.0–91.1% sensitivity and 75.6–89.0% specificity [6,11,12]. In terms of specificity, our system demonstrated more optimal results, although, in terms of sensitivity, EasyScan GO outperforms *iMAGING*. Other similar devices, such as miLAB^{TM}, an automated microscope for the detection of *Plasmodium* parasites in thin blood smears, were evaluated for detecting the parasites in samples from symptomatic patients at point-of-care in Sudan [12]. Accuracy results demonstrate a 91.1% sensitivity and 66.7% specificity in the automated mode, although specificity increased to 96.2% with operator intervention [12]. The cited devices demonstrate the applicability of automated tools, such as *iMAGING*, as supportive tools for point-of-care malaria diagnosis. However, AI-systems comparison should be pursued with the same samples and in the same laboratory conditions in order to reliably compare the diagnostic potential of each device.

The two-step diagnostic procedure allows the system to avoid possible false-positive results by the YOLOv5s model. YOLOv5s was executed by the smartphone device; therefore, its computational potential and performance were lower in comparison with YOLOv5x (computer). All sample scanning analyses were stopped by the ≥15 possible parasites detected criteria (Figure 1) and screened via the YOLOv5x model to discard false-positive results. However, less than 100 FoV were observed in each sample, causing negative results to depend on the reliability of the observed fields. Although the proposed methodology significantly reduces the time of analysis for each sample, equaling that of a microscopist.



Another determinant factor for the diagnostic evaluation is the previous development validation of the diagnostic system, as performed with the *iMAGING* system [3]. Neural network validation should be performed before a diagnostic validation. Convolutional Neural Network (CNN) performance is crucial to obtain a reliable diagnosis, although optimal CNN metrics are not sufficient to evaluate a diagnostic system, as they provide information about the accuracy of the detection of objects in digital images and not about the final diagnosis of the analyzed sample. Several studies demonstrate optimal CNN metrics for malaria detection in thick and thin blood smear samples [13–15]. However, diagnostic validations should be the next step in order to evaluate detection software as a supportive novel diagnostic tool. Artificial intelligence-based systems could be a suitable supportive tool for malaria diagnosis in non-endemic settings, although low parasite-density infections continue to challenge diagnostic accuracy [16]. The implementation of this type of technology in real clinical diagnostic conditions must be accompanied by microscopists, and the results should be interpreted by observing the digital images displayed by the system. In addition, we could avoid the number of false-positive and false-negative results due to errors in image analysis by means of CNNs.

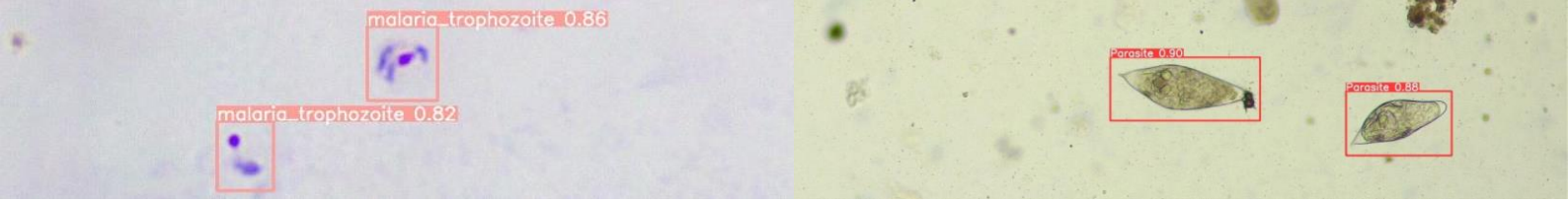
This is the first diagnostic evaluation study of the *iMAGING* system. Therefore, further validation studies will be needed in reference laboratories and in the field. Preliminary results demonstrate that the *iMAGING* device could be a suitable supportive solution for the diagnosis of malaria in cases of medium and high *Plasmodium* parasite density samples and negative samples, although it should be supervised by laboratory technicians to detect low parasite densities. The diagnostic system algorithm could be improved by modifying the threshold value, by adding more digital images to our dataset, or by improving image quality [17,18]. These strategies could be the future steps for the *iMAGING* validation in non-endemic settings.

Some of the challenges of our study were (i) to increase the sample size (n = 54) employed for diagnostic evaluation, (ii) the non-identification by the AI system of *Plasmodium* species, (iii) smartphone camera (image resolution), and (iv) the false-negative results in some of the low parasitized samples. Thus, further studies in endemic areas should be pursued in order to evaluate the robustness of the AI-based system diagnostics in other laboratories with a larger number of patients and samples and with low parasite densities. Retraining AI algorithms and improving the smartphone camera resolution, or the 3D pieces of the device, could also positively improve image analysis. Moreover, Giemsa staining, laboratory infrastructure, and microscope lenses could affect the image acquisition procedure and, therefore, the final diagnosis [3]. *Plasmodium* identification models should also be integrated into the system to perform a complete malaria diagnosis, as published in other similar studies [19,20]. The availability of electricity and the diagnostic protocols of each country or region can also be significant when implementing this type of device.

5. Conclusions

To conclude, the *iMAGING* system has demonstrated a reliable diagnostic correlation with the gold standard methodology. The improvement of the system via the retraining of CNN models and the validation in other laboratory settings are needed to optimize the system, especially for low-parasitized samples. Furthermore, the training of microscopists remains a global priority, and in order not to lose awareness, strategies could be generated based on accessible digital tools. We believe that this novel diagnostic system will join the global effort to fight against malaria and poverty-related diseases, breaking the digital gap between the north and the south.

Author Contributions: Conceptualization: C.R.M., E.S. (Elena Sulleiro), E.S. (Elisa Sayrol), D.L.-C. and J.J.-M.; methodology, C.R.M., A.D.d.O., F.Z., E.S. (Elena Sulleiro), E.S. (Elisa Sayrol), D.L.-C.



and J.J.-M.; software, A.D.d.O. and A.A.; data analysis: C.R.M. and A.D.d.O.; resources: E.S. (Elena Sulleiro), E.S. (Elisa Sayrol), D.L.-C., A.V., A.A. and J.J.-M.; writing the original draft of the manuscript and figures and tables design: C.R.M. and J.J.-M.; all authors have revised the manuscript and provided valuable feedback; experimental work supervision, E.S. (Elena Sulleiro), E.S. (Elisa Sayrol), D.L.-C. and J.J.-M. All authors contributed to the article and approved the submitted version. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: This study was conducted in accordance with the Declaration of Helsinki and approved by the Clinical Research Ethics Committee (CEIm) of the Vall d'Hebron University Hospital/Vall d'Hebron Research Institute with reference number PR(AG)40/2023.

Informed Consent Statement: Patient consent was waived due to the retrospective design of the study and was approved by the Clinical Research Ethics Committee (CEIm) of the Vall d'Hebron University Hospital/Vall d'Hebron Research Institute.

Data Availability Statement: The original contributions presented in the study are included in the article; further inquiries can be directed to the corresponding author.

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Conflicts of Interest: The authors declare no conflicts of interest.

Appendix A

Table A1. Comparison of the diagnostic performance of the *iMAGING* system with the 54 analyzed samples in comparison with the gold standard diagnosis (International Health Unit Vall d'Hebron-Drassanes). All negative samples were also confirmed with *RT-PCR (RealStar® Malaria, Altona)*.

Sample Codification	Light Microscopy (Reference Laboratory)	AI System (Number of FoV Observed)
1	POSITIVE (*P. falciparum*; +10,000 parasites/µL)	POSITIVE (2)
2	POSITIVE (*P. falciparum*; +10,000 parasites/µL)	POSITIVE (3)
3	POSITIVE (*P. falciparum*; +160 parasites/µL)	NEGATIVE (26)
4	POSITIVE (*P. falciparum*; +160 parasites/µL)	POSITIVE (13)
5	POSITIVE (*P. falciparum*; +640 parasites/µL)	NEGATIVE (43)
6	POSITIVE (*P. falciparum*; +7600 parasites/µL)	POSITIVE (24)
7	NEGATIVE	NEGATIVE (45)
8	NEGATIVE	NEGATIVE (18)
9	NEGATIVE	NEGATIVE (8)
10	NEGATIVE	NEGATIVE (15)

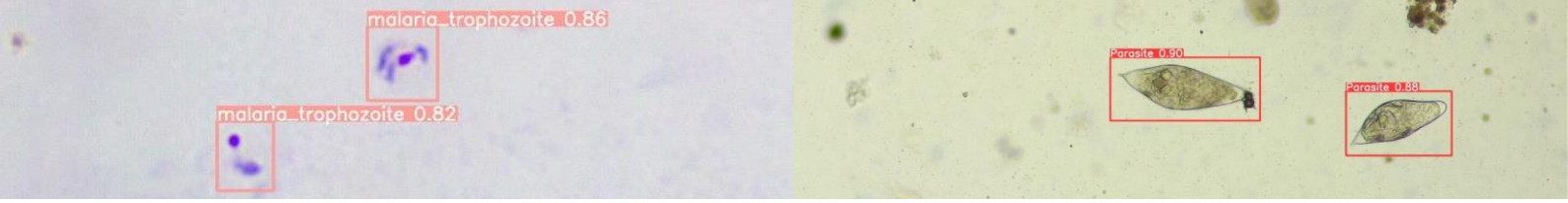

Table A1. *Cont.*

Sample Codification	Light Microscopy (Reference Laboratory)	AI System (Number of FoV Observed)
11	NEGATIVE	NEGATIVE (16)
12	NEGATIVE	NEGATIVE (7)
13	NEGATIVE	NEGATIVE (9)
14	NEGATIVE	NEGATIVE (20)
15	NEGATIVE	NEGATIVE (17)
16	NEGATIVE	POSITIVE (33)
17	NEGATIVE	NEGATIVE (5)
18	NEGATIVE	NEGATIVE (22)
19	NEGATIVE	NEGATIVE (88)
20	NEGATIVE	POSITIVE (21)
21	NEGATIVE	POSITIVE (27)
22	POSITIVE (*P. vivax*; +640 parasites/µL)	NEGATIVE (32)
23	POSITIVE (*P. vivax*; +640 parasites/µL)	POSITIVE (12)
24	POSITIVE (*P. falciparum*; +10,000 parasites/µL)	POSITIVE (1)
25	POSITIVE (*P. falciparum*; +10,000 parasites/µL)	POSITIVE (14)
26	NEGATIVE	NEGATIVE (18)
27	NEGATIVE	NEGATIVE (6)
28	NEGATIVE	NEGATIVE (29)
29	NEGATIVE	NEGATIVE (31)
30	POSITIVE (*P. falciparum*; 80 parasites/µL)	POSITIVE (5)
31	POSITIVE (*P. falciparum*; 640 parasites/µL)	POSITIVE (4)
32	NEGATIVE	NEGATIVE (7)
33	NEGATIVE	NEGATIVE (7)
34	NEGATIVE	NEGATIVE (18)
35	NEGATIVE	NEGATIVE (17)
36	NEGATIVE	NEGATIVE (16)
37	NEGATIVE	NEGATIVE (93)
38	NEGATIVE	NEGATIVE (45)
39	NEGATIVE	NEGATIVE (22)
40	NEGATIVE	NEGATIVE (10)
41	NEGATIVE	NEGATIVE (15)
42	NEGATIVE	NEGATIVE (7)
43	NEGATIVE	NEGATIVE (21)
44	NEGATIVE	NEGATIVE (12)
45	POSITIVE (*P. ovale*; +10,000 parasites/µL)	POSITIVE (2)

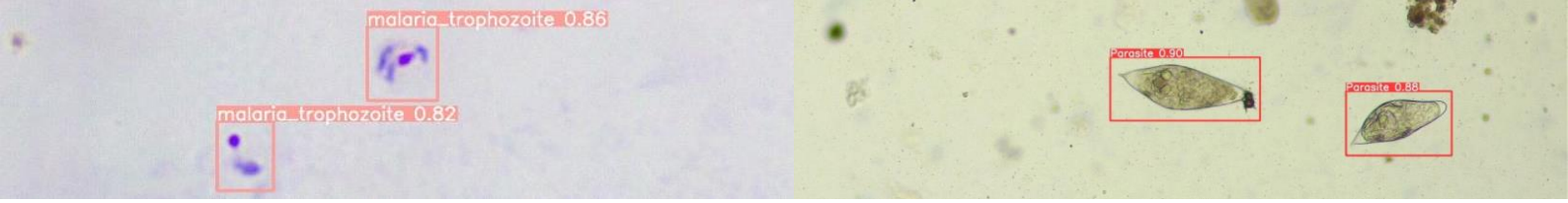

Table A1. *Cont.*

Sample Codification	Light Microscopy (Reference Laboratory)	AI System (Number of FoV Observed)
46	POSITIVE (*P. ovale*; +8200 parasites/µL)	POSITIVE (6)
47	NEGATIVE	NEGATIVE (53)
48	NEGATIVE	NEGATIVE (36)
49	NEGATIVE	NEGATIVE (9)
50	NEGATIVE	NEGATIVE (10)
51	POSITIVE (*P. malariae*; +5600 parasites/µL)	POSITIVE (6)
52	POSITIVE (*P. malariae*; +5400 parasites/µL)	POSITIVE (4)
53	NEGATIVE	NEGATIVE (9)
54	NEGATIVE	NEGATIVE (4)

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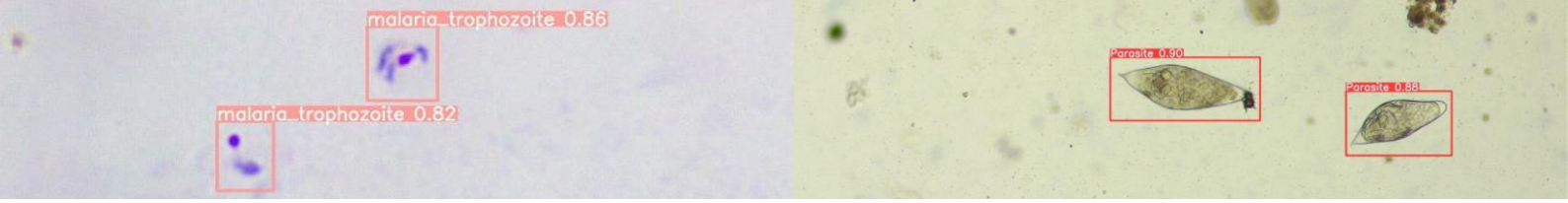

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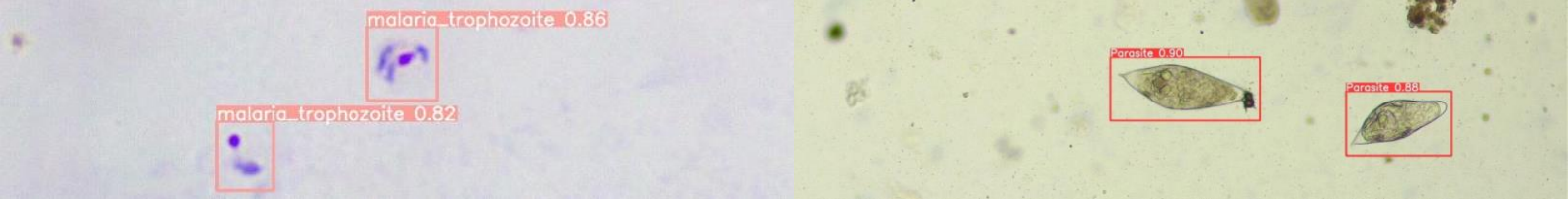


4.2. Altres treballs

4.2.1. Advances and challenges in automated malaria diagnosis using digital microscopy imaging with artificial intelligence tools: A review

Revista: Frontiers in Microbiology

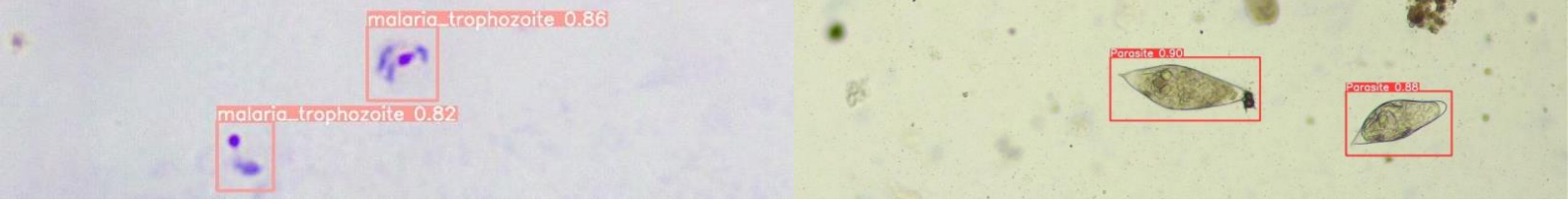
Impacte (any de publicació): Factor d'impacte 5.2 ; Quartil 1 (Q1) Microbiologia

Data de publicació: 15 de novembre de 2022

Tipus d'article: Article de revisió (*Review*)

Resum del treball:

La malària és una malaltia infecciosa causada per paràsits del gènere *Plasmodium*. Es transmet als humans per la picada d'un mosquit femella infectat del gènere *Anopheles*. És la malaltia més comuna en entorns amb pocs recursos, amb 241 milions de casos de malària reportats l'any 2020 segons l'Organització Mundial de la Salut. L'examen microscòpic de gotes de sang és la tècnica *gold standard* pel diagnòstic de la malària; no obstant això, és un mètode que requereix molt de temps i es necessita un microscopista ben entrenat per realitzar el diagnòstic microbiològic. Les noves tècniques basades en l'anàlisi d'imatges digitals mitjançant l'aprenentatge profund i els mètodes d'intel·ligència artificial són una eina alternativa desafiadora per al diagnòstic de malalties infeccioses. En particular, els sistemes basats en xarxes neuronals convolucionals per a la detecció d'imatges dels paràsits de la malària emulen la visualització de microscòpia d'un expert. L'automatització de microscopis proporciona un diagnòstic ràpid i de baix cost, que requereix menys supervisió. Els telèfons intel·ligents són una opció adequada per al diagnòstic microscòpic, que permet la captura d'imatges i la identificació dels paràsits. A més, les tècniques d'anàlisi d'imatges podrien ser una solució ràpida i òptima per al diagnòstic de la malària, la tuberculosi o les malalties tropicals desateses en zones endèmiques amb baixos recursos. La implementació del diagnòstic automatitzat mitjançant l'ús d'aplicacions per a telèfons intel·ligents, i noves tecnologies d'anàlisi d'imatge digital en zones amb pocs recursos és un repte. A més, automatitzar els moviments del microscopi i l'enfocament automàtic de les imatges sistematitzaria el procediment. Aquestes noves eines diagnòstiques se sumen a l'esforç global per lluitar contra la malària i altres malalties infeccioses relacionades amb la pobresa.

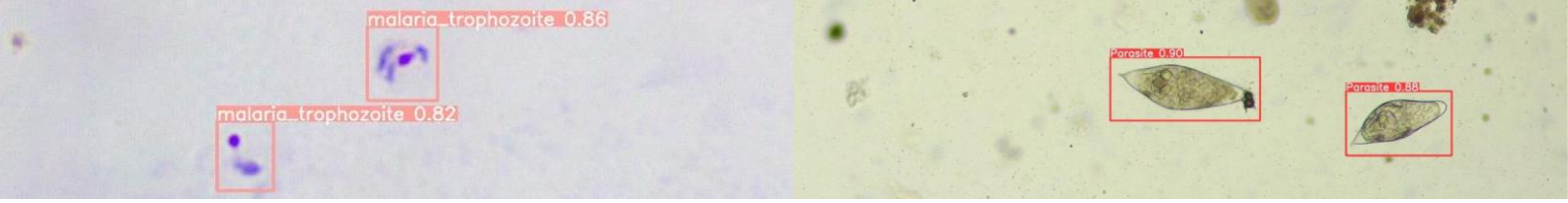


5. Discussió

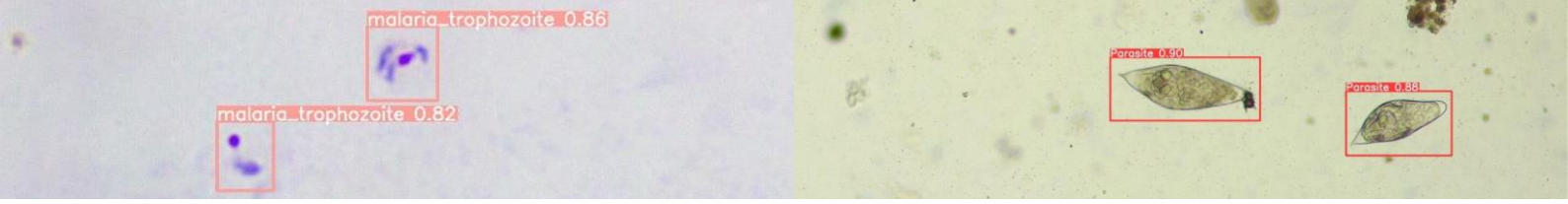
El diagnòstic de les malalties tropicals, especialment el de la malària i les MTDs, continua sent un repte de salut global. Les dades epidemiològiques de l'OMS sobre el nombre de casos estimats de malària i l'esquistosomiasi a nivell mundial, descriuen la problemàtica actual, amb especial rellevància en regions amb pocs recursos. La falta de recursos tècnics i de personal qualificat per la realització del diagnòstic microscòpic és un problema en moltes de les regions on aquestes malalties són endèmiques. La globalització, l'alta mobilitat de les persones i el canvi climàtic contribueixen a l'expansió de malalties tropicals en regions prèviament no endèmiques, ja que afavoreixen la presència i la dispersió de vectors com els mosquits *Anopheles*, responsables de la transmissió de la malària, i d'hostes intermediaris com els cargols *Bulinus*, implicats en el cicle de vida de l'esquistosomiasi urogenital. Per tant, aquests factors contribueixen a corroborar el concepte de salut global i *One Health*, molt importants per l'epidemiologia i la salut pública a nivell mundial [154].

Les alternatives al diagnòstic per microscòpia convencional, considerada la tècnica *gold standard* tant per la malària com l'esquistosomiasi urogenital, segueixen sent millorables, i requereix un grau d'expertesa alt per poder realitzar correctament la interpretació diagnòstica de les mostres biològiques. La visualització de múltiples mostres durant la jornada de treball, especialment en regions endèmiques amb gran incidència, també poden derivar en fatiga i errors per part del personal de laboratori, especialment en el cas de la malària. Degut a la casuística descrita, les alternatives al diagnòstic tradicional són motiu d'estudi i desenvolupament durant els últims anys. En concret, les eines d'anàlisi d'imatges digitals basades en la intel·ligència artificial es postulen com a opcions eficients per donar suport i optimitzar el *gold standard* diagnòstic actual.

El sistema proposat a la tesi doctoral és innovador, i integra diversos àmbits del coneixement en una sola aplicació per a telèfons intel·ligents. S'ha desenvolupat i validat un sistema universal de diagnòstic automatitzat per la malària basat en IA i un sistema de robotització microscòpica de baix cost per analitzar mostres de gota gruixuda de sang tenyides amb Giemsa. El sistema també ha estat desenvolupat per la detecció d'ous de *S. haematobium* en mostres d'orina. El dispositiu és capaç d'autoenfocar les mostres biològiques, i escanejar-les automàticament emulant els moviments de la platina del microscopi. Mitjançant una aplicació per a telèfons intel·ligents anomenada "*iMAGING*", s'han integrat els models entrenats de XNC i el control dels moviments del microscopi amb un sistema d'*Arduino* i servo motors. D'altres grups de recerca i/o empreses

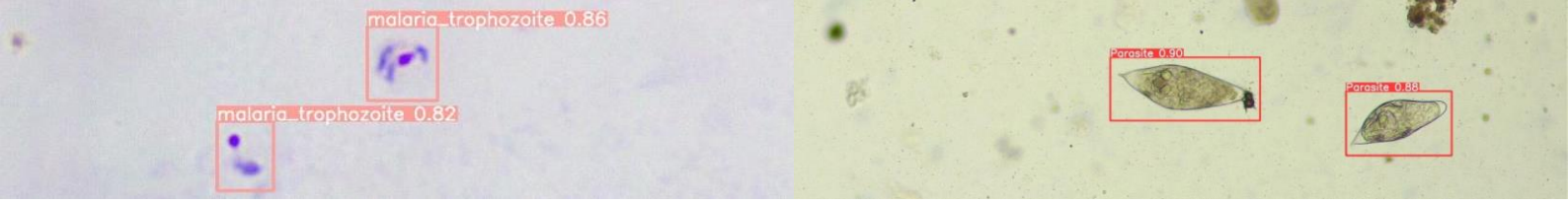


estan treballant en la mateixa direcció, fent propostes similars pel diagnòstic que poden ajudar a millorar l'estat de l'art d'aquest àmbit d'estudi. Actualment, s'estan desenvolupant i validant multitud de tècniques complementàries a la microscòpia convencional, no solament per la malària i l'esquistosomiasi urogenital, sinó també per altres malalties parasitàries com, la filariasi o la malaltia de Chagas [155–157]. La irrupció de la intel·ligència artificial durant la dècada del 2010 fins a l'actualitat, i la millora de la tecnologia a nivell computacional estan permetent generar i entrenar algoritmes més potents capaços de realitzar tasques cada cop més complexes. Concretament, les xarxes neuronals profundes han permès fer grans avenços en l'àmbit del reconeixement d'objectes en imatges digitals [158]. Les XNC estan revolucionant aquest tipus de tasques, així com d'altres metodologies com els *transformers* [138,139,159–161], l'augment de dades [162,163] o els models d'atenció [164,165].



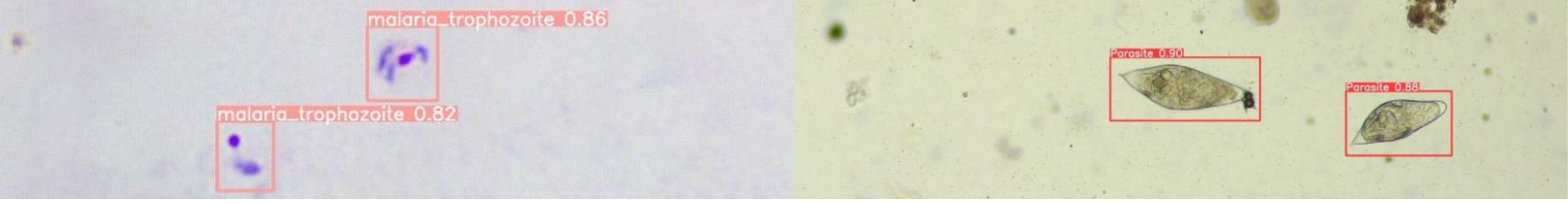
5.1. Diagnòstic de la malària amb intel·ligència artificial

Diversos estudis demostren que les XNC són òptimes per la detecció de *Plasmodium* en mostres de gota gruixuda i extensió fina de sang [166–169]. En concret, la detecció de *Plasmodium* en mostres d'extensió fina és la tasca més utilitzada en aquest tipus d'investigacions. Estudis recents, han realitzat comparatives de diversos models de xarxes per la detecció d'eritròcits infectats o no infectats en mostres d'extensió fina de sang [170]. En canvi, l'anàlisi de paràsits de malària en gota gruixuda de sang mitjançant l'ús de tècniques d'imatge amb IA és menys freqüent, degut al major nombre d'artefactes i la dificultat per detectar-los respecte a l'extensió fina. *Nakasi* et al. 2020, va desenvolupar un sistema d'anàlisi d'imatges basat en la xarxa neuronal Faster R-CNN capaç de detectar paràsits de la malària i leucòcits amb un valor de *mAP0.5* de 94.48% i un temps d'inferència de 2.24 mil·lisegons [171]. El nostre sistema va demostrar els millors resultats (94.40%) comparatius de *mAP0.5* amb el model YOLOv5, encara que amb la Faster R-CNN es van obtenir resultats (94.12%) molt similars als presentats per *Nakasi* et al. 2020. Altres estudis també demostren la possibilitat de distingir les diferents espècies de *Plasmodium* en mostres d'extensió fina, fet principal en el diagnòstic diferencial d'aquesta malaltia. *Wang* et al. 2023 proposa un sistema per detectar paràsits de *P. falciparum, P. vivax, P. ovale, P. malariae, P. knowlesi i P. cynomolgi* mitjançant el model YOLOv7 [172]. Encara així, no especifica si el sistema també és capaç de classificar i diferenciar els paràsits segons l'espècie, o únicament detectar-los. *Malaria Screener* és una solució assequible i efectiva per a la detecció automàtica de la malària mitjançant una aplicació per telèfons intel·ligents [173], un concepte molt similar al sistema *iMAGING* descrit a la tesi doctoral. Combina l'adquisició d'imatges, l'anàlisi i la visualització de resultats. Es tracta d'un sistema semiautomatitzat basat en imatges digitals i models de XNC per predir la presència de paràsits de *P. falciparum*, en ambdós tipus de mostres, extensió fina i gota gruixuda. En el cas del nostre sistema *iMAGING,* la diferenciació de les espècies de *Plasmodium* encara no està integrada a l'aplicació, però s'han realitzat els primers entrenaments per la diferenciació de les espècies *P. falciparum*, *P. vivax/ovale* i *P. malariae* mitjançant l'entrenament de la xarxa neuronal YOLOv11n. La xarxa neuronal ha demostrat una precisió de 87.8%, *recall* de 88.2%, valor F de 87.9% i *mAP0.5* de 92.2% (resultats no publicats) per la diferenciació de les espècies de *Plasmodium* mitjançant el nostre sistema en mostres d'extensió fina de sang.



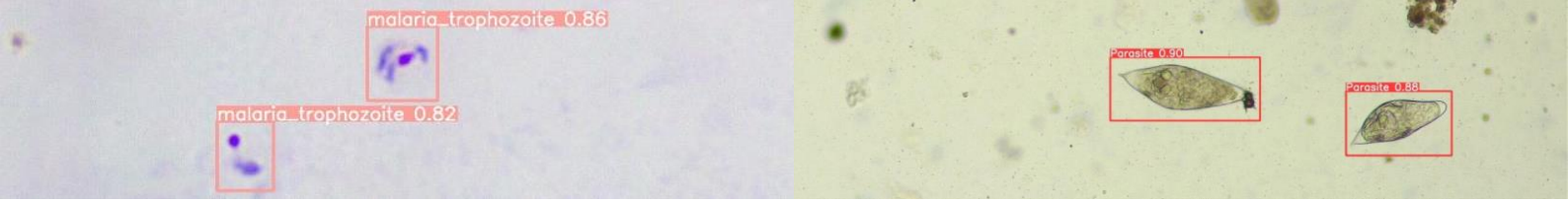
S'han desenvolupat altres aplicacions per combinar la detecció automàtica de paràsits de malària a través d'un prototip d'ampliació òptica amb un dispositiu de telèfon intel·ligent que realitza processament i anàlisi d'imatges. El model de classificació VGG16-CNN, o altres models de XNC, es van integrar en aplicacions de telèfons intel·ligents per detectar automàticament la presència de patògens com la malària o infeccions pulmonars bacterianes [174–176]. Un altre sistema és l'anomenat *EasyScan GO,* un escàner digital que ha estat testejat per realitzar el diagnòstic de la malària mitjançant l'anàlisi d'imatges digitals amb XNC de mostres de gotes grosses i extensions fines de sang. L'estudi es va realitzar en viatgers retornats al Regne Unit (UK), demostrant una sensibilitat diagnòstica del 88% i una especificitat del 89% [177]. Aquests resultats diagnòstics són molt similars als obtinguts a la validació diagnòstica del sistema *iMAGING,* 81.25% sensibilitat i 92.11% especificitat, però la mida mostral de l'estudi que utilitza *EasyScan GO* és superior (n=1202). En el diagnòstic de la malària el recompte de la parasitèmia és crucial per determinar la gravetat de la malaltia. El nostre sistema és capaç de comptabilitzar el nombre de paràsits de *Plasmodium* en funció del nombre de leucòcits per determinar la parasitèmia, seguint els protocols de l'OMS [178]. El *EasyScan GO* té la funcionalitat de fer quantificacions de parasitèmia en gotes gruixudes de sang i donar els resultats en paràsits/µL de sang [148], amb un procediment molt similar al del nostre sistema. El sistema *EasyScan GO* també va demostrar resultats satisfactoris de quantificació de la parasitèmia en mostres amb >2000 paràsits/µL, encara que la concordança amb els comptatges de parasitèmia per microscòpia convencional baixaven fins al 30% en mostres amb un rang de 200-2000 paràsits/µL [177]. *Nagendra* et al. 2024 va testejar el dispositiu miLab MAL, un microscopi digital, capaç de fer comptatges automatitzats de la parasitèmia de la malària en mostres d'extensió fina, mostrant el resultat en percentatge en funció del número d'eritròcits [179]. El mateix dispositiu també va ser emprat per *Ewnetu* et al. 2024 per avaluar mostres de pacients amb simptomatologia compatible amb malària a Gondar, Etiòpia [180]. El sistema va demostrar resultats de sensibilitat del 94.3% en mostres amb >200 parasits/µL i ha demostrat, a diferència dels tests antigènics de diagnòstic ràpid, que és una tècnica que no es veu afectada per les delecions dels gens *pfhrp2/3* [180].

La intel·ligència artificial s'utilitza majoritàriament per aplicacions de diagnòstic o detecció indirecta de la malària, encara així hi ha d'altres funcionalitats centrades en la predicció de nous brots o la prevenció. *Nazir* et al. 2024 va dissenyar un model predictiu per anticipar-se a l'aparició de brots de malària a regions del Pakistan, Bangladesh i l'Índia [181]. El model matemàtic multidimensional va predir la incidència de malària amb un percentatge d'encert

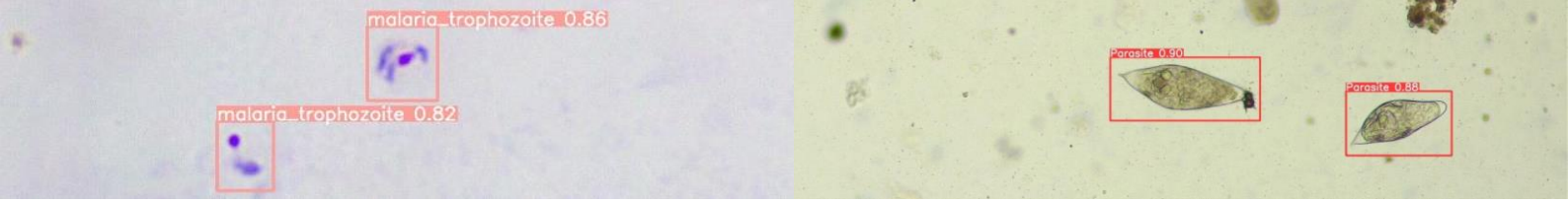


superior al 75%, mitjançant dades geo-ambientals, demogràfiques, sanitàries i nutricionals a nivell de districte.

La validació dels sistemes de diagnòstic basats en l'anàlisi d'imatges per intel·ligència artificial s'ha de realitzar en diversos laboratoris, per tenir en compte la variabilitat de les mostres degut a la seva preparació i/o tinció. Les diferències morfològiques a escala d'imatges són crucials per avaluar el rendiment d'aquest tipus de sistemes en diferents laboratoris [182]. L'avaluació preliminar del nostre dispositiu, pel diagnòstic de la malària mitjançant la visualització de gotes gruixudes tenyides amb Giemsa al Laboratori de Microbiologia del Centre Drassanes-Vall d'Hebron, va mostrar un 81.25% de sensibilitat i un 92.11% d'especificitat diagnòstiques. També s'han realitzat proves de concepte prèvies a la validació al laboratori de l'Hospital Nossa Senhora da Paz (Angola), demostrant un valor de concordança diagnòstica amb la microscòpia *gold standard* del 64.75%, i d'un 92.62% quan s'aplica la revisió de les imatges per part de l'operador degut a la confirmació de falsos positius i negatius en les imatges digitals analitzades. Els resultats diagnòstics obtinguts a Angola són preliminars, encara així l'estudi ha demostrat la viabilitat d'implementar el sistema en un laboratori amb pocs recursos. D'altres estudis també han realitzar validacions multicèntriques per avaluar el rendiment dels sistemes d'anàlisi d'imatges en diferents indrets. *Das* et al. 2022 va avaluar el rendiment diagnòstic del sistema *EasyScan Go* a 11 països amb diferents prevalences i espècies de *Plasmodium* (Brasil, Senegal, Burkina Faso, República Democràtica del Congo, Uganda, Kenya, Sud-àfrica, Nepal, Bangladesh, Tailàndia i Cambodja) [148]. El sistema es va testejar tant amb mostres de gota gruixuda com d'extensió fina de sang. El dispositiu va demostrar diferents resultats de sensibilitat i especificitat diagnòstiques, segons la qualitat i infraestructura dels laboratoris testejats. En laboratoris amb bona qualitat de la mostra, segons els criteris descrits a la publicació, el sistema va demostrar un 89.1% de sensibilitat i un 85.1% d'especificitat, mentre que amb mostres de mala qualitat es va observar un 94.2% de sensibilitat i un 51.5% d'especificitat [148]. Aquests resultats estan en concordança amb els del nostre sistema *iMAGING*, encara que el nombre de mostres emprat per validar els dispositius és més gran en el cas del *EasyScan Go* (n=2152) que en el del *iMAGING* (n=176). Aquest tipus de validacions no són exclusives dels sistemes d'imatge per emular la microscòpia. *Lam* et al. 2022 va realitzar una validació multicèntrica d'un sistema d'aprenentatge profund per detectar la síndrome inflamatòria multisistèmica i la malaltia de Kawasaki als Estats Units [183]. *Suzuki* et al. 2022, en una validació prospectiva multicèntrica, va avaluar un algoritme d'intel·ligència artificial per classificar electroencefalogrames capaços de diferenciar la demència provocada pels cossos de Lewy o per l'Alzheimer [184].



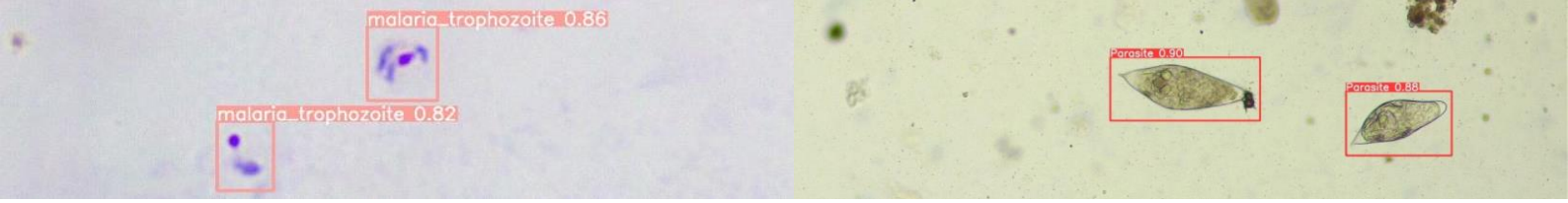
Alguns dels punts febles de l'estudi que es poden aplicar tant al sistema desenvolupat pel diagnòstic de la malària com de l'esquistosomiasi urogenital son: (i) robustesa de l'algoritme per obtenir resultats diagnòstics en diferents situacions i entorns, i (ii) la disponibilitat de bases de dades públiques i de qualitat per poder avaluar les xarxes neuronals amb informació de centres externs. En ambdós casos, tant la qualitat de la imatge capturada, com la tinció emprada o microscopi utilitzat poden afectar a la morfologia de la imatge i per tant a la qualitat final del diagnòstic. Per tant, s'han d'optimitzar els models de xarxes neuronals amb imatges d'altres centres per poder obtenir algoritmes el més robustos possibles, i conseqüentment implementables en la majoria de laboratoris d'arreu, minimitzant els possibles biaixos de rendiment segons l'entorn.



5.2. Diagnòstic de l'esquistosomiasi urogenital amb intel·ligència artificial

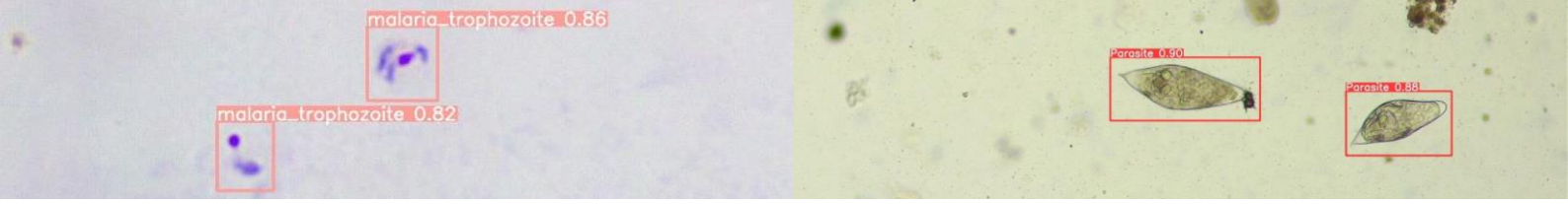
En el cas de l'esquistosomiasi urogenital, hi ha menys aplicacions d'IA per la detecció dels paràsits en orina, en comparació amb tot el que s'ha desenvolupat per malària. Les tècniques clàssiques d'anàlisi d'imatges, com l'anàlisi binaria basat en les MVS, ja es van utilitzar per detectar ous de *S. haematobium* en imatges digitals d'orina [185]. El mètode implicava el preprocessament de la imatge i l'extracció de característiques mitjançant el protocol de les tècniques tradicionals de processament d'imatge.

Amb la irrupció de les noves tècniques d'IA, *Peñas* et al. 2020 va utilitzar el model YOLOv3 per la detecció automatitzada d'ous d'helmints en mostres de femtes, entre els quals destacaven les espècies *S. mansoni* i *S. intercalatum* [186]. El model de xarxa neuronal va demostrar un 95.31% de precisió per la detecció d'ous de *Schistosoma* [186], resultats lleugerament inferiors als del nostre sistema (99.30% de precisió), però tenint en consideració que es van emprar mostres fecals. Com a altre exemple de sistema de detecció basat en XNC, *Oyibo* et al. 2022 va desenvolupar un sistema anomenat *Schistoscope* [187]. El sistema és capaç de detectar ous de *S. haematobium* en imatges digitals de mostres d'orina mitjançant XNC [187]. Es tracta d'un dispositiu òptic mecanitzat capaç d'escanejar i analitzar les mostres. Es diferencia del nostre sistema, ja que en el nostre cas utilitzem microscopis òptics convencionals automatitzats, mentre que en el cas de l'estudi *Oyibo* et al. 2020 han dissenyat un aparell independent per realitzar els diagnòstics. El sistema ha estat validat en estudis realitzats en àrees rurals del Gabon, demostrant una sensibilitat diagnòstica del 83.1% i una especificitat del 96.3% [188]. Per altra banda, *Makau-Barasa* et al. 2023 va avaluar el rendiment d'un sistema de multi diagnòstic anomenat *AiDx* per la detecció de l'esquistosomiasi a Abuja, Nigeria [189]. El sistema completament automatitzat (autoenfocament, escaneig de la mostra, processament i anàlisi d'imatge) va demostrar una sensibilitat i especificitat diagnòstiques del 89% i del 99% respectivament [189]. Com a exemple d'ús de models de xarxes neuronals per la identificació de *Schistosoma, Tallam* et al. 2021 va realitzar una prova de concepte d'una aplicació capaç de diferenciar els cargols *Bulinus spp*. i diverses fases del cicle de vida de *Schistosoma* en humans [190]. A diferència del nostre sistema, no està dissenyat pel diagnòstic, però pot ser d'utilitat pel control ecològic dels cargols en regions endèmiques de la malaltia. En relació amb la detecció dels cargols, *Liu* et al. 2022 va proposar un sistema d'aprenentatge profund (U-Net) basat en la



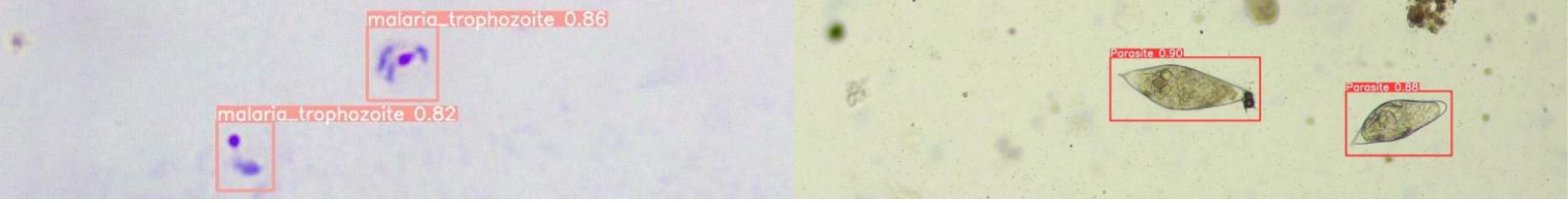
segmentació d'imatges satèl·lit per identificar vegetació aquàtica associada amb la presència dels hostes intermediaris, en el riu Basin del Senegal [191].

També trobem aplicacions, fora de l'àmbit del diagnòstic, centrades en el descobriment de nous fàrmacs mitjançant algoritmes d'IA. *Zorn* et al. 2021 va utilitzar dades de pantalles fenotípiques contra l'esquistosòmula i les etapes adultes de *S. mansoni* per desenvolupar models d'aprenentatge automàtic. Van generar vuit models d'aprenentatge automàtic bayesians basats en cada etapa del desenvolupament del paràsit. Posteriorment, es van utilitzar els models generats per predir l'activitat de diversos compostos farmacològics. Finalment, es van seleccionar 40 compostos predits com a actius i 16 compostos predits com a inactius i es van comprar per a assajos fenotípics *in vitro* contra l'esquistosòmula i estadis adults de *S. mansoni* [192,193].



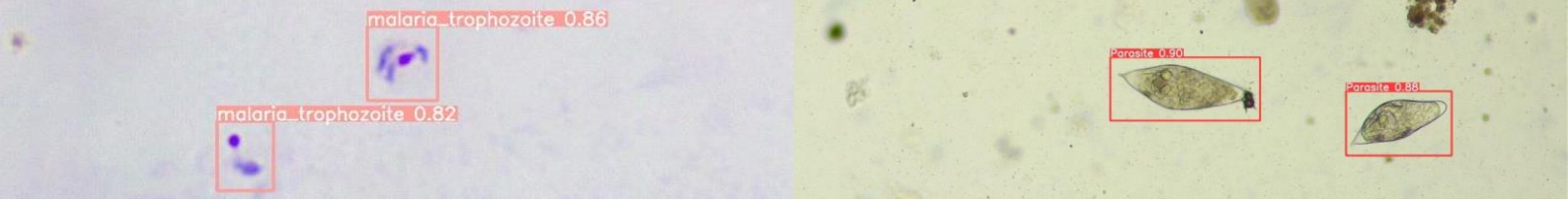
5.3. Bases de dades d'imatges

Un dels factors limitants més importants a l'hora de generar i entrenar algoritmes d'IA són les dades. En el cas del nostre projecte, les dades emprades per aquesta funció han estat imatges digitals etiquetades de mostres clíniques de sang i orina de pacients. Actualment, no es disposa de moltes imatges en format *open source* perquè la comunitat científica i investigadora pugui accedir i treballar amb elles. Com a exemple, la web *Roboflow* permet desenvolupar i entrenar algoritmes de visió per computador mitjançant bases de dades amb llicències *Creative Commons* (CC) proporcionades pels usuaris particulars [194]. Encara així, la qualitat de les bases de dades d'imatges depèn de l'usuari que les ha publicat, i no tenen cap control de qualitat que asseguri que estan correctament etiquetades. D'altra banda, *ImageNet* és una base de dades d'imatges públiques organitzada d'acord amb la jerarquia *WordNet*, en la qual cada node de la jerarquia és representat per centenars i milers d'imatges [195]. Les dades estan disponibles de forma gratuïta per als investigadors d'arreu i per a ús no comercial. Aquest tipus de plataformes en format obert permeten realitzar avenços a nivell global gràcies a la disponibilitat de dades per a usos exclusivament de recerca. Un altre format de publicació i que garanteix la qualitat de les imatges i el seu etiquetatge és el de publicació en revistes de dades. L'Institut d'Enginyers Elèctrics i Electrònics (IEEE) disposa del repositori *IEEE DataPort* [134]. El portal és una plataforma de dades de recerca dissenyada per donar accés a les dades científiques obertament, i ajudar els investigadors i institucions a compartir la recerca, gestionar les seves dades i col·laborar [134]. En el cas de la malària hi ha bases de dades del portal IEEE tant per gota gruixuda [196] com per extensió fina de sang [197]. Les bases de dades de països endèmics són crucials per obtenir informació diversa i de qualitat. *Nakasi* et al. 2024 va publicar una base de dades d'imatges de l'Hospital Regional de Kampala (Uganda), amb 3000 imatges etiquetades de gota gruixuda i 1000 imatges etiquetades d'extensió fina de sang capturades amb un telèfon intel·ligent [198]. També, existeix l'anomenat *Lacuna Dataset,* una base de dades de 3925 d'imatges digitals de mostres de gota gruixuda tenyides amb Giemsa i capturades amb dispositius mòbils [199]. La base de dades té la funció de ser emprada per millorar models d'aprenentatge automàtic, i ser aplicats en el diagnòstic de la malària a regions endèmiques amb pocs recursos [199]. En el cas de l'esquistosomiasi, els autors *Oyibo* et al. 2023 van publicar a l'Institut Nacional d'Al·lergologia i Malalties Infeccioses (NIAID Data Ecosystem, USA) una base de dades que conté 12.051 imatges microscòpiques etiquetades de 103 mostres de sediment urinari en format *open source* [200]. Com a exemple de recerca emprant bases de dades obertes,



Boit et al. 2024 va entrenar el model EfficientNetB2-Dense-Residual-Inception amb la base de dades de malària del NIH, que conté 27.558 etiquetes [201].

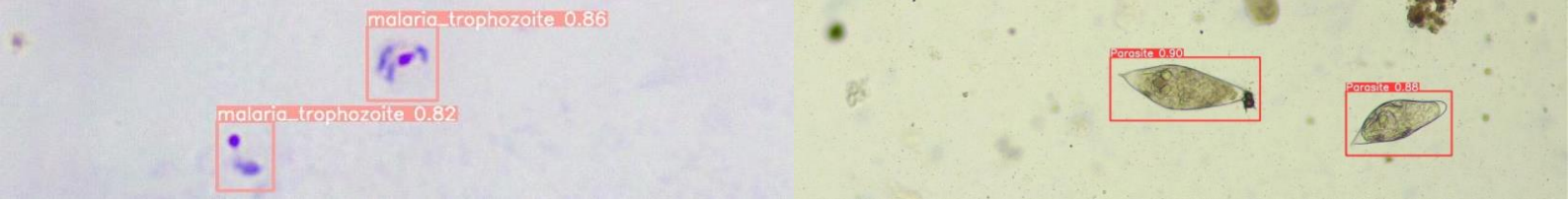
Les dades obertes, no solament en el cas de les imatges, poden ser de gran utilitat per generar algoritmes amb inputs de diferents laboratoris a nivell global, molt més eficients i complets que aquells generats amb dades locals. En el cas dels estudis publicats per la tesi doctoral, hem utilitzat imatges de diversos laboratoris per obtenir algoritmes de detecció més robustos. Així i tot, el futur de les dades obertes pot ser el primer pas per globalitzar aquest tipus de sistemes i col·laborar entre institucions pel benefici mutu. Informes de la Unió Europea ja descriuen aquesta casuística i pronostiquen un futur prometedor a causa dels avenços en IA que s'estan produint en els últims anys i els beneficis de treballar amb dades d'accés obert [202].



5.4. Automatització i digitalització de la microscòpia

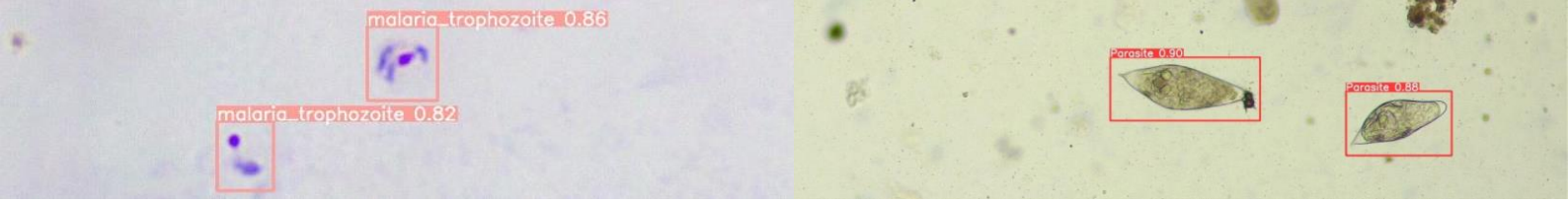
En l'àmbit de l'automatització, el nostre sistema ha estat dissenyat per ser universal i adaptable a la majoria dels microscopis òptics convencionals, i per ser de baix cost. El sistema, també, és en format *open source,* i per tant el codi de programació i els dissenys 3D de les peces estan disponibles per a tothom qui els requereixi. El procés d'automatització es va realitzar amb controladors *Arduino* i servo motors de baix cost per emular els moviments X-Y de la platina del microscopi, i l'autoenfocament de la mostra. Actualment, es disposa de microscopis robotitzats capaços d'escanejar mostres biològiques per la seva digitalització. Un exemple podria ser l'empresa Hamamatsu®, encarregada de desenvolupar microscopis òptics per l'escaneig i digitalització de mostres biològiques [203]. Les empreses Bruker®, Zeiss® o Nikon® també disposen d'aquest tipus de tecnologies, però els preus solen ser molt elevats i no solen estar pensats per la seva implementació en entorns amb pocs recursos. Com a alternativa, d'altres sistemes sorgits de treballs de recerca s'han desenvolupat i utilitzat per funcions similars. *Lien* et al. 2019 va desenvolupar un sistema *open source* i de baix cost per la digitalització automatitzada d'imatges de plantes del gènere *Arabidopsis thaliana* [204]. El sistema no està pensat per digitalitzar imatges microscòpiques, però l'automatització i el concepte de l'estudi és molt similar al desenvolupat en les nostres publicacions. En l'àmbit de la microscòpia, *García-Villena* et al. 2021, van desenvolupar un sistema amb impressió 3D pel diagnòstic microscòpic de malalties tropicals desateses i hematològiques mitjançant telèfons intel·ligents [205]. El sistema està pensat per la seva implementació en entorns amb pocs recursos, però es tracta d'un dispositiu independent no adaptable als microscopis. Requereix un enfocament manual i ha estat testejat satisfactòriament amb mostres histològiques i de femtes per la seva implementació. El sistema també està pensat per treballar amb una plataforma de telemedicina, que mitjançant XNC, sigui capaç d'analitzar les imatges i donar suport al diagnòstic [205]. L'anteriorment mencionat *EasyScan Go* també és un bon exemple de sistema automatitzat pensat per la seva implementació en laboratoris del terreny. El dispositiu també està dissenyat per utilitzar algoritmes d'IA capaços de detectar les formes d'interès en imatges digitals microscòpiques de mostres biològiques [148]. En aquest cas, el sistema és capaç d'autoenfocar la mostra i realitzar un escaneig complet d'aquesta.

Un dels factors més importants a l'hora d'autoenfocar mostres com la gota gruixuda i l'extensió fina de sang és el relleu de la preparació. Les mostres no són perfectament planes, i per tant és important ajustar o revisar el focus en cada un dels camps a analitzar, per així evitar possibles



errors d'enfocament i identificació per part de les XNC. En el nostre cas vam emprar l'algoritme de Variança Laplaciana, capaç de detectar la imatge més enfocada mitjançant l'assignació d'un valor amb l'anàlisi del gradient d'entropia. És un mètode de referència àmpliament contrastat que s'ha emprat en multitud d'estudis similars [206–208]. Altres metodologies, com el Harris Corner Response Measure (HCRM), es basen en la detecció dels canvis d'intensitat dels píxels de les vores de les imatges [209]. *Sanz* et al. 2022 va realitzar un estudi comparatiu de diverses metodologies d'anàlisi d'imatge per l'autoenfocament, demostrant que la de millor rendiment va ser el *Squared Gradient* seguit del *Brener Gradient* [210].

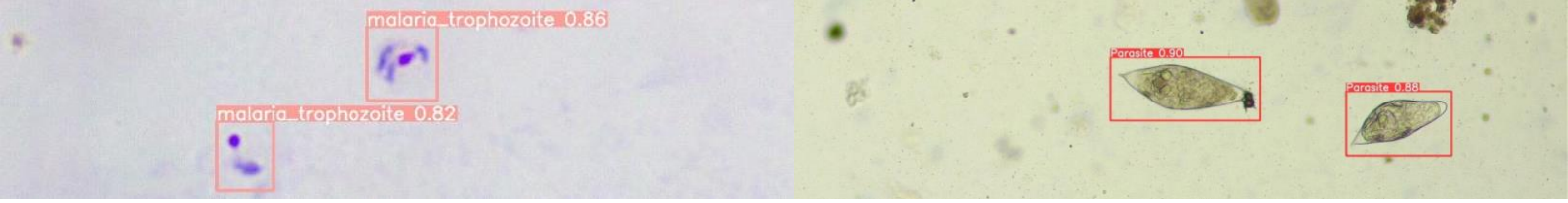
El desenvolupament de sistemes basats en IA i automatització pel diagnòstic de malalties tropicals ha de tenir en consideració els recursos i infraestructura de la majoria dels centres de salut de les regions endèmiques. Sistemes automatitzats de baix cost, amb requisits energètics baixos i sense necessitat de connectivitat a internet són característiques òptimes per aquest tipus de contextos. La claredat dels resultats i el fàcil maneig a l'hora d'usar aquests sistemes també són punts crucials per implementar-los a laboratoris del terreny. Els dispositius *point-of-care* solen ser solucions adients per realitzar diagnòstics *in situ*, sense necessitat d'enviar mostres a regions amb major infraestructura i endarrerir l'informe de resultats [211]. Més enllà del diagnòstic, la globalització dels telèfons intel·ligents, la informatització dels laboratoris, el desenvolupament de sistemes de dades sociodemogràfiques per generar algoritmes de suport al diagnòstic i la prevenció, i les aplicacions de salut digital; poden arribar a tenir un gran impacte en regions amb pocs recursos [212]. Actualment, la disponibilitat dels sistemes informàtics no és global, i per tant les eines integrades en *softwares* per telèfons intel·ligents poden ser alternatives per arribar arreu del món. Encara així, segons informes de les Nacions Unides, l'escletxa digital entre països del Sud-global és un problema, i s'han d'adaptar tots aquells sistemes que vulguin ser implementats a les necessitats i requeriments de cada situació [213].



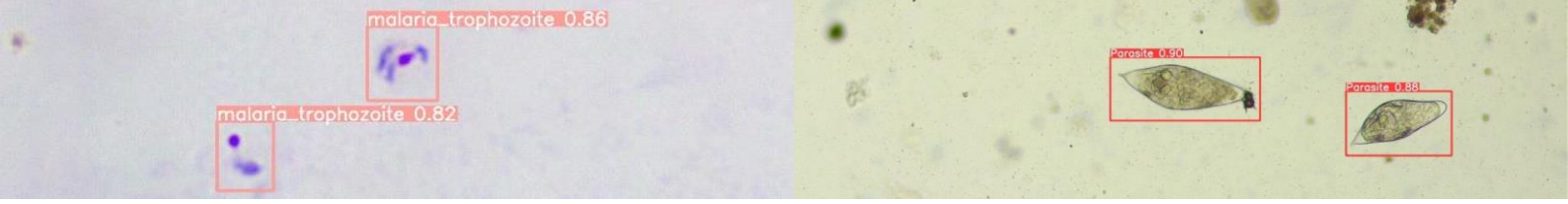
5.5. Ètica i regulació actual de la intel·ligència artificial pel diagnòstic

Recentment, la Unió Europea ha posat a disposició un document legal (Regulació EU 2024/1689) per la regulació de la IA i els seus potencials avantatges i riscos al continent Europeu [214]. El document també té la finalitat de reduir les regulacions administratives a l'hora de desenvolupar i implementar sistemes d'IA, especialment per a empreses. L'objectiu general de la regulació és garantir la seguretat, paritat i els drets fonamentals de les persones centrats en l'aplicació de la IA. La regulació defineix 4 nivells de risc dels sistemes d'IA, des d'un risc mínim fins a un risc inacceptable (prohibit el seu ús a la Unió Europea). Algunes de les funcionalitats considerades d'alt risc són: aplicacions de transport autònom, puntuació automàtica d'exàmens, cirurgies assistides per robots, aplicacions pel control de migrants, i aplicacions en l'àmbit judicial [214]. Tenint en compte el projecte de la tesi doctoral, el suport al diagnòstic es menciona com a activitat d'alt risc. En els apartats 47 i 50 del document es menciona textualment "*en el sector sanitari, on pot haver-hi repercussions importants en la vida i la salut, els sistemes de diagnòstic i de suport, han de ser fiables i precisos*" i "*s'escau classificar-los com d'alt risc en virtut del present reglament [...] productes sanitaris pel diagnòstic*" [214]. El reglament no prohibeix l'ús d'aquest tipus de sistemes a la Unió Europea, però obliga a validar-los seguint els estaments europeus, abans d'implementar-los com a eines de suport al diagnòstic al laboratori.

D'altres documents oficials descriuen l'ètica de l'ús de la IA en els àmbits quotidians, i concretament en el de la salut. L'OMS ha publicat dues guies que parlen de l'ètica de la IA en salut. L'any 2021 es va publicar la guia "Ètica i governança de la intel·ligència artificial per la salut" [215], i l'any 2024, amb l'aparició dels models multimodals es va publicar la guia " Ètica i governança de la intel·ligència artificial per la salut: Guia pels grans models multimodals" [216]. En ambdós casos, es menciona la necessitat de validar els models en diverses ubicacions, per tenir en compte les necessitats i limitacions de cada un d'aquests llocs, i no esbiaixar els resultats segons la població d'estudi. El suport i implementació d'eines d'IA en localitzacions on no es disposa de personal, han de venir acompanyats de la formació per a les persones. Un altre dels temes importants tractats en els documents és la paritat de les dades. Els models d'IA haurien de ser entrenats amb dades que respectin les diferències entre les persones, i sempre seguint el codi ètic i dels drets humans fonamentals. En el cas del nostre projecte i d'altres projectes publicats a la literatura, el codi ètic és crucial pel desenvolupament i implementació d'aquest tipus d'eines, que s'han d'adaptar a la legislació i al codi ètic internacional.



Les aplicacions per a telèfons intel·ligents són una opció adequada per integrar la tecnologia de diagnòstic en un sol dispositiu i conferir als laboratoris una nova eina pel diagnòstic de la malària i altres malalties. Nous avenços i millores en l'àmbit de la IA i la computació poden ser la fita final per a l'optimització i implementació de la tecnologia de diagnòstic per la imatge a nivell global. En conclusió, cada vegada estem més a prop de desenvolupar noves eines de diagnòstic ràpides i eficients per a la detecció automatitzada de paràsits de la malària i esquistosomiasi disponibles per a laboratoris d'arreu. Aquest tipus de sistemes estan demostrant el seu potencial en el diagnòstic microbiològic de les malalties infeccioses, i seran, en un futur pròxim, tècniques de rutina en els laboratoris clínics. La coalescència de les tècniques d'anàlisi d'imatges digitals i la robotització microscòpica confereixen autonomia per realitzar els diagnòstics, sempre amb la supervisió del operador i/o del personal de laboratori.



6. Conclusions

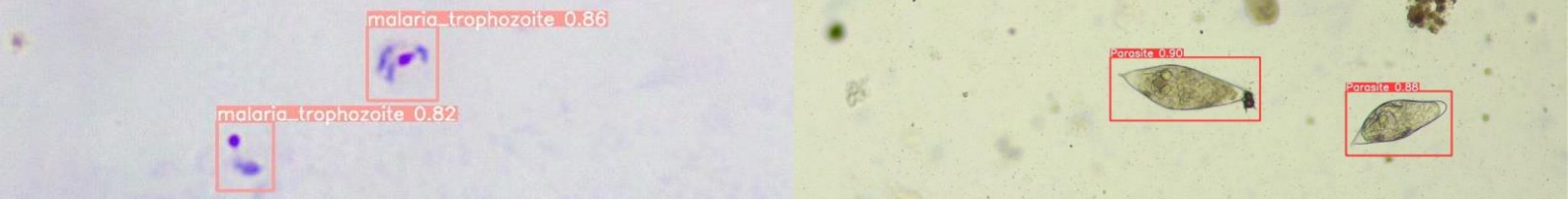
Aquesta tesi doctoral ha abordat els reptes associats al diagnòstic microscòpic convencional, com la dependència de personal especialitzat, la variabilitat en la interpretació microscòpica i l'accés limitat a recursos en entorns empobrits. Durant el projecte de tesi s'ha desenvolupat un sistema automatitzat integral basat en una aplicació per a telèfons intel·ligents que combina algoritmes d'IA, com les xarxes neuronals YOLOv5 i YOLOv8, amb un microscopi robotitzat universal de baix cost, fabricat mitjançant peces 3D i *open source*. Amb aquest sistema s'ha permès automatitzar el diagnòstic microbiològic de la malària i l'esquistosomiasi urogenital mitjançant l'adquisició d'imatges, l'autoenfocament, l'escaneig de mostres biològiques i la interpretació diagnòstica, reduint la dependència de l'expertesa humana i donant suport al diagnòstic tradicional.

Les conclusions de la present tesi doctoral són:

(i) La base de dades d'imatges etiquetades permet l'entrenament de xarxes neuronals per tasques de detecció d'objectes, la formació de professionals de laboratori i la docència. La base de dades d'imatges actual (31 de març de 2025) conté un total de 7684 imatges digitals. En detall conté: 2803 imatges etiquetades de mostres de gota gruixuda sang (Drassanes), 1028 imatges de mostres de gota gruixuda (Hospital Nossa Senhora da Paz, Cubal, Angola), 1503 imatges etiquetades de mostres d'extensió fina de sang (Drassanes), i 2350 imatges etiquetades de sediments urinaris.

(ii) Els algoritmes d'intel·ligència artificial han demostrat ser òptims per la detecció de paràsits de *Plasmodium* i de *S. haematobium* en imatges digitals de mostres biològiques de sang i d'orina respectivament capturades amb el telèfon intel·ligent i la càmera integrada del microscopi. Els models d'anàlisi d'imatges basats en IA van assolir un valor F del 92.79% per la detecció de la malària, i del 99.30% per la detecció de l'esquistosomiasi urogenital.

(iii) Els sistemes automatitzats desenvolupats demostren un gran potencial per millorar el diagnòstic de la malària i l'esquistosomiasi urogenital. No obstant, la incorporació de *transformers*, tècniques de *domain adaptation*, aprenentatge semisupervisat, estratègies de normalització, i tècniques d'augment de dades més robustes podrien optimitzar els paràmetres de precisió i reduir la dependència de dades d'imatges etiquetades per generar algoritmes de diagnòstic més eficients.



(iv) Els models de xarxes neuronals entrenats han permès generar algoritmes d'anàlisi d'imatges implementables no solament en dispositius de baix cost, sinó que poden tenir utilitat en sistemes automatitzats que s'empren en els països industrialitzats per automatitzar el diagnòstic microscòpic.

(v) Els resultats de la validació diagnòstica demostren un bon rendiment del sistema pel diagnòstic de la malària en gota gruixuda de sang i permeten la seva optimització en futures validacions. La validació diagnòstica realitzada al laboratori de Microbiologia de l'Hospital Vall d'Hebron i al Centre de Salut Internacional Drassanes Vall d'Hebron va demostrar una sensibilitat del 81.25% i una especificitat del 92.11% en mostres de gota gruixuda tenyides amb Giemsa.

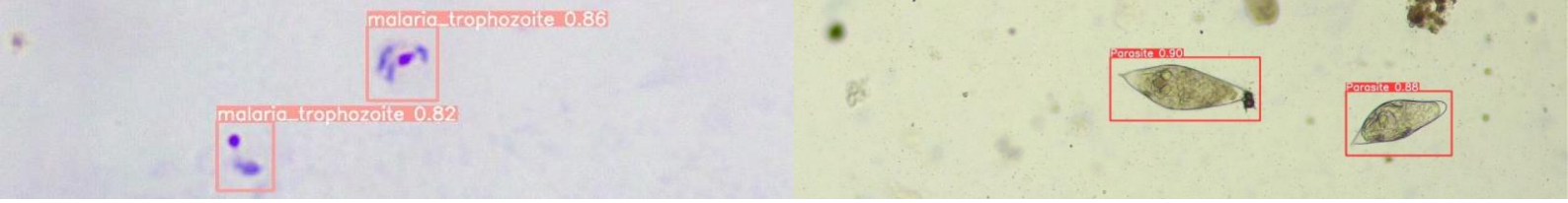
(vi) La integració del sistema en una aplicació mòbil i l'ús de telèfons intel·ligents com a interfície el converteixen en una eina viable i accessible per a laboratoris amb recursos limitats. Aquestes característiques permeten la descentralització del diagnòstic i una major cobertura del diagnòstic en zones endèmiques i llocs remots. Tanmateix, la robotització de microscopis convencionals mitjançant components de baix cost elimina la necessitat d'equips comercials d'alt cost, fent la tecnologia accessible a regions amb pocs recursos.

(vii) Les diferències en el processament de les mostres biològiques i el procediment de tinció poden afectar la qualitat de les preparacions i, per tant, el rendiment dels models d'IA basats en l'anàlisi d'imatges. És adient estandarditzar el procediment de preparació i tinció de les mostres biològiques per la seva visualització mitjançant IA, i així evitar diferències que poden afectar els resultats finals del diagnòstic.

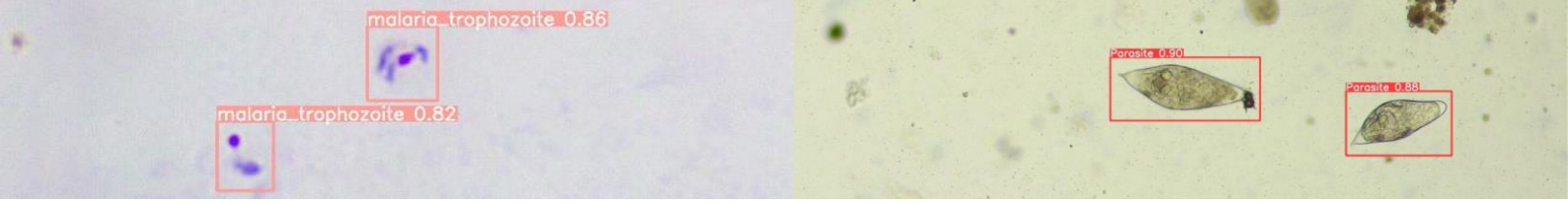
(viii) La implementació d'aquest sistema de diagnòstic requereix adaptar-se a les infraestructures locals, incloent-hi la formació del personal, el manteniment tècnic del sistema i la compatibilitat amb els protocols de diagnòstic existents a cada laboratori, i els plans nacionals de lluita contra aquestes malalties.

(ix) Les col·laboracions amb governs, organitzacions no governamentals, i organitzacions de base comunitària són clau per a la validació i implementació del sistema de diagnòstic, i la distribució equitativa de la tecnologia permetent la sostenibilitat i l'escalabilitat del producte.

La tesi doctoral demostra que la intel·ligència artificial i la robotització de baix cost són eines útils per millorar el diagnòstic de la malària i l'esquistosomiasi urogenital mitjançant la microscòpia. El sistema no només ajuda a millorar l'eficiència i l'accessibilitat del diagnòstic sinó que també contribueix a la justícia global en salut, reduint les desigualtats en regions amb pocs



recursos. Els resultats obtinguts obren un camí prometedor cap a l'erradicació de malalties que afecten milions de persones, alineant-se amb els Objectius de Desenvolupament Sostenible (ODS) de les Nacions Unides i l'Estratègia Global per la Salut Digital de l'OMS [217]. El futur de la microbiologia clínica passa per la fusió de la innovació tecnològica, la cooperació internacional i un compromís ètic ferm amb les comunitats més vulnerables.



7. Perspectives de futur

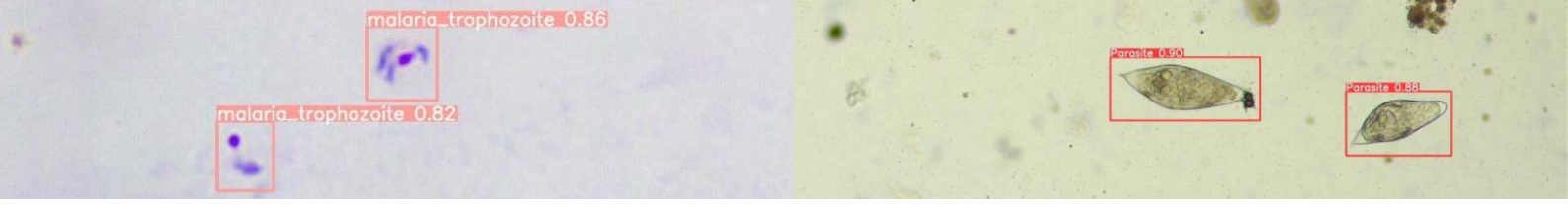
Creiem que la tecnologia desenvolupada pot tenir un gran impacte en salut global en un futur immediat, no solament pel diagnòstic automatitzat de les malalties tropicals, sinó també en altres aplicacions i funcionalitats. El projecte té un caràcter transversal que pot permetre realitzar d'altres projectes relacionats en àmbits diferents del presentat.

A continuació esmenem possibles perspectives de futur relacionades amb el projecte presentat a la tesi doctoral:

(I) **Optimització del diagnòstic de malària**: actualment el sistema ha estat desenvolupat i validat pel diagnòstic de la malària mitjançant la gota gruixuda de sang i, per tant, el diagnòstic diferencial per espècies no està integrat. El grup de recerca està treballant en el desenvolupament d'un algoritme, mitjançant la xarxa neuronal YOLOv11, per la detecció i diferenciació de les espècies *P. falciparum*, *P. vivax*, *P. ovale* i *P. malariae* en mostres d'extensió fina de sang. El sistema és capaç també de diferenciar les etapes del desenvolupament del *Plasmodium*, així com els trofozoïts joves, trofozoïts madurs, esquizonts i gametòcits. Els resultats preliminars demostren una precisió de 87.80%, un *recall* de 88.20%, un valor F de 87.99%, i una *mAP0.5* de 92.20% per a la detecció global de les espècies esmenades.

(II) **Implementació del diagnòstic d'altres malalties**: el diagnòstic de la tuberculosi mitjançant la tècnica del Ziehl-Neelsen, l'anàlisi de mostres tenyides amb Gram pel diagnòstic de la vaginitis bacteriana, l'anàlisi hematològica o qualsevol altra aplicació que empri la visualització de mostres biològiques mitjançant la microscòpia poden ser considerades per aquest tipus de tecnologia. L'entrenament de noves xarxes neuronals per realitzar aquest tipus de tasques no diferiria de la metodologia emprada pel diagnòstic de la malària o l'esquistosomiasi. Per tant, la IA per identificació d'imatges digitals pot aportar noves aplicacions si es disposa de bases de dades d'imatges de qualitat, i pot ser una eina funcional pels laboratoris d'arreu. D'altres grups de recerca i empreses estan treballant en aquest àmbit, i s'estan començant a comercialitzar i implementar aquest tipus de tecnologies.

(III) **Validació i implementació en regions endèmiques**: s'ha realitzat una prova de concepte al laboratori de Microbiologia de l'Hospital Nossa Senhora da Paz,



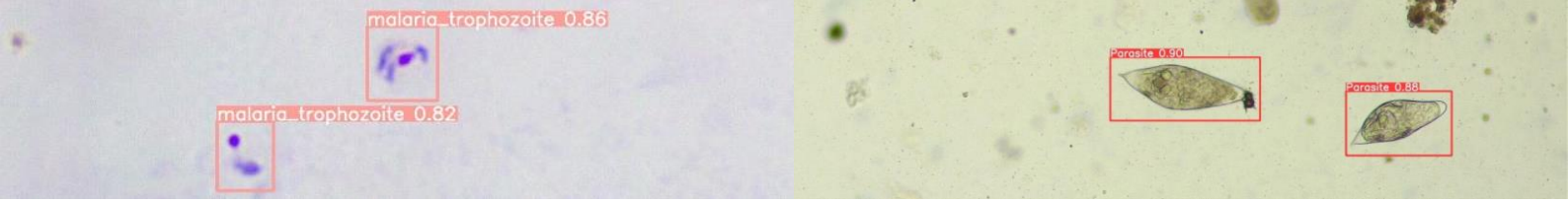
Cubal (Angola), on s'ha pogut demostrar la viabilitat d'implementació del sistema i s'ha avaluat el rendiment de les xarxes neuronals amb mostres biològiques de gota gruixuda. També, s'ha realitzat una prova de concepte a Georgetown (Guyana), per un grup de recerca extern que va muntar i testejar el sistema pel diagnòstic de malària. Encara així, validacions diagnòstiques amb nombres de mostres estadísticament significatius s'han de realitzar per avaluar rigorosament el rendiment diagnòstic del sistema en regions endèmiques.

(IV) **Control de qualitat**: el control de qualitat dels diagnòstics per microscòpia es podria realitzar mitjançant tècniques d'anàlisi d'imatges basades en IA. La detecció de les formes d'interès mitjançant IA ens permetria avaluar la qualitat dels diagnòstics, i comparar-los amb els de la microscòpia convencional. També, es podrien utilitzar tècniques d'imatge per valorar la qualitat de les mostres i/o de les tincions, i millorar el processament de la mostra realitzat al laboratori.

(V) **Formació**: la digitalització de les mostres biològiques, la detecció automàtica dels paràsits i la creació d'eines didàctiques digitals poden ser d'utilitat per la formació de microscopistes d'arreu. Les aplicacions de telèfons intel·ligents i/o d'ordinador són eines de fàcil maneig que poden aportar coneixements sobre conceptes de laboratori, i permetre la formació mitjançant la visualització de les imatges digitals.

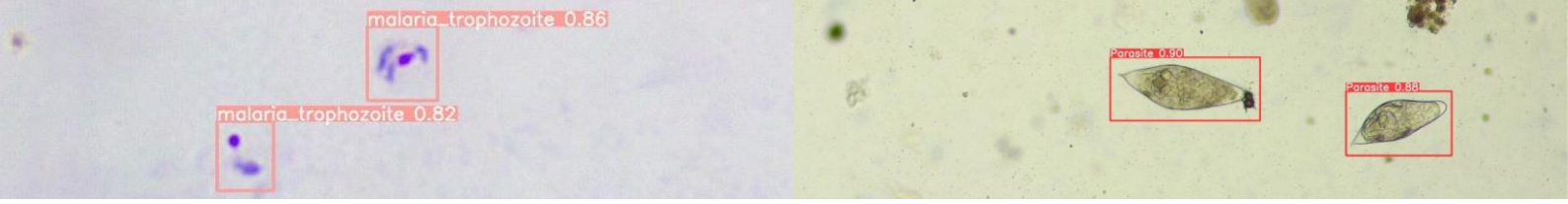
(VI) **Epidemiologia**: molts dels laboratoris del terreny no disposen de dades epidemiològiques fiables, ni d'un sistema informàtic per gestionar les dades. Els telèfons intel·ligents, degut a la seva portabilitat i computació, són capaços de reportar i registrar els casos. Els resultats del diagnòstic automàtic mitjançant IA es poden integrar en *softwares* de gestió de laboratori, i per tant així obtenir dades epidemiològiques reals. També poden ser emprades per tasques de vigilància epidemiològica, com per exemple reportar casos en zones molt remotes, on únicament es pugui disposar d'aquest tipus de dispositius.

(VII) **Bases de dades**: la digitalització i informatització de les dades de laboratori clínic permeten generar grans quantitats d'informació clínica i epidemiològica. En la tesi presentada, s'ha creat una base de dades d'imatges digitals etiquetades que es pretén publicar seguint les directrius de l'Hospital Universitari Vall d'Hebron / Vall d'Hebron Institut de Recerca. La base de dades d'imatges estarà en accés obert, i permetrà entrenar algoritmes d'IA capaços de realitzar tasques



específiques per donar suport al diagnòstic i a la formació de professionals de laboratori, excloent els beneficis comercials per a empreses i tercers. La robotització i automatització de la microscòpia també pot ser d'utilitat per digitalitzar mostres biològiques, i generar bases de dades. Tanmateix, les bases de dades haurien de ser obertes i accessibles, per contribuir a l'avenç de la recerca i poder entrenar i generar algoritmes cada cop més precisos degut a l'heterogeneïtat de la d'informació emprada.

(VIII) **Escalatge i transferència tecnològica**: el sistema de diagnòstic ha de ser escalat fins a la fase de producte, per poder ser cedit o transferit a les institucions i parts interessades. Conjuntament amb els departaments d'innovació del Vall d'Hebron Institut de Recerca i de la Universitat Politècnica de Catalunya s'ha acordat realitzar la transferència tecnològica del sistema mitjançant una spin-off sense ànim de lucre. Els algoritmes de diagnòstic generats podrien permetre la sinergia amb empreses del sector tecnològic, per ser transferits i implementats en sistemes de diagnòstic més sofisticats i que disposin d'equipament d'escaneig professional. Per tant, aquest tipus de transferència també pot generar com a parts interessades als països desenvolupats, que requereixin l'automatització dels diagnòstics microscòpics convencionals.



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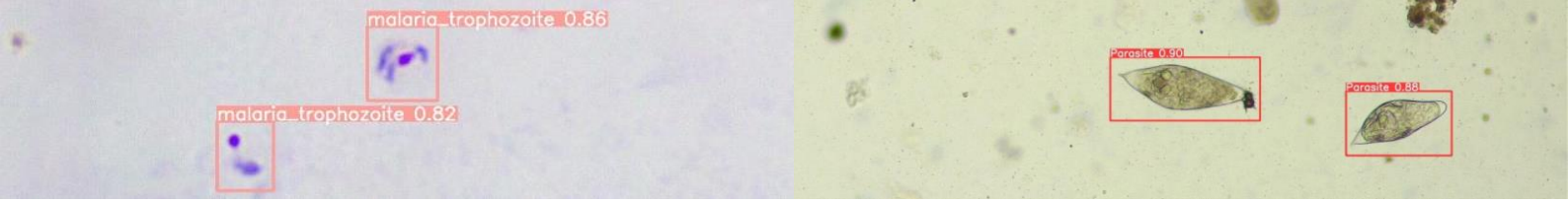

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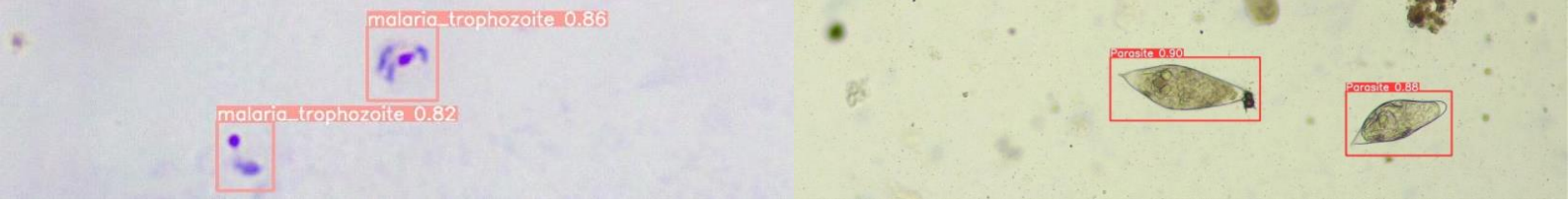

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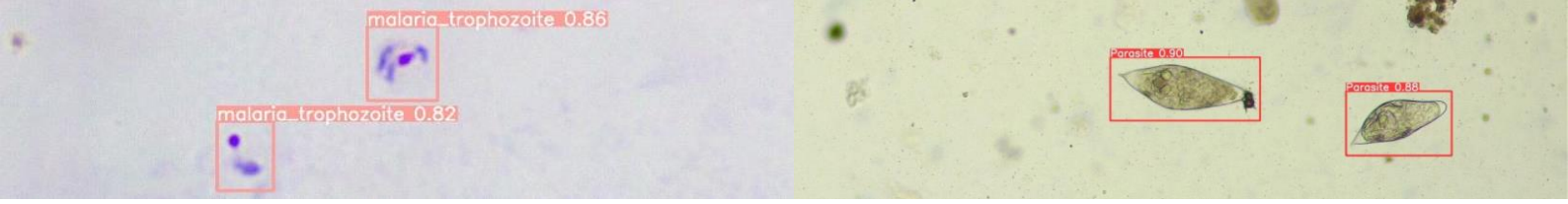

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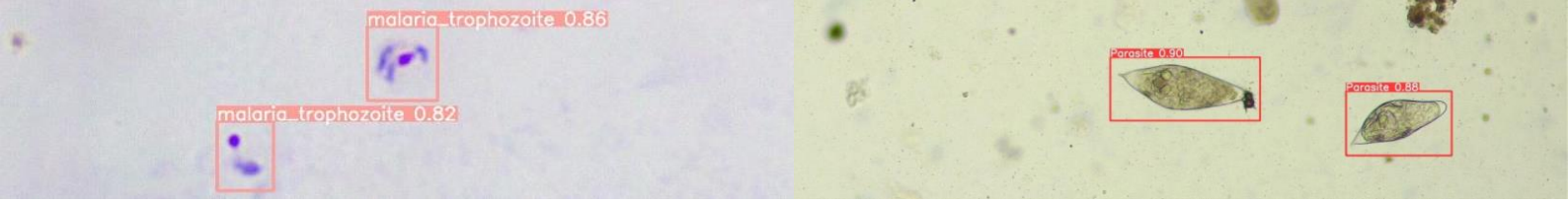

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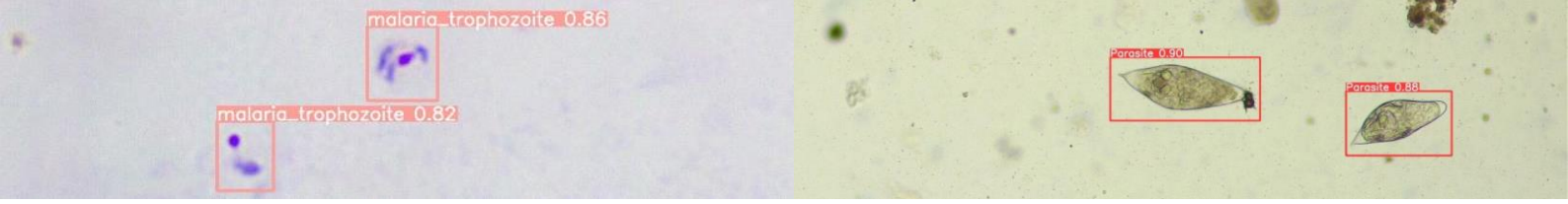

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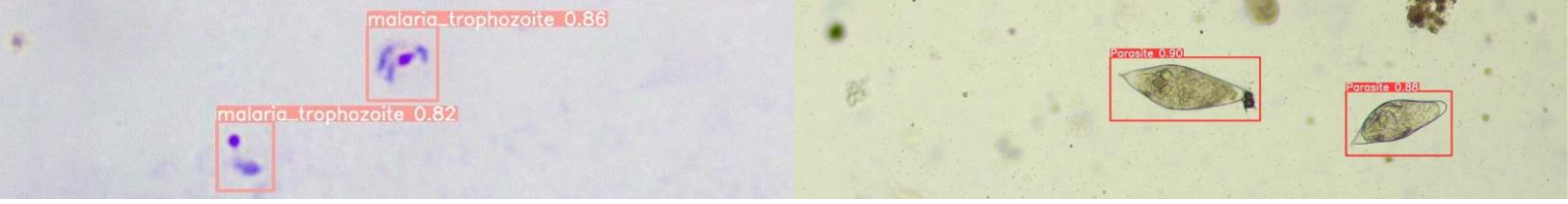

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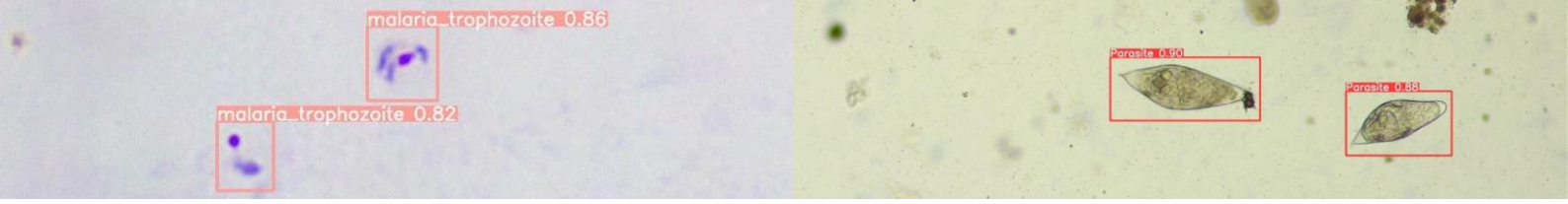

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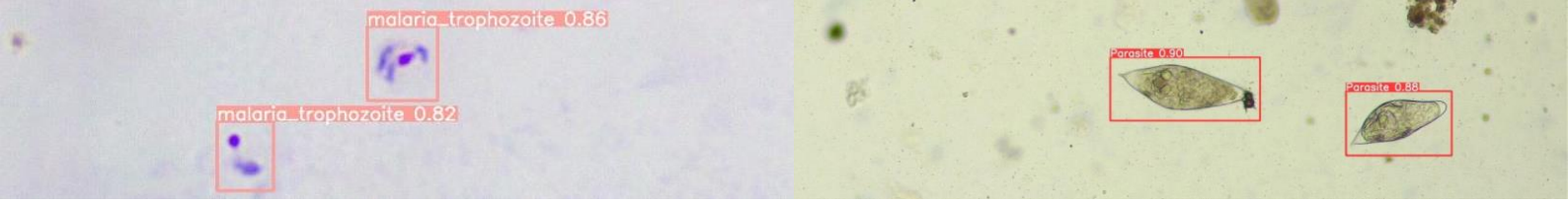

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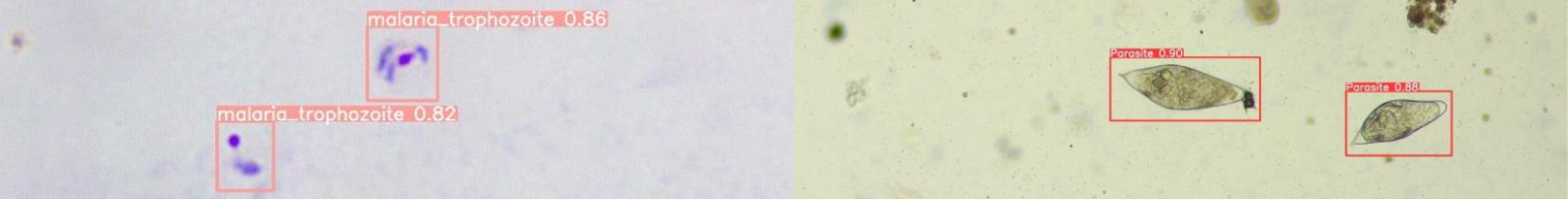

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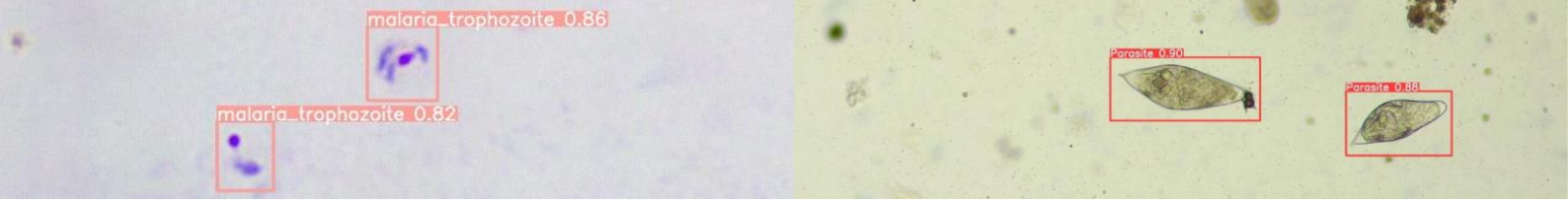

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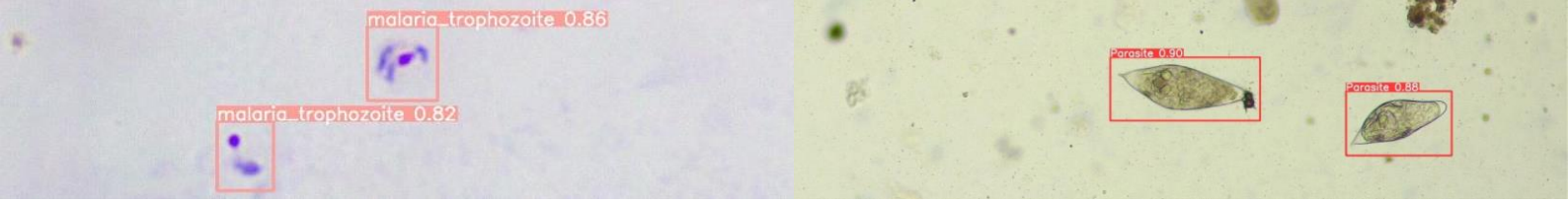

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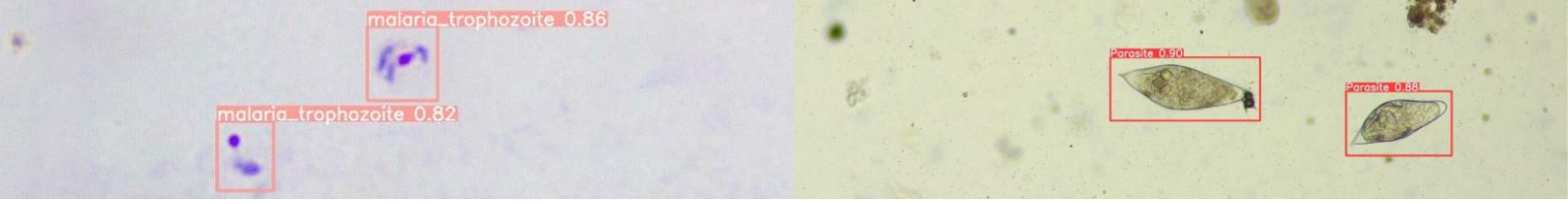

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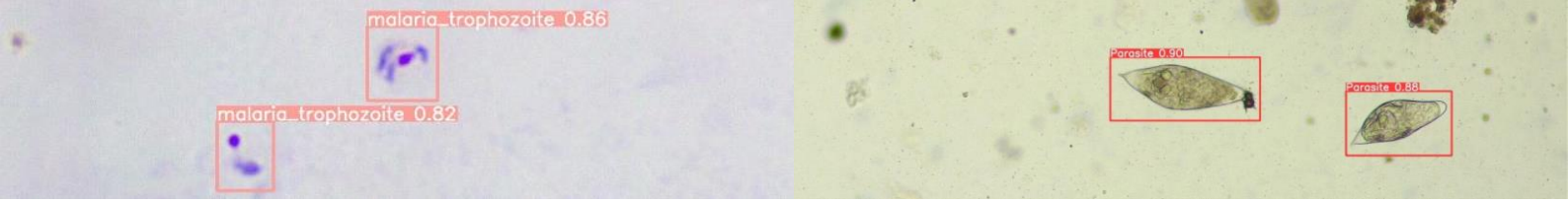

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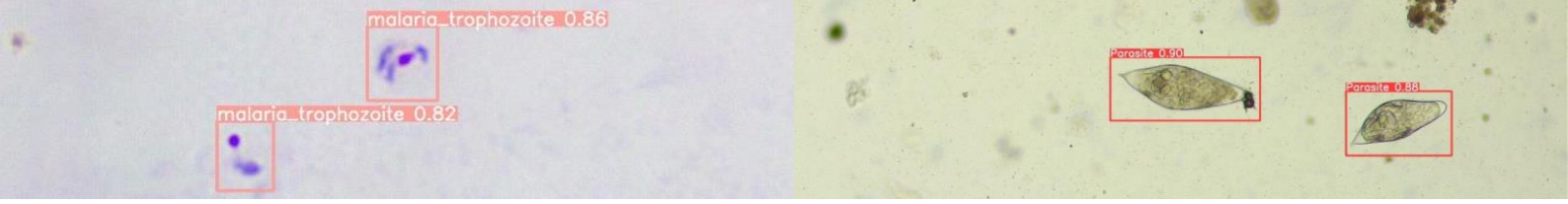

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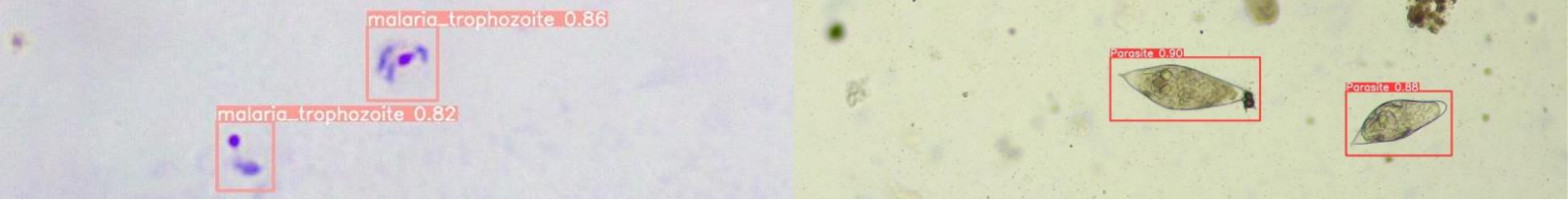

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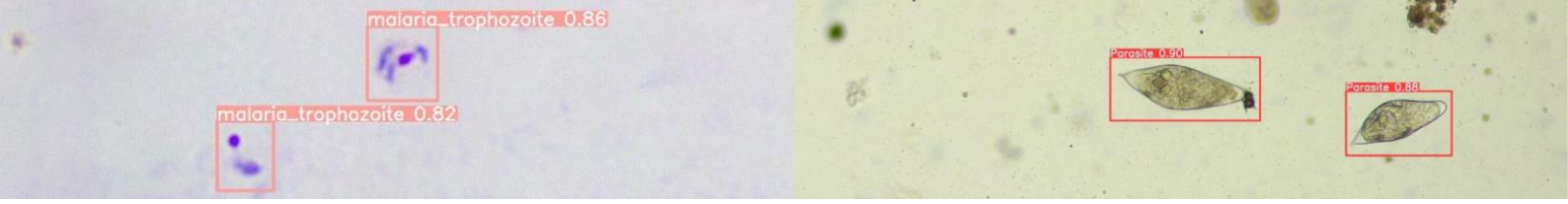

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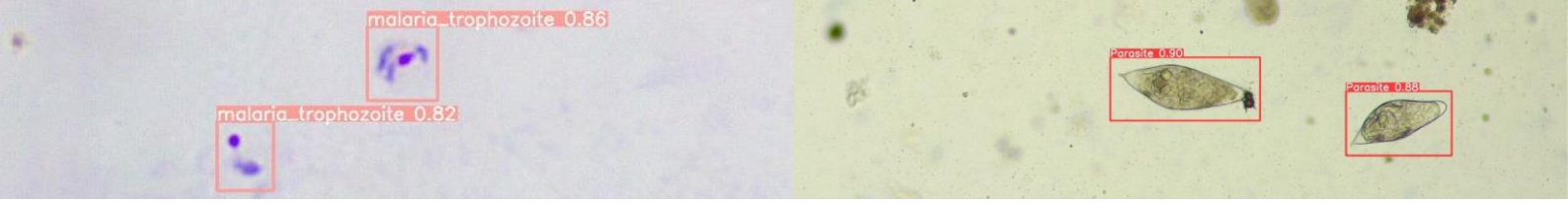

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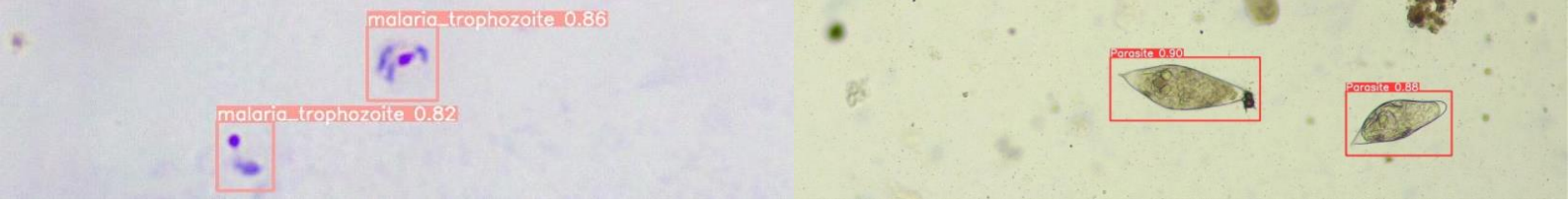

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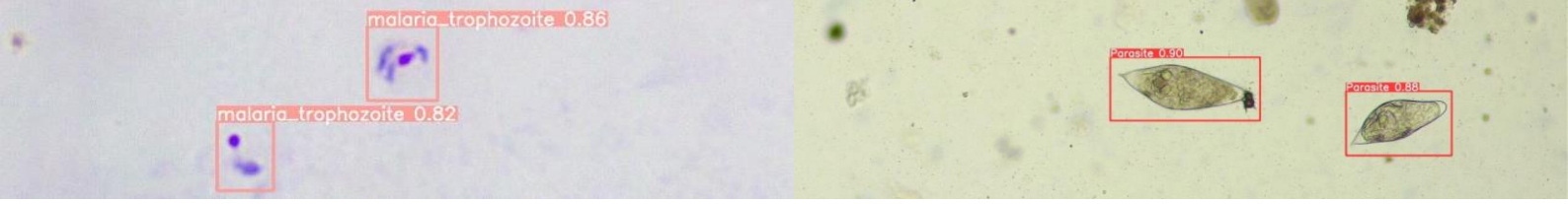

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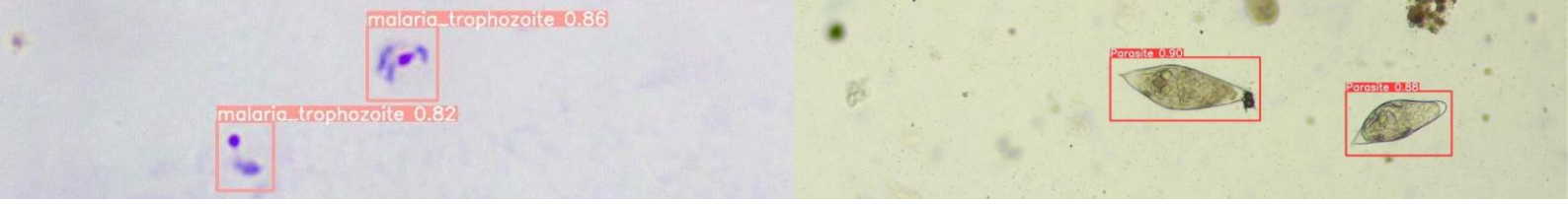

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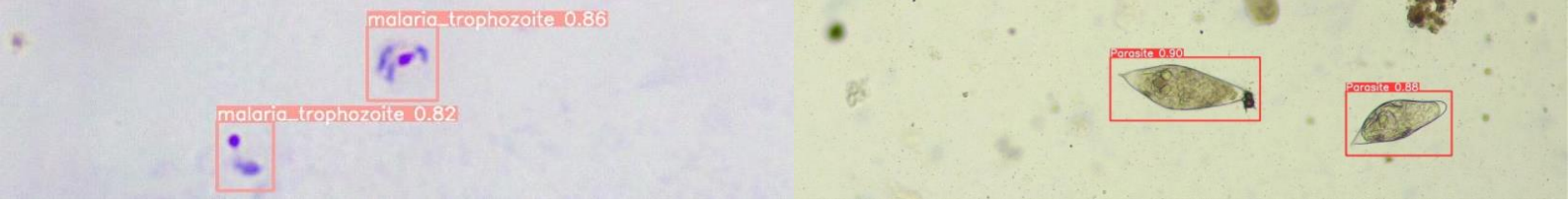

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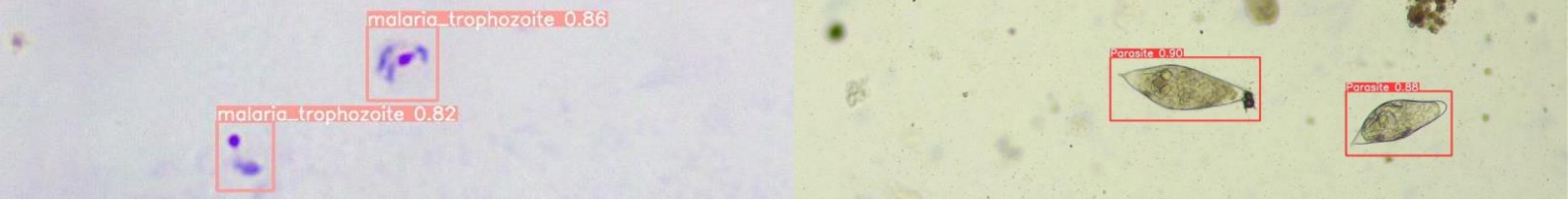

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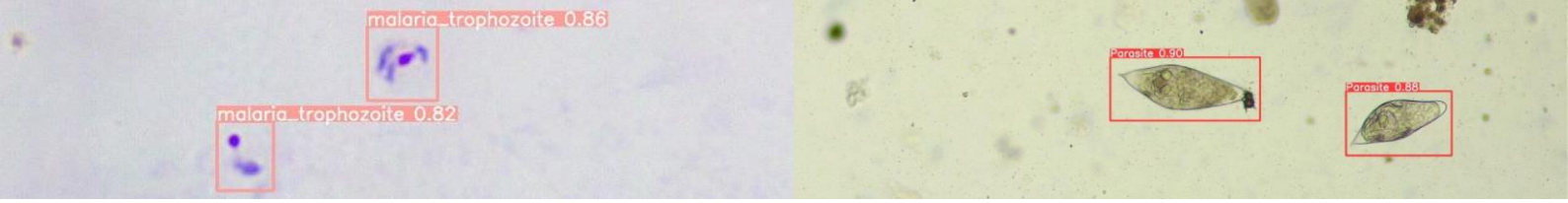

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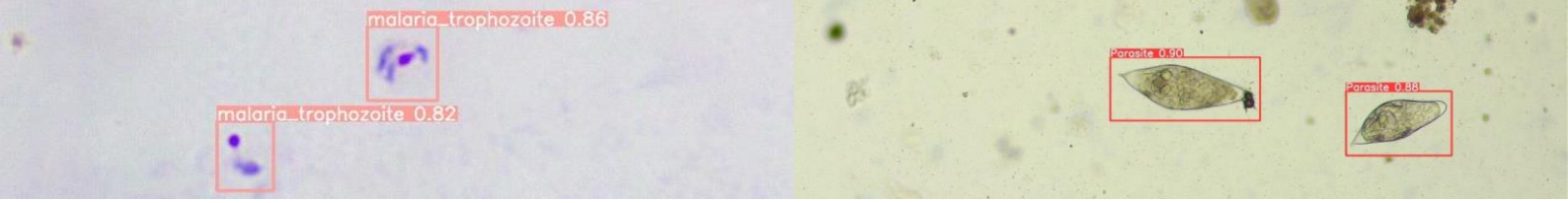

9. Annex

Advances and challenges in automated malaria diagnosis using digital microscopy imaging with artificial intelligence tools: A review

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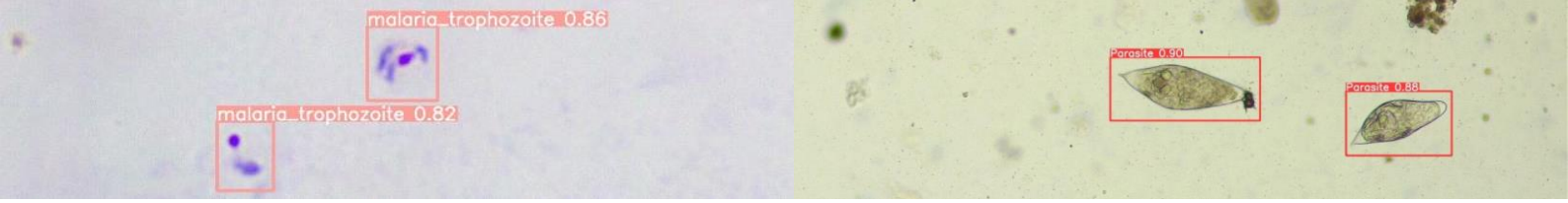

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Advances and challenges in automated malaria diagnosis using digital microscopy imaging with artificial intelligence tools: A review

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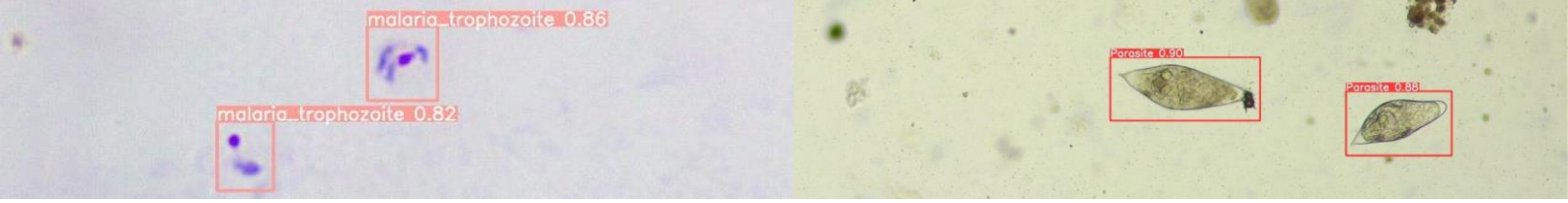
Malaria is an infectious disease caused by parasites of the genus *Plasmodium* spp. It is transmitted to humans by the bite of an infected female *Anopheles* mosquito. It is the most common disease in resource-poor settings, with 241 million malaria cases reported in 2020 according to the World Health Organization. Optical microscopy examination of blood smears is the gold standard technique for malaria diagnosis; however, it is a time-consuming method and a well-trained microscopist is needed to perform the microbiological diagnosis. New techniques based on digital imaging analysis by deep learning and artificial intelligence methods are a challenging alternative tool for the diagnosis of infectious diseases. In particular, systems based on Convolutional Neural Networks for image detection of the malaria parasites emulate the microscopy visualization of an expert. Microscope automation provides a fast and low-cost diagnosis, requiring less supervision. Smartphones are a suitable option for microscopic diagnosis, allowing image capture and software identification of parasites. In addition, image analysis techniques could be a fast and optimal solution for the diagnosis of malaria, tuberculosis, or Neglected Tropical Diseases in endemic areas with low resources. The implementation of automated diagnosis by using smartphone applications and new digital imaging technologies in low-income areas is a challenge to achieve. Moreover, automating the movement of the microscope slide and image autofocusing of the samples by hardware implementation would systemize the procedure. These new diagnostic tools would join the



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global effort to fight against pandemic malaria and other infectious and poverty-related diseases.

KEYWORDS

malaria diagnosis, digital imaging techniques, deep learning, artificial intelligence, microscopic examination, smartphone application, malaria

Introduction

Malaria is one of the most common infectious diseases worldwide. It is caused by *Plasmodium* parasites and transmitted to humans by the bite of an infected female mosquito of the *Anopheles* genus. Over 241 million malaria cases were estimated in 2020, an increase from the 227 million of 2019 according to the World Health Organization (WHO) (*World Malaria Report* WHO 2021). Malaria is endemic in 85 countries and caused 627,000 deaths in 2020. Africa is the most affected continent with 95% of all malaria cases reported and 96% of all deaths (Talapko et al., 2019; *World Malaria Report* WHO 2021). Low-income countries with non-accessible healthcare resources are the most affected regions and malaria-related mortality has a high correlation with poverty rates (Ren, 2019). Socioeconomic data were collected in several studies to demonstrate the aforementioned correlation, describing the global health situation of malaria in low-income countries (Ricci, 2012; Konishi et al., 2016). An early diagnosis, suitable treatment, and prevention strategies such as vaccination or mosquito net control are crucial to fighting the infection. Due to its high global health impact, this infectious disease is still a global issue. In addition, the COVID-19 pandemic has increased the number of malaria deaths and cases from previous years, due to the high impact of this pandemic on the administration of healthcare resources worldwide (Heuschen et al., 2021).

Plasmodium infection is produced by several protozoan parasites of the genus *Plasmodium* spp. (Tangpukdee et al., 2009). Five species of malaria cause infection in humans: *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae,* and *P. knowlesi*. *P. falciparum* is the most virulent species and produces the vast majority of deaths from severe malaria (Heide et al., 2019). The life cycle of *Plasmodium* parasites is represented in Figure 1. The life and infective cycle of the five species are similar, and their morphology and biology are analogous (Talapko et al., 2019).

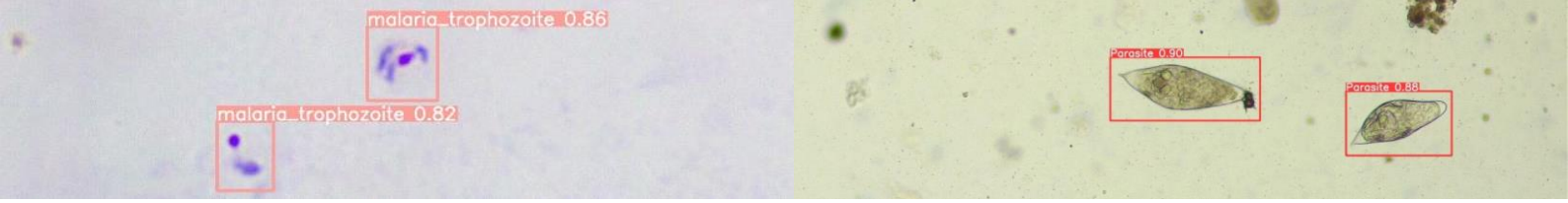
Malaria treatment is crucial to reducing mortality. Prompt treatment is recommended, within 24 h of the onset of fever, and is fundamental for the reduction of mortality among children <5 years of age (Simba et al., 2018). After confirmation of *Plasmodium* infection by laboratory diagnostic techniques, such as Rapid Diagnostic Tests (RDT) or microscopy, anti-malarial drugs are administered. The treatment used should be determined by *Plasmodium* species, parasitaemia density, drug-resistant pattern where the infection was acquired, signs of severe malaria, and patient tolerance of oral medication (Griffith et al., 2007).

The implementation of early detection systems for malaria epidemics is a high priority in Sub-Saharan African regions (Guintran et al., 2006). New advances in the regulation and development of malaria vaccines, such as the RTS, S/ASO1 vaccine recommendation by the WHO, can reinvigorate the fight against malaria (*WHO recommends groundbreaking malaria vaccine for children at risk*, 2021). Laboratory techniques for malaria diagnosis by detecting *Plasmodium* parasites are extensively used worldwide; microscopic visualization of thin and thick blood smears is the gold standard technique for malaria diagnosis. RDTs are also used as recommended diagnostic tools and could be an affordable complement for a precise diagnosis due to their rapidness and easy handling. Both microscopic visualization and RDTs have their limitations and new diagnostic techniques are emerging to complement the tools used nowadays. As a breakthrough, new image analysis techniques based on deep learning, a subfield in artificial intelligence (AI), are being developed for the automated diagnosis of blood slides. Distinguishing between erythrocytes infected or uninfected with malaria parasites is possible with deep learning detection-based models. Image analysis techniques allow the detection of malaria parasites in digital images by pre-trained deep learning models with large image datasets. This process would emulate the optical microscope visualization of thick and thin blood smear samples and automate the procedure. Smartphone applications could integrate image analysis technology based on AI and would be an affordable option for resource-poor environments in endemic areas.

Identification of the different parasite morphologies in the whole *Plasmodium* life cycle is crucial to perform a correct diagnosis by microscopic examination of blood smears. The life cycle must be considered when experts perform manual labelling of digital images. Immature *P. falciparum* trophozoites (ring stage), White Blood Cells (WBCs), and erythrocytes are commonly labelled in malaria thick and thin blood smear digital images (Manescu et al., 2020). The labelled data would be used to train deep neural network models and create AI algorithms capable of detecting parasites and cells.

Malaria diagnosis

Malaria diagnosis is crucial to treat and eradicate *Plasmodium* infections. An early diagnosis is determinant in effectively fighting



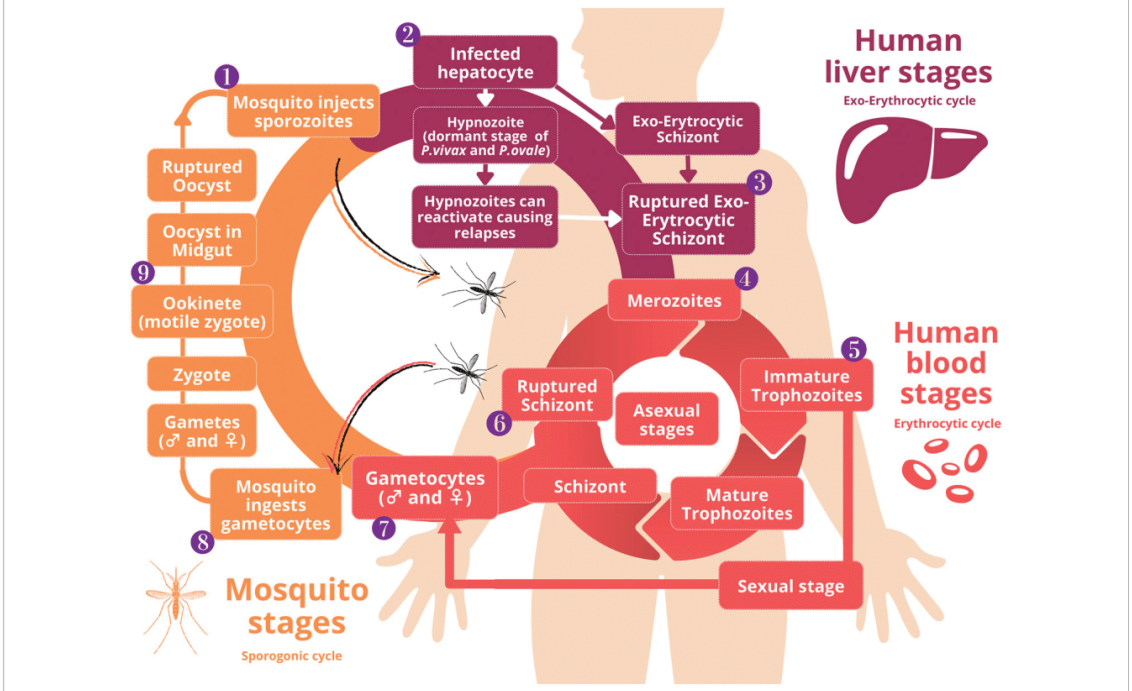


FIGURE 1
Life cycle of the *Plasmodium* parasite. Mosquito, human liver, and human blood stages are represented. **(1)** Mosquito injects sporozoites; **(2)** Infected hepatocyte; **(3)** Ruptured exo-erytrocytic schizont; **(4)** Merozoites in peripheral blood; **(5)** Immature trophozoites in peripheral blood; **(6)** Ruptured schizont; **(7)** Some immature trophozoites develop into sexual precursor cells named gametocytes; **(9)** Ookinete (motile zygote).

against infection. Laboratory diagnosis is accepted worldwide and recommended for malaria detection (Tangpukdee et al. 2009; *World Malaria Report* WHO, 2021). Diagnostic methods for infectious diseases should be fast, accurate, simple, and affordable (Vila et al., 2017). Several techniques are available and used to directly or indirectly detect the presence of malaria parasites in blood. Table 1 shows the advantages and disadvantages of the most important diagnostic methods for malaria parasite detection.

Clinical diagnosis

Clinical diagnosis is the least expensive option for malaria diagnosis (Wongsrichanalai et al., 2007), although the non-specific symptomatology and possible confusion with other infections or diseases with similar manifestations could overlap with the final diagnosis. Patient origin, malaria season, and age group are important aspects to consider. Clinical symptomatology could vary depending on the phase of the disease and the *Plasmodium* parasite species producing the infection. *Plasmodium* infection could produce asymptomatic, placental, uncomplicated, and severe malaria depending on the symptomatology and infection phase (Molyneux, 1989; Bartoloni and Zammarchi, 2012; Phillips et al., 2017).

Clinical symptomatology should be complemented with laboratory diagnostic techniques to confirm the presence of *Plasmodium* parasites. Blood smear samples are used in the vast majority of diagnostic techniques.

Microscopic examination of blood smears

Direct microscopic examination of blood smears to observe malaria parasites is the gold standard technique for malaria diagnosis (Guintran et al., 2006; Collins and Jeffery, 2007; Heide et al., 2019). Prior to examination, the specimen is mostly stained with Giemsa or Leishman staining (Bejon et al., 2014), to afford the parasites a distinctive appearance (*Malaria diagnosis and treatment CDC*, 2019). The protocol for Giemsa staining of malaria blood films is a simple and fast technique to visualize the active form of parasites in blood (Turrientes and López, 2016). Malaria microscopy standard operating procedure is the protocol recommended by the WHO (*Giemsa staining of malaria blood films* WHO, 2016). The blood smear examination procedure is shown in Figure 2.

Knowing the life cycle of *Plasmodium* parasites (Figure 1) is important to perform a correct identification of the different

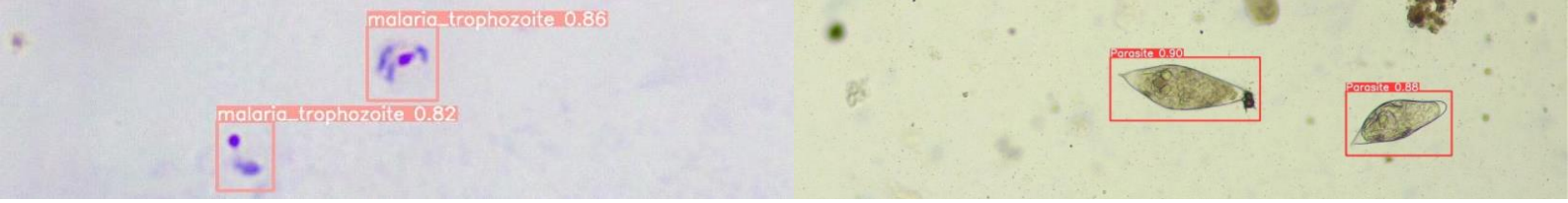


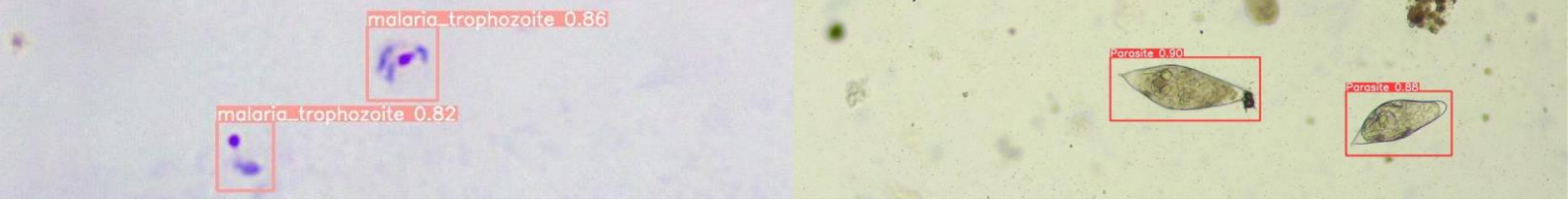
TABLE 1 Advantages and disadvantages of malaria diagnostic techniques.

Diagnostic technique	Advantages	Disadvantages	References
Microscopic examination	(i) Availability (ii) Low-cost diagnosis (iii) Parasite level calculations (iv) Species identification	(i) Requires expert personnel (ii) Results are expert-dependent	Dowling and Shute (1966), Collins and Jeffery, (2007), Guintran et al. (2006), Wangai et al. (2011), Poostchi et al. (2018), Heide et al. (2019), *Malaria diagnosis and treatment* CDC, 2019
Quantitative Buffy Coat (QBC)	(i) Fast preparation and diagnosis results (ii) High sensitivity	(i) Requires expert personnel (ii) Requires fluorescent microscopy (iii) Specialized instrumentation	QBC Malaria Test, 2007, Tangpukdee et al. (2009), Siciliano and Alano (2015), *About Malaria* CDC, 2019, Shujatullah et al., 2006
Rapid Diagnostic Tests (RDTs)	(i) Fast preparation and diagnosis results (ii) Easy handling (iii) Low-cost diagnosis (iv) Species identification (usually *P. falciparum* from non-*P. falciparum* species)	(i) *pfHRP2/3* gene deletions (ii) Low sensitivity with low parasite levels (iii) Low sensitivity with *P. ovale* and *P. malariae* species. (iv) Cross-reactivity (v) Prozone effect	Wongsrichanalai et al. (2007), Gillet et al. (2009), Murray and Bennett (2009), Tangpukdee et al. (2009), Bejon et al. (2014), Nima et al. (2017), Orish et al. (2018), Cunningham et al. (2019), *Response plan to phrp2 gene deletions* (WHO, 2019), Ajakaye and Ibukunoluwa (2020), Kavanaugh et al. (2021), Kavanaugh et al. (2021)
PCR	(i) High sensitivity and specificity (ii) Species identification (iii) Reference tool for comparative studies	(i) Specialized instrumentation (ii) Difficult implementation in endemic areas (iii) Expensive diagnosis	Johnston et al. (2006), Li et al. (2014), Poostchi et al. (2018), Siwal et al. (2018), Haanshuus et al. (2019), Eshag et al. (2020), Leski et al. (2020), Feufack-Donfack et al. (2021), Mwenda et al. (2021)
LAMP	(i) High sensitivity and specificity (ii) Species identification (iii) No thermocyclers needed	(i) Specialized instrumentation (ii) Expensive diagnosis	Ocker et al. (2016), Selvarajah et al. (2020), Morris and Aydin-Schmidt (2021)
Serology	(i) Seroprevalence (ii) Malaria transmission	(i) Non-reliable diagnostic technique (ii) Not indicative of active infection	Tangpukdee et al. (2009), Oviedo et al. (2020)
Flow cytometry	(i) Quantification of infected erythrocytes (ii) Automated parasite level calculations	(i) Low sensitivity (ii) Specialized instrumentation (iii) Difficult implementation in endemic areas	Poostchi et al. (2018), Khartabil et al. (2022)
Biomarkers	(i) High diagnostic potential (ii) Easy handling	(i) Specialized instrumentation	Jain et al. (2014), Krampa et al. (2017))

QBC: Quantitative Buffy Coat, RDTs: Rapid Diagnostic Tests, PCR: Polymerase Chain Reaction, LAMP: Loop-Mediated Isothermal Amplification, Serology, Flow cytometry, and biomarkers.

developmental stages of the parasites and the species for diagnosis. *P. falciparum* usually causes higher parasite levels and produces most malaria deaths in Africa (*World Malaria Report* WHO, 2021). Maurer dots, poly-infected erythrocytes, and the characteristic banana shape of gametocytes are distinctive traits of *P. falciparum* infection (Zekar and Sharman, 2021). *P. vivax* and *P. ovale* are species sharing some similarities in the shape of parasites and quiescent liver forms. Both species infect young erythrocytes, have Schüffner's dots, tend not to have multiple rings per cell, and contain malarial pigment. *P. malariae* usually causes lower parasite levels, due to its 72-h development cycle (24 h longer than *P. falciparum* and *P. vivax*), the lower production of merozoites per erythrocytic cycle, the predilection of parasites to develop inside old erythrocytes and the earlier development of immunity due to the combination of the previous factors (Collins and Jeffery, 2007). *P. knowlesi* is mostly present in Southeast Asia and was originally known as simian malaria. Due to its 24-h development asexual cycle, *P. knowlesi* infection can rapidly progress into severe malaria. Ring stage forms of *P. knowlesi* resemble *P. falciparum* and mature trophozoites and schizonts are similar to *P. malariae* forms (Amir et al., 2018). Gametocytes, the sexual stage of the parasite, are not responsible for clinical symptoms (*Treatment of malaria CDC*, 2013).

Microscopic visualization of thin blood smears allows the *Plasmodium* species identification from erythrocyte morphology and the distinctive features depending on the type of specimen infection. Thick blood smears are more efficient and provide higher sensitivity than thin blood smears (Wangai et al., 2011). The combination of both methods allows experts to determine the type and severity of the infection with a precise diagnosis. Parasite level calculations are performed manually in both types of samples. Direct microscopy observation is a tedious and time-consuming technique that requires experience and training. Continuous visualization of blood smears could trigger diagnostic errors due



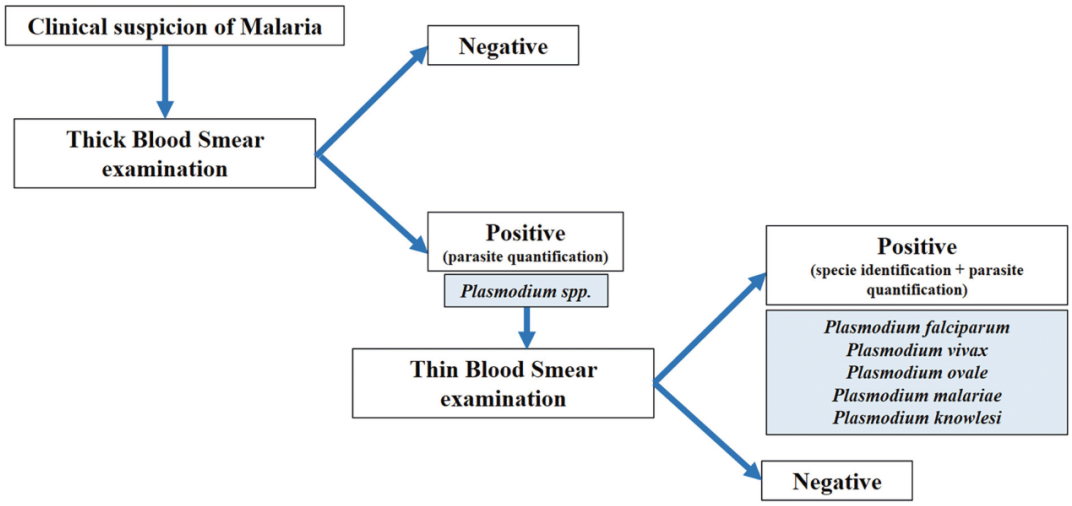


FIGURE 2
Blood smear microscopic examination procedure. Thick blood smear is first examined to determine the presence of malaria parasites. If the sample is positive, a thin blood smear is examined to determine *Plasmodium* species identification. Parasite quantification is performed to determine severity of the infection.

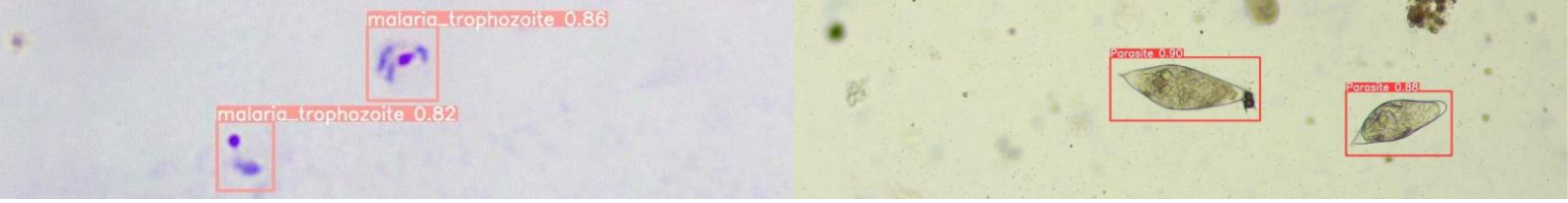
to the difficulty of the procedure (Dowling and Shute, 1966). The quality of the microscope and the staining reagents are also limiting factors (*Malaria diagnosis and treatment CDC*, 2019). False-negative cases lead to the unnecessary use of antibiotics, other consultations and, in some cases, progression to severe malaria. False-positive cases imply a misdiagnosis, unnecessary use of anti-malaria drugs, and suffering their potential side effects (Poostchi et al., 2018). However, microscopic examination of thin and thick blood smears is commonly used in endemic areas and resource-poor settings, due to its availability and easy handling. Other diagnostic techniques could complement and improve traditional microscopic examination and resolve its limitations.

Quantitative Buffy Coat

The Quantitative Buffy Coat (QBC) test is a qualitative screening method for rapidly detecting the presence of malaria parasites in centrifuged capillary and venous blood (*QBC Malaria Test*, 2007). Blood is centrifuged in specially coated QBC tubes and visualized by optical fluorescence microscopy. The technique is based on a density gradient that separates the blood cells and allows the identification of parasitic forms by fluorescent microscopic observation of the capillary tube. The dye commonly used is acridine orange, which allows the identification of parasites between the erythrocyte and leukocyte areas. The QBC tubes also have an anticoagulant for the correct visualization of the sample and to avoid artefacts due to blood clotting (*QBC Malaria Test*, 2007). QBC presents higher sensitivity and specificity than conventional thick blood smear diagnosis due to the additional concentration of parasites in the narrow zone of the blood tubes (Siciliano and Alano, 2015; *About Malaria CDC*, 2019). This technique requires well-trained personnel, specialized instrumentation, is costlier than conventional light microscopy, and is difficult to determine the species and number of parasites (Tangpukdee et al., 2009).

Rapid diagnostic tests

Rapid Diagnostic Tests (RDTs) are a suitable option and complement for detecting *Plasmodium* infection. RDTs are lateral-flow immunoassays that allow visualization of specific antigen–antibody recognition events (*Response plan to phrp2 gene deletions WHO*, 2019). They confer a qualitative diagnosis with a fast response time of less than 30 min (Cunningham et al., 2019). RDTs depend on the observation of a visible band on a nitrocellulose strip produced by the capture of dye-labelled antibodies. A drop of peripheral blood and a buffer solution are usually used to perform the diagnosis on the RDT device by detecting specific *Plasmodium* antigens. The majority of RDTs are based on the detection of the *P. falciparum*-specific protein histidine-rich protein II (HRP2) or universal antigen target for all malaria parasites, such as *Plasmodium* lactate dehydrogenase (p-LDH) or aldolase (Tangpukdee et al., 2009). HRP2 is localized in the cytoplasm of *P. falciparum* and on the surface membrane of infected erythrocytes (Murray and Bennett, 2009). Gene deletions of the parasite target gene *pfhrp2* are observed in several studies in endemic areas such as Ethiopia and Bangladesh (Wongsrichanalai et al., 2007; Bejon et al., 2014; *Giemsa staining*



of malaria blood films WHO, 2016; *Treatment of malaria CDC*, 2013; Nima et al. 2017). False-negative results due to *pfhrp2/3* gene mutation could trigger an incorrect diagnosis. Low parasite density, incorrect interpretation of results, or *P. malariae* and *P. ovale* infections are also causes of false-negative results and reasons for an incorrect diagnosis by RDTs (Kavanaugh et al., 2021). A prozone effect due to excess antigen could trigger an incorrect diagnosis, although it is not a common event (Gillet et al., 2009). False-positive results are less common and can also trigger an incorrect diagnosis. Cross-reactivity due to high parasite levels or the presence of other disease antigens are the main causes of false-positive results (Orish et al., 2018; Kavanaugh et al., 2021; Pyle-Eilola et al., 2021). RDTs are a useful diagnostic support feature for conventional diagnosis, however, they cannot substitute microscopy examination (Ajakaye and Ibukunoluwa, 2020).

Polymerase chain reaction

Polymerase Chain Reaction (PCR) diagnosis is a suitable alternative to conventional techniques. It is based on the amplification of *Plasmodium* DNA, and has high sensitivity, specificity and relatively low complexity (Leski et al., 2020). It is more sensitive than microscopy and capable of identifying malaria parasites at the species level when conventional methods are not able to detect the parasite (Johnston et al., 2006). In addition, the determination of *Plasmodium* species by PCR assay allows the unequivocal diagnosis in mixed species infection (Siwal et al., 2018) or low parasite levels (Haanshuus et al., 2019), which are difficult to detect by microscopic examination. Some of the main disadvantages of PCR diagnosis are the implementation of a non-routine technique in remote areas, the long-time (2–3 h) needed for diagnosis, and the high cost of the technology (Poostchi et al., 2018). Nowadays, PCR is being implemented as a diagnostic technique for malaria, although it is not the gold standard procedure and is not more widely used in endemic countries. Molecular techniques are useful to detect asymptomatic patients or those with very low parasite levels; their performance with this casuistry is considerably better than the other diagnostic techniques employed (Mwenda et al., 2021). This molecular diagnosis technique is commonly used in high-income countries or regions to perform epidemiological studies (Li et al., 2014; Eshag et al., 2020; Feufack-Donfack et al., 2021). As an example, novel PCR assay, such as MC004 RT-PCR, is demonstrated to be a useful tool for clinical settings and has a high degree of sensitivity and specificity (Beyene et al., 2022).

Loop-mediated isothermal amplification

Loop-Mediated Isothermal Amplification (LAMP) is a molecular technique based on the amplification of nucleic acids employing *Bacillus stearothermophilus* DNA polymerase (Morris and Aydin-Schmidt, 2021). It has a 99% sensitivity and 93% specificity for malaria parasite detection compared with microscopy and does not require thermocyclers (Ocker et al., 2016). A fluorescence spectrophotometer is usually needed to read-out diagnostic results, which restricts the applicability in rural areas. However, new LAMP assays are designed with a fluorescence readout unit in order to detect *P. falciparum* parasites (Puri et al., 2022). It is not widely implemented as a diagnostic method, although it is postulated as an interesting alternative to conventional PCR methods and could be progressively implemented in resource-poor settings (Selvarajah et al., 2020).

Other diagnostic techniques

Serology is based on the detection of antibodies against blood-stage malaria parasites. It is not commonly used for a rapid malaria diagnosis, although it is mainly used to perform seroprevalence studies of the disease. As an example, Immunofluorescence Antibody Testing (IFA) uses specific antigens for the quantification of IgG and IgM antibodies in serum samples (Tangpukdee et al., 2009). Combined strategies using serological, antigen detection, and DNA data are used to estimate malaria transmission and perform epidemiological studies (Oviedo et al., 2020).

Flow cytometry is a laser-based cell counting method that allows the quantification of erythrocytes infected by malaria parasites. It offers automated parasite level counts and has a low sensitivity (Poostchi et al., 2018). New advances based on fluorescence flow cytometry have shown that the Sysmex XN-31 device can determine the *Plasmodium* species and quantify parasites in blood. However, it can generate false positive results in case of abnormal erythrocytes cell morphology and the device was tested in a non-endemic region (Khartabil et al., 2022).

Biomarkers are cellular, biochemical, or molecular alterations that indicate the presence of biological, pathogenic, or therapeutic responses, with a high potential for diagnosis (Jain et al., 2014). The development of malaria biomarker detection, multiplex biomarkers for multiple *Plasmodium* parasite infections, and biosensors are new improvements to be considered as diagnostic tools (Krampa et al., 2017).

Diagnostic methods comparison

To perform a comparison between the different diagnostic methods for malaria parasite detection it is important to consider the parasite levels. Low parasite levels are related to lower sensitivity values due to the less number of parasites in blood. Higher parasite levels are easier to detect with all the aforementioned techniques, although in some specific cases, a prozone effect could trigger antigen detection issues by RDTs (Gillet et al., 2009). The commercial brand of the techniques

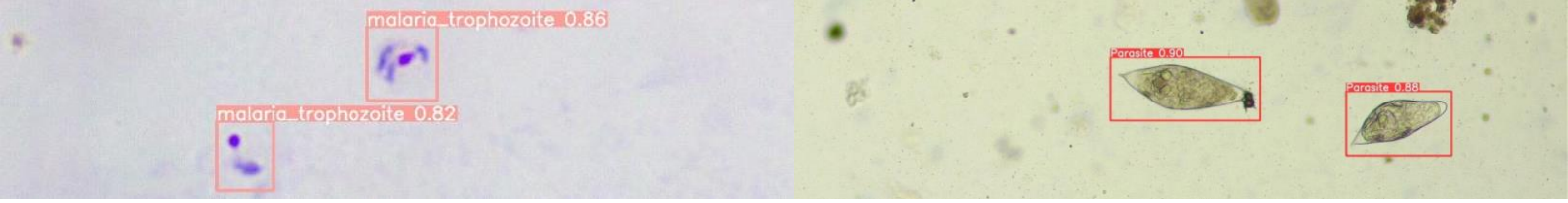


TABLE 2 Sensitivity and specificity of malaria diagnostic methods.

Diagnostic methods	Sensitivity	Specificity	Specifications	References
Microscopy	75.20%	97.12%	Comparative study.**Thick blood** films are 20–40 times more sensitive than **thin blood** films. Parasite density interferes with the final result and is crucial to obtain a reliable conclusion.	Bejon et al. (2006), Wangai et al. (2011), Feleke et al. (2021)
QBC	55.9%	88.8%	Lagos State University Teaching Hospital.	Adeoye and Nga (2007)
	70.5%	92.1%	University College Hospital, Ibadan, Oyo State, Nigeria.	Ifeorah et al. (2017)
RDTs	84.2%	99.8%	BinaxNOW test.	DiMaio et al. (2012)
	63.4–100%	53.4–99.9%	Mixed brands (Comparative study).	Boyce and O'Meara (2017)
	84.2%	95.2%	University College Hospital, Ibadan, Oyo State, Nigeria / pLDH RDT Optimal.	Ifeorah et al. (2017)
	37–88% (37 and 51% in asympt.)	93–100% (28% in one outlier)	Mixed brands (Comparative study).	Feleke et al. (2021)
LAMP	100%	86–99%	LAMP compared with PCR.	Feleke et al. (2021), Puri et al. (2022)
	95–98%	91–99%	LAMP compared with PCR.	Morris and Aydin-Schmidt (2021)
PCR	Considered 100%	Considered 100%	Used as a reference to be compared with other techniques.	Feleke et al. (2021), Mwenda et al. (2021)

QBC: Quantitative Buffy Coat, RDTs: Rapid Diagnostic Tests, pLDH: *Plasmodium* lactose dehydrogenase, LAMP: Loop-Mediated Isothermal Amplification, PCR: Polymerase Chain Reaction, asympt: Asymptomatic.

(QBC, RDTs, and PCR), RDT storage conditions, and response time are crucial for the correct interpretation of diagnostic results and could affect the final outcome. In the case of thick and thin blood smears, the expertise of the microscopist is determinant. The reference technique used as the standard against which others are compared to evaluate the quality of the method is also decisive (Feleke et al., 2021). Table 2 shows the diagnostic methods most commonly used for malaria parasite detection in terms of sensitivity and specificity. In some cases, no differentiation between thick and thin blood smear samples was observed in comparative studies and meta-analyses to determine sensitivity and specificity. However, thick blood smears provide a higher sensitivity than thin blood smear samples (Wangai et al., 2011). PCR is considered to have 100% sensitivity and specificity and is usually used as the reference method.

Novel diagnostic tools by using image analysis techniques

The global health impact of malaria has accelerated the development and implementation of novel diagnostic strategies to fight against the disease. Novel diagnostic techniques based on image analysis and AI are being developed for malaria parasite detection; an emulation of microscopic visualization by image capturing and processing could be a fast and efficient alternative to performing the diagnosis. In the last years, computational microscopic imaging methods for object detection have held higher importance in medical and biomedical studies (Das et al., 2015). Several software applications and tools are being developed to detect malaria parasites in thick and thin blood smear sample images using conventional light microscopy (Luengo-Oroz et al., 2012; Dallet et al., 2014; Das et al., 2015; Pirnstill and Coté, 2015; Bashir et al., 2017; Oliveira et al., 2017; Laketa, 2018; Manescu et al., 2020; Yang et al., 2020; Yu et al., 2020).

Deep learning is a set of computational AI processes and methodologies that allow automated learning and the generation of algorithms by emulating the human brain. It is based on databases information, and uses artificial neural networks with multiple layers to train and generate AI algorithms (Alzubaidi et al., 2021). Deep learning has, in many aspects, boosted and improved the procedure for traditional computer vision imaging techniques (MK. Georgieff, 2016). Convolutional Neural Networks (CNN) are artificial neural networks widely used as trained classifier models to detect objects in images or videos by deep learning algorithms. Specifically, CNN classification is applied in medical diagnosis to analyse and extract efficient features from images as an AI healthcare tool (Sarvamangala and Kulkarni, 2021). Imaging radiology techniques for early diagnosis and treatment of emerging infectious diseases such as Zika, Ebola, or Chikungunya are other image analysis applications (Jardon et al., 2019). Microscope image analysis using a U-Net (convolutional network architecture) to segment and detect Leishmaniosis (Górriz et al., 2018) is a representative study of the wide variety of possibilities of CNNs. The high computing capacity achieved over the past years and the increased amount of training data for CNNs have boosted the

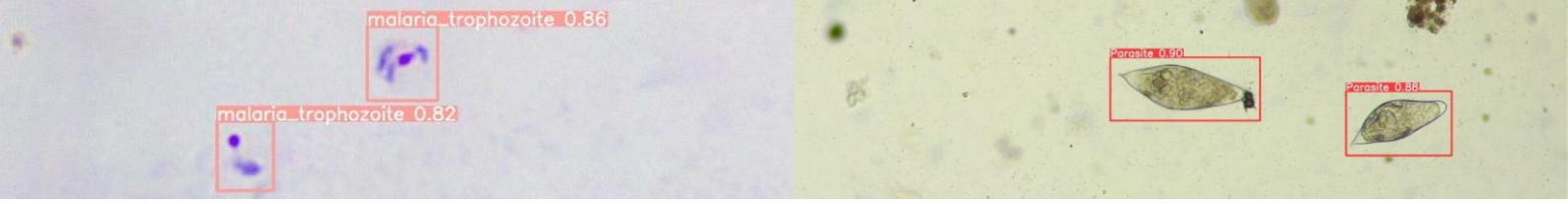
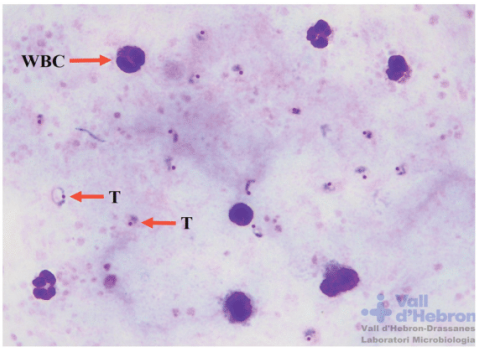
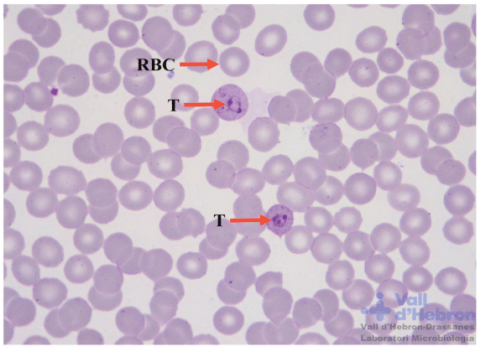


TABLE 3 Visual image differences between thick and thin blood smear samples to distinguish malaria forms by artificial intelligence techniques. (A) Thick blood smear sample 1,000x Giemsa staining. WBC nuclei and immature trophozoites (T) are distinguished with an arrow. (B) Thin blood smear sample 1,000x Giemsa staining. Erythrocytes infected with young trophozoites (T) of *P. falciparum* and uninfected erythrocytes (RBC) distinguished with an arrow. Maurer dots are present in infected erythrocyte morphology.

Thick blood smear sample	Thin blood smear sample
	
(A)	(B)
Main features	Main features
– Positive/Negative diagnosis.	– *Plasmodium* species identification by parasite and erythrocyte morphology.
– Possible to distinguish all development stages of the blood life cycle of the parasites.	– Parasite development stages identification inside erythrocytes.
– Non-species identification (except in the case of *P. falciparum* gametocytes).	– High specificity.
– Haemolysis of erythrocyte cells.	– Fewer artefacts and confusion forms.
– Amorphous morphology of immature *Plasmodium* trophozoite cytoplasm.	– Fixing sample with methanol in Giemsa staining technique.
– High sensitivity.	– Erythrocyte and staining artefacts.
– Common appearance of Giemsa artefacts.	
– Thick blood smear malaria parasite detection by artificial intelligence imaging tools (Rosado et al., 2016; Xiong et al., 2019; Manescu et al., 2020; Yang et al., 2020; Yu et al., 2020).	– Thin blood smear malaria parasite detection by artificial intelligence imaging tools (Ross et al., 2006; Tek et al., 2010; Dallet et al., 2014; Kareem et al., 2012; Mushabe et al., 2013; Oliveira et al., 2017; Rosado et al., 2017; Sankaran et al., 2017; Dantas Oliveira et al., 2018; Pillay et al., 2019; Pardede et al., 2020; Yu et al., 2020; Abubakar et al., 2021; Davidson et al., 2021).

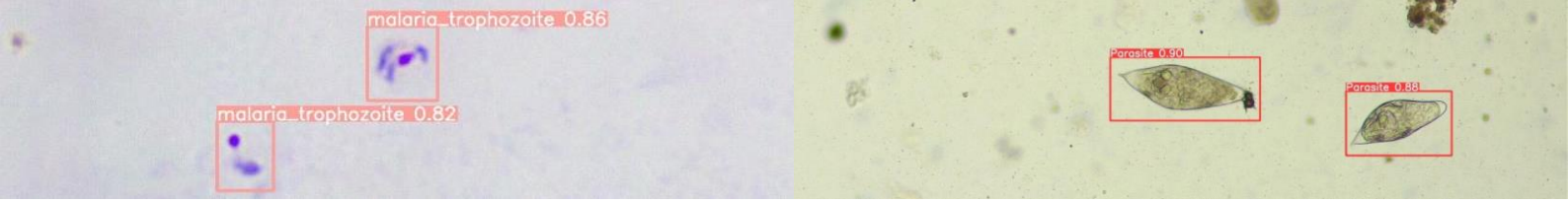
use of this technology for medical applications (O'Mahony et al., 2020).

In particular, automated microscopy imaging analysis could also be an alternative to conventional microscopy examination for malaria diagnosis. The preparation and type of sample are important facts to consider to perform the correct identification of biological features. Table 3 summarizes the visual image differences between thick and thin blood smears and their analysis by AI techniques (Ross et al., 2006; Mushabe et al., 2013; Dallet et al., 2014; Oliveira et al., 2017; Sankaran et al., 2017; Dantas Oliveira et al., 2018). Thick blood smear examination is crucial for a correct diagnosis of malaria, allowing the consequent visualization of thin blood smears for species identification (Figure 2). Thick blood smears are more sensitive and appropriate for low malaria parasite levels (Dowling and Shute, 1966). Nevertheless, the frequency of artefacts observed in this type of sample is higher in comparison with thin blood smears (Prairie, 2012).

CNNs for the detection of malaria parasites in thick blood smears are less used in comparison with thin blood smears. New automated parasite detection in thick blood smears based on deep learning and neural networks is an optimal alternative to traditional parasite microscopy visualization, as demonstrated in several studies (Xiong et al., 2019; Manescu et al., 2020; Yang et al., 2020). Other important factors for the visualization of thick blood smears are erythrocyte haemolysis, WBC cytoplasm rupture, and the variable shapes of ring-stage trophozoites. Most of the methods published for malaria parasite identification are based on supervised procedures that require a previous manual labelling procedure of microscopic images. Malaria digital images of thick and thin blood smears need to be labelled to create a dataset large enough to allow the generation of an optimal detection model (Shambhu et al., 2022). This process requires to manually define the bounding box of each parasite of a set of images to train the neural network model.

Image acquisition

Image capturing/acquisition is the first step towards generating an image database for future analysis and identification. Acquisition depends on the equipment and infrastructure of the laboratories. Microscope-integrated cameras are often used to acquire digital



images with conventional light microscopy. However, smartphone cameras with an adapter bracket are an affordable alternative for automated malaria diagnosis applications (Srikanth et al., 2008; Dallet et al., 2014; Rosado et al., 2016, 2017; Oliveira et al., 2017; Yu et al., 2020). Thus, in low-income countries, smartphone cameras would be a useful tool for acquiring digital images and replacing integrated microscope cameras, which are usually more expensive. The quality and resolution of the digital image, pixel morphology and density would determine future image processing and analysis. Other types of techniques for acquiring malaria parasite images with different microscopes are also used, such as fluorescent microscopy, binocular microscopy, or polarized microscopy (Poostchi et al., 2018). Nevertheless, image acquisition with conventional light microscopy is the most similar procedure to emulate conventional microscopic malaria diagnosis in endemic countries. Image acquisition is the first step for both traditional image processing techniques and deep learning methods (Hegde et al., 2019).

Traditional image processing techniques for malaria parasite detection

Image pre-processing is used in traditional computer vision techniques to automatically detect parasites and allows the preparation of acquired images to improve further analysis. Most studies perform noise reduction, enhancement of image contrast, and image resizing. These modifications would facilitate future procedures of feature extraction. As an example, Gaussian average filters or low-pass filters are used to reduce the noise of malaria microscopy images (Fatima and Farid, 2020). Moreover, background image assumption and colour normalization and correction to reduce the effects of illumination is an affordable solution to reduce image errors (Tek et al., 2016). Colour normalization and grey world-based colour normalization are pre-processing methods to minimise sample staining issues that could trigger image artefacts. Pre-processing imaging methods for smartphone image acquisition by colour normalization and background removal are useful tools to prepare images for the automated diagnosis of leishmaniasis or bartonellosis in remote locations (Cesario et al., 2012). Image resolution and quality are decisive to perform a correct and precise diagnosis *via* imaging methods.

Image segmentation is very often required to extract features. Segmentation consists of classifying each pixel as part of the objects in the original image. Morphological operations, Hough transform, K-means clustering, watershed algorithm, edge-based segmentation algorithms, rule-based segmentation, template matching, and marker-controlled watershed are segmentation techniques used for thin and thick blood smear images, among other applications (Poostchi et al., 2018). Many of these are complemented with thresholding techniques as a final step to extract and define the different segmented regions.

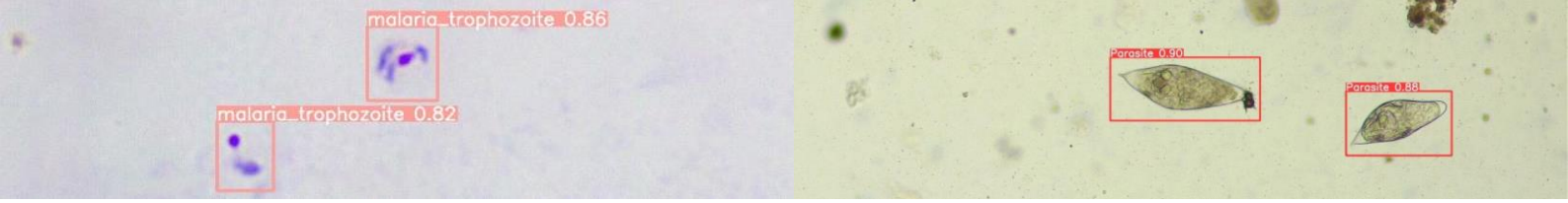
Feature extraction is the next procedure. The characterization of thin and thick blood smear images by features such as staining colours, cell texture, and morphology are carefully chosen (Poostchi et al., 2018). For example, erythrocyte feature calculations in thin blood smear images are performed by open-source platforms such as PyRadiomics 2.2.0 (Savkare and Narote, 2015). Feature extraction facilitates the subsequent learning and classification steps by providing quantitative information on certain image parameters.

Machine learning or pattern recognition is the final step of the image analysis procedure before identification. Classification methods are used for the identification of parasites and WBCs in thick blood smear samples, or infected and uninfected erythrocytes in thin blood smears. It is important to distinguish between the parasite identification procedures for the two sample types. In both cases, the performance of the technology developed should be optimized in terms of accuracy, sensitivity, and specificity (Poostchi et al., 2018). Most articles published on the identification of malaria parasites in thick blood smears are for *P. falciparum* infections (Yang et al., 2020). Thin blood smear parasite identification is used to distinguish between erythrocytes infected or uninfected with malaria parasites. In addition, parasite species identification and the development stage of the parasite in thin blood smears are detected by using traditional pattern recognition techniques that include, for example, Support Vector Machine (SVM) or logistic regression classifiers (Tek et al., 2010). Response time depends on the computational complexity of the predictive model. Complexity increases the time of response, although an evaluation between complexity and time is crucial to perform a correct and sufficiently fast identification (Freire et al., 2021).

Convolutional neural networks for malaria parasite detection

Convolutional neural networks are computational systems inspired by biological neurons designed to process data (Anwar et al., 2018). Image input is analysed to recognize visual patterns and complete the future identification of objects as an output. Neurons in deep networks are controlled by an activation function, which is responsible for controlling the output. Operations such as pooling and regularizers, with L1, L2 norms, batch normalization, or dropout are key elements to make the predictive models learn better and faster (Goodfellow et al., 2016; Anwar et al., 2018). Overfitting issue due to a memorization of data instead of learning could interfere in the final training outcome and obtaining of robust final predictions (Demšar and Zupan, 2021).

An important fact to consider when training CNNs is to have sufficient representative data. Data is commonly distributed into three sets: training, validation, and testing. The prediction model learns from the multiple examples of the dataset and the same training data is fed into the CNN repeatedly in an iterative procedure. During training, the validation dataset allows hyperparameter tuning and model evaluation by a continuous optimization. Finally, a test dataset is used to assess the model



after completing the training process with unseen data (Xu and Goodacre, 2018).

Object detection deep learning models are able to identify and locate objects of a certain class in images and videos (Jiao et al., 2019). During the last few years, object detection models have been improved and most of the state-of-the-art object detectors use deep learning networks. Usually, raw images need a simple pre-processing to resize them and fed them into the network. The model itself decides and computes the appropriate features and provides an output that leads to the identification and location of objects. Among other uses, medical imaging may benefit from object detection techniques, in particular, it could be a useful alternative to malaria parasite detection (Jiao et al., 2019).

Object detectors are classified as two-stage or one-stage. Two-stage detectors have high localization and object recognition accuracy, whereas one-stage detectors achieve high inference speed (Jiao et al., 2019). The most representative two-stage detector is Faster R-CNN (Ren et al., 2017) and one-stage object characteristic detectors are YOLO (Redmon et al., 2016) and SSD (Konishi et al., 2016).

In most cases, manually labelled data is required to perform all the aforementioned processes. Unsupervised training is an alternative, although most medical imaging studies are performed with supervised training data. Supervised learning based on image annotation is diverse and several strategies have been described (Sarangi, 2014). Whole-image classification is the annotation of the whole image as a type or class. Non-discerning objects are detected in the image, so the whole image is classified as a type. Object detection using bounding boxes within each image is another option when solving classification tasks. It requires a more time-consuming supervised annotation procedure of the different objects in the image. CNNs use the dataset and identify every bounding box as an object class (Ibrahem et al., 2022).

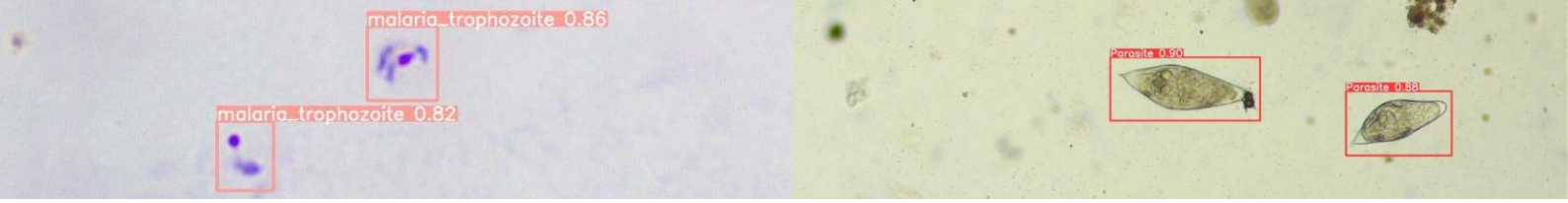
In the case of image segmentation, the identification of objects is based on a pixel-by-level classification. Each pixel is classified as a class object with its own value and annotations are manually added to images. However, it is an even higher time-consuming task for large databases, therefore automatic annotation procedures are being developed. Thus, other conventional machine learning methods and deep learning procedures are used to automatically annotate images (Murthy et al., 2015; Cao et al., 2020).

Furthermore, CNNs need large datasets with annotated data. ImageNet is one of the largest available datasets of universal images for researchers and non-commercial use (ImageNet, 2021). In the particular case of malaria, a sufficiently large dataset of malaria annotated images is needed to train CNN models and perform an automated identification of parasites. Malaria thick blood smears from the Institute of Electrical and Electronics Engineers (IEEE) DataPort is an open-source image dataset (*Malaria Thick Blood Smears | IEEE DataPort*, 2021). Strikingly, there are not many publicly available datasets of malaria thick and thin blood smear images. Data augmentation techniques, to artificially enlarge image datasets and obtain better performances, is nowadays used with promising results as DACNN model demonstrates (Oyewola et al., 2022).

CNNs have been shown to have optimum performance with computer-aided image diagnosis applications in specific fields of study and can be generalised for other medical imaging tasks (Shin et al., 2016). Object detection models, such as YOLOv3, YOLOv4 and YOLOv5 are used for malaria parasite detection (Abdurahman et al., 2021; Rocha et al., 2022). Feature scale and addition of detection layers are modifications that provide better performances than state-of-the-art articles. Moreover, Faster R-CNN (Hung and Carpenter, 2017; Ren et al., 2017) and SPPnet (Zhou et al., 2018) are optimized neural networks used to speed up and enhance identification time. Recent studies demonstrate the potential of CNNs for malaria parasite detection with promising results, such as VGG-19 model by transfer learning mechanism (Alnussairi and İbrahim, 2022; Jameela et al., 2022) or transformer-based models to obtain optimized performance parameters (Islam et al., 2022). The general procedure for malaria parasite detection using deep learning imaging methods is represented in the bottom part of Figure 3. Nowadays, CNNs have improved and replaced the use of traditional methods.

Automated malaria parasite level calculations using deep learning methods

Automated parasitaemia counting by image analysis is a useful tool that could overcome and provide support to manual parasite level quantification. Conventional malaria parasite level calculations by microscopy visualization of thick and thin blood smears are not precise and difficult to reproduce. An estimation is usually performed and, when parasite levels are high, is tedious and time-consuming. Thick blood smear parasite level quantification is routinely performed by counting the number of parasites and leukocytes in a blood smear sample (WHO and Regional Office for the Western Pacific, 2016). Thin blood smear quantification is based on the counting of infected erythrocyte cells in each microscopy field (WHO and Regional Office for the Western Pacific, 2016). Quantification of parasite levels *via* digital image analysis techniques would require the shortest period of time. An image analysis software was developed to perform this function automatically with thick blood smear images (Arco et al., 2015). For thin blood smear automated parasitaemia calculations there are image analysis tools available to improve conventional manual counting. Determination of malaria *P. vivax* parasite concentration is possible using image processing techniques (Prasad et al., 2020). *Plasmodium* AutoCount is a digital image analysis tool to perform an automatic count of parasites in Giemsa-stained thin blood smears (Ma et al., 2010). Other image processing tools based on OpenCV software libraries were satisfactory in determining parasite levels in thin blood smear samples (Swain et al., 2018). Previously mentioned methods used image processing techniques, such as noise reduction with filters and binary transformations, to determine the presence of malaria



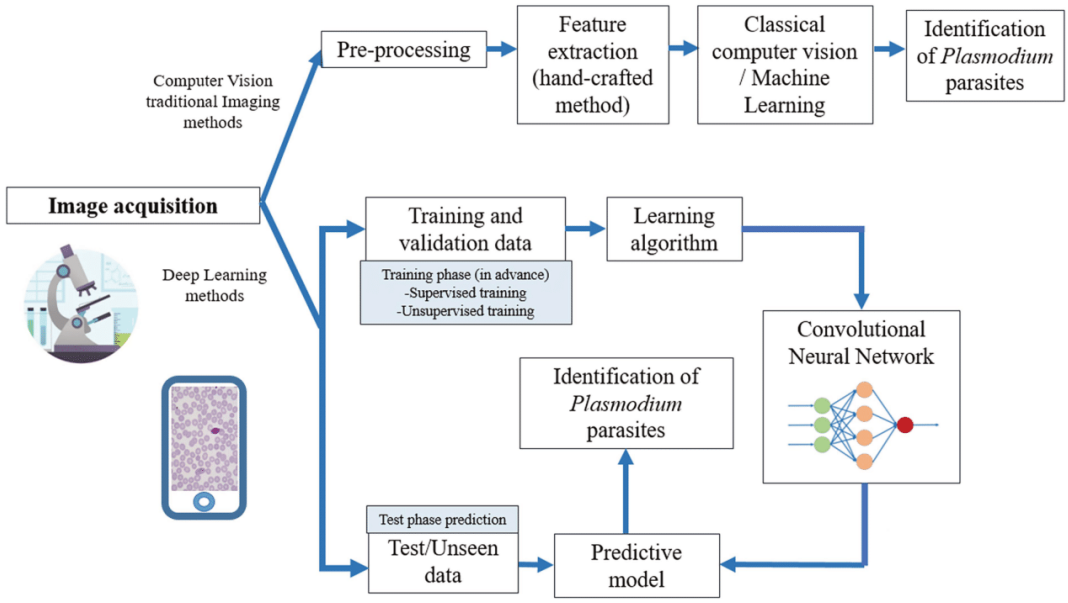


FIGURE 3
Representation of the different procedures using Traditional Computer Vision Imaging methods or Deep Learning methods (Convolutional Neural Networks) for malaria parasite identification in thick and thin blood smear samples.

parasites inside erythrocytes and perform a final parasite level calculation.

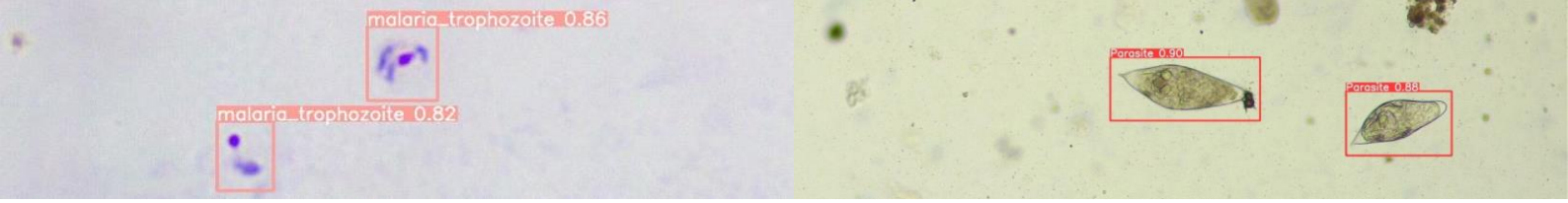
Mobile phone applications for malaria parasite detection

Mobile phone applications are being developed for the automatic detection of malaria parasites (Cesario et al., 2012; Rosado et al., 2016, 2017; Oliveira et al., 2017; Yu et al., 2020; Zhao et al., 2020). Smartphone image capturing is a suitable and easy alternative for the acquisition of blood smear images through the microscope lens. Only an optical microscope, a mobile adapter, and a conventional smartphone are needed to perform an imaging diagnosis. Mobile phone cameras could substitute integrated or external microscopy cameras and perform an optimum diagnosis by image analysis.

The integration of CNN predictive models in a smartphone software application is possible. Adapted CNN models perform the entire diagnosis in a single device. The coalescence between malaria automated diagnosis and smartphone software is a milestone and challenge for future implementation in worldwide laboratories. Image analysis and deep learning procedures allow smartphones to be one of the best alternatives for the implementation of automated malaria parasite detection. Even in resource-poor settings, smartphones are an available and relatively cheap option. One of the main problems of smartphone cameras was image quality and adaptation to microscopy lenses. Nowadays, smartphone cameras provide high image quality, although adaptation to the microscope is not as good as expected. Images could be disturbed by light microscopy issues, lens adaptation to smartphone cameras, or image quality downgrades related to image focus. Microscope image auto-focus is also an issue to solve. The technology to fully automate the entire procedure of image focusing, image acquisition, and parasite identification by an independent device is still required.

As an example, Malaria Screener is an affordable and effective solution for automatic malaria parasite detection by a mobile phone application (Yu et al., 2020). It combines image acquisition, smear image analysis, and result visualization. It is a semi-automated system based on digital images and CNN models to predict the presence of malaria *P. falciparum* parasites in thin and thick blood smears. Other applications were developed to combine automatic detection of malaria parasites *via* an optical magnification prototype with a smartphone device that performs image processing and analysis (Rosado et al., 2016). VGG16 classification CNN, or other CNN models, were integrated into smartphone applications to automatically detect the presence of malaria parasites inside erythrocytes in thin blood smear samples (Zhao et al., 2020).

Gamification of the technology for the identification of malaria parasites in digital images is also an innovative application. As an example, a web-based game where online volunteers analyse thick blood smear images to detect malaria parasites was



developed for the creation of an annotated image database (Luengo-Oroz et al., 2012).

To sum up, smartphone applications might be the future for autonomous image acquisition and analysis by AI technologies, and a suitable alternative for malaria and Neglected Tropical Disease (NTDs) diagnosis. The possibility to integrate predictive models and image acquisition in a single device confers a wide range of applications in the field of image analysis for diagnostics.

Microscopy automation linked to smartphone software technology

Microscopy automation to move blood smear samples and capture focused images automatically is a challenging approach. Automation would solve the limitations related to the non-fully autonomous diagnosis procedure performed. Image processing methods allow automation of the diagnosis, although a person is still needed to move the X-Y axis and issue focus of the microscope. A few studies have implemented automatic hardware devices to solve this problem and optimise the automation of malaria diagnosis (Kaewkamnerd et al., 2012; Gopakumar et al., 2018; Muthumbi et al., 2019). Microscopy adaptation is crucial to fully implement the aforementioned technology in real clinical and diagnosis practices. Low-cost hardware optimization with 3D printing models to manufacture specific parts or pieces of the microscope would be a suitable option in resource-poor settings with endemic malaria. A 3D-Printed portable robotic mobile-based microscope for the diagnosis of global health diseases is an example of the potential of this technology (García-Villena et al., 2021). As mentioned before, some studies present the possibility of developing an optical device that emulates or substitutes an optical microscope. An optical prototype with 1,000x magnification adapts to the smartphone camera and avoids possible light issues (Rosado et al., 2016). Nevertheless, conventional optical microscopy adaptation is the most suitable technique for image acquisition and analysis by smartphone applications.

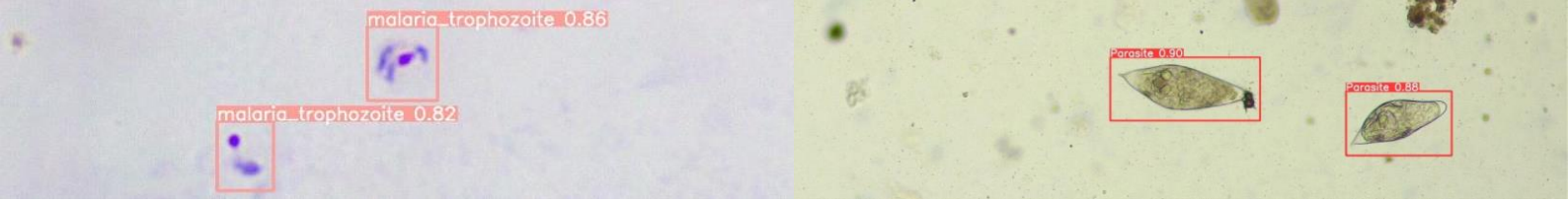
Implementation of malaria digital microscope imaging diagnosis in resource-poor settings

More than 90% of severe malaria produced by *P. falciparum* is estimated to affect young children under 5 years old in Sub-Saharan Africa, in areas with resource-poor settings (Schumacher and Spinelli, 2012; Gitta and Kilian, 2020). The gold standard method for malaria diagnosis by the WHO is still microscopy, although this is dependent on laboratory resources and could result in diagnostic errors due to a lack of instrumentation, medical devices, or well-trained laboratory staff. Microscopic examination of blood smears and RDTs are the techniques most used for malaria diagnosis and improvements aimed at the development of new and better diagnostic techniques are being implemented in endemic areas (Gitta and Kilian, 2020). The increase of RDT usage in malaria-endemic areas is replacing microscopic examination of blood smears due to the lack of resources and well-trained personnel. In addition, the biosocial situation of mothers and children in resource-poor regions, such as Imo State in south-eastern Nigeria, has an impact on the increased appearance of complicated malaria cases (Iloh et al., 2013). A non-precise diagnosis or treatment due to the low availability of resources is a serious issue in endemic areas. Consequently, the implementation of new and affordable diagnostic imaging techniques could help solve this problem.

Smartphones are a portable and suitable alternative for malaria diagnosis *via* imaging techniques, which could be implemented in resource-poor settings and remote endemic areas. They could improve and automate malaria diagnosis with less need for resources and personnel. CNN models could be integrated inside smartphone software and an internet connection would not be required. The provision of health centres with mobile devices by governmental organisations and national programs against malaria would be a determinant factor for the correct implementation of this novel technology for malaria diagnosis. However, due to the constraints specific to many malaria endemic areas, this may be a major problem to be addressed in the coming years by political willingness. The benefits of smartphones for diagnostics can be of significant value, not only for malaria, but also for the diagnosis of many other tropical diseases or NTDs (Vasiman et al., 2019). Due to that, implementation in regional hospitals or small healthcare centres would be a challenge for future studies. New object detection models trained with smartphone camera images are suitable for malaria diagnosis deployment in resource-poor settings (Abdurahman et al., 2021).

Diagnostic performance studies to validate the technology are a must for the future implementation of a tool. The performance evaluation should be carried out under ideal and resource-poor conditions to determine its effectiveness in different environments. There are many barriers to overcome in order to transition a product or technology from development to introduction and implementation. Some of the main barriers are the adjustment to the health and laboratory systems necessary to ensure effective adoption and implementation, demonstration of the technology's value, evaluation of operational viability, policy and regulatory requirements of government organizations, operation research to evaluate the net effect of the technology in the field, distribution, service and repair, and quality assurance and control (Palamountain et al., 2012).

The implementation of new diagnostic techniques in laboratory environments has to be regulated and controlled by the Food and Drug Administration and WHO protocols (Palamountain et al., 2012). The technology should be validated and accepted by international and national authorities as described (Mugambi et al., 2018). Most efforts to implement new diagnostic tools in resource-poor settings are focused on



infectious diseases such as HIV, Tuberculosis, and Malaria. Deep understanding and coordination of the stakeholders involved in the diagnostic development and implementation are milestones for the success of diagnostic interventions (Mugambi et al., 2018).

Discussion and concluding remarks

Epidemic malaria is very prevalent in Sub-Saharan Africa and tropical regions with low resources. It is still a global health issue that should be solved by mosquito control strategies, rapid and accurate diagnosis, and correct treatment (*World Malaria Report* WHO, 2021). Therefore, diagnosis is crucial for the eradication of the disease and to reduce mortality in prevalent regions. However, the recurrent problems in these environments with conventional microscopic examination due to lack of resources and experimented microscopists (Ngasala and Bushukatale, 2019), and the increasing failure of RDTs mainly due to gene mutations (Golassa et al., 2020), reinforces the necessity of developing new, affordable, and accessible diagnostic methods for *Plasmodium* infection.

Advances in image analysis and processing allow and postulate the implementation of automated malaria diagnosis as a new diagnostic tool. Thick and thin blood smears would be the samples analysed by the new technology. Traditional image analysis techniques were used to automatically detect malaria parasites in thin and thick blood smears (Turrientes and López, 2016), as demonstrated in several studies (Tek et al., 2010). The irruption of deep learning methodologies with CNNs has boosted and improved the results for the identification of malaria parasites in comparison with traditional computer vision techniques. For CNN models and, specifically medical image processing and analysis, it is crucial to have a large image dataset to obtain reliable results. Unfortunately, open-source image datasets are not globally available and are usually used for individual CNN training.

Open image availability would be a beneficial resource for the scientific community. The ImageNet (ImageNet, 2021), parasite image (Li and Zhang, 2020), and malaria thick blood smear (*Malaria Thick Blood Smears | IEEE DataPort*, 2021) databases are representative examples. Neural networks such as YOLO are used as CNN models to detect malaria parasites in blood smears (Abdurahman et al., 2021).

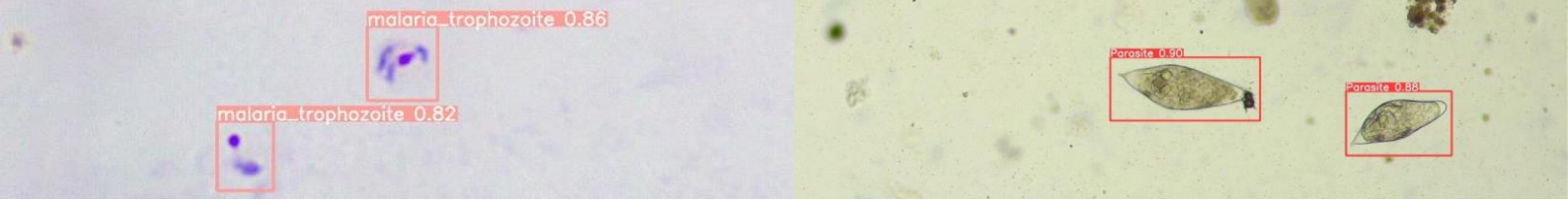
In addition, the integration of CNN models into smartphone software is possible. Thus, the implementation of digital image analysis-based diagnostic tools in endemic areas with smartphone applications could improve and automate malaria diagnosis by the emulation of the gold standard microscopy examination technique. As an example, fully automated systems, such as the slide screening microscope EasyScan GO, evaluate their performance against WHO slide samples with promising results (Horning et al., 2021). CNN-based models are widely used as predictive models with the capacity to distinguish parasite forms and blood cells and could be implemented in low-resource settings (Zhao et al., 2020). Automated parasite detection, parasite level calculations, and faster diagnosis are some of the main advantages of image analysis for malaria diagnosis. This technology could be used as a fast and precise tool to perform parasite level calculations (Ma et al., 2010). Overall, the use of smartphones and artificial intelligence techniques for diagnosis might help the global goal of malaria eradication in the coming years. The support and enhancement of traditional microscopy-based diagnostic techniques through the use of AI, the upgrading of laboratory infrastructures in malaria endemic areas and the improvement of computer technology over the years may help to implement such techniques in most remote areas. Integrating innovations into the current microscopy method would reinforce malaria elimination (Nema et al., 2022).

Hardware automation is still in the process of optimization to complete the goal of independent predictive and mechanized diagnostics. Other limitations such as image quality dependence, laboratory infrastructure requirement, local regulatory organization permissions, or the necessity to create a standardized protocol for the final diagnosis should be addressed. Nevertheless, several studies are improving predictive models, pre-processing techniques, microscope automation, and faster detections (Sriporn et al., 2020; Masud et al., 2020). Artificial intelligence improvements and better predictive algorithms due to computing power evolution could be an advance in terms of automatic image diagnosis with optimized predictive results in the following years. In conclusion, with diagnostic techniques based on image analysis, the samples used are the same (thick and thin blood smear) and the procedure of sample preparation, parasite observation and interpretation would be very similar to conventional microscopy. In addition, it would provide technical support to health professionals and help to automate the process in order to increase its efficiency.

In this review, we have summarised the main advances, challenges, and limitations in the automation of malaria diagnosis using digital image analysis by AI tools. Smartphone applications are a suitable option to integrate diagnosis technology into a single device and confer laboratories a new tool for malaria and other disease diagnoses. New advances and improvements in AI would be the final milestone for the optimisation and implementation of the technology worldwide. In conclusion, we are ever closer to developing a fast, efficient, and optimum new diagnosis tool for malaria parasite detection available for laboratories located in malaria-endemic regions worldwide.

Author contributions

CRM, ES, and JJ-M: conceptualized and drafted the manuscript, wrote the manuscript and designed the figures, AdO provided advice about artificial intelligence topics and designed the figures. FZ and ES provided continuous



intellectual feedback about malaria diagnosis and the protocols used nowadays. JJ-M, ES, FZ, ES, DL-C, SN, BB, MB, AV, ME, AA, and TP revised the manuscript and provided valuable feedback. ES and JJ-M edited and revised the overall manuscript. All authors agreed to be accountable for the content of the work.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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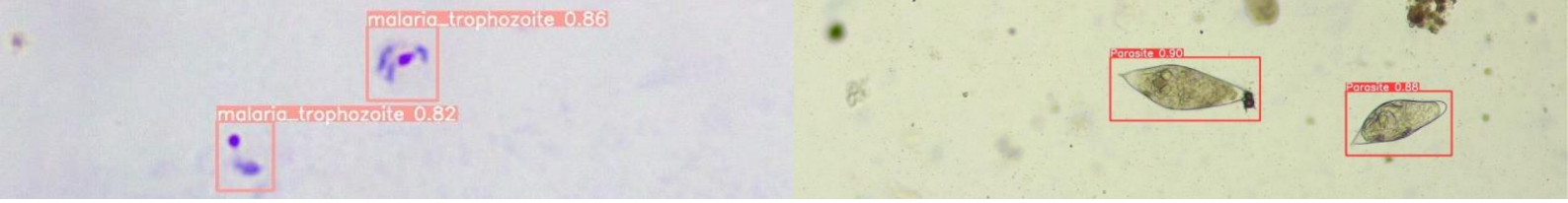

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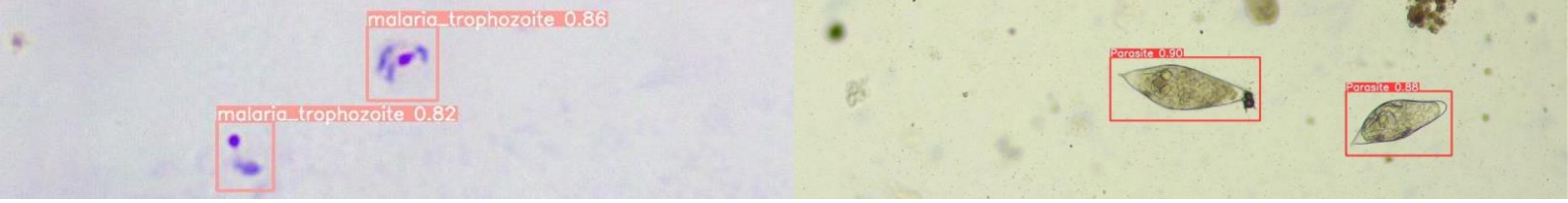

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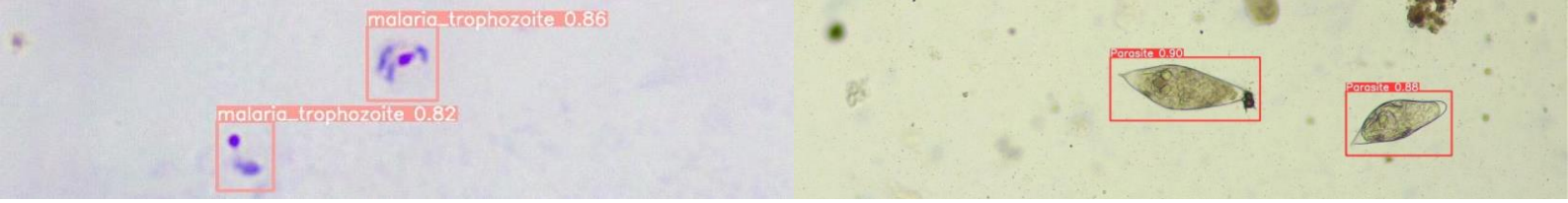

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