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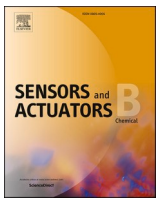

Mycobacterium detection method combining filtration, immunomagnetic separation, and electrochemical readout in a portable biosensing device

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ABSTRACT

This study addresses a straightforward and highly sensitive approach for detecting *Mycobacterium fortuitum*. The method involves a combination of filtration and direct immunomagnetic separation to isolate the bacteria retained on filters for further electrochemical magneto immunosensing in a handheld device. Unlike conventional methods involving pre-enrichment by culturing, this approach employs a simplified preconcentration technique, which includes filtration of large water samples, up to 100 mL, followed by a one-step process of immunomagnetic separation and labeling. After a 60-min, during which the filter with the retained bacteria and all the reagents (including modified magnetic particles and enzymatic conjugates) are incubated, the resulting product is directly drop into a cartridge, capable of performing magnetic actuation and washing. The electrochemical readout is carried out on a portable battery-operated device within 30 seconds. Remarkably, the immunosensor demonstrates an outstanding limit of detection of 5 CFU mL^{-1} in hemodialysis water processing 100 mL of sample. This achievement is remarkable considering the short and simplified analytical procedure, compared to the traditional isolation and culturing of mycobacteria, which typically takes 2 weeks.

1. Introduction

The genus *Mycobacterium* contains more than 170 species of non-tuberculous mycobacteria (NTM) that are commonly referred to as environmental mycobacteria [1,2]. Unlike *Mycobacterium tuberculosis complex* are the major responsible of infections, opportunistic NTM infections are increasingly prevalent worldwide [3,4], particularly among individuals with compromised immune systems or underlying lung diseases. Pathogenic environmental mycobacteria (PEM) frequently cause infections, often transmitted through water [5–8], via ingestion and inhalation, particularly from aerosols [7,9,10]. Rapidly growing PEM, including *Mycobacterium abscessus*, *Mycobacterium chelonae*, *Mycobacterium mucogenicum* and *Mycobacterium fortuitum* can eventually progress to disseminated bacteremia, meningitis, or other syndromes. Accordingly, [7]. Rapidly growing PEM is also responsible for health care-related outbreaks by contamination of potable water. An example is infections in hemodialysis patients, highlighting the need to identify reservoirs and minimize proliferation to prevent outbreaks [8,11]. Traditional detection of PEM in water relies on culturing [7]. Rapidly growing PEM takes 7 days to produce observable colonies, whereas slowly growing PEM need several additional days. Before culturing, water samples are decontaminated with alkali or acid solutions to eliminate other bacteria and fungi that could mask the PEM colonies, which can also have an impact on mycobacterial viability. Traditional

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culture-based PEM detection is time-consuming, labor-intensive, and potentially inefficient due to loss or underestimation of PEM. Nowadays, molecular techniques play an important role for detecting mycobacterial species in water samples. These methods involve identifying distinct mycobacterial DNA sequences, often employing culture enrichment or direct DNA isolation, followed by PCR amplification. Given the typically low concentration of mycobacterial DNA in environmental samples, PCR amplification is commonly employed [7,12,13]. Predominant reports on *Mycobacterium* detection are mostly focused on *M. tuberculosis* complex. While reports have increased due to the emergence of NTM infections, research on PEM detection remains limited. Given PEM's significant role in environmental systems and potential health risks, there is a need for a straightforward, rapid, and user-friendly PEM monitoring approach, especially in low-resource settings. In this work, an electrochemical magneto-immunosensor is introduced for quantifying *M. fortuitum*, serving as a PEM model in this study. According to the low concentration of this bacteria in water, the collection of 100 up to 1000 mL of sample is typical for further filtration or centrifugation, as a preconcentration step [7]. A preconcentration strategy relying on the combination of filtration and immunomagnetic separation (IMS) is used in this procedure to pull out the bacteria directly from the filter. Moreover, an in order to simplify the analytical procedure, the filter is incubated at the same time with the magnetic particles modified with anti-lipoarabinomannan antibodies and the enzymatic conjugate, performing a one-step incubation in only 60 min. Furthermore, the electrochemical detection takes place in an easy-to-use and handheld device with a novel cartridge, in which the magnetic actuation is performed, while the excess of sample and reagents are removed.

2. Experimental section

2.1. Instrumentation

The electrochemical measurements were performed on a disposable cartridge (Fig. 1, panel C) for magneto-actuation, washing and reading (BioEclosion SL, Spain). The cartridge can be either inserted to a handheld device operated by batteries (BioEclosion, Spain) which includes the external magnet, for the quantitative amperometric readout directly on the display in 30 s, or in a bipotentiostat DRP-STAT200 operated by DropView 2.2 for instrument control and data acquisition (Dropsens, Spain). A complete filtration system of 25 mm (Product no. 073–0Q7724, Scharlab) was used to assess polycarbonate track-etched membranes (Catalogue no. 10417106, Whatman), nylon (Catalogue no. 7404–004, Whatman), cellulose acetate (Catalogue no. 10404006, Whatman), cellulose nitrate (Catalogue no. 10401106, Whatman) and mixed cellulose ester (Ref. HAWP02500, Merck Millipore Ltd), in all instances 0.45 μm pore size and 25 mm diameter. The cyclopore polycarbonate track-etched membranes of 0.2 μm pore size were used as a SEM support (Catalogue no. 10417606, Whatman). The SEM images were taken with the scanning electron microscope EVO MA-10 (with EDS Detector, Oxford LINCA). The confocal images were collected on the microscope Leica, TCS SP5 (Leica Microsystems, Germany).

2.2. Chemicals and biochemicals

Different set of buffers were used for specific procedures in the experiments, and their composition is described in S1 (Supp. Data). All buffers were prepared from analytical grade purchased from Merck and Sigma and using milliQ water. The monoclonal anti-LAM antibody from rabbit (Catalogue no. MA533311, ThermoFisher Scientific) was immobilized on tosyl activated magnetic particles (Dynabeads™ M-450 Tosylactivated, Catalogue no. 14013, Invitrogen). The anti-Mycobacterium polyclonal antibody (anti-TBS) from rabbit (Catalogue no. PAB29798, Abnova) was biotinylated with EZ-Link NHS-Biotin Kit (Catalogue no. 20217, ThermoFisher Scientific) following the manufacturer's protocol, as detailed in S2 (Supp. Data), providing a biotinylation degree of 6 biotin per IgG molecules (anti-TBS-BIO). The excess of biotin tag was removed by desalting using the Zeba™ spin and desalting columns of 7 K cut-off (Catalogue no. 89882, Pierce Rockford, IL). An enzymatic conjugate of anti-TBS-BIO and streptavidin-HRP (Catalogue no. 11089153001, Roche) was used for labelling, and previously prepared by incubation for 45 min at 37ºC.

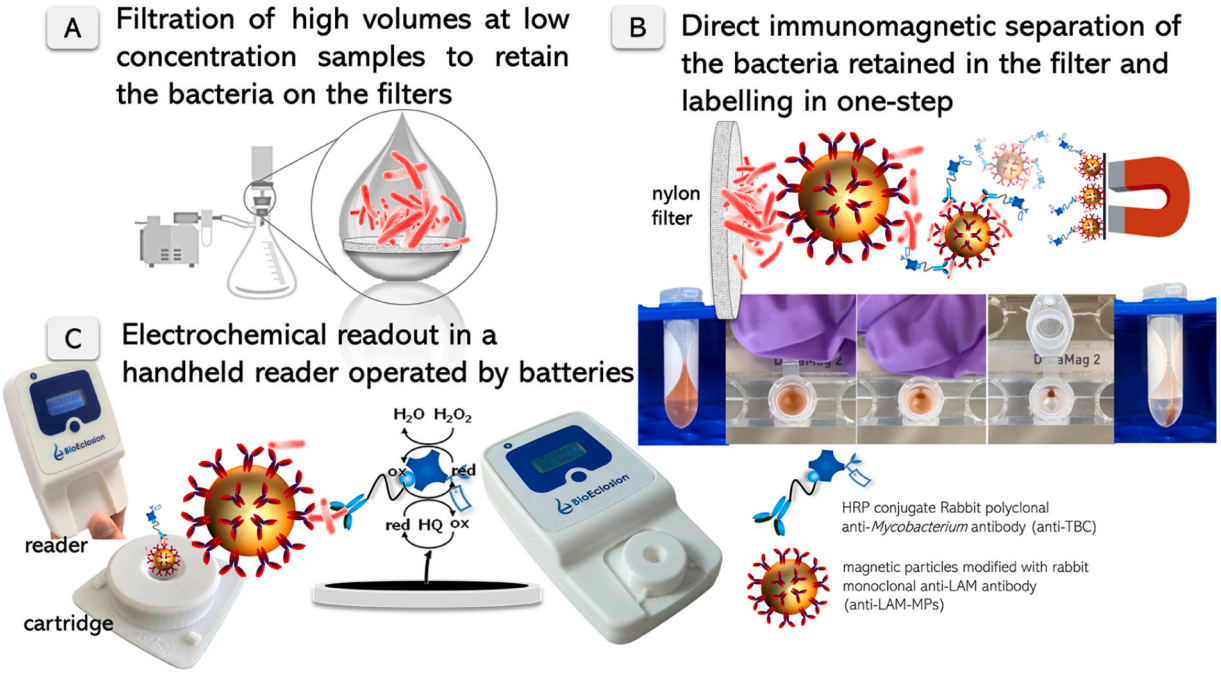

Fig. 1. Schematic representation of A) filtration, B) immunomagnetic separation of the bacteria retained in the filters and labelling in one-step, followed by C) electrochemical readout for the detection of *M. fortuitum* in hemodialysis water performed in a cartridge and handheld device. In Panel B, experimental details are presented, showing how the filter is positioned on the Eppendorf tube for immunomagnetic separation. The photos in Panel B were captured in 1-second frame sequences. More details are presented in S8 (Sup. Materials).

2.3. Culturing Mycobacterium fortuitum and other strains

The strain of *Mycobacterium fortuitum* ATCC 6841 was growth in Tryptic Soy Broth (TSB) medium supplemented with 0.015 % (v/v) of tyloxapol at 37 ºC for 72 h in continuous agitation, until reaching an OD of 1.000 AU at 550 nm. The liquid culture was aliquoted and preserved supplemented with 20 % (v/v) of glycerol at −40 ºC. The concentration of the stock was calculated by CFU counting in Tryptic Soy Agar (TSA) plates, after the incubation for 5–7 days at 37 ºC. The concentration of viable cells was quantified for each experiment by CFU counting, as shown in Fig. S1 (Supp data). The strains for the specificity study were: *E. coli (CECT 405, ATCC* 10536), *L. Pneumophila (serogroup 1, Philadelphia 1, ATCC 33152)*, *P. Aeruginosa* (CECT116, ATCC 15542), and *M. fortuitum*. The concentrations of the viable bacteria were determined in all the experiments by the gold standard culturing method in solid media and CFU counting, as detailed in Supp data.

2.4. Covalent immobilization of anti-LAM antibodies

The tailored modification of the MPs with anti-LAM antibodies was performed on tosyl activated magnetic particles as reported previously [11], following by blocking the unreacted tosyl groups with glycine buffer. The outline of the procedure is schematically represented in Fig. S3, panel A (Supp. data). The coupling efficiency was determined as 98.3 %, as detailed in Fig. S3, panel B (Supp. Data). The final concentration of the anti-LAM-MPs was 1 x 10^8 MP mL^{-1} and were previously washed three times with washing buffer before used in each experiment.

2.5. Evaluation of the immunomagnetic separation of M. fortuitum by scanning electron and confocal microscopy. Specificity study

To assess the performance anti-LAM-MPs for binding *M. fortuitum*, scanning electron microscopy was performed. A volume of 100 μL of anti-LAM-MPs at 10^7 MP mL^{-1} was incubated with 500 μL of *M. fortuitum* at 2.2 × 10^8 CFU mL^{-1} for 1 h at 750 rpm. Afterwards, the sample was filtered in a polycarbonate membrane. Then, the filter was treated as previously described [14]. In order to visualize the interaction in a sandwich format of *M. fortuitum* with both anti-LAM-MPs and anti-TBS-BIO antibody, confocal fluorescence microscopy was performed. To provide blue fluorescence to the bacteria, *M. fortuitum* 2.2 × 10^8 CFU mL^{-1} was incubated with 5 μL of Hoechst for 30 min at 750 rpm. To provide green fluorescence to the MPs, 100 μL of anti-LAM-MPs at 10^7 MP mL^{-1} was preincubated with an anti-rabbit-ALEXA488 for 30 min. The anti-LAM-MPs were then incubated with 100 μL of *M. fortuitum* for 1 h at 750 rpm, and then, with 200 μL conjugate streptavidin-Cy5/anti-TBS-BIO (80 μg mL^{-1}) for 1 h. Confocal images were thereafter collected with the laser line of Hoechst (350–461 nm excitation, 461 nm emission), anti-rabbit Alexa Fluor® 488 (anti-rabbit-ALEXA488) (495 nm excitation, 518 nm emission) and streptavidin-Cy5 (600–650 nm excitation, 670 nm emission).

The specificity study was performed by magneto-actuated immunoassay as detailed described in S4 (Supp Data) at the optimized concentration of reagents, as shown in Fig. S4 (Supp Data). In this study, bacteria commonly found in contaminated water were included at a high concentration range: *E. coli* 3.6 × 10^8 CFU mL^{-1}, *L. Pneumophila* 7.2 × 10^7 CFU mL^{-1}, *P. Aeruginosa* 4.1 × 10^8 CFU mL^{-1}, and *M. fortuitum* 2.1 × 10^5 CFU mL^{-1}. The bacteria grew in solid plates were inoculated in water, adjusting the absorbance to 0.200 AU at 550 nm.

2.6. Electrochemical magneto immunosensing for the quantification of M. fortuitum

The electrochemical magneto immunosensing procedure involves i) the immunomagnetic separation of *M. fortuitum* with the anti-LAM-MPs and incubation with the enzymatic conjugate anti-TBS-BIO/strep-HRP in a one-step incubation and ii) the electrochemical readout in 30 s. Briefly, 100 μL of sample containing *M. fortuitum* was incubated with anti-LAM-MPs at 10^7 MP mL^{-1} and enzymatic conjugate (40 μg mL^{-1} anti-TBS-BIO and 5 U mL^{-1} strep-HRP), in a volume of 1 mL in with PBST for 60 min under gentle rotation, as optimized in S4, S5 and S6 (Supp. Data). The product is introduced in the cartridge in the "OPEN position", in which the magnetic actuation is performed, while the excess of sample and reagents are removed. 3 drops (150 μL) of the cartridge solution are added. The cartridge is then put in "CLOSE position", and 3 drops (150 μL) added again for the further amperometric measurement under enzyme saturation conditions at − 0.15 V and 30 s. All the cartridge operation as well as the electrochemical readout were performed following the product specifications provided by the manufacturer. More details about the amperometric measurements are provided in S7 (Supp. Data). The LOD of the electrochemical magneto immunosensor was calculated by processing a calibration plot ranging from 0 to 2.2 × 10^6 CFU mL^{-1}.

2.7. Novel preconcentration method. Filtration, immunomagnetic separation and electrochemical immunosensing in handheld device. Study of the filtering material

A preconcentration strategy is integrated to the electrochemical immunosensing to improve de LOD, by filtering high volume of samples and further immunomagnetic separation to pull-out the bacteria from the filter for downstream analysis. Briefly, the procedure combines three steps as described in Fig. 1 and Fig. S8 (Supp. data): i) Filtration of large volumes (typically 100 mL) of sample followed by ii) the immunomagnetic separation of *M. fortuitum* retained in the filters with the anti-LAM-MPs and incubation with the enzymatic conjugate anti-TBS-BIO/strep-HRP in a one-step incubation and ii) the electrochemical readout in 30 s. The experimental details are provided in S8 (Supp. data) and the figure therein. After filtration from 100 mL sample under vacuum, the 25 mm diameter filter was placed on a 2.0 mL tube. 100 μL of anti-LAM-MPs at 10^7 MP mL^{-1}, 200 μL of the enzymatic conjugate anti-TBS-BIO/streptavidin-HRP were added and PBST buffer to complete a final volume of 1 mL, to fully cover the filter and to ensure complete interaction of the MP with the bacteria retained in the filters. All reagents were used in the optimized concentrations (as described in S6, Supp. data), and incubated under gentle rotation at RT for 60 min. Then, the MPs labelled complexes with the captured bacteria were recovered under magnetic actuation and resuspended in 200 μL of washing buffer for further transfer to the cartridge. Then, the electrochemical readout was performed as detailed described in S7 (Supp. data).

In order to validate the approach, different materials were assessed. Polycarbonate (PC), nylon (NY), cellulose acetate (CA), cellulose nitrate (CN), and mixed cellulose ester membranes (MCE) were selected because of their widely use in microbiological filtration techniques with a 0.45 μm porous size and 25 mm diameter. For this study, 100 mL *M. fortuitum* samples at the same concentration (2.4 ×10^3 CFU mL^{-1}) as well as the negative control were filtered using each membrane and submitted to downstream analysis, in this instance magneto-actuated immunoassays described in S4 and S5 (Supp. Data).

2.8. Electrochemical magneto immunosensing for the quantification of M. fortuitum in hemodialisys water samples

M. fortuitum is one of the most common mycobacteria in contaminated hemodialysis waters [15] which can put immunocompromised patients at risk. Hence, it is important to continuously monitor water supplies in hemodialysis centers, to minimize the exposure of renal transplantation and immunocompromised individuals to contaminated sources. In fact, a magneto-actuated immunoassay for the detection of *M. fortuitum* in hemodialysis water samples was reported previously by our group [11]. Therefore, a calibration curve with different concentrations of the bacteria in hemodialysis water samples was performed and the LOD was calculated.

2.9. Biosafety considerations

All experiments were performed in a Biosafety Class 2 environment required for the handling of *M. fortuitum*, *P. aeruginosa* and *L. pneumophila*. All biological waste generated from the experiments were disposed in accordance with the local regulations for handling biohazards.

2.10. Statistical analysis

The statistical analyses were performed using Prism v 10.0.1 (GraphPad, San Diego, USA). The calibration curves were adjusted to a non-linear regression fitting, using the four-parameter logistic curve, abbreviated 4PL. The limit of detection was calculated considering the number of negative controls for each curve, using the one-tailed test with a significance level of $p > 0.05$ (t Student, $p > 0.05$).

3. Results and discussion

3.1. Evaluation of the immunomagnetic separation of M. fortuitum by scanning electron and confocal microscopy. Specificity study

Following the incubation of the *Mycobacterium* -containing sample with anti-LAM-MPs, the sample was observed by scanning electron microscopy (SEM) to confirm the effectiveness of the immunomagnetic separation. In Fig. 2, it is confirmed that tosyl-activated magnetic particles were coated with anti-LAM monoclonal antibodies to enhance the interaction between the particles and the bacteria. Fig. 2, panel A clearly demonstrate the successful binding of the modified magnetic particles to the *M. fortuitum* bacteria, resulting in the formation of clusters due to the polyvalency of both the bacteria and the magnetic particles. Large corded bacterial aggregates are also observed in Fig. 2, panel B, since they are known to form clumps associated with biofilm formation in the natural environment [16]. In order to visualize not only the ability of the anti-LAM-MPs in capturing mycobacteria, but also anti-TBS-BIO to detect them in a sandwich-like format, confocal fluorescence microscopy was performed using streptavidin-Cy5 for labelling (emitting far-red-fluorescence at 670 nm). According to Fig. 2, panel C, anti-LAM-MPs (incubated with ant-rabbit-ALEXA488 antibodies emitting green at 518 nm) are capable of capturing aggregates of mycobacterium which appears in blue due to the Hoechst staining of the DNA at 461 nm recovered by red due to anti-TBS-BIO/streptavidin-Cy5 conjugate. The monoclonal anti-LAM antibody was selected for capturing in the MPs considering that the lipoarabinomannan is a lipoglycan and major virulence factor in the bacteria genus *Mycobacterium*. It is thus expected that other bacteria will not be detected, as demonstrated in Fig. 2, panel D, when challenged with a high concentration of gram-negative bacteria. The signal of the negative control containing 0 CFU mL-1 of *M. fortuitum* was found to be comparable with *E. coli, L. Pneumophila, P. Aeruginosa* ($p < 0.05$), suggesting a good specificity for the assay.

3.2. Electrochemical magneto immunosensing for the quantification of Mycobacterium fortuitum

The primary objective of this study is to achieve analytical simplification, aimed at minimizing user intervention while maintaining analytical performance. The optimized assay conditions were thoroughly tested in Sections S5 and S6 (Supp. Data). This work is also focused on detecting *M. fortuitum* through an amperometric readout using a user-friendly handheld electrochemical device with an integrated internal potentiostat. The disposable cartridge encompasses both the microfluidic system and the electrode, enabling magnetic actuation and efficient removal of excess samples and reagents. Notably, the same

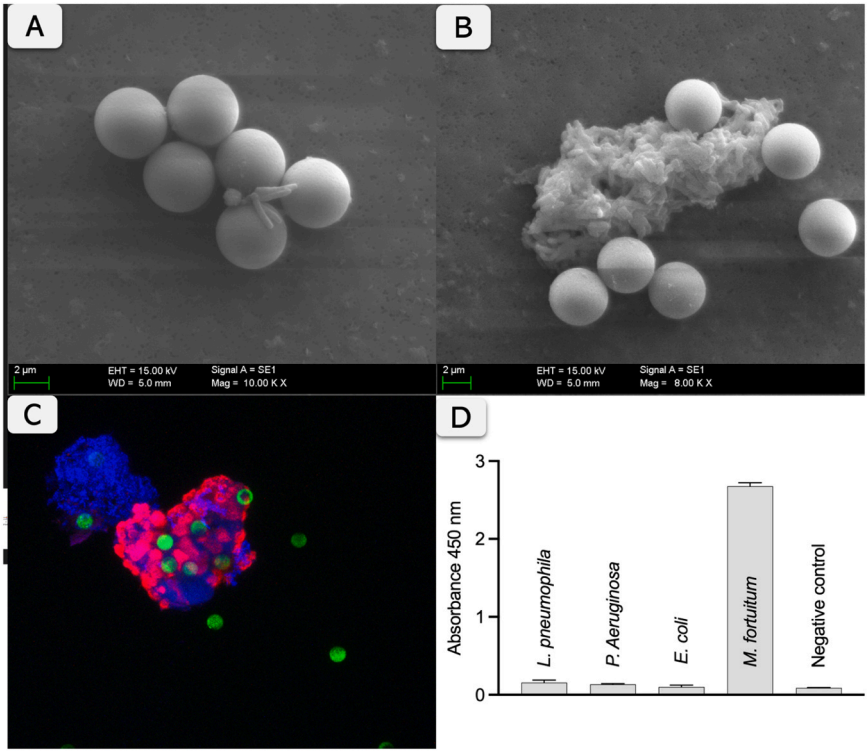

Fig. 2. Panel A and B. Evaluation of the IMS by SEM at a *M. fortuitum* concentration of 2.2×10^8 CFU mL-1. In all cases, identical acceleration voltage (15 KV) was used. Panel C. Confocal microscopy study of the sandwich-like format. Anti-LAM-MPs are shown in green, capturing a cluster of *M. fortuitum* that was stained in blue by Hoechst, and superimposed with the specific red fluorescence from anti-TBS-BIO/ streptavidin-Cy5 conjugates. Panel D. Specificity study for *M. fortuitum* 2.1×10^5 CFU mL^{-1}, *E. coli* at 3.6×10^8 CFU mL^{-1}, *P. Aeruginosa* at 4.1×10^8 CFU mL^{-1} and *L. pneumophila* 7.2×10^7 CFU mL^{-1}. The error bars show the standard deviation for n = 3.

solution serves a dual role in both washing and catalyzing the reaction through the HRP enzyme (referred to as the cartridge solution). As a result, the necessity for employing diverse buffers is eliminate, leading to reduced time in a simpler approach for the end-user. Additionally, the cartridge design allows for the use of a small solution volume, up to 300 µL. Fig. 3, Panel A shows the calibration plot from 0 to 2.2×10^6 CFU mL^{-1} for the detection of the *M. fortuitum* with the electrochemical immunosensor without the integration of the novel preconcentration method. The data was fitted with a non-linear regression (Sigmoidal 4PL, GraphPad Prism Software v 10.0.1, $R^2 = 0.9987$) and the limit of detection were calculated, resulting in a value of $4{,}3\times10^3$ CFU mL^{-1}, by processing 0.1 mL of sample. This LOD is comparable with a magneto-actuated immunosensor previously reported by our research group but for *Salmonella*, in which a LOD of 7.5×10^3 CFU mL^{-1} in milk was achieved in 50 min without any pretreatment. [17]. In order to further improve the LODs, a new approach to handle higher volume of sample, combining filtration and IMS, was presented in the next section.

3.3. Filtration, magnetic actuation and electrochemical immunosensing. Study of the filtering material

The LOD achieved by the electrochemical magneto immunosensor ($4{,}3\times10^3$ CFU mL^{-1}) by processing 0.1 mL of sample are above the limits required by most legislations. In order to improve the LODs, a new approach to handle higher volume of sample (up to 100 mL), combining filtration and direct immunomagnetic separation (IMS) of the bacteria retained in the filters, is proposed in this work, as depicted in Fig. 1. Selecting the appropriate filter material is crucial to ensure a swift filtration workflow, a good removing of the bacteria retained in the filters by the MPS, while minimizing nonspecific adsorption of all the reagents (including the enzymatic conjugate) during the one-step incubation on the filters.

A collection of filter materials frequently employed in microbiology, such as polycarbonate, nylon, cellulose acetate, cellulose nitrate, and mixed cellulose esters, was chosen. These materials share a hydrophilic surface to prevent excessive retention and thereby facilitate immunomagnetic separation and further labelling. The results obtained from filtering 100 mL water containing *M. fortuitum* and negative controls were compared for each material, as illustrated in Fig. 4, panel A. While the negative controls yielded similar results across all materials, nylon emerged as the choice due to its significant difference in signal compared to other materials. In this study, nylon filters exhibited the highest signal intensity, thereby promoting interaction among different biorecognition elements during the one-step incubation and yielding optimal recovery rates for the bacteria.

The calibration plot integrating the novel preconcentration method in a one-step incubation (by filtering 100 mL of hemodialysis water), and further electrochemical immunosensor is shown in Fig. 4, panel B as green line, for the detection of the *M. fortuitum* from 0 to 8.7 x 10^4 CFU mL^{-1}. The data was fitted with a non-linear regression (Sigmoidal 4PL, GraphPad Prism Software v 10.0.1, $R^2 = 0.9906$) and the LOD was calculated ($n = 9$), resulting in a value of as low as 5 CFU mL^{-1}. Compared with the electrochemical biosensor without the integration of the preconcentration method (Fig. 4, blue solid line), an impressive 10^3 fold improvement was achieved, from 4.8×10^3 CFU mL^{-1} to 5 CFU mL^{-1}. Moreover, the LODs for the magneto-actuated electrochemical immunosensor in water (Fig. 3) and hemodialysis water was found to be the same, 4.8×10^3 ($n = 8$).

This approach allows the detection of low concentrations in high-volume samples that is one of the bottlenecks in the detection of environmental bacteria, in around 60 min (including filtration, which takes only 2 min). A similar LOD (13 CFU mL-1) was previously reported by our research group in a magneto-ELISA, but in 3 h [11]. However, the new method offers several key advantages over to time-consuming and labor-intensive ELISAs. It streamlines the process by integrating immunomagnetic separation and labeling into a single step, significantly reducing the analysis time and minimizing washing steps. User intervention as pipetting is minimized through a user-friendly cartridge design, enhancing accessibility and reproducibility. The incorporation of handheld electrochemical readout makes on-site testing possible, offering portability and immediacy, crucial for point-of-care and field-based applications. The readout time is further reduced through several steps: the direct introduction of the modified-MPs particle conjugate into the cartridge, the subsequent washing, the addition of enzymatic substrates (using the same solution) within the cartridge, and the subsequent electrochemical measurement, accomplished in less than 30 seconds. This represents a 10-fold reduction in time in comparison with an immunoassay with optical readout, where the substrates require a minimum incubation time between 15 and 30 min [11]. The table S9 (Supp data) provides a more comprehensive overview of the proposed method in relation to other methods including standard culturing and PCR, enhancing the main features. The preconcentration strategy not only eliminates preenrichment and DNA extraction or amplification techniques but also enables detection of intact mycobacteria in a streamline process. In earlier studies, we explored several strategies to improve LOD, such as incorporating a short preerichment step [17] or amplifying the genetic material after the IMS [14]. In the current study, we have achieved a remarkable 10^3-fold improvement in the LOD,

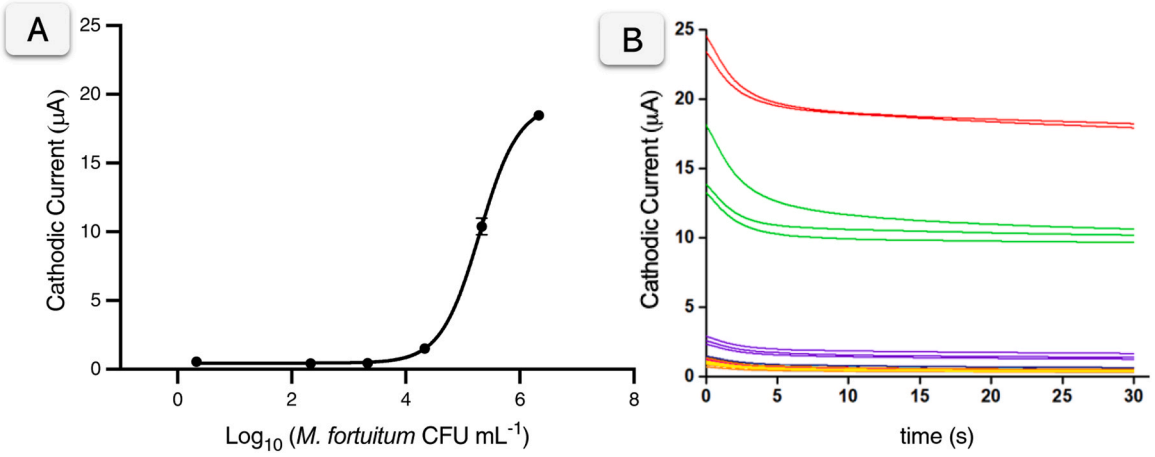

Fig. 3. Panel A. Calibration plot for the for the detection of *M. fortuitum* 0 to 2.2×10^6 CFU mL^{-1} with the magneto-actuated electrochemical immunosensor in water samples, without the integration of the novel preconcentration method ($R^2 = 0.9987$). ($n = 3$). Panel B. Raw data obtained from the amperometric measurements, performed at applied potential = −0.150 V (vs. Ag/AgCl). The current value at the steady current (30 s) was used for the calibration plot.

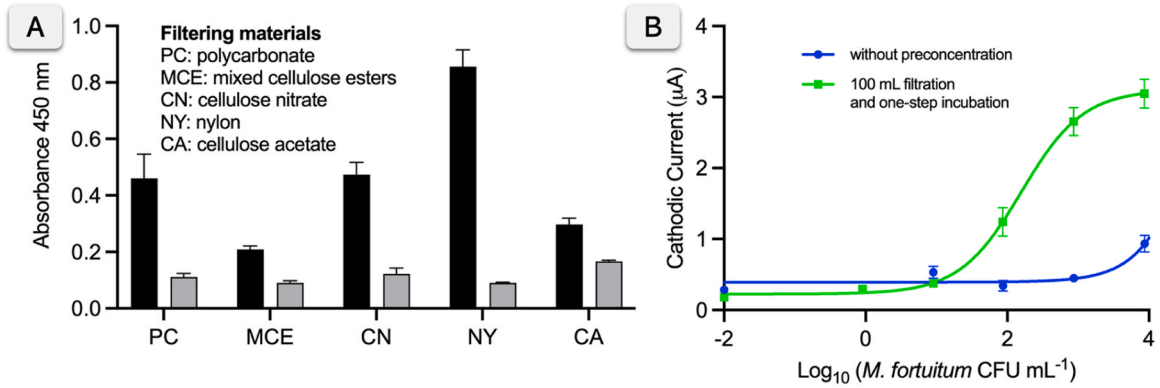

Fig. 4. Panel A. Study of the filtering material by the filtration of 100 mL samples containing *M. fortuitum* (2.4 x 10^2 CFU mL^{-1}) as well as the negative controls (0 CFU mL^{-1}). All the filters were 0.45 μm pore size and 25 mm of diameter. The filtering materials were polycarbonate (PC), mixed cellulose ester (MCE), cellulose nitrate (CN), nylon (NY) and cellulose acetate (CA). Panel B) Calibration plot for the magneto-actuated electrochemical immunosensor in hemodialysis samples. The blue solid line shows the calibration plot ranging from 0 to 8.7×10^4 CFU mL^{-1} without the integration of the novel preconcentration method (R^2 = 0.9967). The green solid line shows the calibration plot ranging from 0 to 8.7×10^4 CFU mL^{-1} (R^2 = 0.9906) with preconcentration by filtering 100 mL hemodialysis samples with nylon membranes. In all cases, a one one-step incubation of the reagents and the electrochemical readout in the cartridge with the handheld device was performed. The error bars show the standard deviation for n = 3.

reducing it from 4.8×10^3 CFU mL^{-1} to 5 CFU mL^{-1}. The Advancement Medical Instrumentation (AAMI) [18], the European Pharmacopeia (EP) (ISO13959, 2014) or the Japanese Society for Dialysis Therapy (JSDT) [19] establish as maximum level of heterotrophic bacteria 100 CFU mL-1 of total viable counts (TVC) in conventional hemodialysis water. On the other hand, the Centers for Medicare and Medicaid Services (CMS), Department of Health and Human Services set the regulations in 200 CFU mL-1, while urgent action should be taken at 50 CFU/mL [20]. These values underscore the effectiveness of the method as a monitoring tool for conventional dialysis water and dialysis fluids. Notably, the calculated limit of detection is as low as 5 CFU mL^{-1}, and a remarkable signal-to-background ratio of 6.8 is achieved for 86 CFU mL^{-1}. This is achieved using a portable device powered by batteries in only 1 h and requiring minimal intervention from the end user.

4. Conclusions

The presence of non-tuberculous mycobacteria in water distribution systems constitutes a critical global health concern, owing to its link with an increased incidence of NTM-associated pulmonary diseases and related complications. Instances of these bacteria being detected in drinking water and hemodialysis systems were reported several times [5,21–24]. The innovative aspects of the strategy presented here for *M. fortuitum* integrates key components aiming to achieve simplification and portability while maintaining outstanding analytical performance. In conclusion, the method offers a preconcentration strategy that combines magnetic particles and filtration, facilitating efficient capture and concentration of bacteria via immunomagnetic separation. In the standard microbiological method, after sample filtration, the retained bacteria are resuspended or extracted from the filter using special buffers, vortexing, centrifugation or ultrasonication. In this study, the preconcentration strategy is integrated to the electrochemical biosensing. By filtering high volumes of samples and subsequently employing magnetic actuation to pull out the bacteria directly from the filter, the need for resuspension or additional steps for downstream analysis was eliminated. Other further source of analytical simplification relies on the integration of immunomagnetic separation and labeling into a single step in only 60 min, significantly reducing analysis time in contrast to the conventional two-week timeframe needed for traditional isolation and culturing methods. The user-friendly cartridge design minimizes user intervention, enhancing ease of use and reproducibility. Moreover, the handheld electrochemical readout powered by batteries enables on-site and point-of-care testing, marking a significant advance in electrochemical biosensing applications.

CRediT authorship contribution statement

Melania Mesas Gómez: Data curation, Investigation, Validation, Formal analysis, Writing – original draft. Bárbara Molina-Moya: Investigation, Validation. Bárbara. Camila de Araújo: Investigation, Validation. Arnau Pallarès-Rusiñol: validation. Jofre Ferrer-Dalmau: validation. Maria Valnice Boldrin Zanoni: Supervision. José. Domínguez: Supervision. Esther Julian: Conceptualization, Methodology, Supervision. María Isabel Pividori: Conceptualization, Methodology, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The datasets generated during this study are available in the CORA RDR repository https://dataverse.csuc.cat.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.snb.2023.135211.

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