Title: Efficacy of the FilmArray Blood Culture Identification panel for direct molecular diagnosis of infectious diseases from samples other than blood

Running title: FilmArray BCID Panel efficacy in samples other than blood

Contents Category: Diagnostics, typing and identification

Miquel Micó[1], Ferran Navarro[1,2,3]*, Daniela de Miniac[1], Yésica González[1], Albert Brell[1], Cristina López[1], Ferran Sánchez-Reus[1,2,3], Beatriz Mirelis[1,2,3], Pere Coll[1,2,3].

[1]Servei de Microbiologia. Hospital de la Santa Creu i Sant Pau Barcelona, Spain; [2]Institut d'Investigació Biomèdica Sant Pau (IIB Sant Pau), Barcelona, Spain and Universitat Autònoma de Barcelona, Barcelona, Spain; [3]Red Española de Investigación en Patología Infecciosa (REIPI),

*Corresponding Author: C/ Sant Quintí, 89. 08041 Barcelona Spain; fnavarror@santpau.cat; Phone: +34935537297; Fax: +34935537287

SUMMARY:

Molecular-based techniques reduce the delay in diagnosing infectious diseases and therefore contribute to better patient outcomes. We assessed the FilmArray Blood Culture Identification (Biofire Diagnostics Inc. bioMérieux) panel directly on clinical specimens other than blood: cerebrospinal, joint, pleural and ascitic fluids, bronchoscopy samples, and abscesses. We compared the results from 88 samples obtained by culture-based techniques. The percentage of agreement between the two methods was 75% with a Cohen's kappa of 0.51. Global sensitivity and specificity using the FilmArray Blood Culture Identification (BCID) panel were 71% and 97%, respectively. Sensitivity was poorer in samples with a low bacterial load, such as ascitic and pleural fluids (25%), whereas sensitivity for abscess samples was high (89%). These findings suggest that the FilmArray BCID panel could be useful to perform microbiological diagnosis directly from samples other than positive blood cultures, as it offers acceptable sensitivity and moderate agreement with conventional microbiological methods. Nevertheless, cost-benefit studies should be performed before introducing this method into algorithms for microbiological diagnostics.

INTRODUCTION

Time is crucial when managing infectious diseases. Conventional culture-based methods are slow to give definitive results. Rapid culture-independent methods contribute to a better initial management of patients and more efficient use of antimicrobials (Picard & Bergeron, 2002; Bissonnette & Bergeron, 2006; Pozzetto *et al.,* 2010). Culture-independent methods such as nucleic acid amplification tests are being increasingly used for diagnosis of infectious diseases. The FilmArray platform is one of the few commercially available systems using nucleic acid amplification tests to diagnose infectious diseases (Poritz *et al.,* 2011). Briefly, it is a nested multiplex PCR system that performs all the assay steps, from nucleic acid extraction to interpretation of amplification data, in a closed system using a single panel on a minimally processed clinical sample in a completely automated manner. Most importantly, it gives results in just one hour. Additionally, the laboratory procedures are not technologically complex and no special training is required. Accordingly, this technology has the potential to significantly improve the management of several infectious diseases.

Three FilmArray panels are commercially available, a respiratory panel, a gastrointestinal panel, and a blood culture identification (BCID) panel (Poritz *et* al., 2011; Blaschke *et al.,* 2012; Khare *et al.,* 2014). The FilmArray BCID panel was designed to detect 24 pathogens associated with bloodstream infections, and three antimicrobial resistance genes (*vanA/B*, *mecA*, *bla*$_{KPC}$) (Table 1). These pathogens included in the FilmArray BCID panel cause many other infectious processes (Almuhayawi *et al.,* 2014; Bhatti *et al.,* 2014). Accordingly,

we hypothesized that the off-label use of the FilmArray BCID panel directly on clinical specimens other than blood could improve the management of other severe infections, such as meningitis or pneumonia.

In this study, we assessed the applicability of the FilmArray BCID panel to diagnose infectious diseases other than sepsis directly from samples in a totally culture-independent manner.

METHODS

This study was performed in the microbiology laboratory at Hospital de la Santa Creu i Sant Pau, a tertiary hospital in Barcelona, Spain. The Research Institute of Hospital de la Santa Creu i Sant Pau (IIB Sant Pau) and the Clinical Ethics Committee approved this study (IIBSP-FIL-2013-57; 18/2013). Informed consent was waived because the study was prospective without interaction with patients and all patient personal information was de-identified prior to analysis.

The flow chart in Figure 1 illustrates sample selection and processing criteria. Only those samples with higher probability of positive outcome were included: 1) fluids from normally sterile sites (cerebrospinal [CSF], joint, ascitic and pleural fluids) with microorganisms or polymorphonuclear cells observed on a Gram stain; 2) lower respiratory tract samples obtained by bronchoscopy (protected specimen brush [PSB] and bronchoalveolar lavage fluid [BAL]) from immunocompetent patients; and 3) abscess samples with microorganisms on a Gram stain. Samples processed for this study were collected prospectively at our laboratory on working days from 8 a.m. to 8 p.m. from April to October

2013. As samples were processed immediately after arrival at our laboratory, no storage was needed.

After Gram staining, all samples selected for this study were processed using the FilmArray BCID panel as well as conventional culture-based methods, at the same time. Results obtained by the FilmArray BCID panel were blinded. For the FilmArray BCID panel, we followed the manufacturer's indications as recommended for blood cultures, using 100µl of well-mixed samples directly without previous centrifugation and with no special adaptation to the samples. The FilmArray BCID panel contains all the required reagents for sample preparation, reverse transcription-PCR, PCR, and detection in a freeze-dried, room temperature stable format. The samples and hydration solution is injected into the pouch prior to the run. The panel is then introduced into the device and results are obtained in one hour.

For the conventional culture-based laboratory methods, we performed our standard procedures: Gram stain, culture, and identification of isolates. All microorganisms isolated by culture-based methods were identified with Vitek 2 identification cards (bioMérieux) with the exception of anaerobes, which were identified with RapID™ ANA II System identification panels (Remel, Thermo Scientific). Microorganisms with no consistent results using conventional methods were identified by 16S rDNA sequencing (Esparcia *et al*. 2011). Antimicrobial susceptibility testing was performed according to the guidelines of the Clinical and Laboratory Standards Institute (Clinical and Laboratory Standards Institute, 2012). The turnaround time for standard methods is 24-48 hours. All strains isolated in cultures were stored for further analyses. We

performed the FilmArray panel with those strains isolated from samples FilmArray negative/culture positive to confirm the functionality of the molecular design of the FilmArray.

To confirm the clinical significance of the results, we collected clinical data (pre-existing pathology, demographic data, clinical outcome, and empirical and directed treatment) from the clinical records and evaluated the results from other samples if available (blood cultures, antigen detection and other cultures). This additional information was useful to decide the clinical relevance of the culture and FilmArray panel results. We considered a positive result to be clinically significant when the microbiological results from the FilmArray panel or the culture-based methods, or both, were concordant with clinical data (Isenberg. 2004). Physicians were not informed of the results obtained by FilmArray.

Statistical analyses (percentage of agreement, Chi square analyses and the confidence intervals) were performed using the Vassar Stats website (http://vassarstats.net/). In all cases, the 95% confidence intervals (CI) are shown. Cohen's k values of 0.41–0.60 were considered of moderate agreement and values of 0.21–0.40 were considered of fair agreement.

RESULTS

During the six months of the study, a total of 88 samples were processed using both the FilmArray BCID panel and conventional culture-based methods. These samples were 41 fluids from normally sterile sites (19 CSF, 8 pleural fluids, 7 ascitic fluids and 7 joint fluids), 36 lower respiratory tract samples (15

bronchoalveolar lavage fluids and 21 protected specimen brushes) and 11 abscess samples.

Global results for the FilmArray BCID panel and culture-based methods: From the total of 88 samples, 57 (65%) specimens were positive (Table 2): 35 by culture and FilmArray, 16 by culture only, and 6 by FilmArray only. The percentage of agreement between the two methods was 75% (CI: 64-83%) with a Cohen's kappa: 0.51 (CI: 0.33-0.68), indicating a moderate agreement.

Detection of organisms and antibiotic resistance markers: We isolated 86 different microorganisms from the 51 samples that were positive using standard culture methods (Table 3). Sixteen of these 51 samples were positive for more than one microorganism. Seven of these 16 samples were from abscesses, six were from respiratory samples (four PSB and two BAL), two were ascitic fluid, and one was pleural fluid. Seventy-two of the 86 (84%) were pathogens included in the FilmArray BCID panel. The 14 microorganisms not included in the panel were four *Bacteroides* spp., three *Fusobacterium* spp., three *Propionibacterium acnes*, one *Clostridium perfringens,* one *Burkholderia gladioli,* one *Bordetella bronchiseptica* and one *Stenotrophomonas maltophilia*. The FilmArray detected 57 (79%) of the 72 microorganisms isolated by culture and included in the FilmArray BCID panel. Eighteen of the 51 samples were polymicrobial using FilmArray: six samples were from abscesses, 11 were respiratory (seven PSB and four BAL) and one was ascitic fluid.

We did not detect any MRSA, vancomycin-resistant enterococci strains, or *bla*$_{KPC}$–carrying strains. There were no pouch failures.

Results according to clinical assessment: Fifty-six of the 88 (64%) specimens were considered true infections (Table 4).

FilmArray BCID panel-positive discrepant results: from the 56 samples considered true infections, five were culture-negative/FilmArray BCID panel - positive. Three of these were *Streptococcus pneumoniae* (two from CSF and one from PSB), one was coagulase-negative *Staphylococcus* (CoNS) from CSF from a ventriculoperitoneal (VP) shunt, and one was *Candida glabrata* from PSB. The two *S. pneumoniae* from CSF were also detected by immunochromatography in CSF (BinaxNow, Alere). One of the two *S. pneumoniae* isolated from CSF and the *S. pneumoniae* isolated from PSB were also isolated from blood cultures. The CoNS was also isolated from the VP shunt catheter tip culture. Finally, *C. glabrata* was also isolated from blood culture.

Culture-positive discrepant results: from the 56 samples considered true infections, 16 were culture-positive/FA-negative. These correspond to three ascitic fluids (one *Escherichia coli*, two viridans group streptococci and one *C. perfringens*), three pleural fluids (culture-positive for one viridans group streptococci and one *Enterobacter cloacae* complex, while one was polymicrobial with *Corynebacterium* spp., *Bacteroides* spp. and *Fusobacterium* spp.), three BAL (one *B. gladioli*, one *B. bronchiseptica* and one *S. maltophilia*), three joint fluids (*Serratia marcescens*, viridans group streptococci and *Morganella morganii*), three CSF (VP shunt) (two *Propionibacterium acnes* and one *Pseudomonas aeruginosa*) and one abscess sample with one *Staphylococcus epidermidis* (Table 3). One viridans group streptococcus from an ascitic fluid sample was also isolated from two blood cultures. The viridans

group streptococci from a pleural fluid was also isolated from a PSB from the same patient. The *S. marcescens* obtained from a joint fluid was also isolated from another joint fluid from the same patient. The *M. morganii* isolated from a joint fluid was also isolated from several surgical samples from the same patient. The *B. bronchiseptica* was also isolated from several BALs from the same patient. The *P. aeruginosa* isolated from a CSF was also isolated from the VP shunt catheter tip culture. Finally, one of the two *P. acnes* obtained from CSF was also isolated from other CSFs obtained from the same patient.

Table 5 summarizes the results for each specimen. Global sensitivity and specificity of the culture were 91% (CI: 80-97%) and 100% (CI: 87-100%), respectively, whereas for FilmArray BCID panel they were 71% (CI: 58-82%) and 97% (CI: 82-100%), respectively.

Sensitivity of the FilmArray BCID panel was lower for sterile fluids (between 25% for ascitic and pleural fluids and 73% for CSF) than for culture-based methods. In the respiratory samples, sensitivity ranged from 70% for BAL to 100% for PSB. Sensitivity was 89% for purulent samples. The specificity of the FilmArray BCID panel ranged from 80% to 100%.

DISCUSSION

Our findings indicate that the FilmArray BCID panel could be useful for the diagnosis of several infectious processes besides those in the blood stream. Nevertheless, in view of the cost of the platform, a strict algorithm would be required to select samples in order to maximize profitability and decrease general costs. The percentage of agreement between the molecular approach with the FilmArray BCID panel and conventional culture methods was 75%, with

a Cohen's kappa: 0.51, indicating a moderate agreement. A similar Cohen's kappa value is frequently observed with new molecular approaches (Carrara *et al.,* 2013). There are several possible explanations: first, not all microorganisms are included in the panel of microorganisms detected by the molecular approach; second, viable microorganisms are studied using the conventional culture approach but molecular approaches for DNA; and third, the sample volume processed for each method differs (Esparcia *et al.,* 2011; Ginocchio & McAdam 2011; Carrara, *et al.,* 2013).

Another crucial point in validating new molecular approaches is the selection of the gold standard. When we took the culture results as a gold standard, we obtained a global analytical sensitivity for the FilmArray BCID panel of 78%. When we took molecular and culture results into consideration together with other analytical and clinical results to obtain the gold standard, the global sensitivity and specificity of the culture were 91% (CI: 80-97%) and 100% (CI: 87-100%), respectively, whereas for the FilmArray BCID panel they were 71% (CI: 58-82%) and 97% (CI: 82-100%), respectively. These values are in concordance with many other molecular approaches and emphasize, once again, that the accuracy of a specific molecular approach differs depending on the sample, the microorganisms involved, the expected bacterial load, and the presence of nucleic acids from non-pathogenic bacteria (commensal microbiota) (Esparcia *et al.,* 2011; Soriano *et al.,* 2011). On the other hand, sensitivity and specificity were lower than those obtained when the FilmArray BCID panel was used in positive blood cultures (Poritz *et al.,* 2011; Altun *et al.,* 2013).

We should emphasize that the FilmArray BCID panel has been approved and commercialized for positive blood cultures only. Accordingly, there are no data regarding its use with samples other than blood cultures. Sensitivity of the FilmArray BCID panel for sterile fluids was poor in our study, ranging from 25% in ascitic and pleural fluids to 73% in cerebrospinal fluid. These FilmArray BCID false negative results correspond to three ascitic and three pleural fluids. Two of the ascitic fluids were positive only from blood culture bottles (where five ml of sample were inoculated). The remaining ascitic fluid was positive for less than 200 cfu/mL in blood agar plate. Two of the pleural fluid samples were positive for about 2,000 cfu/mL. Finally, the remaining pleural fluid sample was positive for microorganisms not included in the FilmArray BCID panel. We consider these low percentages were due to the characteristic low colony count in most ascitic and pleural fluid infections (Tassi *et al.,* 2010; Lippi *et al.,* 2014). The use of a larger sample would likely increase the sensitivity in this kind of samples. Another possible explanation for the discrepant FilmArray BCID panel-negative/culture-positive results could be the low sensitivity of primers and probes. Nevertheless, we ruled this possibility out by performing the assay directly from the growing isolates, instead of from the specimen. In all cases, the FilmArray BCID panel was positive when performed from the isolate. Finally we should take in consideration the possibility of microorganisms not included in the panel.

We hypothesize that the low sensitivity of the test could be related to the volume of sample used and the bacterial load, as mentioned above (Carrara *et al.,* 2013). FilmArray BCID panel sensitivity for abscess samples was high (89%). To our knowledge, there are no data in the literature about using

molecular techniques for such samples. In all the aforementioned cases, specificity was 100%. Respiratory samples differed considerably from the other samples evaluated (sterile fluids and abscesses): sensitivity ranged from 70% for BAL to 100% for PSB, whereas specificity ranged from 80% for BAL to 100% for PSB. Among the 36 LRT samples, the FilmArray BCID panel detected 10 microorganisms that were not detected by culture. An important point that could be controversial is that seven of these respiratory samples were considered as possibly contaminated by DNA from oral flora [*Haemophilus influenzae* (5) and *S. pneumoniae* (2)]. Accordingly, the FilmArray BCID panel seems to be more sensitive than culture. However, it should be pointed out that most molecular approaches can detect DNA from microorganisms other than those involved in the infectious process, which could complicate the clinical interpretation of results. The detection of DNA from microorganisms not involved in the infectious process, or at least from those not able to grow in conventional cultures, has already been observed in other clinical situations, such as in the diagnosis of peritonitis (Esparcia *et al.,* 2011) and particularly in viral respiratory infections in children (Ginocchio & McAdam 2011). Finally, we should mention that the low negative predictive value of FilmArray (67%) is mainly due to the relatively high number of samples with microorganisms not included in the FilmArray BCID panel. Most of these microorganisms are anaerobes or non-fermenting Gram-negative bacilli.

Detection of resistance genes is a valuable advantage of these new molecular technologies, but in the present study, we did not detect any of the resistance genes tested (*van*, *mecA* or *bla*$_{KPC}$). This is in concordance with the prevalence of these antimicrobial resistance genes in our environment

(http://www.santpau.es/santpau/activitats/inicioMicrobiologia.htm). Accordingly, a possible addition of other resistance markers within the panel, consistent with the resistance prevalence, should be taken in consideration. The absence of *van*, *mecA* or *bla*$_{KPC}$ antimicrobial resistance genes in our environment is an important limitation of this study to evaluate the accuracy of the FilmArray BCID panel in detecting these resistance genes.

In conclusion, this study assessed the applicability and viability of the Multiplex PCR-based FilmArray BCID panel. This panel could be recommended as a routine diagnostic method for direct use on samples other than positive blood cultures as it offers acceptable sensitivity and moderate agreement with conventional microbiological methods. Indeed, the manufacturer, Biofire, is currently developing a new meningitis/encephalitis panel to detect most of the viruses and bacteria involved in these pathologies. Cost-benefit studies will be needed, however, before it can be introduced into algorithms for microbiological diagnosis.

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Table 1. Target pathogens and resistance genes included in FilmArray Blood Culture Identification panel.

Gram-positive bacteria	
Enterococcus	*Streptococcus*
Listeria monocytogenes	*Streptococcus agalactiae*
Staphylococcus	*Streptococcus pneumoniae*
Staphylococcus aureus	*Streptococcus pyogenes*
Gram-negative bacteria	
Acinetobacter baumanii	*Enterobacteriaceae*
Haemophilus influenzae	*Enterobacter cloacae* complex
Neisseria meningitidis	*Escherichia coli*
Pseudomonas aeruginosa	*Klebsiella oxytoca*
	Klebsiella pneumoniae
	Proteus
	Serratia marcescens
Yeast	
Candida albicans	*Candida parapsilosis*
Candida glabrata	*Candida tropicalis*
Candida krusei	
Antimicrobial resistance genes	
mecA	bla_{KPC}
vanA/B	

Table 2. Global results comparing culture-based methods with FilmArray Blood Culture Identification panel (FA).

	FA-positive	***FA-negative***	*Total*
Culture-positive	*35*	*16*	*51*
Culture-negative	*6*	*31*	*37*
Total	*41*	*47*	*88*

The percentage of agreement between the two methods was 75% (64%-83%), with a Cohen's κ: 0.51 (CI: 0.33-0.68); indicating a moderate agreement.

409 **Table 3.** Results of culture and FilmArray Blood Culture Identification panel (FA) for each microorganism.

Organism (n)	Culture	FA	Cult+/FA-	Cult-/FA+	Cult+/FA+	Samples (n)
Total microrganisms (104)	86	75	29	18	57	
All Cult+/FA+						
Enterococcus sp. (7)	7	7	0	0	7	AS(5), BAL(1), PSB(1)
K. oxytoca (1)	1	1	0	0	1	AS
K pneumoniae (2)	2	2	0	0	2	PSB(2)
Proteus sp.(1)	1	1	0	0	1	AS
C. tropicalis (1)	1	1	0	0	1	BAL
Cult+/FA+ Dominant						
S. aureus (8)	8	7	1	0	7	PSB(3), CSF(2), JF(2), BAL(1)
CoNS (10)	7	9	1	3	6	AS(5), CSF(2), JF(1), BAL(1), PSB(1)
E. coli (6)	6	5	1	0	5	AS(4), AF(1), BAL(1)
E. cloacae (4)	4	3	1	0	3	PF(2), AS81), AF(1)
P. aeruginosa (11)	11	9	2	0	9	PSB(4), AS(3), BAL(2), CSF(1), AF(1)
C. albicans (7)	6	6	1	1	5	BAL(3), AS(3), PSB(1)
C. glabrata (3)	2	3	0	1	2	AF(1), PSB(1), AS(1)
Cult+/FA-						
Serratia sp. (1)	1	0	1	0	0	JF
*M. morganii** (1)	1	0	1	0	0	JF
Cult+/FA- Dominant						
Streptococcus sp.(11)	11	5	6	0	5	AS(4), AF(2), PF(2), JF(1), CSF(1), PSB(1)
Cult-/FA+						
S. agalactiae (1)	0	1	0	1	0	PSB
Cult-/FA+ Dominant						
H. influenzae (9)	2	9	0	7	2	PSB(4), BAL(3), CSF(1), AS(1)

S. pneumoniae (6)	1	6	0	5	1	CSF(2), BAL(2), PSB(1), JF(1)	410
							411
Not in panel†	14	-	14	-	-		412
Negative	49	60	-	-	-		413
							414
Total	135	135					415

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420 Cult: Culture; FA: FilmArray Blood Culture Identification panel; JF: joint fluid; AF: ascitic fluid; PF: pleural fluid; CSF: cerebrospinal fluid; BAL:
421 bronchoalveolar lavage fluid; PSB: protected specimen brush; AS: abscess samples; CoNS: coagulase-negative staphylococci.
422 * FilmArray was negative even for probes for *Enterobacteriaceae*.
423 † four *Bacteroides* sp., three *Fusobacterium* sp., three *Propionibacterium acnes*, one *Clostridium perfringens*, one *Burkholderia gladioli*, one
424 *Bordetella bronchiseptica* and one *Stenotrophomonas maltophilia*.

Table 4. Results of the FilmArray Blood Culture Identification panel (FA) and culture compared to clinical criteria

	TI	Non-TI	Total
Culture			
Positive	51	0	51
Negative	5	32	37
Total	56	32	88
FA			
Positive	41	1	42
Negative	15	31	46
Total	56	32	88

TI: True infection. We considered a positive result to be clinically significant when the microbiological results from the FilmArray panel or the culture-based methods, or both, were concordant with clinical data (Isenberg. 2004).
Culture results: sensitivity 91% (80-97%), specificity 100% (87-100%), positive predictive value 100% (91-100%), negative predictive value 86% (70-95%).
FA results: sensitivity 73% (59-84%), specificity 97% (82-100%), positive predictive value 98% (86-100%), negative predictive value 67% (52-80%). The only specimen with a non-TI positive FA was considered a contaminated specimen.
95% confidence intervals are shown in brackets.

Table 5. Results of culture and FilmArray Blood Culture Identification panel (FA) for each type of sample

									Culture		FilmArray BCID panel		
	N	**TI**	**Cult +**	**FA +**	**TI/C+**	**TI/C-**	**TI/FA+**	**TI/FA-**	**% Sensitivity**	**% Specificity**	**% Sensitivity**	**% Analytical sens.**	**% Specificity**
Total	**88**	**56**	**51**	**41**	51	5	40	16	91 (80-97)	100 (87-100)	71 (58-82)	78 (64-88)	97(82-100)
Joint F	7	7	7	4	7	0	4	3	100 (56-100)	N/A	57 (20-88)	57 (20-88)	N/A
Ascitic F	7	4	4	1	4	0	1	3	100 (40-100)	100 (31-100)	25 (1-78)	25 (1-78)	100 (31-100)
Pleural F	8	4	4	1	4	0	1	3	100 (40-100)	100 (40-100)	25 (1-78)	25 (1-78)	100 (40-100)
CSF	19	11	8	8	8	3	8	3	73 (39-93)	100 (60-100)	73 (39-93)	89 (51-99)	100 (60-100)
Total SF	41	26	23	14	23	3	14	12	88 (69-97)	100 (75-100)	54 (34-73)	58 (37-77)	100 (75-100)
BAL	15	10	10	8	10	0	7	3	100 (66-100)	100 (46-100)	70 (35-92)	100 (56-100)	80 (30-99)
PSB	21	11	9	11	9	2	11	0	82 (48-97)	100 (66-100)	100 (68-100)	100 (68-100)	100 (66-100)
Total LRTS	36	21	19	19	19	2	18	3	90 (68-98)	100 (66-100)	86 (63-96)	100 (76-100)	93 (66-100)
Total AS	11	9	9	8	9	0	8	1	100 (63-100)	100 (20-100)	89 (51-99)	89 (51-99)	100 (20-100)

Cult: Culture; FA: FilmArray Blood Culture Identification panel; TI: True infection; Sens: sensitivity; F: fluid; CSF: cerebrospinal fluid; SF: fluids from normally sterile sites; BAL: bronchoalveolar lavage fluid; PSB: protected specimen brush; LRTS: lower respiratory tract samples; AS: abscess samples

Figure
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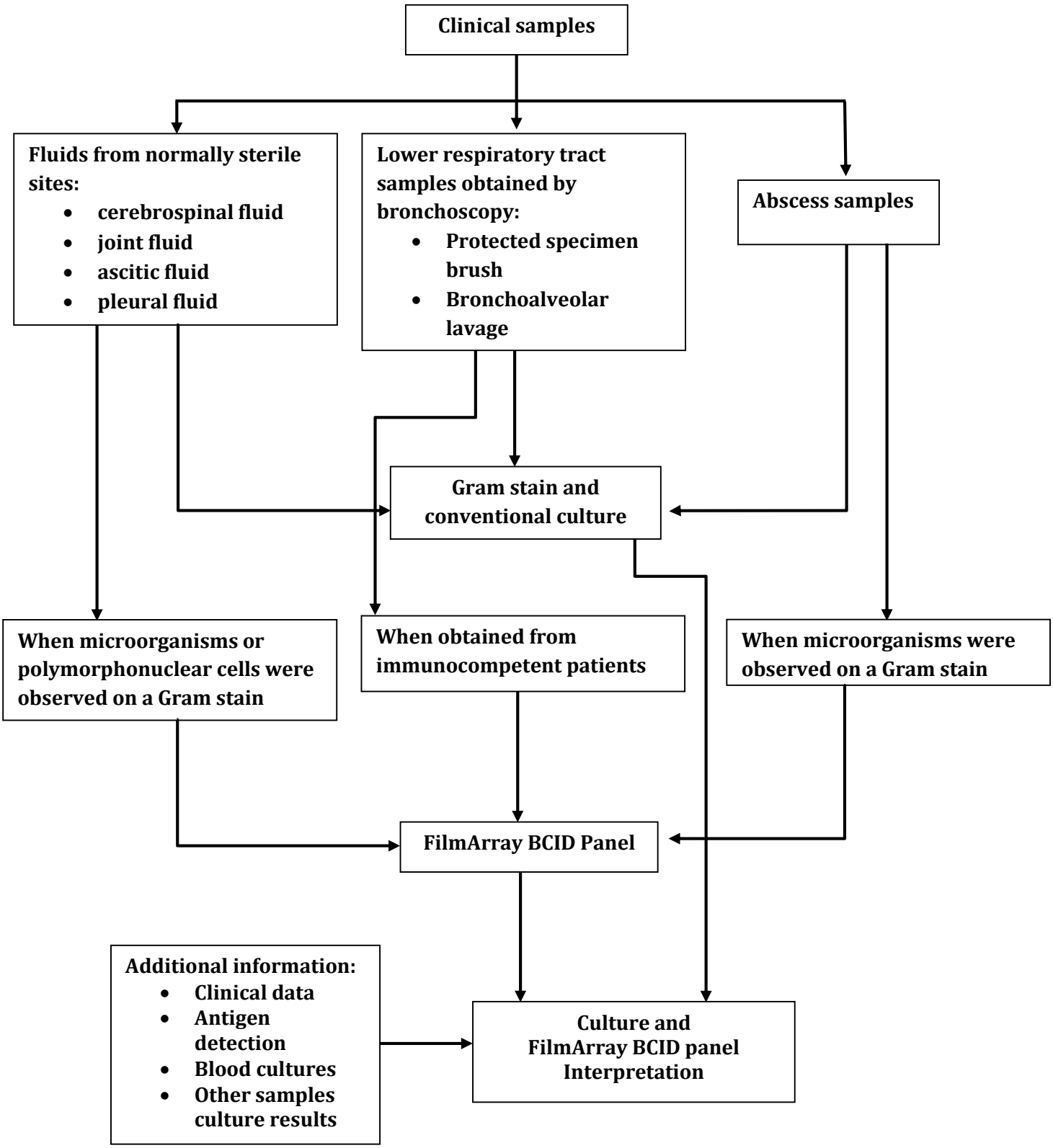
Figure 1. Flow chart for sample processing.



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