

# Biofire FilmArray Meningitis/Encephalitis panel for the aetiological diagnosis of central nervous system infections: A systematic review and diagnostic test accuracy meta-analysis

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## Summary

**Background** The FilmArray Meningitis/Encephalitis (FA/ME) panel brings benefits in clinical practice, but its diagnostic test accuracy (DTA) remains unclear. We aimed to determine the DTA of FA/ME for the aetiological diagnosis in patients with suspected central nervous system (CNS) infection.

**Methods** We performed a systematic review with DTA meta-analysis (PROSPERO: CRD42020139285). We searched Embase, Medline (Ovid), and Web of Science from inception until September 1st, 2021. We assessed the study-level risk of bias with the QUADAS-2 tool and applied the GRADE approach to assess the certainty of the synthesised evidence. We included studies that simultaneously measured the reference test (CSF/blood culture for bacteria, and specific polymerase chain reaction for viruses) and the FA/ME in patients with suspected CNS infection. We performed random-effects bivariate meta-analysis models of combined sensitivity and specificity using CSF/blood cultures (reference test 1) and a final diagnosis adjudication based on clinical/laboratory criteria (reference test 2).

**Findings** We included 19 studies (11,351 participants). For all bacteria with reference test 1 (16 studies/6183 patients) sensitivity was estimated at 89.5% (95%CI 81.1–94.4), and specificity at 97.4% (95%CI 94–98.9). With reference test 2 (15 studies/5,524 patients), sensitivity was estimated at 92.1% (95%CI 86.8–95.3) and specificity at 99.2 (95%CI 98.3–99.6). For herpes simplex virus-2 (HSV-2), enteroviruses, and Varicella-Zoster virus (VZV), we obtained sensitivities between 75.5 and 93.8%, and specificities above 99% (reference test 1). Certainty of the evidence was low.

**Interpretation** FA/ME may have acceptable-to-high sensitivities and high specificities for identifying bacteria, especially for *S.pneumoniae*, and viruses, especially for HSV-2, and enteroviruses. Sensitivities for *L.monocytogenes*, *H.influenzae*, *E.coli*, and HSV-1 were suboptimal.

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**Keywords:** CNS infection; Meningitis; Encephalitis; Film array; Multiplex PCR; Meta-analysis; Diagnostic accuracy

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### Research in context

#### *Evidence before this study*

The FilmArray Meningitis/Encephalitis panel (BioFire Diagnostics<sup>®</sup>) (FA/ME) simultaneously detects 14 pathogens in cerebrospinal fluid (CSF). A previous systematic review identified in MEDLINE searches concluded that the entire FA/ME panel has a sensitivity of 90% and a specificity of 97% for identifying any microorganism in the CSF. However, the review did not assess the certainty of the evidence, only included 13 studies, considered evidence from studies in which the reference and the index tests were not performed simultaneously and independently, did not perform meta-analyses according to specific microorganisms (viruses and bacteria) or based on specific clinical subgroups.

#### *Added value of this study*

This review is the most updated and rigorous systematic review on the diagnostic test accuracy of the FA/ME. We used state-of-art methods for conducting diagnostic test accuracy meta-analysis (including sensitivity and subgroup analyses), and we provide the certainty of the evidence for both reference tests. We provide the DTA measures of the FA/ME panel discriminated by the most important bacteria and viruses causing central nervous system (CNS) infections in immunocompetent patients, and we identified those microorganisms in which the panel may have better diagnostic accuracy.

#### *Implications of all the available evidence*

We found moderate sensitivities and high specificities for the diagnosis of bacterial CNS infection (mainly for identifying any bacteria and *S. pneumoniae*) and of enterovirus, HSV-1, and HSV-2, in immunocompetent patients. The validity for identifying *L.monocytogenes*, *H. influenzae* and *E.coli* was found to be suboptimal. In general, the FA/ME test seems to be an excellent tool for ruling in but very limited for ruling out CNS infections.

## Introduction

Central Nervous system (CNS) infections are a major cause of morbidity and mortality.<sup>1,2</sup> A timely and accurate aetiological diagnosis in CNS infections is the cornerstone for targeted, appropriate treatments that will allow better clinical outcomes such as a lower hospital stay and mortality and lower health care expenses.<sup>3–5</sup> However, appropriate aetiological diagnoses of meningitis and encephalitis with traditional microbiological methods, i.e., cultures, is challenging due to low diagnostic performance, long time to complete, and the previous use of antimicrobials.<sup>6</sup> Consequently, different molecular biology techniques have recently been developed.<sup>7</sup> The use of molecular panels for the simultaneous diagnosis of several microorganisms employing

multiplex PCR has opened up new possibilities and new challenges.<sup>8</sup>

The FilmArray Meningitis/Encephalitis panel (BioFire Diagnostics<sup>®</sup>) (FA/ME), approved by the Food and Drug Administration in 2015, is a panel that simultaneously detects 14 pathogens in cerebrospinal fluid (CSF). A recent meta-analysis reported that FA/ME has a sensitivity of 90% and a specificity of 97% for detecting microorganisms.<sup>9</sup> However, this review has some limitations. Authors included some studies in which the reference and the index tests were not performed simultaneously and independently, they did not perform combined analyses according to specific microorganisms, and they did not present sensitivity and specificity values for some microorganisms such as *N. meningitidis* and *L.monocytogenes* for which the largest studies did not have enough information to establish the accuracy for these pathogens,<sup>10</sup> and they did not assess the certainty of the summarized body of the evidence. Due to the gaps that still exist in the literature and practice regarding the use of this panel, we decided to conduct this systematic review to determine the diagnostic validity of FA/ME in immunocompetent patients for identifying any microorganism, and for identifying specific bacteria and viruses in suspected acute meningitis and encephalitis.

## Methods

We performed a systematic review and meta-analysis of diagnostic test accuracy (DTA) studies. We registered the protocol in the PROSPERO database (CRD42020139285), and a copy of this register is provided as supplemental material. This manuscript follows the reporting guidelines of the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) extension for DTA studies.<sup>11</sup>

### Literature search

Two authors performed searches (JT,IF) with liaison with an experienced librarian in Embase, Medline, and Web of Science. In addition, we conducted manual searches using references of the included studies, and gray literature through WorldWideScience, National Technical Information Service (NTIS), and OpenGrey databases. The search was carried out until September 1st, 2021, and we present it in the Supplemental Material Appendix 1

### Eligibility criteria

Prospective or retrospective studies with a diagnostic test or cross-sectional design were included. Studies had to simultaneously apply the reference test (CSF/blood culture for bacteria, specific PCR, or Laboratory Developed Test for viruses) and the index test (i.e. FA/

ME), in patients with suspected CNS infection. Studies also had to include reports of the detected microorganisms and sufficient data to calculate the DTA measures, i.e., true positives (TP), true negatives (TN), false positives (FP) and false negatives (FN). We excluded studies with no clear information and whose authors did not respond to e-mail contact; included only immunocompromised patients; or patients with ventriculoperitoneal shunts (or other intracranial devices).

### Study selection

Two researchers (JT,MJ) independently and in duplicate reviewed the titles and abstracts. References considered eligible by at least one reviewer were selected for full-text review. The full-texts obtained were independently reviewed in duplicate by two reviewers (JT,MJ). Reviewers resolved discrepancies through discussion.

### Data extraction

Two researchers (JT,IF) performed the data extraction independently and in duplicate, using a piloted extraction form in a Microsoft Excel sheet. We extracted the following information: publication year, study design, inclusion criteria, mean participants age, number of participants/sample size, funding source, reference tests per microorganism, diagnosis adjudication methods (additional laboratory test, CSF cytochemical, or clinical analysis result), and the required data for estimating TP, TN, FP, and FN.

### Tests' definition and results

The index test was the FA/ME. We focused on the most frequent microorganisms involved in acute CNS infections (Table 1). For bacteria, since CSF/blood cultures results can be affected by the quality of the samples or by previous antimicrobials use,<sup>6,12</sup> they can be of limited value in some cases. Thus, some authors have considered a combination of additional factors to adjudicate the presence of infection. For example, in cases with positive FA/ME and negative culture (disagreement), an additional test or a final diagnosis adjudication following a clinical analysis by the researchers was applied. We, therefore, performed two analyses, using a reference test 1 (positive CSF/blood culture and viral PCR for bacteria and viruses, respectively) and a reference test 2 (final diagnosis was adjudicated following an additional test or a clinical analyses of the cases). Table 1 provides details of both reference tests and the definitions used for TP, TN, FP, and FN.

### Risk of bias assessment

We assessed the risk of bias (RoB) of the included studies with the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2)<sup>13</sup> by two researchers (JT,IF). In

Appendix 2 (Supplemental Material), we detail the methods used the RoB assessment with the QUADAS-2 tool

### Diagnostic test accuracy measures

For both reference tests, we created  $2 \times 2$  contingency tables of the number of TP, TN, FP, and FN for "all bacteria" and for each individual microorganism. In the studies that only applied CSF culture (and not alternative viral tests to all samples regardless of the FA/ME result), only the accuracy for bacteria was analysed. Additionally, in those studies in which the data was very clear for only a subgroup of participants, and not for others, we only analysed the information for the former, and discarded the rest of the data if we could not guarantee the other subgroups met our eligibility criteria. We performed analyses for both reference tests 1 and 2 for "all bacteria" (any of our eligible bacteria), for each individual bacterium and virus.

### Statistical analyses

We used the bivariate random-effects model to estimate a summary sensitivity and specificity with their corresponding 95% confidence intervals (95%CI).<sup>14</sup> We present study-specific sensitivities and specificities in forest plots and crosshair plots, study-specific positive (LR+), and negative likelihood ratios (LR-), with their 95%CI for all the defined groups in scatterplots, and summary sensitivity and specificity estimate in summary receiver operating characteristic (SROC) plots.<sup>14</sup> Likelihood ratios were calculated from the combined sensitivity and specificities. LR+ was calculated as the combined sensitivity divided by 1- specificity. LR- was calculated as 1- the combined sensitivity divided by the combined specificity.

We assessed between-study heterogeneity through visual inspection of forest plots for sensitivity and specificity separately. We also visually inspected the study-specific effects in a ROC plot (1-specificity against sensitivity), in which the higher the scatter of the study-specific effects, the larger the prediction ellipse, and hence the higher the heterogeneity. We attempted to explore whether visual variability was based on study characteristics, including sample size and test variations. Analyses for both, reference tests 1 and 2, were performed for "all bacteria" (any bacteria), for each of the six bacteria, and for four viruses. Lastly, we performed the  $\chi^2$ -test and corresponding p-values to assess the presence of statistical heterogeneity and consider p-values < 0.05 as significant.

We aimed to assess the influence of some covariates on the DTA measures. We, therefore, conducted subgroup analyses in children/infants, abnormal CSF (according to authors' definitions), and in patients with previous antimicrobial use (defined as  $\geq 70\%$  of patients with previous antimicrobial use). We also

Index test	Reference test 1/RT1 (Cultures or viral PCR)	Reference test 2/ RT2 (Adjudicated diagnosis) <sup>†</sup>	Definitions of results according to reference test.
<b>Bacteria</b> FA/ME positive for: - <i>Streptococcus pneumoniae</i> - <i>Escherichia coli</i> K1 - <i>Haemophilus influenzae</i> - <i>Listeria monocytogenes</i> - <i>Neisseria meningitidis</i> - <i>Streptococcus agalactiae</i>	Aerobic CSF cultures/Blood culture for included microorganisms.  In case of polymicrobial scenarios <i>each microorganism was analyzed separately. For "all bacteria", we considered as positive the isolation of one (or more) microorganism.</i>	Applied only in cases where there was disagreement between FA/ME and reference test 1 (blood/CSF cultures). Final diagnosis adjudication was done through one of the following methods: a. An alternative bacterial molecular test. b. Abnormal CSF analysis (researchers' judgement). c. Clinical presentation suggestive or compatible with bacterial infection (researchers' judgement). d. A combination of previously mentioned criteria.  <b>Note:</b> In all the cases, the final adjudication was not unblinded to FA/ME results.	Reference test 1: - TP: FA/ME (+) and RT1 (+) - FP: FA/ME (+) and RT1 (-) - TN: FA/ME (-) and RT1 (-) - FN: FA/ME (-) and RT1 (+)
<b>Viruses</b> FA/ME positive for: - Enterovirus (EV) - Herpes simplex virus 1 (HSV-1) - Herpes simplex virus 2 (HSV-2) - Varicella-zoster virus (VZV)	- HSV-1 y HSV-2: PCR Simplex a HSV 1&2 Direct (Focus Diagnostics) or MultiCode RTx HSV 1&2 kit (Luminex Corporation, or PCR LDT or PCR in house with previously validated primers. - Enterovirus: Cepheid Xpert EV or with PCR LDT tests or PCR in house with previously validated primers - VZV: PCR LDT tests or PCR in house with previously validated primers.	Applied only in cases where there was disagreement between FA/ME and reference test 1 (viral PCR). Final diagnosis adjudication was done through one of the following methods: a. An alternative viral molecular test. b. Abnormal CSF analysis (researchers' judgement). c. Clinical presentation suggestive or compatible with viral infection (researchers' judgement). d. A combination of previously mentioned criteria.  <b>Note:</b> In all the cases, the final adjudication was not unblinded to FA/ME results.	Reference test 2 <sup>‡</sup> : - TP: FA/ME (+) and RT1 (+) or RT2 (+) - FP: FA/ME (+) and RT1 (-) or RT2 (-) - TN: FA/ME (-) and RT1 and RT2 (-) - FN: FA/ME (-) and RT1 and RT2 (+)

**Table 1: Definition of tests and results used.**

CSF: Cerebrospinal fluid; FA/ME: FilmArray Meningitis/Encephalitis panel; PCR: polymerase chain reaction; RT: Reference Test; TP: True positive; FP: False positive; TN: True Negative; FN: False Negative.

<sup>†</sup> It was applied only in case of disagreement of RT1; in case there is no disagreement between FA/ME y RT1, the same result for RT2 was considered.

<sup>‡</sup> In general, when the FA/ME and reference test 1 matched, this was the same result in reference test 2. When the FA/ME result and reference test 1 did not match, reference test 2 was the final adjudication of the diagnosis through a retrospective analysis of each case by the authors based on additional molecular testing, the findings and clinical evolution, and/or the results of the CSF study.

planned to explore heterogeneity if we obtained more than 10 studies, by adding covariate terms in a meta-regression to assess effects by mean age, but very few studies reported this information. Furthermore, we performed sensitivity analyses to evaluate the impact of high RoB studies in the estimates. We conducted two sensitivity analyses by restricting only to studies that had three or more QUADAS criteria judged as low, and with those studies that were judged as low RoB in all the QUADAS criteria.

All analyses were conducted using the 'mada' package in R software (version 3.3.3).<sup>15</sup> We developed a figure summarizing the calculation of post-test probabilities of having correct or incorrect diagnosis (FP, FN, TP, and TN) according to three different prevalence rates of CNS infection for both reference tests using the interactive version of the summary of findings (GRADEpro GDT: GRADEpro Guideline Development Tool [Software]. McMaster University, 2015 (developed by Evidence Prime, Inc.).

### Certainty of the evidence

We summarised key findings in a Grading of Recommendations Assessment, Development and Evaluation

(GRADE) 'Summary of findings' table indicating the certainty of the evidence for the index test according to both reference tests. The GRADE approach encompasses the assessment of the following criteria: RoB (judged by an overall assessment of QUADAS-2), imprecision, inconsistency (also known as heterogeneity), indirectness and publication bias,<sup>16</sup> and summarises the certainty on the evidence for the pooled sensitivity and specificity. The certainty of the evidence can be one of four levels: high, moderate, low, or very low.

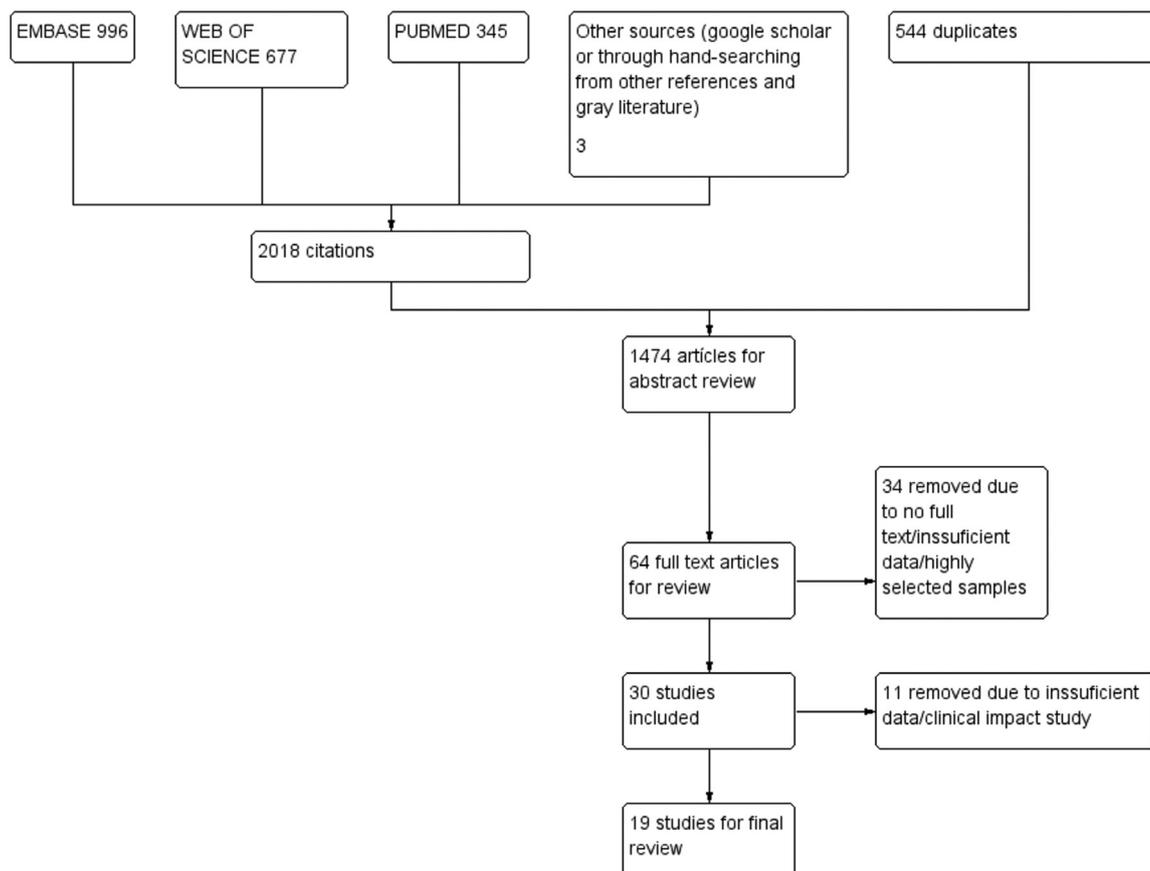
### Role of the funding source

There was no funding source for this study.

## Results

### Study selection

We retrieved 2018 references, and after removing duplicates, 1474 titles and abstracts were screened, of which 64 were selected for full-text review. We excluded 45 studies for multiple reasons (Figure 1), and we included 19 studies. Appendix 3(e-component 3) contains the excluded studies along with reasons for exclusions.



**Figure 1.** Flow diagram for study selection.

**Characteristics of the included studies**

The Appendix 4 in Supplemental Material details the characteristics of the included studies. The 19 studies included 11,351 patients.<sup>10,17–34</sup> Four studies enrolled only children,<sup>18,19,27,28</sup> two included only adults, and the rest included both. Six studies<sup>10,17,18,26,29,32</sup> used a laboratory method to resolve the disagreements between FA/ME and reference test 1, and the rest used clinical information or CSF cytochemical parameters to resolve disagreements. In total, there were 219 bacterial isolates (105 *S.pneumoniae*, 27 *E.coli*, 31 *S.agalactiae*, 24 *H.influenzae*, 21 *N.meningitidis*, and 11 *L.monocytogenes*).

**Risk of bias**

Table 2 depicts the RoB assessment. Four studies<sup>10,17,31,33</sup> were judged as unclear RoB in the patient selection domain due to uncertainty in the definition of suspected CNS infection. Five studies<sup>20,21,23,33,34</sup> were judged as high RoB in the index test criterion due to unclear handling of the sample, storage (recently collected, stored or frozen) or processing of the FA/ME. Six studies were classified as low RoB in the reference test domain,<sup>10,17,18,22,26,29</sup> and the remaining were judged as high RoB since it was not clear whether lumbar puncture and CSF sample

collection occurred before antimicrobial therapy, and they did not use alternative molecular tests for bacteria. Seventeen and two studies were judged as of low and unclear RoB, respectively, in the flow and timing domain.<sup>20,21</sup>

**Main results**

In the meta-analysis of “all bacteria” with reference test 1 (16 studies/6183 patients),<sup>10,17–22,24–27,29–33</sup> we obtained combined sensitivity and specificity of 89.5% (95%CI 81.1–94.4), and 97.4% (95%CI 94–98.9), respectively. With reference test 2 (15 studies/5524 patients),<sup>10,17,29–33,18–22,24,26,27</sup> we obtained combined sensitivity and specificity of 92.1% (95%CI 86.8–95.3), and 99.2(95%CI 98.3–99.6), respectively. Table 3 and Figures 2–5 present all the combined DTA measures and the forest plots and SROC, respectively, for both reference tests. Figures 6 and 7 show the GRADE summary of findings table for sensitivity and specificity for reference tests 1 and 2, respectively, which were in all the cases rated as low.

For *S. pneumoniae* (16 studies/7090 participants)<sup>10,17,29–34,18–22,24–26</sup> we obtained combined sensitivity and specificity of 87.5% (95%CI 77–94), and 98.5% (95%CI 97–99.3), respectively, for

Study	Patient selection	Index Test	Reference standard				Flow and Timing
			Bacteria reference test 1 <sup>†</sup>	Bacteria reference test 2 <sup>‡</sup>	Viruses reference test 1	Viruses reference test 2 <sup>§</sup>	
Arora 2016	Low risk	Low risk	High risk	High risk	Not applicable <sup>¶</sup>	Not applicable	Low risk
Bailu 2019	Low risk	Low risk	Low risk	High risk	Not applicable	Not applicable	Low risk
Barnes 2018	Low risk	Low risk	High risk	High risk	Not applicable	Not applicable	Low risk
Boudet 2019	Low risk	High risk	High risk	High risk	Not applicable	Not applicable	Unclear
Chong 2021	Low Risk	Low Risk	Low Risk	High risk	Not applicable	Not applicable	Low Risk
Domingues 2019	Low risk	High risk	High risk	High risk	Not applicable	Not applicable	Unclear
Eichinger 2019	Low risk	Low risk	High risk	High risk	Not applicable	Not applicable	Low risk
Ena 2021	Low Risk	Unclear	High Risk	High risk	Not applicable	Not applicable	Low Risk
Hanson 2016	Unclear	Unclear	Low risk	High risk	Not applicable	Not applicable	Low risk
Lindstrom 2021	Low Risk	High Risk	Not applicable	Not applicable	Low risk	High risk	Low Risk
Leber 2016	Unclear	Low risk	Low risk	High risk	Low Risk	High Risk	Low risk
Leli 2019	Unclear	Low risk	High risk	High risk	Not applicable	Not applicable	Low risk
Lopez-Amor 2019	Low risk	Unclear	High risk	High risk	Not applicable	Not applicable	Low risk
Peñata 2020	Low Risk	Low Risk	High risk	Not applicable	Not applicable	Not applicable	Low Risk
Piccirilli 2018	Low risk	Low risk	Low risk	High risk	Low Risk	High Risk	Low risk
Radmard 2019	Unclear	High risk	High risk	High risk	Not applicable	Not applicable	Low risk
Tarai 2019	Low risk	High risk	High risk	High risk	Not applicable	Not applicable	Low risk
Vincent 2020	Low risk	Low risk	Low risk	High risk	Low risk	High risk	Low risk

**Table 2: Risk of bias assessments.**

<sup>†</sup> Risk of bias assessment performed with the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool. Reference tests 1 & 2 definitions for bacteria and viruses are detailed in Table 1.

<sup>‡</sup> Reference standard item for reference test 1 for bacterial detection was considered as low risk only when it was clear that the cultures samples were taken before the antimicrobial treatment.

<sup>§</sup> All reference test 2 for both bacteria and viruses were judged as high risk because none of these diagnosis adjudications were conducted blinded to the index test results.

<sup>¶</sup> Items were not applicable when the reference test was not used for viruses.

	Ref. Test 1 <sup>†</sup>					Ref. Test 2 <sup>‡</sup>				
	No. Studies / No. Patients (Ref. studies)	Sensitivity (95%CI) X <sup>2</sup> ; p value <sup>§</sup>	Specificity (95%CI). X <sup>2</sup> ; p value <sup>§</sup>	LR+ (95%CI)	LR- (95%CI)	No. Studies /No. Patients (Ref. studies)	Sensitivity (95%CI). X <sup>2</sup> ; p value <sup>§</sup>	Specificity (95%CI) X <sup>2</sup> ; p value <sup>§</sup>	LR+ (95%CI)	LR- (95%CI)
All bacteria	16/6183 <sup>10,17,22,24–27,29–33</sup>	89.5 (81.1–94.4) 6.00; 0.98	97.4 (94–98.9) 251.9; <0.0001	34 (2.68–137)	0.11 (0.07–3.64)	15/5545 (10,17,29–33,18 –22,24,26,27)	93.5 (87.8–96.6) 2.17; 0.99	99.1 (97.8–99.6) 60.2; <0.0001	104 (7.60–3.98)	0.07 (0.07–3.58)
<i>S. pneumoniae</i>	16/7090 <sup>10,17–22,24–26,30,34</sup>	87.5 (77–94) 3.71; 0.999	98.5 (97–99.3) 144.7; <0.0001	58 (4.63–238)	0.13 (0.07–3.60)	10/5287 (10,17,30,32,33,34, 18,21,22,24)	93 (83.3–97.2) 2.64; 0.91	99.4 (98.2–99.8) 51.2; <0.0001	155 (11–600)	0.07 (0.07–3.58)
<i>H. influenzae</i>	10/4959 <sup>10,17,18,20–22,24,25,30,32</sup>	64.9 (39.5–84) 4.91; 0.842	99.4 (98.9–99.6) 22.4; 0.07	108 (11–601)	0.35 (0.07–3.57)	7/3176 (10,17,18,20,21,24,30)	81.1 (55.6–93.6) 4.97; 0.42	99.8 (99.5–99.9) 5.53; 0.354	405 (28–1889)	0.19 (0.07–3.56)
<i>S. agalactiae</i>	10/5266 <sup>10,17,18,20,22,25–27,31,33</sup>	71.5 (49.6–86.5) 7.67; 0.56	99.5 (98.5–99.9) 7.67; 0.56	143 (13–722)	0.29 (0.07–3.57)	5/2543 10,17,18,20,27	81.4 (52.3–94.6) 6.71; 0.15	99.4 (97.7–99.9) 23.42; <0.0001	136 (11–609)	0.19 (0.07–3.57)
<i>E. coli</i>	11/4743 <sup>10,17–21,25,27,30,32,33</sup>	70.9 (50.2–85.5) 4.93; 0.896	99.6 (99.1–99.8) 25.5; 0.0043	177 (16–909)	0.29 (0.07–3.56)	5/2570 10,18,20,30,32	76.3 (47.6–91.9) 3.56; 0.46	99.6 (98.7–99.9) 12.62; 0.01	191 (15–925)	0.24 (0.07–3.56)
<i>N. meningitidis</i>	10/3501 <sup>17,18,20–22,24,25,29–31</sup>	74.5 (52.9–88.4) 2.26; 0.986	99.1 (98.6–99.5) 20.9; 0.013	83 (7.48–400)	0.26 (0.07–3.58)	5/1950 18,21,22,30,31	84.4 (53.9–96.2) 0.84; 0.838	99.1 (98.8–99.9) 1.17; 0.759	281 (19–1265)	0.16 (0.07–3.56)
<i>L. monocytogenes</i>	7/1332 <sup>18,21,24,25,29,31,32</sup>	70.4 (40–89.5) 0.504; 0.008	98.9 (96.9–99.6) 5.62; 0.22	54 (4.99–280)	0.30 (0.07–3.60)	3/550 18,21,24)	80.4 (40.4–96.1) 0.205; 0.903	99.5 (97.8–99.9) 2.13; 0.344	161 (9.03–796)	0.20 (0.07–3.57)
Enterovirus	3/6883 <sup>10,22,23</sup>	93.8 (87–97.2) 2.91; 0.23	99.3 (98.7–99.7) 28.53; <0.001	313 (22–1209)	0.06 (0.07–3.56)	3/6883 10, 22, 23	99.8 (86.1–97.4) 4.18; 0.123	99.9 (99.7–100) 3.43; 0.179	998 (58–3763)	0.04 (0.07–3.55)
HSV-1	3/6883 <sup>10,22,23</sup>	75.5 (51.2–90.1) 1.18; 0.554	99.9 (94.7–100) 2.55; 0.28	755 (58–3763)	0.25 (0.07–3.55)	3/6883 10, 22, 23	78.2 (58.1–90.3) 0.69; 0.706	99.9 (99.8–100) 1.8; 0.405	782 (58–3763)	0.22 (0.07–3.55)
HSV-2	3/6883 <sup>10,22,23</sup>	94.4 (83.9–98.2) 0.435; 0.804	99.9 (99.7–100) 1.36; 0.507	944 (58–3763)	0.06 (0.07–3.55)	3/6883 10, 22, 23	94.5 (84.2–98.2) 0.46; 0.79	99.9 (99.8–100) 1.36; 0.507	945 (58–3763)	0.06 (0.07–3.55)
VZV	4/6897 <sup>10,21,23,29</sup>	91.4 (78.9–96.9) 0.82; 0.84	99.8 (98.7–100) 23.55; <0.001	457 (32–1832)	0.09 (0.07–3.56)	4/6897 10,21,23,29	93.3 (83.6–97.4) 0.16; 0.91	99.9 (99.6–100) 6.23; 0.004	933 (58–3763)	0.07 (0.07–3.55)

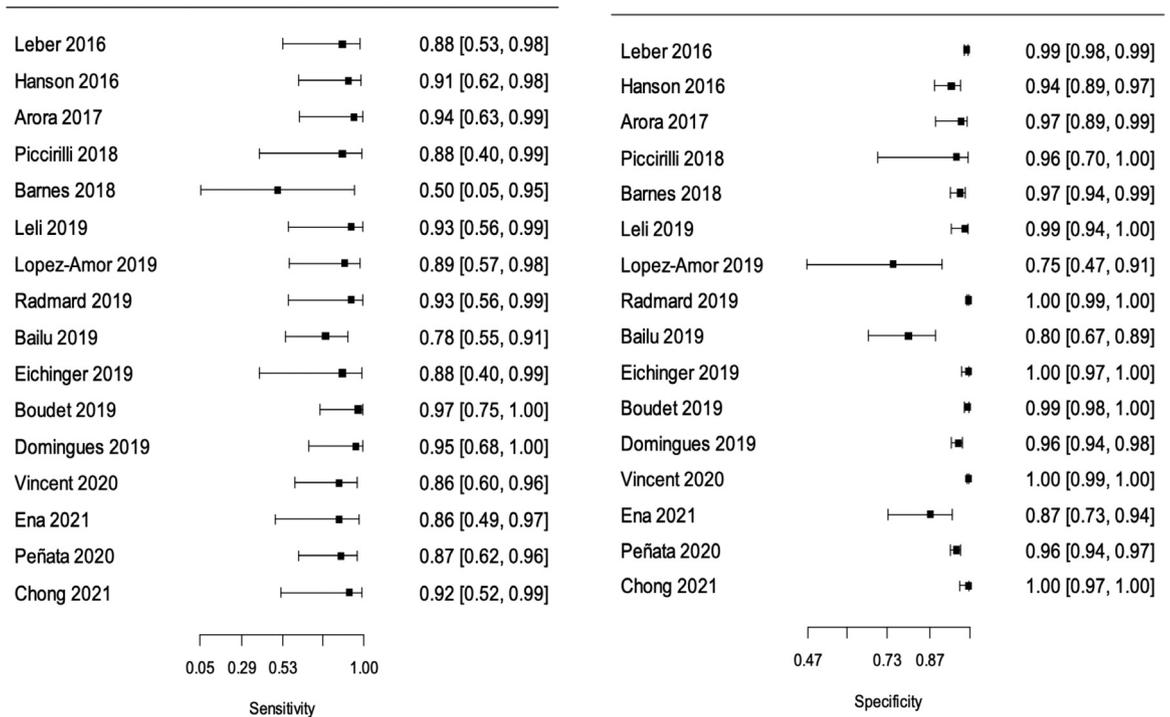
**Table 3: Meta-analyses of all bacteria and per bacteria and viruses, with reference test 1 and reference test 2.**

CSF: Cerebro-Spinal fluid; LR+: Positive Likelihood ratio; LR-: Negative Likelihood ratio; X<sup>2</sup>: Chi-2 test.

<sup>†</sup> Reference Test 1: Aerobic CSF cultures/Blood culture or viral PCR.

<sup>‡</sup> Reference Test 2: It was applied only in case of disagreement of RT1. Final adjudication of the diagnosis through a retrospective analysis of each case by the authors based on additional molecular testing, findings, and clinical evolution, and/or the results of the cerebrospinal fluid study.

<sup>§</sup> Chi2-test and corresponding p-value to assess presence of statistical heterogeneity.



**Figure 2.** Forest Plot for “all bacteria” with reference test 1. Sensitivities (left) and specificities (right) of FA/ME per study for the detection of any bacteria in Cerebrospinal (CSF) fluid when the reference standard was a positive CSF or a blood culture (reference test 1).

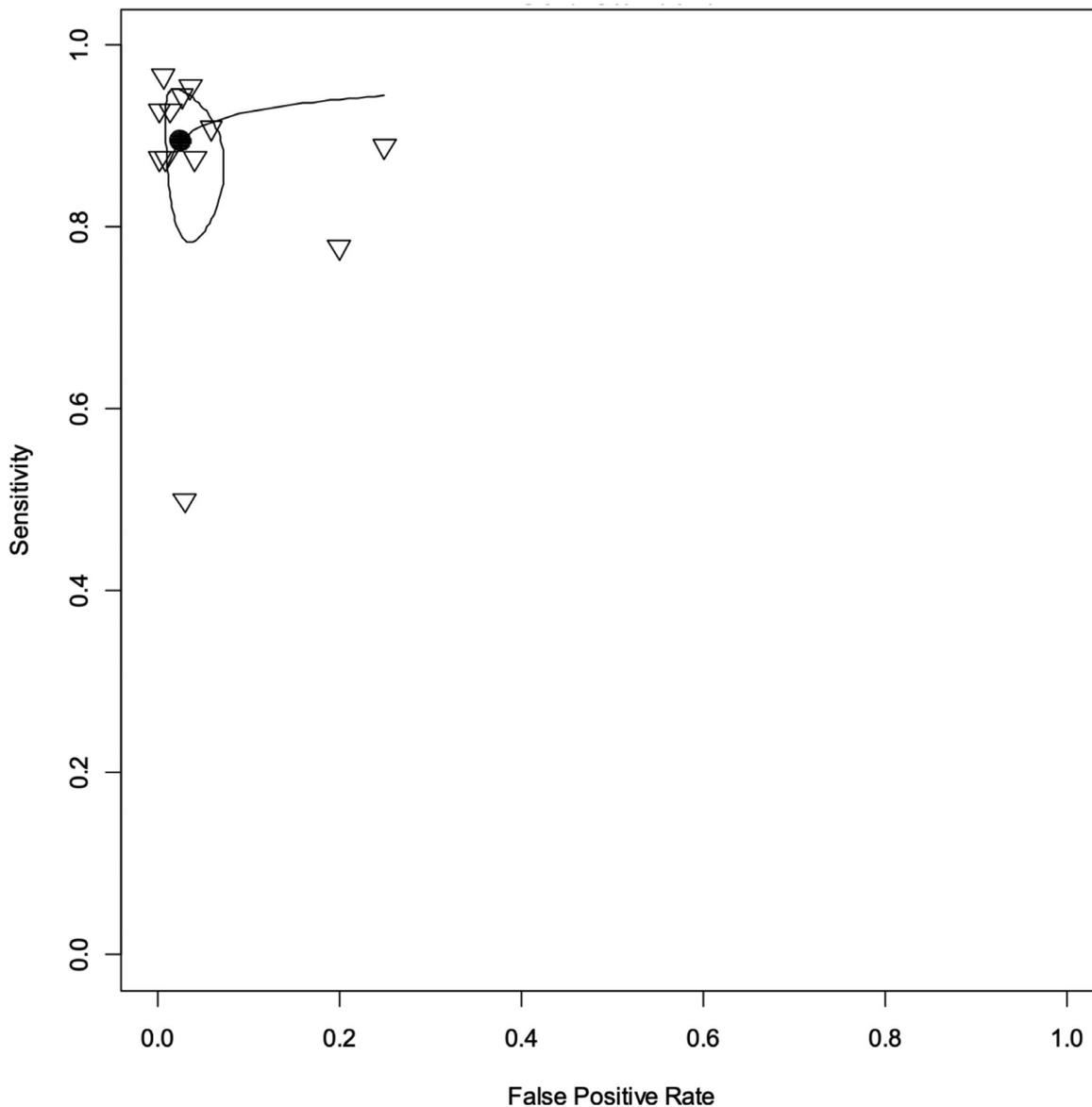
reference test 1, and 93.4% (95%CI 85.4–97.1) and 99.5% (95%CI 98.6–99.8), respectively, for reference test 2. The main DTA measures for the rest of the microorganisms are detailed in Table 3. In general, LR+ were optimal for all the bacteria, being *L.monocytogenes*, *E.coli*, *S.agalactiae* and *H.influenzae*, the ones with higher values (LR+ >100) for reference test 1. The LR- values, on the other hand, were acceptable, and none of the bacteria had very low values (i.e., LR- all > 0.1). The Forest plots and SROC curves for all these microorganisms are presented in the Appendix 5 of the Supplemental Material).

In the Appendix 6 of the Supplemental Material, we discriminate the total positive results for FA/ME by each bacterium and study, along with TP and FP rates for both reference tests. For a total of 211 bacteria (16 studies, 6514 patients) detected by FA/ME or reference test, 113 (53.5%) were considered true positives and 98 (46.4%) false positives based on reference test 1. Based on reference test 2, 191 (90.5%) were considered true positives and 20 (9.4%) false positives. Since predictive values of tests depend on the prevalence of the disease, we have summarised and presented three different clinical scenarios according to different prevalence values (2, 5 and 10%), to display the expected TP, FP, TN, and FN probabilities for both reference tests for bacteria detection, in Figure 8. As expected, the higher the prevalence, the lower the FP rate for both reference tests.

As for the viruses, we performed meta-analyses for the detection of enterovirus, Herpes simplex virus 1 and 2 (HSV-1, HSV-2) and Varicella-Zoster virus (VZV). For enterovirus, HSV-1 and HSV-2, three studies were analysed (6883 patients)<sup>10,22,23</sup> For enterovirus, we obtained a combined sensitivity and specificity of 93.8% (95%CI 87–97.2) and 99.7% (95%CI 98.1%–100%), respectively, for reference test 1, and 99.8 (95%CI 86.1–97.4) and 99.9% (95%CI 99.7–100), for reference test 2. The DTA measures of all the viruses analysed, for both reference tests, are presented in Table 3. Forest plots and the SROC curves for the rest of the viruses are presented in the Appendix 7 of the Supplemental Material.

**Additional analyses**

In the RoB sensitivity analysis 1, for all bacteria, we combined results from 7 studies, and we obtained a lower combined sensitivity and very similar specificity: 84.4% (95%CI 72–92%), and 98% (95%CI 93.5 to 99.4), respectively. In the RoB sensitivity analysis 2, for all bacteria, we combined results from 3 studies, and we also obtained a lower combined sensitivity and a lower specificity: 82.5% (95%CI 65.3–92.3), and 98.7% (95%CI 67.8–99.8), respectively (Appendix 8, Supplemental Material).



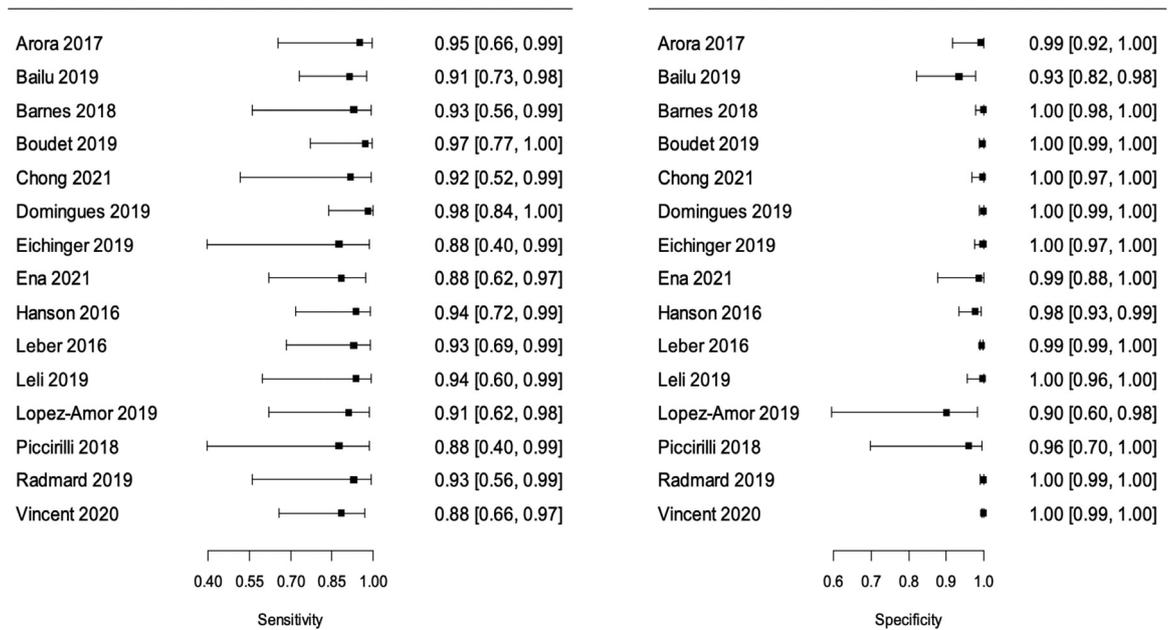
**Figure 3.** Summary receiver operating characteristic (SROC) curve for "all bacteria" with reference test 1. Each study is identified with a small reverse triangle. Back dot denotes the combined sensitivity and specificity. The figure also shows 95% confidence contour and 95% prediction contour. Reference test 1 means the standard was a positive cerebrospinal or a blood culture.

Subgroup analyses are presented in [Table 4](#). In the analysis of studies including only infants and children, we obtained a combined sensitivity and specificity of 83.6% (95%CI 65.6–93.2) and 97.4% (95%CI 84.8–99.6), respectively. In patients with abnormal CSF (defined by the authors as >10 CSF cells in one study and with no clear definition in another study), with two studies, the combined sensitivity and specificity were 94.4% (95%CI 65.6–99.3) and 99.6% (95% CI 93.7–100), respectively. Forest plots for all subgroups analyses are presented in the Appendix 9 of the Supplemental Material. Although LR+ are high for all the

subgroups (except for previous antimicrobials' use), their 95%CI were wide related with high uncertainty. LR- values were acceptable, except in the patients with abnormal CSF which showed to be very low, but with wide 95%CI.

### Discussion

In this systematic review (19 studies; 11,351), we found that the FA/ME panel has moderate sensitivities and very high specificities for identifying the selected bacteria and viruses. However, sensitivity values for bacteria



**Figure 4.** Forest Plot for “all bacteria” with reference test 2. Sensitivities (left) and specificities (right) of FA/ME for the detection of any bacteria with reference test 2 per study. Reference test 2 means the standard (final diagnosis of the infection in cases where cerebrospinal fluids SF/blood cultures or viral tests were negative) was defined by the researchers through a final diagnosis adjudication using molecular tests, an analysis of the clinical manifestations or based on the cerebrospinal fluid findings.

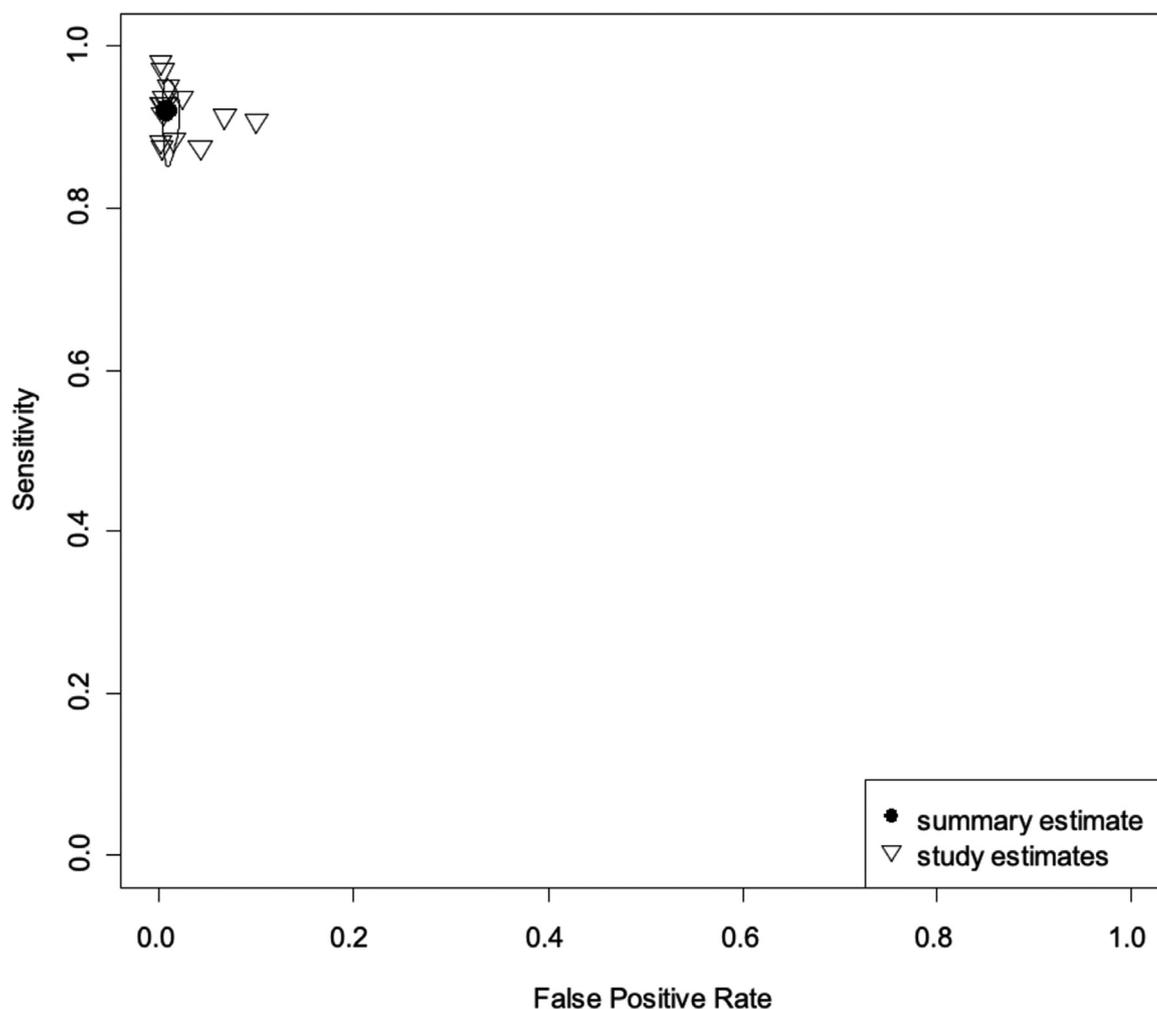
(“all bacteria” and individual bacteria) were lower than 90% in all cases of reference test 1 (CSF/blood culture). These sensitivities increase when clinical and other test analyses complement cultures results (reference test 2). Namely, for the detection of any bacteria, the sensitivity of FA/ME seems to range between 89.5% (reference test 1) and 93.5% (reference test 2). Nonetheless, sensitivity values are lower when we consider only low RoB studies (between 82.5%–84.4%), and the certainty of the evidence was low. The only analyses in which we obtained sensitivity values higher than 90% and LR- lower than 0.1, for bacteria, were “all bacteria” and *S. pneumoniae* with reference test 2. The worst sensitivity values for bacteria were found for *L.monocytogenes*, *H. influenzae* and *E.coli*. Sensitivities and LR- were also sub-optimal for HSV-1 but were high for VZV, HSV-2 and specially, for enteroviruses. Nonetheless, specificity and LR+ values were optimal for bacteria and viruses. Thus, in summary, FA/ME seems to be excellent for ruling in all the analysed bacteria and viruses, very limited for ruling out bacteria (acceptable for ruling out *S. pneumoniae*) HSV-1 and VZV, and excellent for ruling out enterovirus, VZV, and HSV-2.

However, most studies were judged as high RoB due to issues related to the reference test domain. In reference test 1, this is explained by the limitations of the CSF/blood cultures, which are easily affected by previous antimicrobial use and due to limitations in the samples’ management protocols. Reference test 2 would

be an ideal approach as it can incorporate clinical and other tests analyses for diagnosis adjudication. Nonetheless, in all the studies, this adjudication was unblinded to the index test results. Moreover, the criteria used by researchers for the adjudication varied among the studies.

Our planned a priori subgroup analyses showed some interesting findings. Sensitivities for bacteria were lower in children than the obtained in the complete analyses, while specificities remained high. Moreover, the DTA measures in patients with abnormal CSF were remarkably high, which may suggest that using the FA/ME in this population might be an alternative to testing all the patients. While it is true that there are cases of viral CNS infection with normal CSF, such as early stages of herpetic encephalitis,<sup>35–37</sup> and enterovirus meningitis in neonates,<sup>38,39</sup> these are rare and occur in precise clinical settings.<sup>40</sup> Considering the limitations of FA/ME for some microorganisms and its relatively high cost, defining diagnostic algorithms approaches to define the group of patients who can benefit the most from this test is an urgent need.

Very high specificity values were found for all the microorganisms, which shows that the FP of FA/ME may be irrelevant. However, when analysing in detail the proportion of FP of the total of positive results, the former values are not negligible; that is, the positive predictive values (PPV) are not high. For instance, for reference test 1, we found FP of 46.4% (Appendix 29). FP



**Figure 5.** Summary receiver operating characteristic (SROC) curve for "all bacteria" with reference test 2. Each study is identified with a small reverse triangle. Back dot denotes the combined sensitivity and specificity. The figure also shows 95% confidence contour and 95% prediction contour. Reference test 2 means the standard (final diagnosis of the infection in cases where cerebrospinal fluids SF/blood cultures or viral tests were negative) was defined by the researchers through a final diagnosis adjudication using molecular tests, an analysis of the clinical manifestations or based on the CSF findings.

are higher for *S.pneumoniae* and *S.agalactiae*. PPV and negative predictive values (NPV) depend on the disease prevalence in each context, and the positive result should always be analysed in combination with clinical manifestation and additional tests to make a final diagnosis. The prevalence of CNS infections in the complete sample of the meta-analysis for all bacteria was 2%. The low pre-test probability of CNS infections as a result of a significant decrease in recent years,<sup>6,41</sup> explains the low prevalence and a high FP rate. Clinical scenarios where prevalence rates are higher such as 5 or 10% will yield PPV of 33% and NPV of 18%. Causes of FP are related to sample contamination and cross-reactivity with other bacteria.<sup>8,10</sup> Limiting the use of FA/ME to more selected cases with higher pre-test probabilities will yield lower FP.

Furthermore, we found that the FP were much lower (9.4%) with reference test 2 (Appendix 29), which means that the clinical analysis to complement the FA/ME resulted in some cases adjudicated as a CNS infection. However, we should add a caveat here, as the reference standard 2 assessment was judged as of high risk in all the studies because it was unblinded. Therefore, we cannot be certain about how many of those adjudicated cases were due to a real specific infection or could have been biased due to the knowledge of the FA/ME results. For bacteria, we think the true FP rate may be between 9.4 and 46.4%. Further studies would need to apply a reference test 2 in a blinded fashion, so we can obtain more certain DTA measures. Regardless of these results, in the clinical context, the best approach to dealing with cases in which a FA/ME FP is suspected is to

Question: Should BioFire FilmArray ME Panel be used to diagnose Bacterial Meningitis in patients with suspected CNS infection (Reference test 1)?

Sensitivity	0.90 (95% CI: 0.81 to 0.94)
Specificity	0.97 (95% CI: 0.94 to 0.99)

Prevalences	2%	5%	10%
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Outcome	No of studies (No of patients)	Study design	Factors that may decrease certainty of evidence					Effect per 1,000 patients tested			Test accuracy CoE
			Risk of bias	Indirectness	Inconsistency	Imprecision	Publication bias	pre-test probability of 2%	pre-test probability of 5%	pre-test probability of 10%	
<b>True positives</b> (patients with Bacterial Meningitis)	16 studies 6183 patients	cross-sectional (cohort type accuracy study)	serious <sup>a</sup>	not serious	not serious	serious <sup>b</sup>	none	18 (16 to 19)	45 (41 to 47)	90 (81 to 94)	⊕⊕○○ Low
<b>False negatives</b> (patients incorrectly classified as not having Bacterial Meningitis)								2 (1 to 4)	5 (3 to 9)	10 (6 to 19)	
<b>True negatives</b> (patients without Bacterial Meningitis)	16 studies 6183 patients	cross-sectional (cohort type accuracy study)	serious <sup>a</sup>	not serious	serious <sup>c</sup>	not serious	none	955 (921 to 969)	925 (893 to 940)	877 (846 to 890)	⊕⊕○○ Low
<b>False positives</b> (patients incorrectly classified as having Bacterial Meningitis)								25 (11 to 59)	25 (10 to 57)	23 (10 to 54)	

Explanations

- a. Most of the studies had low risk in patient selection, flow and timing, and index test criteria. However, most of them were high risk due to the reference standards criterion.
- b. Wide confidence interval showing a sensitivity of 0.81 in the worst-case scenario and 0.94 in the best-case scenario
- c. Results from individual studies were heterogeneous, and Chi-2 test showed statistical significance (P<0.0001)

**Figure 6.** GRADE Summary of findings table for reference test 1. The table summarises the certainty of the evidence according to the Grading of Recommendations Assessment, Development and Evaluation (GRADE) methodology, also called “quality of evidence”. The certainty can be one of four levels: High, moderate, low, or very low. The interpretation of these levels should be performed as follows; High: we are very confident that the true effect lies close to that of the estimate of the effect we found; Moderate: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect that we found, but there is a possibility that it is substantially different. Low: our confidence in the effect estimate we found is limited: the true effect may be substantially different from the estimate of the effect; Very low: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect we found. The results presented in this table should not be interpreted in isolation from the results of individual included studies contributing to each summary test accuracy measure. Reference test 1 means the standard was a positive CSF or a blood culture.

assess the clinical scenario (i.e., considering clinical manifestations and history of antimicrobial use) and laboratory findings (CSF results).

To date, there is only one systematic review on the diagnostic validity of FA/ME<sup>9</sup>. In this review, the authors found higher sensitivity and specificity for the entire FA/ME panel. Their results differ from ours in several aspects. We provide the DTA measures discriminated by the most important bacteria and viruses, and therefore, we could highlight those microorganisms in which the FA/ME panel may have higher DTA measures, such as enteroviruses, or those with suboptimal performance of the FA/ME, such as *L.monocytogenes*, *H. influenzae* and *E.coli*. Also, in the mentioned review authors included some studies we excluded because they failed to demonstrate they performed index and reference tests independently, an essential element for a high-quality DTA analysis. Moreover, our RoB assessment was performed according to the characteristics of both reference tests, and as a result, the findings from the adjudication diagnosis (reference test 2), were judged to have high RoB. Lastly, we have doubled the number of included studies providing more updated evidence and we have applied cutting-edge methods for DTA synthesis, including conducting sensitivity and subgroup analyses, following the PRISMA-DTA

guidance, and applying the GRADE approach to assess the certainty of the evidence.

Our results have important implications for clinical practice. The FA/ME has the advantages of a higher speed of the results, the ability to test for multiple organisms simultaneously, and it is not affected by previous antimicrobials use. These benefits may translate into targeted and timely treatments to initiate, change, or dismantle an antimicrobial treatment, which in turn can be reflected in fewer adverse effects and shorter hospital stays.<sup>42–47</sup> However, the performance of the test varies among microorganisms and depends on the intended purpose. FA/ME seems better for ruling in, than for ruling out, the disease. Therefore, clinicians should be very cautious about their results given the relatively high LR- values and high FP in scenarios with a low prevalence of CNS infections.

Future DTA studies interested in filling some of the identified gaps need to perform a blind adjudication of the diagnosis to reduce biases associated with the reference test and need to apply the reference test (specific PCR) for each of the viruses, independent of the result of the panel in patients with suspected CNS infection. Likewise, studies focused on specific subpopulations such as neonates, patients with previous antimicrobial use, or with abnormal CSF are required.

Question: Should BioFire FilmArray ME Panel be used to diagnose Bacterial Meningitis in patients with suspected CNS infection (Reference test 2)?

Sensitivity	0.94 (95% CI: 0.88 to 0.97)	Prevalences		
Specificity	0.99 (95% CI: 0.98 to 1.00)	2%	5%	10%

Outcome	No of studies (No of patients)	Study design	Factors that may decrease certainty of evidence					Effect per 1,000 patients tested			Test accuracy CoE
			Risk of bias	Indirectness	Inconsistency	Imprecision	Publication bias	pre-test probability of 2%	pre-test probability of 5%	pre-test probability of 10%	
<b>True positives</b> (patients with Bacterial Meningitis)	15 studies 5545 patients	cross-sectional (cohort type accuracy study)	serious <sup>a</sup>	not serious	not serious	serious <sup>b</sup>	none	19 (18 to 19)	47 (44 to 48)	94 (88 to 97)	⊕⊕○○ Low
<b>False negatives</b> (patients incorrectly classified as not having Bacterial Meningitis)								1 (1 to 2)	3 (2 to 6)	6 (3 to 12)	
<b>True negatives</b> (patients without Bacterial Meningitis)	15 studies 5545 patients	cross-sectional (cohort type accuracy study)	serious <sup>a</sup>	not serious	serious <sup>c</sup>	not serious	none	971 (958 to 976)	941 (929 to 946)	892 (880 to 896)	⊕⊕○○ Low
<b>False positives</b> (patients incorrectly classified as having Bacterial Meningitis)								9 (4 to 22)	9 (4 to 21)	8 (4 to 20)	

**Explanations**

a. In this synthesis, all the studies used as reference test a combination of culture and clinical and additional lab tests criteria (reference test 2). Clinical and other laboratory tests criteria were applied to those cases they defined as disagreement between the culture and the FA/ME panel (former negative and latter positive). Clinical other laboratory analyses were performed in these cases and researchers and experts defined whether the patients should be considered as positive cases. These analyses were not blinded, and as such, the final diagnosis adjudication may have been influenced by the knowledge of the FA/ME test result  
 b. Wide confidence intervals, including a sensitivity of 88% in the worst-case scenario and 97% in the best-case scenario  
 c. Heterogeneity identified. Inconsistency test: X<sup>2</sup>=5.9, p<0.0001

**Figure 7.** GRADE Summary of findings table for reference test 2. The table summarises the certainty of the evidence according to the Grading of Recommendations Assessment, Development and Evaluation (GRADE) methodology, also called “quality of evidence”. The certainty can be one of four levels: High, moderate, low or very low. The interpretation of these levels should be performed as follows; High: we are very confident that the true effect lies close to that of the estimate of the effect we found; Moderate: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect that we found, but there is a possibility that it is substantially different. Low: our confidence in the effect estimate we found is limited: the true effect may be substantially different from the estimate of the effect; Very low: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect we found. The results presented in this table should not be interpreted in isolation from the results of individual included studies contributing to each summary test accuracy measure. Reference test 2 means the standard (final diagnosis of the infection in cases where cerebrospinal fluids SF/blood cultures or viral tests were negative) was defined by the researchers through a final diagnosis adjudication using molecular tests, an analysis of the clinical manifestations or based on the CSF findings.

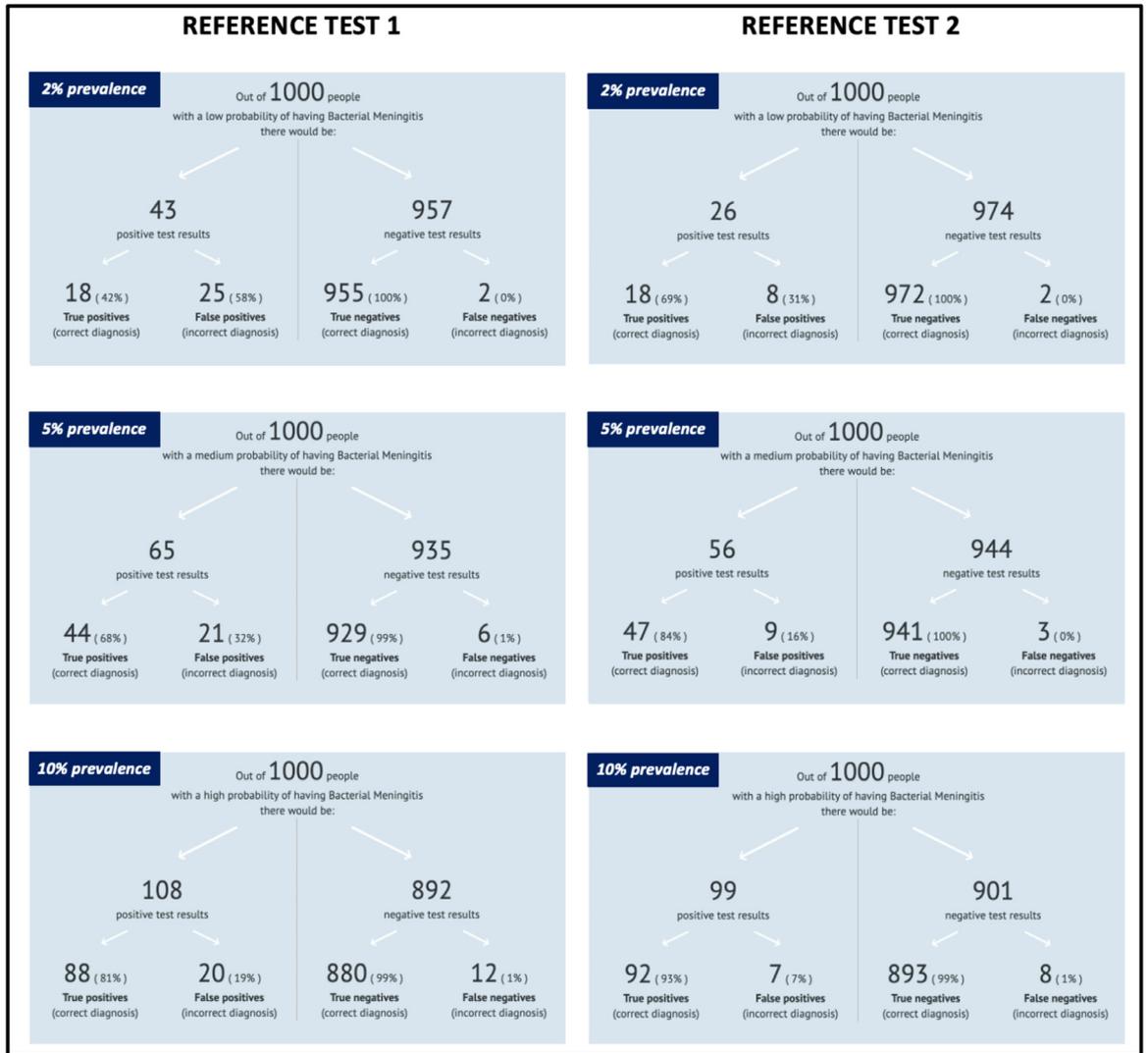
Our review has several strengths. This is the first review that presents DTA measures discriminated by microorganisms (six bacteria and four viruses). As mentioned above, we followed the highest methodological standards for a systematic review and DTA meta-analysis, and followed the PRISMA-DTA guidelines.<sup>11</sup> We only included studies that performed both index and reference tests simultaneously, i.e., we did not include studies in which FA/ME was performed after results from the cultures or other molecular tests were known. We conducted analyses by type of reference test, type of microorganism, sensitivity analyses, and by some subgroups, we assessed the certainty of the evidence with the GRADE approach, and we present figures that facilitate results interpretation and contextualization for users according to different pre-test probabilities.

Our study is not free of limitations. The criteria used for resolving disagreements between the FA/ME and the cultures varied among the studies, which may have introduced some heterogeneity. We did not consider other microorganisms included in the FA/ME panel, such as cytomegalovirus, HSV-6, human parechovirus and *Cryptococcus neoformans/gattii* because their role in

CNS infections in immunocompetent patients is not clear. We are, therefore, unable to provide conclusions of the performance of the test in immunocompromised patients.

In conclusion, the FA/ME panel may be a valid diagnostic tool to identify different microorganisms in CNS infections. FA/ME may have acceptable to high sensitivity, and high specificity for identifying bacteria and viruses in CNS infections, in immunocompetent patients. However, the certainty of the results was low mostly due to the high RoB of the studies. Moreover, in the context of low and high prevalence rates of bacterial infections, the FP and the FN, respectively, can be relevant. As a result, the FA/ME validity may be reduced in some scenarios (e.g., low prevalence rates) as the only diagnostic tool, and clinicians should apply an integral analysis, including the CSF findings and the clinical manifestations.

FA/ME diagnostic validity was high for most of the studied microorganisms. Nonetheless, attention should be put, mainly, into those microorganisms with less available evidence, that had the less suboptimal DTA measures. The diagnostic validity of FA/ME to detect L.



**Figure 8.** Post-test probabilities of correct or incorrect diagnoses of meningitis according to three prevalence scenarios for both reference tests. The figure display three potential scenarios based on three different meningitis prevalence rates, for each reference test. Readers could choose a potential prevalence (low 2%, medium 5%, or high 10%) of meningitis in a patient with suspected meningitis and based on an example of 1000 patients in which we would apply the FA/ME test, the figure shows the expected positive and negative results, and the correspondent true and false positives and negatives. The larger the prevalence, the fewer the expected false positives.

*monocytogenes*, *H.influenzae*, *E.coli*, and HSV-1, may be suboptimal. More targeted DTA research on these microorganisms, on other viruses, and in specific sub-groups (children and previous antimicrobial use) and with blinded appropriate diagnosis adjudication are encouraged. Furthermore, there is an urgent need for developing protocols and diagnosis algorithms to determine the patients who can benefit the most from this FA/ME, and to conduct economic evaluations to optimize its usefulness and relevance in different contexts.

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None.

**Contributors**

JT and IDF conceptualised and designed the study; IDF supervised the project; JTG and MJJ screened literature, JT and IDF extracted data and performed the RoB and the certainty of the evidence assessment. JT and IDF had access to and verified the data. ST and AAV did the

	Ref. Test 1					Ref. Test 2				
	No. studies/ No. patients (Ref studies)	Sensitivity (95%CI) $\chi^2$ ; p value <sup>§</sup>	Specificity (95%CI). $\chi^2$ ; p value <sup>§</sup>	LR+ (95%CI)	LR- (95%CI)	No. studies/ No. patients (Ref studies)	Sensitivity (95%CI) $\chi^2$ ; p value <sup>§</sup>	Specificity (95%CI). $\chi^2$ ; p value <sup>§</sup>	LR+ (95%CI)	LR- (95%CI)
Infants and children (All bacteria)	4/462 <sup>18,19,27,28</sup>	83.6 (65.6–93.2) 1.99; 0.57	97.4 (84.8–99.6) 54.64; <0.0001	28 (2.13–122)	0.18 (0.07–3.66)	4/462 (18,19,27,28)	90.7 (69.4–97.7) 3.39; 0.33	98.4 (93.6–99.6) 11.6; 0.008	45 (3.04–186)	0.1 (0.07–3.63)
Infants and children ( <i>S. pneumoniae</i> )	3/400 <sup>18,19,28</sup>	76.6 (39.2–94.3) 0.51; 0.77	98.3 (90.7–99.7) 12.05; 0.00247	38 (2.98–187)	0.24 (0.07–3.63)	3/400 (18,19,28)	82 (41.7–96.6) 0; 1	98.8 (95.8–99.6) 0; 1	41 (2.61–193)	0.18 (0.07–3.63)
0–3 months (All bacteria)	2/207 <sup>27,28</sup>	86.4 (38.8–98.5) 0; 1.0	98.3 (94.9–99.5) 0.0546; 0.815	43 (2.59–194)	0.14 (0.07–3.63)	NA	NA	NA	NA	NA
Patients with abnormal CSF (All bacteria) <sup>†</sup>	2/482 <sup>21,24</sup>	94.4 (65.6–99.3) 0.26; 0.6	99.6 (93.7–100) 0; 1.0	94 (5.38–383)	0.06 (0.07–3.7)	2/482 (21,24)	94.4 (65.6–99.3) 0.26; 0.6	99.6 (93.7–100) 0; 1.0	94 (5.38–383)	0.06 (0.07–3.7)
Patients with previous use of antibiotics <sup>‡</sup> (All bacteria)	2/130 <sup>18,27</sup>	87.6 (53.7–97.8) 0.3; 0.584	91.5 (55.6–98.9) 6.33; 0.0118	10 (0.82–42)	0.14 (0.08–3.88)	2/130 (18,27)	92.2 (76.5–97.7) 0; 1	95 (86.6–98.2) 0; 0.32	18 (1.37–72)	0.08 (0.07–3.74)

**Table 4: Subgroup analyses for bacterial microorganisms.**

CSF: Cerebro-spinal fluid; LR+: Positive Likelihood ratio; LR-: Negative Likelihood ratio NA: Not applicable;  $\chi^2$ : Chi-2 test.

<sup>§</sup> Chi2-test and corresponding p-value to assess presence of statistical heterogeneity.

<sup>†</sup> Defined by the authors as >10 CSF cells in one study (21) and with no definition in the other study (24).

<sup>‡</sup> We defined it as studies with more than 70% of patients with previous antimicrobial therapy.

statistical analysis. JT, IF and ST and AAV interpreted the data. JT and IDF wrote the first draft of the manuscript, and all authors provided critical review and revision of the text and approved the final version. JT and IDF had final responsibility for the decision to submit for publication.

#### Declaration of interests

Authors declare no competing interests.

#### Data sharing statement

All data relevant to the study come from published studies and are included in the article or uploaded as supplementary information.

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#### Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.eclinm.2022.101275.

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