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FULL PAPER

Infectious meningitis/encephalitis: evaluation of a rapid and fully automated multiplex PCR in the microbiological diagnostic workup

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SUMMARY

Infectious diseases of the central nervous system (CNS) such as meningitis/encephalitis (ME) require rapid identification of causative pathogens for effective treatment. This study evaluated the analytical performance and clinical utility of a fully automated multiplex PCR test to improve the microbiological diagnostic workup of ME.

Seventy-seven cerebrospinal fluid (CSF) samples from 77 patients with suspected ME were studied. The samples were tested by FilmArray™ (FA) ME Panel test and the results were compared with those obtained using conventional microbiological procedures (CMP). Furthermore, the assay's validity was evaluated testing 5 pooled CSF samples positive for different pathogens.

The data showed a good concordance (90.9%) between the FA ME panel test and CMP results. Discrepant results were observed in CSF samples with low viral load (5/77) and in samples of patients (2/77) undergoing antimicrobial therapy for fungal infection. The ability of the FA ME panel test to correctly detect the target pathogens was confirmed. Faster microbiological diagnosis was obtained by the FA ME test in comparison to CMP for both bacterial and viral analytes (P<0.001).

Implementation of microbiological diagnostic workup with FA ME panel test may improve the management of patients with suspected CNS infection.

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INTRODUCTION

Infections that involve the central nervous system (CNS), such as meningitis/encephalitis (ME), are severe clinical conditions associated with high rates of morbidity and mortality as well as significant long-term sequelae (Leber *et al.*, 2016). ME may be caused by a wide variety of pathogens, including bacteria, viruses and fungi; clinical symptoms may vary (e.g. fever, headache to altered consciousness, neck stiffness and seizures) and often overlap with various infectious agents (Hanson, 2016; Leber *et al.,* 2016). Early identification of ME causative pathogens has been proven to enable timely and appropriate treatment thereby reducing death or permanent neurological damage (such as problems with vision and hearing, cognitive deficits, seizures and behavioral changes) (Bianchi *et al.,* 2015; de Crom *et al.,* 2012; Leber *et al.,* 2016; Messacar *et al.,* 2016; Shin *et al.,* 2012). Cerebrospinal fluid (CSF) analysis is crucial in the diagnosis of CNS infection (Greig *et al.,* 2006). Currently, in order to identify a potential causative pathogen, microbiological diagnosis in combination with cellular and chemistry parameters in CSF (some findings may suggest the general category of the causative agent, e.g. bacterial versus viral or fungal) are evaluated (Hanson, 2016). In particular, traditional tests such as Gram stain (GS) with culture and pathogen-specific molecular method on CSF samples are used for the diagnosis of acute bacterial/fungal and viral CNS infections, respectively. However, this conventional approach is conditioned by a low diagnostic yield and slow turnaround time. The sensitivity of GS and culture is relatively low and could further be reduced in patients who have received empiric therapy (Messacar *et al.,* 2016). In addition, in ME cases, an etiology is not always identified. This could also be due to the lack of targeted testing

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and the low volume of CSF samples (Leber *et al.,* 2016; Wootton *et al.,* 2016). To overcome these limitations, interest focused on the development of standardized molecular diagnostic tests for simultaneous detection of the most common agents of infectious ME requiring a small volume of CSF.

In order to improve the microbiological diagnostic workup in the identification of the ME causative pathogen, this study evaluated the analytical performance and clinical utility of a fully automated multiplex PCR test, FilmArray™ (FA) ME Panel test (BioFire Diagnostics LLC, Salt Lake City, UT - a bioMérieux Company).

MATERIAL AND METHODS

Study population and clinical samples

A total of 77 CSF samples from 77 patients with suspected ME (*Table 1*) were analyzed using the FA ME Panel test. In particular, 63 CSF residual samples from routine analysis for microbiological investigation performed at the Operative Unit of Microbiology, St. Orsola-Malpighi University Hospital of Bologna, were retrospectively analyzed using the FA ME Panel test to evaluate the analytical performance of this assay. The CSF samples were stored at -80°C until testing and were selected based on microbiological results obtained by conventional microbiological procedures (CMP). Specifically, 25 out of the 63 CSF samples were routinely tested for bacterial (16/25 resulted positive), 37 for viral (25/37 resulted positive) and 1 sample for fungal (resulted positive) analytes. Among the CSF samples positive for bacterial analytes, GS-negative samples were mainly selected (14/16 samples). The remaining 14 out of 77 CSF samples were prospectively analyzed using the CMP and the new molecular test simultaneously in order to evaluate the analytical performance and clinical utility of the FA ME Panel test. The results obtained were analyzed based on clinical data. The management of patients with suspected CNS infection was routinely performed as previously described (Viale et al., 2015).

Furthermore, to evaluate the validity of the test, i.e. its ability to detect the pathogens that it purports to identify (Chien PF *at al.,* 2001), 5 additional pooled CSF samples positive for different pathogens were tested. In particular, 4 CSF samples containing pathogens targeted by assay were pooled, as described in *Table 2*. In the last pooled CSF sample non-target analytes such as organisms closely related to target species (*Klebsiella oxytoca*, *Haemophilus parainfluenzae*, *Neisseria cinerea, Streptococcus mitis*), other clinically relevant pathogens (West Nile virus, Toscana virus, *Staphylococcus aureus*) and an organism that may be a contaminant in a CSF sample (*Staphylococcus epidermidis*) were included. The concentration of each bacterium and virus in the sample was equal to $1.5x10^4$ CFU/mL and approximately $3x10^4$ copies/mL, respectively. In order to confirm the validity of the test, the CSF sample containing all non-target pathogens should result negative, and CSF sample with target analytes should result positive correctly identifying pathogens.

Finally, 2-fold serial dilutions of a sample spiked with herpes simplex virus type 1 (HSV-1) and varicella zoster virus (VZV) were tested in triplicate to evaluate the ability of the FA ME Panel test to detect low viral loads in the CSF samples. These viral targets were selected because they are the most clinically relevant and treatable causes of viral ME (Hasbun *et al.,* 2017; Granerod *et al.,* 2010). Thus from a clinical point of view, a high sensitivity of the test for such viruses is crucial.

Table 1 - *Characteristics of the study population and specimens analysed.*

	Retrospective study	*Prospective study*
N. of patients		
Adult	58	13
Paediatric	5	1
Total	63	14
Gender		
Male/Female	32/31	8/6
Immune status		
Immunocompetent patient	58	13
Immunocompromised patient	5	1
Origin unit of specimens		
High-Intensity Care and Emergency Units:		
Intensive Care Unit	7	2
Pediatric Emergency Unit	4	0
General Emergency Unit	20	1
Infectious Disease Unit	8	7
Low-Intensity Care Units:		
General Internal Medicine Division	8	2
Geriatric Unit	4	0
Pediatric Unit	1	1
Neurology Unit	11	1

Conventional microbiological procedures (CMP)

CSF specimens were prepared for GS and culture examinations by centrifugation at 3,000 rpm for 10 minutes at room temperature. In order to perform the GS, the smear was prepared by placing 1-2 drops of the well-mixed CSF sediment on the slide. When dry, the smear was fixed,

Table 2 - *Composition and volume of the 4 pooled samples.*

	CSF1	*CSF2*	*CSF3*	*CSF4*	*CSF5*	*CSF6*	*Total volume*
	HSV-1 [$1.0x10^5$ copies/mL]	*HSV-2 [$6.9x10^4$ copies/mL]*	*VZV [$2.2x10^3$ copies/mL]*	*EV [$5.2x10^4$ copies/mL]*	*CMV [$1.3x10^4$ copies/mL]*	*S. agalactiae*	
PS-1	125 µl	125 µl	/	/	/	/	250 µl
PS-2	/	/	/	125 µl	/	125 µl	250 µl
PS-3	/	/	125 µl	/	125 µl	/	250 µl
PS-4	40 µl	40 µl	40 µl	40 µl	40 µl	50 µl	250 µl

CSF: cerebrospinal fluid; PS: pooled sample, HSV-1: herpes simplex virus 1; HSV-2: herpes simplex virus 2; VZV: varicella-zoster virus; EV: enterovirus; CMV: cytomegalovirus.

stained and finally observed by light microscopy with a 100X objective. Culture was performed by inoculating 1-2 drops of CSF sediment directly onto each of the following agar plates: horse-blood agar, Thayer-Martin agar, chocolate agar and Sabouraud agar. Moreover, a broth tube (brain-heart infusion, BHI) was inoculated with one drop of the sediment. Agar plates and broth were incubated for 1 to 5 days at 35-37°C (with ~5% CO_2, or in a candle-jar, for Thayer-Martin and chocolate agar). For positive cultures, the isolate identification was performed by MALDI-TOF mass spectrometry.

In addition to GS and culture methods, the rapid and reliable identification of *Streptococcus pneumoniae* was also assessed using an immunochromatographic (IC) test (Binax NOW *Streptococcus pneumoniae* antigen test, Binax, Inc., Portland, ME, USA), which detects the C polysaccharide cell wall antigen (common to all pneumococcal serotypes) on CSF samples before the centrifugation for microscopic and culture examination. *Cryptococcus neoformans* was detected by India ink test, culture and latex agglutination test (LAT). LAT assay was performed on 25 µl of CSF samples using Latex-Cryptococcus antigen detection system kit (IMMY, Norman, OK, USA) according to the manufacturer's instructions.

In accordance with the protocol applied in the Operative Unit of Microbiology at the St. Orsola-Malpighi University Hospital, all CSF samples obtained from patients with clinical and/or physical-chemical signs of bacterial invasive disease that resulted negative at microscopic examination underwent molecular testing. In particular, qualitative Real Time PCR using the EuSepScreen® Lattanti and EuSepScreen® kits (Eurospital, Trieste, Italy) was performed for the detection of *Neisseria meningitidis, Streptococcus pneumoniae, Haemophilus influenzae, Streptococcus agalactiae, Listeria monocytogenes*, *Escherichia coli and Klebsiella pneumoniae.* DNA was extracted from 200 µl of CSF using NucliSENS® easyMAG® system (bioMerieux SA, Marcy l'Etoile, France) and the amplification was carried out according to the manufacturer's instructions.

Finally molecular routine tests were performed on CSF in order to identify and quantify herpes simplex virus (HSV-1 and HSV-2), VZV, human herpes virus 6 (HHV-6), cytomegalovirus (CMV), Epstein-Barr virus (EBV), enterovirus (EV) and human adenovirus (HAdV). Molecular tests to identify other viral pathogens are performed when additional viral agents (e.g. Toscana virus, TOSV and West Nile virus) are suspected (based on anamnestic data, clinical signs, etc.) or during the arbovirus surveillance period with the highest vector activity. The nucleic acids were purified following two protocols (starting - elution volume: 200 µl - 110 µl and 1000 µl - 25 µl, to identify DNA and RNA viruses, respectively) using the easyMAG® system. Quantitative singleplex real-time PCR for each virus except Toscana virus was carried out on an ABI PRISM® 7300 system (Applied Biosystems, Foster City, CA, USA) using ELITe MGB® Kits (ELITech Group, Italy) according to the manufacturer's protocol after reverse transcription for RNA viruses using RT-Kit Plus (ELITech Group, Italy). For Toscana virus detection, the method described by Vocale *et al.* was used (Vocale *et al.,* 2012).

Film Array ME multiplex PCR system

A total of 200 µl for each CSF sample were analyzed using the CE marking, FDA approved FA ME Panel test according to the manufacturer's instructions. The assay simultaneously detected 14 pathogens directly from CSF samples: 6 bacteria (*E. coli K1*, *H. influenzae*, *L. monocytogenes*, *N. meningitidis*, *S. agalactiae*, *S. pneumoniae*) 7 viruses (CMV, EV, HSV-1, HSV-2, VZV, HHV-6, HPeV-Human Parechovirus) and 1 yeast (*C. neoformans*/*gattii*). Sample extraction and multiplex-nested PCR was performed in an enclosed pouch on the FA instrument. For each pathogen, the results were generated using endpoint melting curve analysis.

Statistical analysis

Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the FA ME Panel test were evaluated comparing the results with those obtained by CMP used as comparator assay. Nonparametric Mann-Whitney test was used to compare the differences of:

1) median time for microbiological diagnosis obtained by FA ME Panel test and CMP;
2) WBC count, protein CSF levels and ratio of CSF glucose/blood glucose between bacterial and viral CNS infections.

RESULTS

Analytical performance of the FA ME Panel test on retrospectively tested clinical CSF samples

Among the 63 CSF samples retrospectively tested by FA ME test, 25 (39.7%), 37 (58.7%) and 1 (1.6%) samples were selected on the basis of the results obtained by CMP for the detection of bacteria, viruses and yeast, respectively. The overall performance of the FA ME Panel test in the detection of pathogens is summarized in *Table 3*.

Bacterial analytes. The FA ME Panel test provided 100% sensitivity and specificity compared to combined CMP used according to the protocol described in the previous section. The results of 16 CSF samples positive for bacteria obtained by CMP and FA ME Panel test are reported in *Table 4*.

Viral analytes. The FA ME Panel test showed 100% spec-

Table 3 - *Comparison of results obtained by FA ME Panel test and CMP used as comparator assays on 63 CSF samples.*

		Comparator assays					
		BACTERIA (n=25)		*VIRUSES (n=37)*		*YEAST (n=1)*	
		Positive	*Negative*	*Positive*	*Negative*	*Positive*	*Negative*
FA ME Panel test	Positive	16	0	20	0	0	0
	Negative	0	9	5	12	1	0
	Sensitivity - Specificity	100% - 100%		80% - 100%		N.E.	
	PPV - NPV	100% - 100%		100% - 70.6%		N.E.	

PPV: positive predictive value; NPV: negative predictive value, N.E.: not evaluable.

Table 4 - *Results obtained with conventional microbiological procedures (CMP) and FA ME Panel on 16 CSF samples positive for bacteria.*

Bacteria	*N. of samples*	*GS*	*Culture*	*IC*	*Real Time -PCR*	*FA ME Panel test*
L. monocytogenes	1	Negative	Positive	nd	Positive	Positive
N. meningitidis	4	Negative	Negative	nd	Positive	Positive
	1	Negative	Positive	nd	Positive	Positive
	1	Gram-negative cocci	Positive	nd	nd	Positive
N. of positive results (%)		1 (16.7%)	2 (33.3%)	-	5 (100%)	6 (100%)
S. pneumoniae	2	Gram-positive cocci	Positive	Positive	nd	Positive
	1	Negative	Negative	Positive	Positive	Positive
N. of positive results (%)		2 (66.7%)	2 (66.7%)	3 (100%)	1 (100%)	3 (100%)
S. agalactiae	2	Negative	Positive	nd	Positive	Positive
	1	Negative	Negative	nd	Positive	Positive
N. of positive results (%)		-	2 (66.7%)	-	3 (100%)	3 (100%)
H. influenzae	2	Negative	Negative	nd	Positive	Positive
	1	Negative	Positive	nd	Positive	Positive
N. of positive results (%)		-	1 (33.3%)	-	3 (100%)	3 (100%)

IC: immunochromatographic; nd: not done.

ificity in the detection of all viral targets and sensitivity ranged from 50% to 100% (*Table 5*). Discrepant results were obtained in 5/25 (20%) CSF samples resulted positive by CMP and negative using FA ME Panel test. In all five cases (1 positive for EV, 1 for HSV-1, 2 for HHV-6 and 1 for VZV), a low viral load (<500 copies/mL) was quantified by Real-time PCR.

Fungal analyte. The selected positive sample for *C. neoformans* was positive by LAT and negative by microscopic, culture methods and FA ME Panel test (*Table 4*). The specimen was from an HIV-infected patient undergoing fungal treatment at the time of lumbar puncture for clinical suspicion of fungal infection.

Overall, for 57/63 (90.5%) CSF samples the results obtained by CMP were confirmed by the FA ME Panel test and no further pathogen was detected in each sample. Discordant data were observed in 6/63 (9.5%) samples that were positive by CMP (5 for viral and 1 for fungal analytes) and negative by the FA ME Panel test. These samples were retested using the CMP and all 6 positive results were confirmed.

Table 5 - *Comparison of results for individual pathogens obtained by FA ME Panel test and CMP.*

Analytes *FA ME Panel*	*Positive results* *FA ME Panel test /CMP*
*E. coli K1**	/
H. influenzae	3/3
L. monocytogenes	1/1
N. meningitidis	6/6
S. agalactiae	3/3
S. pneumoniae	3/3
CMV	4/4
EV	4/5
HSV-1	3/4
HSV-2	4/4
HPeV*	/
HHV-6	2/4
VZV	3/4
C. neoformans/gattii	0/1

*No positive samples by CMP were available to be tested by FA ME Panel test.

Analytical performance of the FA ME Panel test on prospectively tested clinical CSF samples and clinical utility in the microbiological diagnosis

Among the 14 CSF samples, positive results were obtained in 9 (64.3%) and 7 (50%) cases by CMP and the FA ME Panel test, respectively (*Table 6*). Discordant results were obtained for two CSF samples. One of these (case ID 145) resulted positive for *C. neoformans* by LAT (microscopic and culture test were negative) and the sample was from an allogeneic hematopoietic stem cell transplant recipient on antifungal prophylaxis with posaconazole. The other case (case ID 150) was positive for TOSV by molecular method conventionally used and the sample was from a patient reported as a suspected TOSV case by the public health department during the surveillance period. The patient was living in central Italy, where TOSV confirmed cases were documented. In addition, IgG and IgM specific for TOSV were detected by serological diagnosis. The 3 CSF samples positive for VZV were from VZV-seropositive patients. In all 3 cases, negative results were obtained for the detection of VZV DNA in whole blood samples. For the patient with a positive result for *S. pneumoniae* and HHV-6 (ID case 149), the HHV6-DNA was detected in a whole blood sample with a viral load equal to $5.5x10^5$ copies/mL. A chromosomally integrated form (ciHHV-6) was suspected and the detection of HHV-6 DNA in hair follicle cells confirmed the ciHHV-6 for the patient. Regarding the 4 cases for which bacterial analytes were identified, 3 were positive by both microscopic and culture methods and one case (case ID 144) by culture and molecular method conventionally used. In addition, in 3 out of 4 cases (case ID: 144, 149 and 151), the blood culture was performed and the same pathogen detected in the respective CSF sample was identified.

In those patients with bacterial positive results, the median WBC count and protein level in CSF samples were higher than in those detected in patients with viral posi-

Table 6. *Laboratory, clinical and treatment characteristics of 9 CSF positive samples prospectively analyzed by FA ME Panel Test*

ID Cases	*CMP Results*	*FA ME Panel test Results*	*WBC Count (0-5 cells/mm³)*	*Ratio of CSF glucose/ blood glucose*	*Protein (15-45 mg/dL)*	*Clinical Symptoms*	*Therapy after microbiological diagnosis*	*Outcome*
142	VZV (< $5x10^2$ copies/mL)	VZV	440	0.41	583	Polyradiculoneuropathy in long-stay patient with bronchopneumonia	ACV (10mg/kg every 8h for 10 days)	Died for intestinal perforation (acute abdomen)
143	*N. meningitidis*	*N. meningitidis*	620	0.2	585	Fever, sensory impairment, neck pain, vomit, headache in immunocompetent patient	CRO (2 g twice a day for 9 days) LVX (500 mg twice a day for 9 days) DEX (0.15 mg/kg every 6h for 7 days)	Alive without neurologic sequelae
144	*L. monocytogenes*	*L. monocytogenes*	29	N.A.	200	N.A.	N.A.	N.A.
145	C. neoformans	Negative	2	0.52	68	Headaches, reduced general condition in HSCT patient with acute PTLD	AMB (0.7-1 mg/kg/day for 14 days)	Alive without neurologic sequelae
147	VZV ($3.2x10^4$ copies/mL)	VZV	64	0.55	78	Fever and persistent headaches in immunocompetent patient	ACV (10 mg/kg every 8h for 10 days) AMP (2 g every 4h for 6 days)	Alive without neurologic sequelae
148	VZV ($5.1x10^4$ copies/mL)	VZV	290	0.47	120	Fever, persistent headaches, vomiting in immunocompetent patient	ACV (10 mg/kg every 8h for 9 days) AMP (2 g every 4h for 5 days)	Alive without neurologic sequelae
149	*S. pneumoniae* HHV6 ($1.2x10^6$ copies/mL)	*S. pneumoniae* HHV6	1120	0.00	974	Fever, stiff neck, positivity by Lasègue's test, sensory impairment in immunocompetent patient	CTX (2 g every 6h for 14 days) LVX (500 mg twice a day for 10 days)	Alive with neurologic sequelae
150	Toscana virus	Negative	877	0.46	169	Fever and persistent headaches in immunocompetent patient	ACV (10 mg/kg every 8h for 5 days) AMP (2 g every 4h for 5 days	Alive without neurologic sequelae
151	*S. pneumoniae*	*S. pneumoniae*	1061	0.00	269	Fever, stiff neck, sphincter dysfunction, sensory impairment in immunocompetent patient	CTX (2 g every 6h for 12 days) LVX (500 mg twice a day for 10 days)	Died for bacterial meningitis

N.A.: not available; WBC: white blood cells; HSCT: hematopoietic stem cell transplant; PTLD: post-transplant lymphoproliferative disorder.
Normal values/ranges for WBC and protein are given in parentheses; ACV: acyclovir; CRO: Ceftriaxone; LVX: Levofloxacin; DEX: Dexamethasone; AMB: liposomal amphotericin B; AMP: Ampicillin; CTX: Cefotaxime.

tive results (WBC: 840.5 cells/mm^3 vs 365 cells/mm^3 and protein: 427 mg/dL vs 144.5 mg/dL, respectively). However, the difference was not statistically significant for both (z=-0.87; p=0.386 for WBC count and z=-1.73; p=0.083 for protein level). A difference in median ratio of CSF glucose/blood glucose was observed between cases positive for bacteria and viruses (0.00 vs. 0.54, respectively; Mann-Whitney test z=2.141, p=0.032).
Using the FA ME Panel test, the microbiological diagnosis for each case was reported to the clinical physician within 2 hours of the arrival of the specimen in the laboratory (median time: 1 hour and 45 min, range: 1 hour and 25 min - 2 hours); patient management was assessed based on these data. The microbiological diagnosis by CMP (including culture methods) was available after a median timespan of 3.5 days (range: 48-120 hours) and 5 hours (range 4-9 hours) for bacterial and viral analytes, respectively.
The difference of time for microbiological diagnosis obtained by the FA ME Panel test compared with CMP was statistically significant for both bacterial and viral analytes detection (Mann-Whitney test z=-3.58, p<0.001 and z=-3.95, p<0.001, respectively).

Analytical performance of the FA ME Panel test to detect pathogens on pooled CSF samples

All the different pathogens contained in the 4 CSF samples pooled as described in *Table 2* were detected by the FA ME Panel test. No positive results were obtained for the pooled sample containing non target analytes.

Analytical performance of the FA ME Panel test to detect HSV-1 and VZV with low viral loads in the CSF samples

Table 7 shows the results obtained for the 2-fold serial dilutions of a sample spiked with HSV-1 and VZV by FA ME Panel test. The assay was able to detect HSV-1 and VZV genome in all triplicates in the dilutions of spiked CSF samples containing viral load ranging from 3.3x10^3 to 8.2x10^2 copies/mL and from 3x10^3 to 7.5x10^2, respectively.

DISCUSSION

CNS infections have a wide spectrum of causes and clinical presentations. Microbiological diagnosis is essential to identify the causative pathogen of the clinical condition (Big *et al.,* 2009, Leber *et al.,* 2016). Despite some cases where the etiology remains unknown, the advent of multiplex molecular testing has improved the sensitivity of pathogen detection in CSF samples (Leber *et al.,* 2016).
This study evaluated the analytical performance and clinical utility of the multiplex method FA ME Panel test on clinical CSF samples from patients with CNS infection. The results obtained showed an overall concordance equal to 90.9% (90.5% and 92.8% on retrospective and prospective samples) compared with those obtained by CMP. In 7/77 samples (9.1%) discrepant results were observed for viral and fungal analytes. In particular, in 5 samples low viral load (<500 copies/mL) for EV (n=1), HSV-1 (n=1), HHV-6 (n=2) and VZV (n=1) was detected only by the quantitative molecular CMP. We showed that the assay was able to detect the genome of HSV-1 and VZV in all triplicates of CSF samples containing viral load equal to 8.2x10^2 and 7.5x10^2 copies/mL, respectively. As described by other authors, the ability of the FA ME Panel test to detect viral targets could be somewhat less than by singleplex assay (Hanson, 2016, Messacar *et al.,* 2016). However, for these samples, the false negative results could also be due to sampling variability caused by a low concentration of pathogens in the CSF specimen below/near the limit of detection (LoD) of FA ME Panel test (Bowman *et al.,* 2015; Leber *et al.,* 2016). Other studies suggest that low positive results for herpesvirus targets should be evaluated carefully in combination with the clinical context (Bhaskaran *et al.,* 2013; Labská *et al.,* 2015; Parisi *et al.,* 2016; Razonable *et al.,* 2013). In fact, human herpesviruses, such as HHV-6, are able to establish latent state of infection, and positive results could be due to detection of latent virus in peripheral lymphocytes cells present in the CSF (Pellett *et al.,* 2012). In addition, HHV-6 is able to integrate its genome into human chromosomes and this condition can be vertically transmitted. The prevalence of ciHHV-6 is approximately 1% in the general population and in these cases the detection of HHV-6 DNA in the cellular clinical sample may lead to erroneous diagnosis of active HHV-6 infection (Parisi *et al.,* 2016; Pellett *et al.,* 2012). In cases with HHV-6 DNA levels in whole blood >5.5 $\log_{10}$ copies/mL, ciHHV-6 should be suspected and a confirmatory test for the detection of HHV-6 genome on hair follicles or nails (only ciHHV-6 individuals have detectable HHV-6 DNA in these tissues) is recommended (Pellett *et al.,* 2012). In this study, among the patients with HHV-6 positive results on CSF samples, one case of suspected ciHHV-6 was identified and confirmed by HHV-6 DNA detection in hair follicle cells.
Low viral loads for HSV-1 and VZV detected in two cases only by conventional molecular method were consistent with the specific antiviral treatment started in these patients before the lumbar puncture on the basis of clinical signs. However, for HSV and VZV a high variability of

Table 7 - *Performance of FA ME Panel test to detect HSV-1 and VZV both with low viral loads in 2-fold serial dilutions of spiked CSF samples.*

2-fold serial dilution of spiked CSF samples	*HSV-1 viral load (copies/mL)*	*VZV viral load (copies/mL)*	*FA ME Panel test results*
1	3.3x10^3	3x10^3	Positive (3/3 triplicates)
2	1.6x10^3	1.5x10^3	Positive (3/3)
3	8.2x10^2	7.5x10^2	Positive (3/3)
4	4.1x10^2	3.7x10^2	Positive (2/3)
5	2.0x10^2	1.9x10^2	Positive (1/3)
6	1.0x10^2	9.4x10^1	Negative
7	5x10^1	4.7x10^1	Negative

viral load in CSF samples was described with absence of a clear correlation between viral load and neurological outcome of CNS infection that may not allow an assessment of the prognosis of disease caused by these viruses (Růzek *et al.,* 2007). For EV, the variations in CSF viral loads in patients with EV meningitis was described as related to genotypic differences in the virus strains involved (Volle *et al.,* 2014).

Discordant results obtained for *C. neoformans* were from immunocompromised patients under antifungal therapy. As already described, in these patients, CSF positive results were obtained only by LAT, most likely due to antigen persistence rather than the presence of live organism (that resulted undetectable by microscopic examination and culture test) after therapy (Leber *et al.,* 2016, Lu *et al.,* 2005).

For bacterial targets, the FA ME Panel test provided 100% sensitivity and specificity in comparison to combined CMP results. In particular, most of CSF samples retrospectively tested resulted negative by microscopic examination. The FA ME Panel test provided concordant results with molecular routine method in 100% of cases and detected pathogens that were missed by microscopic examinations and by culture test in 81.2% and 50% of cases, respectively. Among the samples prospectively tested and positive by the FA ME Panel test, one case was falsely negative by microscopic examination. The lower sensitivity of the traditional methods (GS and culture) compared to molecular tests has already been described by other authors and may result from antibiotic pretreatment prior to lumbar puncture or from fastidious pathogens that are difficult to grow in culture media (Brouwer *et al.,* 2010; Meyer *et al.,* 2014; Welinder-Olsson *et al.,* 2007; Wu *et al.,* 2013).

Other studies have investigated the potential utility of CSF laboratory findings, such as WBC count and protein CSF levels, to distinguish bacterial from viral CNS infection (Águeda *et al.,* 2013; Fitch *et al.,* 2007; Lagi *et al.,* 2016; Leber *et al.,* 2016; Meyer *et al.,* 2014; Neuman *et al.,* 2008; Spanos *et al.,* 1989). Ss confirmed by other authors, our study found no statistically significant difference in WBC count and protein levels for two types of CNS infection among the cases prospectively tested with the FA ME Panel test (Fitch *et al.,* 2007; Leber *et al.,* 2016). A difference in ratio of CSF glucose/blood glucose was observed between cases positive for bacteria and viruses, but the data need to be evaluated due to the small number of cases analyzed.

Of note, the data obtained on CSF samples prospectively tested showed that FA ME Panel testing provided reliable results much sooner than CMP with a difference time for microbiological diagnosis that was statistically significant in positive cases for bacterial and viral analytes ($P<0.001$ and $P<0.001$), respectively. In these cases, fast microbiological diagnosis improved patient management by allowing administration of optimal antimicrobial or antiviral therapy and interruption of empiric treatment.

Finally, other authors have obtained false positive results by FA ME Panel testing likely due to contamination (Hanson, 2016; Leber *et al.,* 2016; Wootton *et al.,* 2016). In our study, no false positive results were observed. In addition, the FA ME panel test was able to correctly identify the target pathogens in 5 pooled samples containing two or more analytes, among which also some not included in the panel.

In conclusion, our data showed that FA ME Panel test is able to quickly detect a vast range of pathogens with good performance compared with CMP. The testing is simple to perform and provides reliable results that could impact:

1) in potential infectious disease emergencies like bacterial meningitis
2) in cases where patients have received antibiotic treatment before lumbar puncture.

The FA ME Panel test cannot supersede CMP as it does not provide any information on antibiotic susceptibility. Furthermore, the panel does not detect all potential microorganisms that cause CNS infections. In fact, in our study the FA ME Panel test was not able to identify the case of TOSV. In addition, for virological analytes, quantitative results should be provided by the following singleplex quantitative PCR to evaluate in the clinical context the relevance of virus detected.

Given that pathogens in low quantities could possibly go undetected, in those cases with elevated clinical suspicion of CNS infection, empiric treatment should still be administered even when FA ME Panel testing yields negative results. Appropriate use of the FA ME Panel tests requires that the results be carefully assessed in relation to the clinical setting, and that clinicians be well aware of the characteristics of the test in a shared clinical-microbiological workup.

The implementation of The microbiological diagnostic workup with sensitive and specific FA ME Panel testing may improve the management of patients with suspected CNS infection by early specific treatment and may be especially useful in cases that require effective prevention measures such as post-exposure prophylaxis of close contacts.

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