

MULTICENTRE EVALUATION OF THE FILMARRAY MENINGITIS / ENCEPHALITIS PANEL

Conflict of interest

- All kits for the evaluation were provided by bioMérieux
- The authors have no commercial interest to declare

Aetiology

- Infection of central nervous system
- Different causative organisms can be involved
viruses, bacteria, fungi, parasites
- Clinical symptoms are similar
fever, headache, neck stiffness, vomiting,...
- Rapid identification is important for patient management and prognosis
- Tests with high accuracy and short turn-around-time are needed

Laboratory diagnosis

- Gram stain
- Culture
 - ❖ Gold standard for bacteria
 - ❖ Results available in >24-48 hours
 - ❖ Some pathogens are difficult or impossible to culture
- Nucleic acid amplification testing (NAAT)
 - ❖ Singleplex
 - ❖ Multiplex
- Antigen testing
 - ❖ *Cryptococcus neoformans*
 - ❖ *Streptococcus pneumoniae*

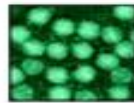
FilmArray[®] (BioFire)

- Fully automated
Cell lysis, Nucleic Acid extraction, nested PCR and detection
- Easy to use (hands-on-time 5')
- Short turn-around-time (approximately 1 hour)
- Multiplex PCR system: targets 14 pathogens simultaneously



Bacteria

Escherichia coli K1
Haemophilus influenzae
Listeria monocytogenes
Neisseria meningitidis
Streptococcus agalactiae
Streptococcus pneumoniae



Viruses

Cytomegalovirus (CMV)
Enterovirus
Herpes simplex virus 1 (HSV-1)
Herpes simplex virus 2 (HSV-2)
Human herpesvirus 6 (HHV-6)
Human parechovirus
Varicella zoster virus (VZV)



Fungi

Cryptococcus neoformans/gattii

Study aim

- Verify the **limit of detection** (LOD) as claimed by the company
- Verify the **accuracy**
- Perform **inter-laboratory evaluation** to guarantee equal results

Study design

□ LOD experiments

- ❖ Bacterial strains (ATCC/NCTC) were quantified by colony counting
- ❖ For viruses, QCMD samples with given consensus concentration were diluted
- ❖ Analysis of each pathogen once at a concentration of 2-3 times LOD
- ❖ Reanalysis of dilution twice if negative result
- ❖ Analysis with higher concentration if 3 times negative result

Remark: Escherichia coli K1 and Cryptococcus neoformans / gattii were not included

Study design

□ Accuracy

- ❖ Retrospective analysis of 65 samples, suspicious for meningitis / encephalitis (ME)
 - 16 clinical CSF samples tested negative with routine analysis
 - 37 clinical CSF samples tested positive with routine analysis
 - 5 EQA samples (QCMD)
 - 7 spiked strains (ATCC/IPH-Belgium)

Study design

- Accuracy
 - ❖ WBC and total protein elevated
 - ❖ Clinical symptoms were combined with other laboratory test results
 - ❖ Discordant results were confirmed with in-house real-time PCR

Study design

- Inter-laboratory evaluation
 - ❖ Analysis of 2 samples by all sites:
 - Physiological salt solution
 - NATtrol ME Controls (Zeptomatrix) pool 1:5 dilution
 - ❖ 3 different time points:
 - Day 0
 - Day 1: samples were stored at room temperature (mimic transport conditions)
 - Day 7: samples were stored at 2-8°C (mimic storage conditions)

Study results: limit of detection

□ LOD **confirmed** for:

- ❖ *Listeria monocytogenes*
- ❖ *Streptococcus pneumoniae*
- ❖ *Neisseria meningitidis*
- ❖ CMV
- ❖ Enterovirus
- ❖ Herpes simplex virus 1 and 2
- ❖ Human herpes virus 6
- ❖ Parechovirus
- ❖ Varicella zoster
- ❖ *Haemophilus influenzae* (ATCC 10211, type b biotype 1)
- ❖ *Haemophilus influenzae* (ATCC 49247, unencapsulated)

□ LOD **not confirmed** for:

- ❖ *Streptococcus agalactiae*
- ❖ *Haemophilus influenzae*
(NCTC 8468, type b biotype 4)

Study results: accuracy

16 suspected negative samples were analysed

- 14/16 were negative with FilmArray ME
- 1/16 was positive for human herpes virus 6 with FilmArray ME
- 1/16 was positive for enterovirus with FilmArray ME

Study results: accuracy

- Sample 1: positive for **human herpes virus 6** with FilmArray ME
 - ❖ Male, 2 months old
 - ❖ Clinical presentation: symptoms of viral meningitis / meningoencephalitis, no roseola
 - ❖ WBC: 3/ μ l, total protein: 92,8 mg/dl
 - ❖ Bacterial culture negative
 - ❖ Viral culture not performed
 - ❖ Singleplex PCR HSV, VZV and enterovirus negative
 - ❖ Positive results confirmed by in-house PCR (4066 copies/ml)
 - ❖ Clinical signs not suggestive for human herpes virus 6 infection

Study results: accuracy

- Sample 2: positive for **enterovirus** with FilmArray ME
 - ❖ Male, 33 years old
 - ❖ Clinical presentation: headache probably caused by spinal problems
 - ❖ WBC: 15/ μ l, total protein: 57,6 mg/dl
 - ❖ Bacterial culture negative
 - ❖ Viral culture not performed
 - ❖ Singleplex PCR HSV, VZV and enterovirus negative
 - ❖ Positive results could not be confirmed by 2 in-house PCRs, nor by GeneXpert (Cepheid)

Study results accuracy

- 37 clinical samples
 - ❖ 34/37 samples were positive with FilmArray ME: concordant results
 - ❖ 3/37 samples were negative with FilmArray ME

Study results accuracy

□ Sample 1:

- ❖ Clinical presentation: Benign meningitis 8 months post HSCT
- ❖ WBC and total protein elevated
- ❖ In-house PCR: human herpes virus 6
viral load 7626 copies/ml

Study results accuracy

- Sample 2 and 3:
 - ❖ Clinical presentation: African HIV positive woman
 - ❖ Cryptococcus Ag 1/16 (agglutination)
 - ❖ Culture yeast negative
 - ❖ WBC and total protein elevated
 - ❖ Follow-up sample after 10 days of therapy
 - ❖ Initial CSF: Cryptococcus Ag 1/1024, FilmArray positive, culture 48h: *Cryptococcus neoformans*, PCR: *Toxoplasma gondii* (Ct 28)
 - ❖ FilmArray is possibly not sensitive enough for **follow-up** of Cryptococcus Ag positive samples¹

¹ Hanson et al. JCM 2016 54: 3 785-787

Study results: accuracy

- 5 EQA QCMD samples
 - ❖ Concordant results for pathogen included in EQA panel
 - ❖ 3/5 samples showed also a positive result for human herpes 6 virus
 - QCMD 2015-01 HSV
 - QCMD 2015-03 VZV
 - QCMD 2014-07 CMV
 - ❖ Result of human herpes 6 virus not confirmed by in-house real-time PCR
 - ❖ Sample related?

Study results: accuracy

- 7 spiked strains (ATCC/IPH-Belgium)
 - ❖ Concordant results for 6 strains:
 - *Haemophilus influenzae* ATCC10211
 - *Listeria monocytogenes* (2)
 - *Streptococcus agalactiae*
 - *Streptococcus pneumoniae*
 - *Cryptococcus neoformans/gattii*
 - ❖ False negative result for *Haemophilus influenzae* ATCC 49247. After repeated analysis: positive result

Study results inter-laboratory evaluation

- All sites produced identical results
- No false positive nor false negative results found
- Transport and storage did not influence results

Conclusion

- The limit of detection as claimed by the company is confirmed except for *Streptococcus agalactiae* and for some strains of *Haemophilus influenzae*.
- Analysis of discordant results in the accuracy experiment shows an overall agreement for 60/65 samples (92 %).
- CSF samples can be transported at room temperature and stored for 7 days at 2-8°C.
- FilmArray ME panel seems a promising and useful tool in the diagnosis of central nervous infections.

Multicentre study

- Jessa Hospital, Clinical Biology, Laboratory for Molecular Diagnostics, Hasselt, Belgium
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