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A multi-center prospective evaluation of the new Filmarray Meningitis/Encephalitis panel for rapid PCR-based diagnostics

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No conflict of interests!

High clinical impact of Meningitis and Encephalitis

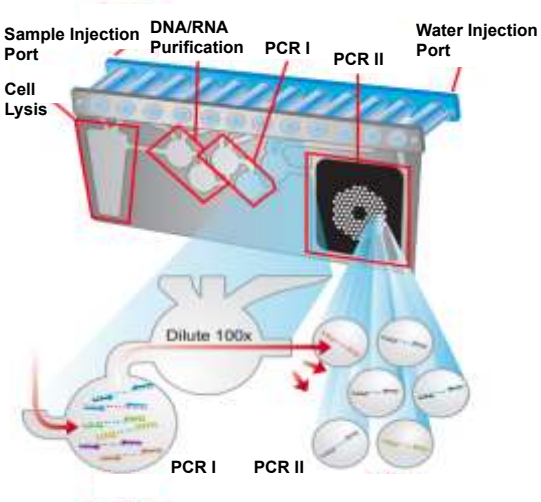
- Meningitis and encephalitis is associated with
  - High morbidity[1,2]
  - High mortality[1,2]
  - Significant health care costs[3]

- Rapid treatment is associated with improved outcome[4,5]

- Current gap in diagnostics: Identification of the causing pathogen[6]
  - Time-consuming (culture based)
  - Due to pre-treatment sometimes unsuccessful.

1 Brouwer MC et al. Clin Microbiol Rev 23:467-492, 2010; 2 Edmond K et al. Lancet ID, 10:317-328, 2010
3 Portnoy A et al. Vaccine, 33:A240-A247, 2014; 4 Viale P, Scudeller L, et al. Ann Pharmacother, 49(9): 978-85, 2015;
6 Giulieri SG, et al. J Clin Virol, 62:58-62, 2015; 7 Hanson KE, J Clin Microbiol, 54(9):2222-4, 2016

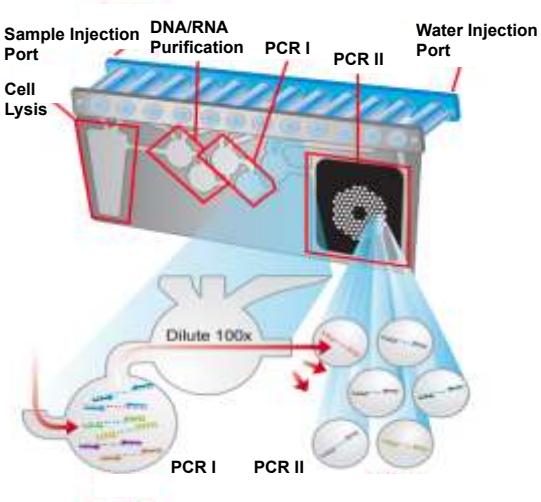
Filmarray: Mengititis/Enzephalitis panel



Filmarray technology

- Bacteria
  - *Escherichia coli* K1
  - *Haemophilus influenzae*
  - *Listeria monocytogenes*
  - *Neisseria meningitidis*
  - *Streptococcus agalactiae*
  - *Streptococcus pneumoniae*
- Yeast
  - *Cryptococcus neoformans/gattii*
- Viruses
  - Cytomegalovirus
  - Enterovirus
  - Herpes simplex virus 1
  - Herpes simplex virus 2
  - Human Herpes virus 6
  - Human paraechovirus
  - Varicella zoster

Filmarray: Mengititis/Enzephalitis panel



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  - Varicella zoster
- Yeast
  - *Cryptococcus neoformans/gattii*

Most common pathogens for acute community acquired meningitis/enzephalitis

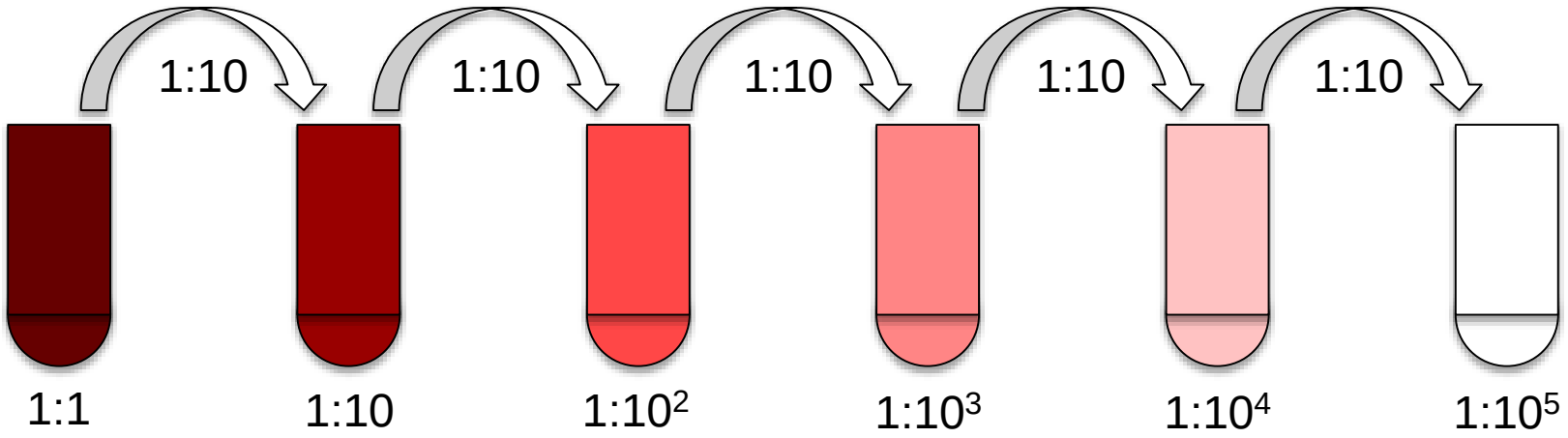
Missing shunt infections e.g. *Staphylococcus aureus*, *CNS*, *P. acnes*

Aims

- Assess the diagnostic performance of a new broad panel PCR system
  - Limit of detection for pathogens
  - Detection of all pathogens included in the panel
  - Sensitivity and specificity
  - Determine the „real world“ time to result

Methods: Limit of detection

- Limit of detection
  - Serial dilution (1:10) of pathogens spiked into cerebrospinal fluid (CSF)



  - Comparison with
    - Plating and determination of colony forming units
    - Microscopy
    - PCR detection

A general low limit of detection could be determined

Pathogen	Filmarray ME	Microscopy[1]	PCR assay
***E. coli* K1**	300 CFU/mL	>3'500 CFU/mL	-
L. monocytogenes	200 CFU/mL	15'000 CFU/mL	-
N. meningitidis	10 CFU/mL	>1'200 CFU/mL	-
S. agalactiae	3'700 CFU/mL	8'500 CFU/mL	-
S. pneumoniae	200 CFU/mL	7'500 CFU/mL	-
C. neoformans	10 CFU/mL	>40 CFU/mL	-

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HSV1	100 TCID50/mL	-	30 GEq/mL
HSV2	pos	-	79 GEq/mL
Enterovirus	55 TCID50/mL	-	Semi-quant. PCR
VZV	pos	-	120 GEq/ml

Methods: Comparison with Goldstandard

- Four different diagnostic laboratories
  - All accredited due to the ISO norm 17025

- Comparison of Filmarray Meningitis/Enzephalitis with
  - Laboratory internal gold standards
  - Culture based diagnostics for bacteria
  - Standard single-PCRs i.e. GeneXpert, real time PCR

Filmarray shows high sensitivity

		Routine assay		
		Positive	Negative	
Filmarray ME	Positive	63	10*	73 (25.1%)
	Negative	0	230	230
		63 (21.7%)	240	291

- **Positive results:**
  - **Filmarray 73/291 = 25.1%**
  - **Routine diagnostic 63/291 = 21.7%**

CMV was not considered in this analysis, as this is not routinely tested in the laboratories

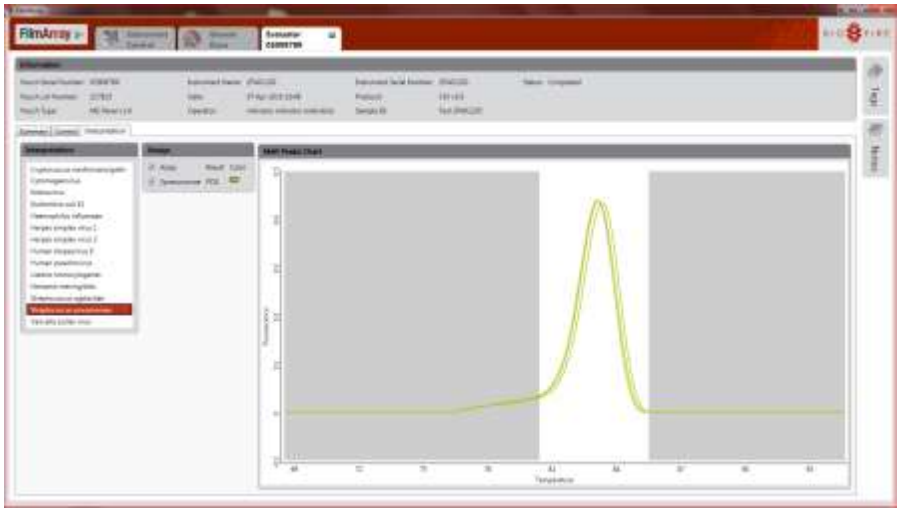
Filmarray shows high sensitivity

		Routine assay		
		Positive	Negative	
Filmarray ME	Positive	63	10*	73 (25.1%)
	Negative	0	230	230
		63 (21.7%)	240	291

- **Sensitivity: 100%**
- **Specificity: 95.8%**
- Positive predictive value : 86.3%
- Negative predictive value : 100%

- *7/10 were confirmed by another test i.e. specific PCR or antigen test
- 3/10 were confirmed “false” positive

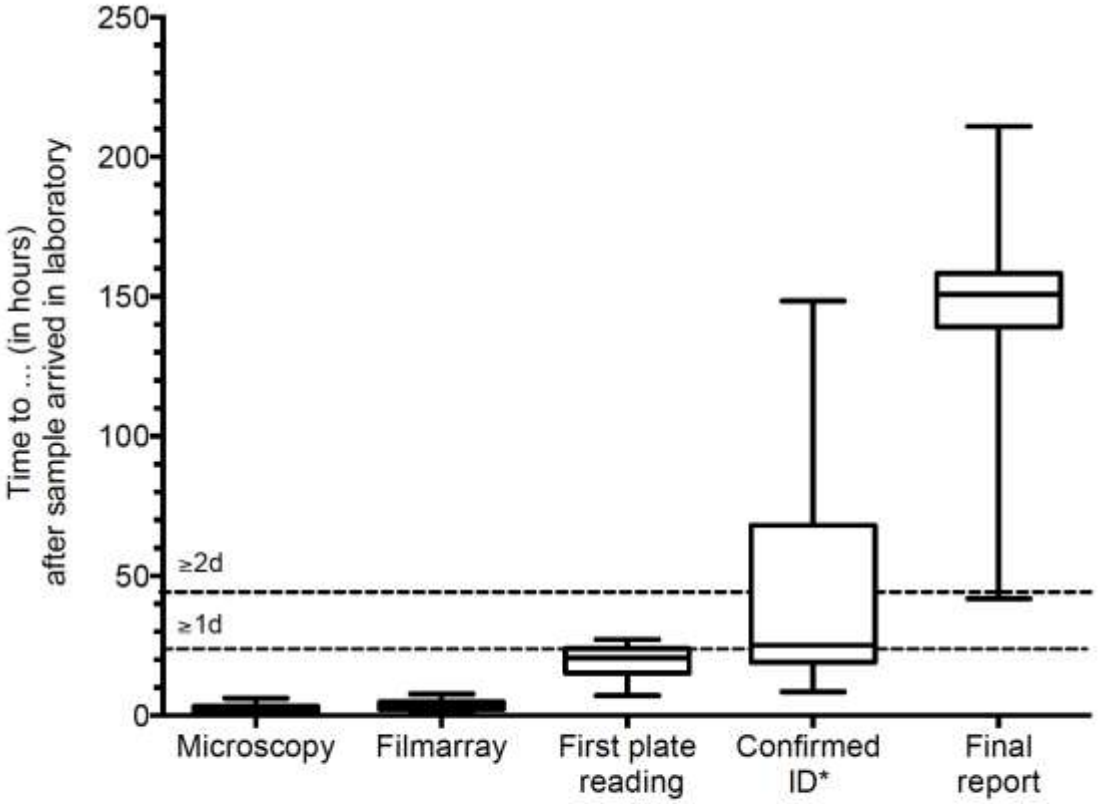
Trouble shooting: ID of false positive



Correct shaped melt curves

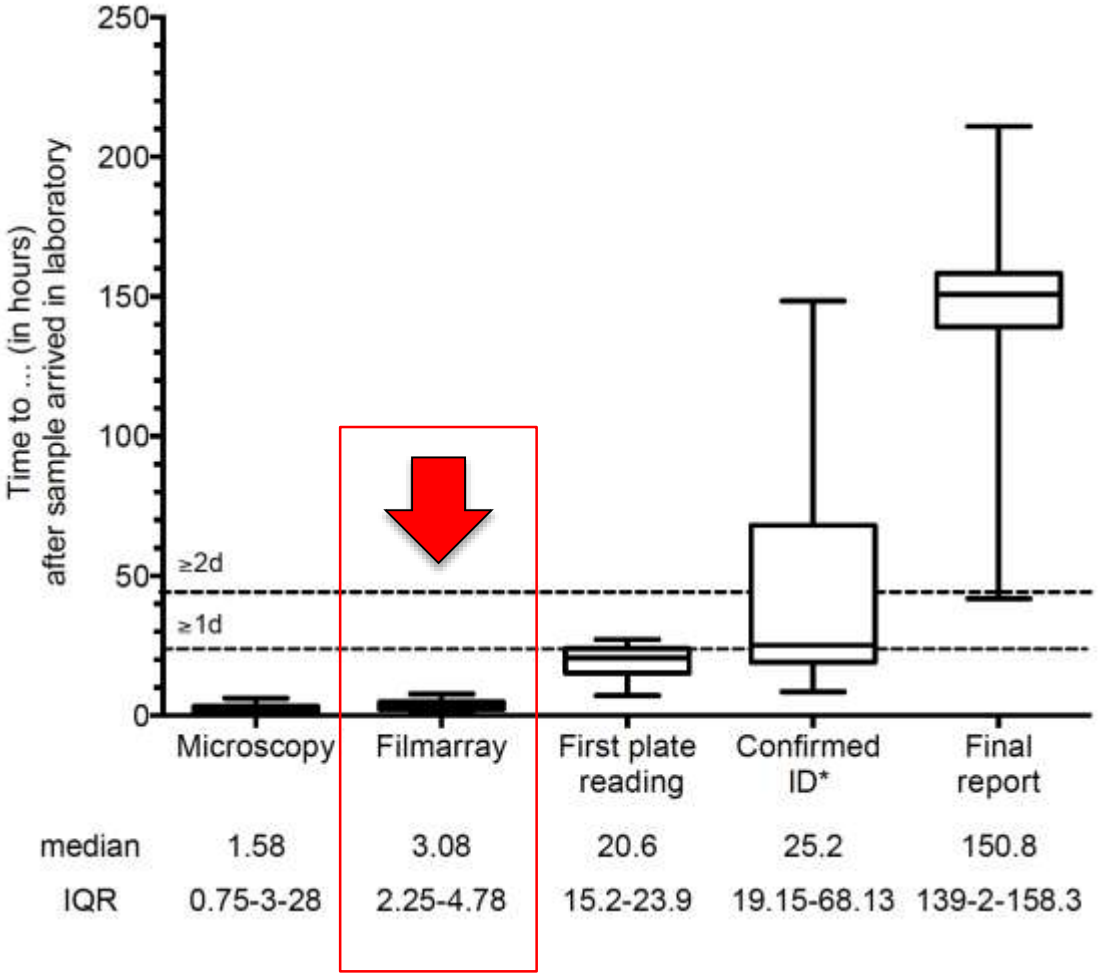
Not correct shaped melt curves

Time to result is around 3h



	Microscopy	Filmarray	First plate reading	Confirmed ID*	Final report
median	1.58	3.08	20.6	25.2	150.8
IQR	0.75-3-28	2.25-4.78	15.2-23.9	19.15-68.13	139-2-158.3

Time to result is around 3h



Summary and conclusion

- High sensitivity with low limit of detection for pathogens
- Be aware of unspecific detections -> check the meltcurve

- Advantages:
  - Short hands-on-time in sample preparation
  - Short assay turn around time, overall about 3h
  - Assessment of the most common community acquired pathogens

- Disadvantages:
  - Diagnostic gaps for pathogens e.g. staphylococci for shunt infections
  - No information on antibiotic resistance
  - No quantification
  - Costs

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Thank you for your attention…
Questions?

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