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Real-time multiplexes for the direct diagnosis on CSF of community-acquired, nosocomial and neonatal bacterial meningitis

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Background: Meningitis is an inflammation of meninges surrounding the central nervous system tissue due to many bacterial or viral pathogens. Bacterial meningitis (BM) is rare but serious compared to viral meningitis, and there is no obvious differential clinical diagnosis between both. BM affects 22 people out of one million, with about 1400 cases/year in France. Two peaks of incidence are observed: one in less than one year old children most often due to maternal-foetal transmission, and the other in adults over 60 mainly due to nosocomial infections. BM is thus often subdivided in three categories: community-acquired BM, maternal-foetal BM, and nosocomial BM.

The purpose of our study was to develop and validate a fast and targeted method in real-time PCR to diagnose the main bacteria involved in BM according to the diagnostic framework, directly on cerebrospinal fluid (CSF) without DNA extraction.

Material/methods: This study presents three new Taqman based multiplexes, Eurobioplex Bacterial Meningitis, designed for the specific detection of the main bacteria responsible for that disease as well as a control of PCR inhibition. The first one concerns community-acquired bacterial meningitis and allows the qualitative detection of *Neisseria meningitidis (NM,* two genes*), Streptococcus pneumoniae (SP)*, and *Haemophilus influenza* (HI). The second multiplex concerns nosocomial meningitis and detects *Staphylococcus aureus* (SA) and *Pseudomonas aeruginosa* (PA). The third detects *Streptococcus agalactiae* (SAg), *Listeria monocytogenes* (LM) and *Escherichia coli* (EC) responsible for neonatal meningitis. PCR and analysis can be carried out in

approximately 1 hour from 5µl of sample using the same protocol for the three multiplexes. These kits are ready to use by mixing two reagents: enzyme and “oligomix” containing all necessary primers and probes, to the DNA. The preliminary step of DNA extraction is optional. This new diagnosis technique was compared to an in-house method routinely used at the French National Reference Center.

Results: Diagnostic performance has been performed on cerebrospinal fluid clinical samples and bacterial strains. Specificity is: 95.5% for the two genes tested for NM (n=50), 95.1% for SP (n=46), 96.8% for SAg (n=36), 97.2% for EC (n=43), and > 98% for HI (n=14), SA (n=48), PA (n=49), and LM (n=43), without any cross reactivity. Sensitivity is >98% for all targets. The detection limit is 10 copies/µl and the linearity stands from 10 to 10^9 copies/µl for each target. Depending on both the multiplex and the channel of fluorescence, the coefficient of variation is 0.54 to 1.89% for intra-experiment reproducibility, and 0.64 to 4.84% for inter-experiments reproducibility.

Conclusions: The Eurobioplex Bacterial Meningitis multiplexes therefore proved to have excellent analytical and diagnostic performance and allow early diagnosis of presumption of bacterial meningitis in humans, directly from CSF. These tests received CE marking.