DIRECT MULTIPLEX REAL-TIME PCR ASSAY FOR THREE MAJOR BACTERIAL MENINGITIS CAUSING AGENTS

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Background:

Bacterial meningitis remains a serious global health problems. Without treatment the case fatality rate can be as high as 70%, and one of the five survivors of bacterial meningitis may be left with permanent sequelae including hearing loss, neurologic disability, or loss of a limb. That's why, rapid diagnostic is the key for timely management of bacterial meningitis. Predominant etiologies of bacterial meningitis are *Streptococcus pneumoniae*, *Heamophilus influenzae and Neisseria meningitidis*. Detection for meningitis is improving day by day. Recent development of direct rt-PCR allows detection from clinical specimens[1]. We developed a multiplex assay to detect all three major bacterial meningitis causing pathogens in one reaction.

Methods:

We used published primer and probe sequences of *Streptococcus pneumoniae* (lytA), *Haemophilus influenzae* (hpd) and *Neisseria meningitidis* (sodC)[2] with three florescence dye combination to design a multiplex reaction. We compared cycle threshold (Ct) value of singleplex and multiplex reactions by using known DNA concentrations. Next, we compared samples with known etiology using both singleplx and multiplex assay and extracted with direct PCR. Finally, we used our assay to detect etiology in CSF specimens of meningitis cases with unknown etiology.

Results:

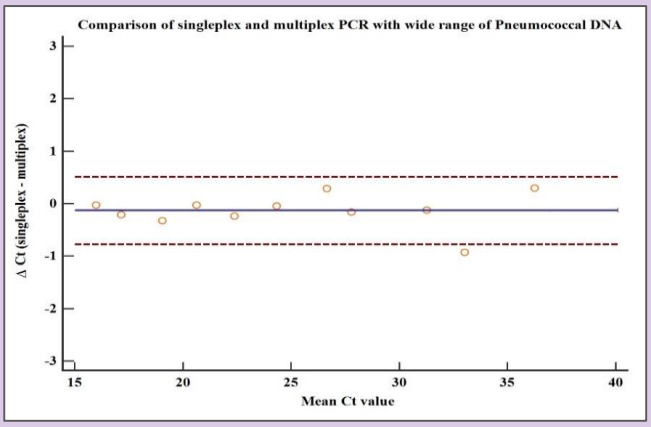
Each assay was 100% specific in detecting the organisms directly from CSF; no DNA extraction was required. No significant difference was observed in Ct values between singleplex and multiplex assays. In the 25 tested clinical specimens, with and without previously known etiology, we found 100% concordance between singleplex and multiplex assays. The proposed multiplex assay requires approximately 3 hours to yield the results.

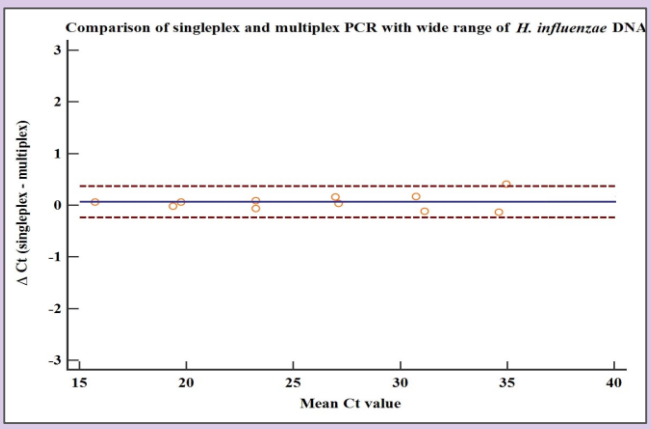
Conclusion:

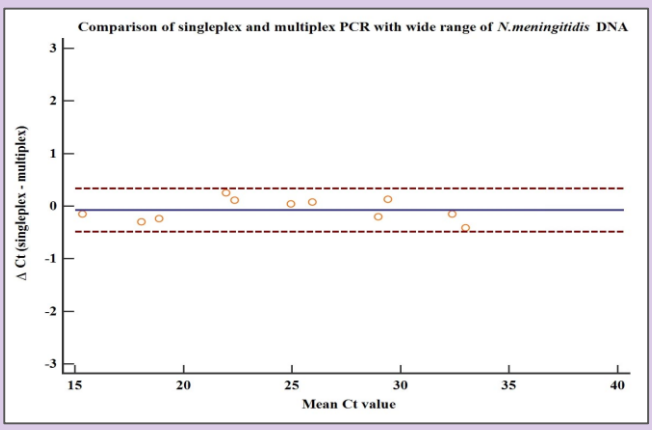
This assay detects three major etiologies of meningitis simultaneously using only 2 µl of CSF. It can be used as a point-of-care diagnostic for rapid etiology detection, specifically in endemic and epidemic areas with high prehospital antibiotic usage and where we need a urgent diagnosis adjacent to the bed.

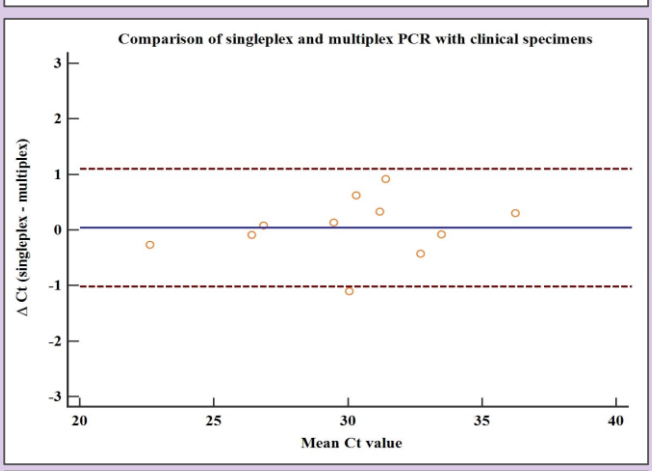
Reference:

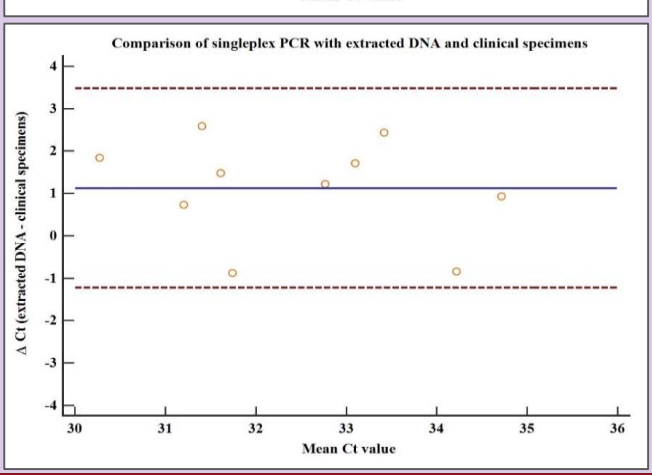
1. Development of Real-Time PCR Methods for the Detection of Bacterial Meningitis Pathogens without DNA Extraction. Jeny Vuong et al, 2016.
2. Laboratory methods for the diagnosis of meningitis caused by N.men, Spn and Hi, WHO manual, 2nd edition, 2011.











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