ABSTRACT

Introduction: Laboratory diagnosis of Meningoencephalitis by conventional methods is cumbersome, time consuming and has limited sensitivity. Therefore a qualitative PCR-HRMA was standardized to simultaneously test for 9 different bacterial and viral pathogens using a small sample of CSF.

Methods & Materials: Nine hundred and fifty consecutive CSF samples received at the Microbiological Laboratory, Coimbatore were included in the study. Processing by conventional methods (Gram Stain & Culture) & Innovative molecular method (PCR-HRMA) were done. Assay validation has been performed by measuring the linearity, repeatability (Inter Assay), reproducibility (Intra Assay), Robustness, time, and limit of detection (LOD) and compared with classical gold standards like culture, Gram Stain and other molecular tests (RT PCR & Sequencing).

Results & Discussion: Pathogen specific primers showed no cross reactivity with tested organisms. Time to detection was three hours. The LOD of the assay ranged from 15 to 162 copies/µl. Sequencing revealed that the amplified product of the validated primers were truly from the expected targets.

PCR-HRMA was positive in 213 cases (22.4%) including Streptococcus pneumoniae (n=73), Hemophilus sp. (n=38), N.meningitidis (n=6), MTB (n=28), HSV-1(n=29), HSV-2(n=3), VZV (n=9), CMV (n=15) and Enterovirus (n=12) respectively. Gram stain and / or culture were positive in 29 (3.8%) cases, all of which were also positive by HRMA. The poor sensitivity of smear & culture is due to empiric antibiotic therapy prior to lumbar puncture.
Conclusions: Study shows the potential value of PCR-HRMA in the diagnosis of Meningoencephalitis. The short turn-around time of the PCR and absence of probe in HRMA makes this technique an ideal rapid test for the diagnosis of meningitis & encephalitis. HRMA can help in the diagnosis even when culture is negative due to prior antibiotic treatment which is often the case in India. Multiplexing the PCR technique could make HRMA even more useful in a clinical setting.