Type: Poster Presentation

Final Abstract Number: 59.027
Session: Diagnosis
Date: Saturday, April 5, 2014
Time: 12:45-14:15
Room: Ballroom

Evaluation of three commercial real-time PCR kits for dengue diagnosis

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Background: Dengue is the most important arthropod-borne viral disease in the world. Since dengue clinical manifestations are similar to those of many other febrile syndromes, the availability of dengue-specific laboratory tests is useful for appropriate management of patients. Dengue is endemoepidemic inMartinique, a French Caribbean island. The island has experienced epidemics caused by DENV-2/DENV-4 in2005, DENV-2 in2007, DENV-1/DENV-4 in2010 and DENV-2/DENV-4 in2013. In our virology unit, RT-PCR (Hemi-nested protocol from Lanciotti et al., J Clin Microbiol 1992) is systematically run for patients consulting at hospital facilities with acute febrile disease and admitted until day 8 of fever onset. This technique is considered as the gold standard but it is time consuming and at high risk of contamination. Moreover there is a need for standardized dengue RT-PCR with internal controls.

Methods & Materials: In the present study, we evaluated the performance of three commercialdengue real-time PCR kits (*Simplexa™ Dengue PCR assay: Focus Diagnostic*, *RealStar® Dengue RT-PCR kit 1.0: Altona Diagnostics, Geno-Sen's Dengue 1-4 Real-time PCR kit: Genome Diagnostics Pvt)* using 162 samples positive with the hemi-nested RT-PCR (46 DENV-1, 37 DENV-2, 33 DENV-3, and 46 DENV-4). In addition, 70 negative serum specimens were used to evaluate specificity. All three kits included an internal control. RNA extraction was performed using the NucliSens easyMAG® (BioMérieux).

Results: Geno-Sen's, RealStar®, and Simplexa™ tests were positive in 138 (85.1%), 135 (83.3%), and 151 (93.2%) samples, respectively. The sensitivity of Simplexa™ was significantly higher than Geno-Sen's (Chi 2, p = 0.009) and RealStar® (p = 0.005). Compared to the in-place reference RT-PCR, the specificity was 100% for Geno-Sen's and Simplexa™ and 98% for Real Star.According to dengue genotypes, the sensitivity was for DENV-1 (91%, 78% and 96%), for DENV-2 (89%, 87%, and 92%) for DENV-3 (91%, 91%, 91%), for DENV-4 (72%, 80% and 94%) by Geno-Sen's, Real Star® and Simplexa™ kits respectively. Simplexa is the only kit providing genotyping of virus.

Conclusion: The good performance of the Simplexa™ Dengue PCR assay at the detection and genotyping level in combination with an automated extraction is suitable for diagnosis during dengue outbreaks in tropical areas.

http://dx.doi.org/10.1016/j.ijid.2014.03.1189

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Diagnosis of extrapulmonary TB: Experience with the Xpert MTB/Rif assay

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Background: South Africa has the 3rd highest number of TB cases in the world and the 5th highest number of drug-resistant cases (WHO, 2011). In 2009, 16% of newly diagnosed cases were extrapulmonary and 9070 cases were confirmed MDR-TB. Current diagnostic methods lack sensitivity and results are only available weeks later. The Xpert MTB/RIF assay provides a rapid diagnosis and a rifampicin susceptibility result. Although Xpert MTB/RIF assay has a high sensitivity for smear negative sputum samples, it has not been validated as yet for the diagnosis of extrapulmonary TB.

In this study, we describe our experience with the Xpert assay on extrapulmonary specimens.

Methods & Materials: All extrapulmonary specimens received at Lancet Laboratories in KZN, for TB diagnostics from November 2011 through to July 2012 were retrospectively evaluated. Xpert MTB/Rif assay and TB culture were performed on all specimens. Positive cultures were confirmed as *Mycobacterium tuberculosis complex* and subjected to drug susceptibility testing using the MGIT system. Data was extracted from the Meditech system.

Results: A total of 3069 extrapulmonary specimens were processed using the Xpert system. Clinical specimens included CSF's (936), pleural fluid (368), lymph nodes (283), urine (219), tissue (204), peritoneal fluid (63), liver (57) and pus (53). 1092 were excluded as TB culture was not performed. Of the remaining 1977 specimens, 252(12.7%) were TB culture positive. Of these, 180(71.4%) tested TB PCR positive and 72(28.6%) tested negative. Of the 1725 TB culture negative samples, 103(5.9%) tested TB PCR positive. Sensitivity of the PCR on extrapulmonary specimens in this study was 71.6% and the specificity was 94%. PPV and NPV were 64.5% and 95.7% respectively.

Conclusion: The sensitivity of the Xpert MTB/Rif assay on extrapulmonary specimens in this study, is similar to that of the gene xpert on a single smear negative sputum specimen. This sensitivity coupled with the rapid turn-around-time, makes the Xpert MTB/RIF assay a suitable addition to the initial testing algorithm in the diagnosis of extra-pulmonary TB.

http://dx.doi.org/10.1016/j.ijid.2014.03.1190