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Evaluation of a new multiplex pcr assay for the diagnosis of sexually transmitted infections pathogens in Rwanda Normal

C.M. Muvunyi[1], N. Dhont[2], R. Verhelst[2], M. Reijans[3], G. Cleays[2], E. Padalko[2]

[1] *University of Rwanda, Huye, Rwanda*
[2] *Ghent University, Ghent, Belgium*
[3] *PathoFinder B.V., Maastricht, Netherlands*

Background: To evaluated a new multiplex PCR (mPCR) "STDFinder assay", a novel multiplex ligation-dependent probe amplification (MLPA) assay for simultaneous detection of seven clinically relevant pathogens of STD, i.e., *Neisseria gonorrhoeae, Chlamydia trachomatis, Trichomonas vaginalis, Mycoplasma genitalium, Treponema pallidum*, and *Herpes Simplex virus* type 1 and2(HSV-1 and HSV-2).

Methods & Materials: A total of 242 vaginal swabs taken from infertile women, seen at an infertility research clinic at the Kigali Teaching Hospital in Rwanda (the largest public hospital in Rwanda) between November 2007 and March 2010. In order to increase the ability of the study to evaluate the performance of the multiplex PCR assay, an additional 80 specimens (31 positive for either *C. trachomatis* or *N. gonorrhoeae* and 49 negative for both pathogens) were added. The DNA was isolated using a QIAamp DNA Mini Kit (QIAGEN, Valencia, CA, USA). The multiplex PCR assay includes primers and probes specific for highly conserved regions of each pathogen and results were confirmed by single real-time PCR using different species-specific targets.

Results: The limits of detection for the multiplex PCR assay varied among the 7 target organisms from 1 to 20 copies/MLPA assay. There were no cross-reactions among any of the probes. Compared to the reference standard, the multiplex PCR assay showed specificities and sensitivities of 100% and 100%, respectively, for *N. gonorrhoeae, C. trachomatis* and *M. genitalium*; 90.2% and 100%, respectively, for *T. vaginalis*; and 96.1% and 100%, respectively, for HSV-2. No specimen was found positive for HSV-1 by both the multiplex PCR assay and the comparator method. Similarly, the sensitivity for *T. pallidum* could not be calculated due to the absence of any *T.pallidum*-positive samples.

Conclusion: The multiplex PCR assays have comparable clinical sensitivity to the conventional mono and duplex real-time PCR assay and are suitable for the routine detection of a broad spectrum of these STDs at relatively low cost due to multiplexing.

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Rapid detection of *Mycobacterium tuberculosis* complex using two molecular assays from pleural biopsy specimens

M.R. Mohlabeng[1], M.R. Lekalakala[1], N. Mbelle[2]

[1] *UNIVERSITY OF PRETORIA, Pretoria, South Africa*
[2] *National health laboratory Services, Johannesburg, South Africa*

Background: Tuberculosis remains the leading cause of morbidity and mortality in HIV infected patients. It is also one of the major public health problem with approximately 8.2 million new cases and 2 million deaths each year despite the use of BCG vaccine, effective antibiotics. This disease often presents a diagnostic challenge because of its diverse clinical manifestation and low acid fast bacilli especially in tissue specimens. Although culture offers a better sensitivity, the incubation time may take up to 6 weeks, hence the need for rapid and sensitive detection of Mycobacterium tuberculosis in tissue samples. Molecular techniques such as StepOneplus real-time PCR assay and Genotype MTBDRplus assay have been evaluated and adopted for diagnosis of TB in sputum samples, but their perfomances in tissue samples still need to be explored.

Methods & Materials: Hundred formalin fixed paraffin embedded tissue specimens from the department of Anatomical pathology were collected over aperiod of three months, of which 56 cases were suspected of TB based on Hispathology and ZN satining, and the remaining 44 were negative. DNA extraction was performed using QIAamp DNA mini kit. The DNA extracts were then analysed using StepOneplus real-time PCR and Genotype MTBDR plus assays.

Results: Of the 56 cases suspected of TB, 33 cases confirmed TB by StepOneplus real-time PCR assay while Genotype MTBDR plus assay detected M. tuberculosis in 15 cases. Amongst the 56 cases, one multidrug resistant case was detected by Genotype MTBDRpplus assay. Of the remaining 44 non-TB samples, three samples were positive by StepOneplus real-time PCR assay while Genotype MTBDR plus assay did not detected M.tuberculosis.

Conclusion: This study showed that Genotype MTBDR plus assay was less sensitive but highly specific as compared to StepOneplus real-time PCR assay. However, StepOneplus real-time PCR may be an efficient diagnostic adjunct to histology for diagnosis of TB, especially for samples where culture is deemed unsuitable.

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