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Comparison of conventional RT-PCR, One step and Two step qRT-PCR for diagnosis of West Nile virus

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Background: West Nile Virus (WNV) is a mosquito-borne flavivirus causing subclinical/encephalitis symptoms. In recent years it has emerged as a leading cause of viral encephalitis throughout the world. WNV is cross reactive with other closely related flavivirus like Japanese encephalitis Virus (JEV). This poses challenge for specific diagnosis and know-how of epidemiological scenario in areas where two or more flavivirus co-circulate. For effective diagnosis, we investigated the performance of conventional reverse transcriptase (RT)-PCR, GoTaq one step quantitative real time (qRT)-PCR and two step SYBR green based qRT-PCR.

Methods & Materials: To compare the detection sensitivities of three techniques, we used standard WNV strain G22886 (India, 1958, lineage 5, GenBank acc. No. AY944241). Three separate dilution series (10^{-1} to 10^{-6}) of standard WNV RNA for one step qRT-PCR and cDNA for RT-PCR and two step qRT-PCR were assayed in a single run. The mean, standard deviation (SD) and coefficient of variation (CV) were calculated separately for each standard RNA dilution in order to confirm the inter-assay and day-to-day variation. The primers were designed targeting the conserved envelop (E) region of WNV. Specificity of the assays was also cross checked against JEV.

Results: The detection limit of one step based qRT-PCR and two step qPCR assay was found to be up to fifth dilution of WNV template, whereas, in conventional end point RT-PCR, the minimum detection limit was found to be up to fourth dilution. The coefficient of determination (R^2) of the standard curve of one step qRT-PCR was 0.957 with a slope value of 2.551 and that of SYBR Green based two step qPCR was 0.984 with slope value 3.044. Mean CV for intra and inter assay variation was found to be < 2% and < 4% in one step based and two step based qRT-PCR.

Conclusion: Both one step and two step qPCR are equally sensitive for diagnosis, however, efficiency of two step qPCR is more as indicated by slope value. Given its combined sensitivity, specificity and speed, qPCR is the preferred assay for the diagnosis of WNV.

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Performance of MALDI-TOF MS in an academic microbiology laboratory in South Africa

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Background: Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) allows for rapid identification of bacteria and yeasts. Currently, microbial identification in clinical diagnostic microbiology laboratories relies on conventional phenotypic methods that are time consuming. This study assessed the performance of a MALDI-TOF MS Analyser in an academic diagnostic microbiology laboratory in South Africa.

Methods & Materials: The study was conducted over a two month period in 3 phases. For phase 1, isolates from clinical specimens were identified by the Microscan Automated ID/AST system and compared with MALDI-TOF MS Bruker Analyser results. The second phase compared isolates from referral laboratories which used the Vitek 2 Automated ID/AST system. The third phase compared isolates from the Bact TAlert Blood Culture system extracted by Bruker Sepsityper Kit to routine diagnostic methods.

Results: A total of 227 bacteria and yeast isolates were collected from the Academic Laboratory (N = 119) and Referral laboratories (N = 108) in Free State Province of South Africa. Sixty-five positive blood cultures for bacteria and yeast were extracted for direct identification. Of the 119 isolates from the Academic Laboratory, 85 were Gram-negatives, 7 Gram-positive and 27 yeasts. From referral laboratories there were 37 Gram-negatives, 33 Gram-positives and 37 yeasts. There were a total of 21 *E.coli* isolates which were reported by the MALDI-TOF as E.coli/Shigella. From the 65 blood culture bottles,7 grew more than one bacterial pathogen and the MALDI-TOF MS identified only one isolate. The blood cultures yielded 21 Gram-negatives, 43 Gram-positives and 1 Candida. From the total of 292 isolates discrepant results were for 4 isolates (1 bacteriium and 3 yeasts). For yeast isolates the Microscan required a longer incubation.

Conclusion: The MALDI-TOF MS proved to be useful for rapid and reliable results for patients with infectious diseases. The difference in time to identification was significant for all isolates. For blood cultures there was a massive difference for turn-around time contributing to great appreciation by intensive care personnel. There was excellent correlation for the MALDI-TOF MS Bruker Analyser with the Microscan and Vitek ID systems as well as for occasional isolates that required the use of the API identification system.

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