IVD_07 - Development of a multiplex RT-PCR molecular assay for Measles and Rubella viruses detection

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Introduction: Measles is one of the main causes of morbidity and mortality among children under 5 years old, especially those who are undernutrition caused by Measles virus (VS). Rubella virus (VR) is a highly contagious agent with epidemiological importance due to the Congenital Rubella Syndrome that affects the fetus during pregnancy and can lead to misbirth, stillbirth, and congenital malformations for newborns such as deafness, heart malformations, eye injuries, and others. The main importance for the development of the Molecular Measles and Rubella Bio-Manguinhos assay is the diagnosis and epidemiological surveillance. Based on a Real Time PCR technology, this assay was developed for a triplex reaction using specific TaqMan probes for detect Measles and Rubella viruses target and the constitutive gene human RNAse P as an internal control.

Objectives: The aim of this study was to evaluate a multiplex methodology for detection of VS and VR with analysis of sensitivity, reproducibility and specificity.

Methodology: All samples used were collected from individuals with clinical indicative of infection by VR or VS, in addition to cultured viruses. The target regions for RT-PCR amplification of each genome virus were: Measles nucleoprotein and Rubella envelope glycoprotein.

Results: 373 samples from individuals with a clinical infection by VS and VR were tested with positive and negative results. For true positive samples, the assay showed 100% agreement and a Pearson correlation of R2 0.01 for Rubella and R2 0.06 for Measles. Two samples identified as negative by the reference laboratory for respiratory viruses and measles (LVRS), which uses the CDC diagnostic protocol, showed a positive result in the Bio-Manguinhos test, the same occurred with 4 measles samples sent by LACEN-PR, also uses the CDC protocol and 1 positive sample in this Lacen was negative in our assay. There was no cross-reaction when analyzing samples for Influenza A, Influenza B, RSV, Adenovirus, HIV, HCV, HBV, Zika, Chikungunya, Dengue, Syphilis, Varicella Zoster. The assay showed an analytical specificity of 100% and a clinical specificity of 99.9%. PROBIT analyzes, considering a positivity rate of 95% and a confidence interval (CI) of 95%, showed an estimated sensitivity of 0.19 copies/µL (1.9 copies/reaction) for the VS target and with a 95% positivity rate and a 95% confidence interval (CI) showed an estimated sensitivity of 0.09 copies/µL (0.9 copies/reaction) for the VR target.

Conclusion: The assay proved to be highly efficient for detection VS and VR, being an important methodology for diagnosis and epidemiological surveillance.

Keywords: Diagnostic, RT-PCR, Measles and Rubella