

VAC.08 - Phylogenetic approaches to evaluate the application of qPCR designed for measles vaccine strains for diagnosis

Gisela Freitas Trindade^{1*}; Xenia Rawena Medeiros Romeu Lemos²; Cristiane Pinheiro Pestana¹; Liliane Monteiro de Moraes¹; Renata Tourinho Santos¹; Juliana Fernandes Amorim da Silva¹; Sheila Maria Barbosa de Lima¹.

1Fiocruz/Bio-Manguinhos;

2Fiocruz/IOC.

Introduction: Measles is a highly contagious viral disease that causes morbidity and mortality, which affects susceptible individuals of all ages and remains a cause of death among young children globally. Almost 2 years after the Pan American Health Organization declared the Americas free of measles, Venezuela's epidemic has disseminated to Brazil, Ecuador, Colombia, and Peru. In this context, vaccination campaigns have been conducted to prevent the disease using live attenuated measles vaccine, administered in combination with mumps and rubella (MMR), produced by Bio-Manguinhos – Fiocruz. The potency of vaccine viruses is determined using cell-based viral quantification assays, which are reference by WHO and Pharmacopeia to determine viral infectious particles. However, they are complex and take a long time to be completed. Instead, molecular approaches could be developed as alternative methods to quantify measles viral load. This tool offers several advantages, including sensitivity, specificity and fast results. In this sense, measles vaccines strains qPCR could be employed in the detection of others measles strains circulating in Brazil.

Objective: Evaluate the application of the qPCR method, previously developed to quantify measles vaccine strains, in the clinical diagnosis using a phylogenetic approach.

Methodology: A qPCR method was previously design to detect part of gene that encode measles nucleoprotein (689-876 genome position) based on Schwartz strain, which is one of the components of MMR vaccine. Evaluating this method for clinical application, we aligned the target gene to 17 WHO reference strains of different genotypes, including B3, D4, D8 circulating in Brazil, by Clustal W. In addition percentage of homology was verified the by DNASTar and MEGA X softwares, using pairwise distance tool.

Results: After editing, all 17 WHO reference strains and measles target gene results in sequences set with 188 bp. Applying DNASTar software, the measles target gene showed more than 96% homology compared to tested sequences. Results obtained from MVi_Manchester GBR-D8, MVi_Montreal CAN-D4 and MVi_NewYork USA-B3 strains revealed 97.9%, 97.3% and 96.8% homology, respectively, (circulating strains in Brazil). Using MEGA X platform, sequence similarity searching demonstrated high identity between tested sequences: 97.9% for MVi_Manchester GBR-D8, 97.3% for MVi_Montreal CAN-D4 and 96.8% for MVi_NewYork USA-B3. Both bioinformatics tools revealed matched results indicating high homology with measles target gene.

Conclusion: These analyses indicate a possible application of this method not only in MMR production but also as diagnosis to detect measles strains of different genotypes. In addition, it is expected to use this qPCR method to test clinical samples from the Reference Laboratory in Respiratory Viruses IOC – Fiocruz for diagnostic purpose.

Keywords: Measles; sequence analyses; qPCR