R6 - A laboratory-developed TaqMan Array Card for simultaneous detection and genotyping of Group A rotavirus.

Irene T. Araujo[1]*; Alexandre M. Fialho[1]; Rosane M. S. Assis[1]; Ediuardo M. Volotão[1]; Darwin J. Operario[2]; Eric R. Houpt[2]; Duncan Steele[3]; Fatima Serhan[4]; Gloria Rey-Benito[5]; Jose Paulo G. Leite[1].

1 - IOC/Fiocruz;
2 - University of Virginia;
3 - CDC;
4 - WHO;
5 - PAHO/WHO.

Introduction:

Group A Rotaviruses (RVA) represent the main cause of acute gastroenteritis in children under five years old worldwide. A sensitive molecular technique is important to ensure reliable results for epidemiological surveys. The TaqMan Array Card (TAC) system is a 384-well singleplex real-time PCR format that allows detection of multiple infection targets. Here we used a TAC that has been developed for detection of 19 enteropathogens, including characterization of eight G genotypes (VP7 gene) and six P genotypes (VP4 gene) of RVA

Objective:

Evaluate the rotavirus detection and genotyping results obtained with the TAC method comparing with the previous results obtained by polyacrylamide gel electrophoresis (PAGE), enzyme immunoassay (EIA) for RVA detection (Ridascreen, R-Biopharm) and RT-PCR for RVA genotyping.

Methodology:

One-hundred and thirty-nine samples were processed by TAC out of a three-hundred total selection. All samples were previously tested by PAGE, EIA and RVA genotyping by RT-PCR. A modified extraction procedure was performed to isolate both DNA and RNA from stool samples using the QIAamp Fast DNA Stool Mini Kit. Extrinsic controls PhHV (*Phocine Herpesvirus)* and MS2 (bacteriophage) were added to samples during the lysate preparation to evaluate extraction and amplification efficiencies. TaqMan Array Cards were used to amplify nucleic acid. RVA detection and genotyping below a quanti-

fication cycle (Cq) of 35 and 40, respectively, were used as a uniform cutoff for test positivity based on a limit of detection.

Results:

Six samples were previously diagnosed as RVA positive by PAGE, EIA and genotyped by RT-PCR as G2P4 (n=3), G?P8 (n=1), G3P8 (n=1) and G1P8 (n=1). Our TAC preliminary results confirmed RVA detection in all six samples and genotyping by TAC confirmed G1P8, G3P8 and G2P4 strains (n=1 each). The other G2P4 strains (n=2) were characterized as mixed infection with P8 and the G?P8 strain (n=1) was characterized as G12.

Conclusion:

The TAC method is a specific and rapid method for simultaneous detection of nucleic acids from viruses, bacteria, protozoa and helminthes, as well as genotyping RVA, in the same test. The specificity of the TAC method compared to RT-PCR for RVA genotyping is suitable, once one non-typed G sample was characterized. To date, few samples were tested for RVA by TAC method yet, but more samples will be processed to allow a reliable evaluation of specificity and sensitivity of TAC results.

Keywords: rotavirus, real-time PCR