Evaluation and re-design of a VP6 probe sequence for the detection of rotaviruses

Sandrama Nadan[1]; Tersia Kruger[1]; Nicola Page[1,2]

[1]Centre for Enteric Diseases, National Institute for Communicable Disease, South Africa, [2]Department of Medical Virology, Faculty of Health Sciences, University of Pretoria, South Africa

Background

A VP6 real-time reverse-transcriptase polymerase chain reaction (RT-PCR) was performed to confirm positivity in query rotaviruses (RV). These query strains were detected with a high OD value by an enzyme-linked immunosorbent assay (ELISA; ProSpecT Rotavirus Microplate Assay) but were negative by molecular detection and characterisation. However, the results observed with the VP6 RT-PCR were inconsistent for both queried and known positive RV strains. In this study the VP6 assay was investigated and a second PCR probe was designed.

Method

Tests were initially carried out to determine why the VP6 real-time RT-PCR was less efficient or negative. The concentration of the probe was investigated as well as the process of cDNA preparation. Available VP6 gene nucleotide sequences (n=121) and the probe sequence were aligned using BioEdit software and an alternate probe was designed. The queried and known positive specimens were tested using the new probe.

Results

The results showed that neither the concentration of the old probe used nor the cDNA synthesis method increased the RV positivity. Nucleotide sequence analysis revealed a single base mismatch in the old probe sequence and the RV strains. A second probe was designed in the region next to the original sequence. Specimens that were poorly detected using the old probe were amplified more efficiently with the newly design probe (8/11). However, some RVs were only detected using the old probe (3/11).

Conclusion

The new probe was able to detect previously undetectable RVs in a more efficient VP6 screening assay. A bias was introduced by analyses of only G1P[8] sequences when designing the new probe. Other genotypes will be examined and the probe sequence may be edited to introduce degenerate bases. The new reaction will also be applied to resolve the ELISA positive/PCR negative specimens. This report recommends that both probes be used for screening RVs.