Título: RAPID DETECTION OF HUMAN ROTAVIRUSES A BY USING A MOLECULAR BEACON ASSAY.

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Resumo:

Rapid, sensitive and specific detection methods are necessary to detect and quantify infectious viruses. The inability to easily cultivate and detect many enteric viruses in cell culture systems difficult the advance of knowledge about virus-induced diarrhea. Detection of rotaviruses (RV) has been conducted by serological methods or molecular biology techniques. However, these techniques only allow the detection of antigens or genome without providing information about the viral infectivity. The molecular beacon technique (MB) has demonstrated efficacy for viral detection by the use of single-stranded nucleic acid molecules that possess a stem-loop structure and are doubly labeled with a fluorophore and a quencher at the 5' and 3' ends, respectively. These probes are capable of generating a specific fluorescent signal, by the hybridization with the target, which forces a structural change in the probe. In this report, the MB technique was applied, for the first time, to detect human rotavirus type A (HuRV-A) in cell culture. MA-104 cells were cultured and infected with HuRV-A strains, HuRV-A Rotateq® Vaccine (MOI 1) and HuRV-A K8 (MOI 0.5 and 1). After 24h, the cells were fixed with 2% paraformaldehyde in TBSS for 20 min at room temperature. Next, the cells were permeabilized with 0.1% Triton X-100 in TBSS for 5 min at 4ºC following the addition of the MB for 1h and subsequent analysis by fluorescence microscopy. The specific MB for HuRV-A VP6 gene used was 5 '[FAM] CGCGATTAGTTCAGTCCAATTCATGCCTCGCG [DABCYL] 3'. The DAPI nuclear stain was used in all conditions tested as control of cell permeabilization. The results demonstrated that non-infected cells with MB showed basal fluorescence. However, infected cells (Rotateq® Vaccine or K8 strains) with MB exhibited increased fluorescence emission. This result confirms the MB hybridization to the viral mRNA target of RV. Our results also showed the increase of fluorescence according to the increase of the number of infectious viral particles per cell (MOI 0.5 to MOI 1). The application of this technique reached its goal that is quick and efficient HuRV-A detection in cell culture without the need of the viral culture for several days or many times until the appearance of cytopathic effect. Besides these results, we need to understand the nuclear localization of the fluorescence emission in cells and perform new approaches to improve the sensitivity of the probe in hybridizing to the target.

Palavras-chave: human rotaviruses, molecular beacon, detection.

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