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Basic Study

Rapid genotyping of human rotavirus using SYBR green real-time reverse transcription-polymerase chain reaction with melting curve analysis

Yupin Tong, Bonita E Lee, Xiaoli L Pang

Abstract

AIM: To develop a real-time reverse transcription-polymerase chain reaction (RT-PCR) assay to genotype rotavirus (G and P) in Alberta from January 2012 to June 2013.

METHODS: We developed and validated a different approach to perform rotavirus G and P genotyping using a two-step SYBR Green real-time reverse transcriptase (RT) PCR (rt-gPCR) by selecting genotype-specific primers of published conventional RTnested PCR (cnRT-PCR) assay and optimizing the amplification conditions. cDNA was first synthesized from total RNA with SuperScriptTM II Reverse Transcriptase kit followed by amplication step using monoplex SYBR Green real-time PCR. After the PCR reaction, melting curve analysis was used to determine specific genotype. Sixteen

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