Real time RT-PCR assays using cRNA standards for quantification of syncytial respiratory virus type A and B

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Quantificazione del virus respiratorio sinciziale di tipo A e B mediante saggi real time RT-PCR con standards a cRNA

INTRODUCTION

Respiratory syncytial virus (RSV) is a major cause of respiratory tract infections in infants, with bronchopneumonia and bronchiolitis (3). In the adult the clinical manifestation is lighter than in children, however, in the case of immunocompromised patients (transplant recipients or patients in immunosuppressive treatment) infection can lead to death. Real time RT-PCR developed in recent years represents an useful tool in the diagnosis of RNA viruses. In order to accurately quantify and normalize a RNA target, efficiency of reverse-transcription must be considered (1, 2). In this study a cRNA-standard based quantitative real time RT-PCR have been developed for RSV quantification. In particular, we developed two quantitative real time RT-PCR assays by production of cRNA standards for RSV type A (RSVA) and B (RSVB) quantification.

METHODS

Large amount of RSVA and RSVB plasmids (pRSVA and pRSVB, respectively) were obtained by using PureLink HiPure Plasmid Midiprep (Invitrogen). Twelve µg of pRSVA and pRSVB were linearized by using SacI restriction enzyme for 2h at 37°C, inactivated for 20min at 65°C, and purified by using Blood & Cell Culture DNA Mini Kit (QIAGEN).

Klenow fragment (Invitrogen) was used to create blunt ends (15min at 22°C). cRNA standards production was carried out at 37°C for 4h using RiboMAX™ Large Scale RNA Production Systems T7 (Promega). One-tenth of Ribomax product was digested with DNAse (Promega) for 15min at room temperature, then inactivated using EDTA for 10min at 65°C. Finally $cRNA_s$ were purified using RNAgent (Promega).

Dilutions of cRNA from 1 to 10^{10} copies were generated. Intra- and inter-test variability, sensitivity

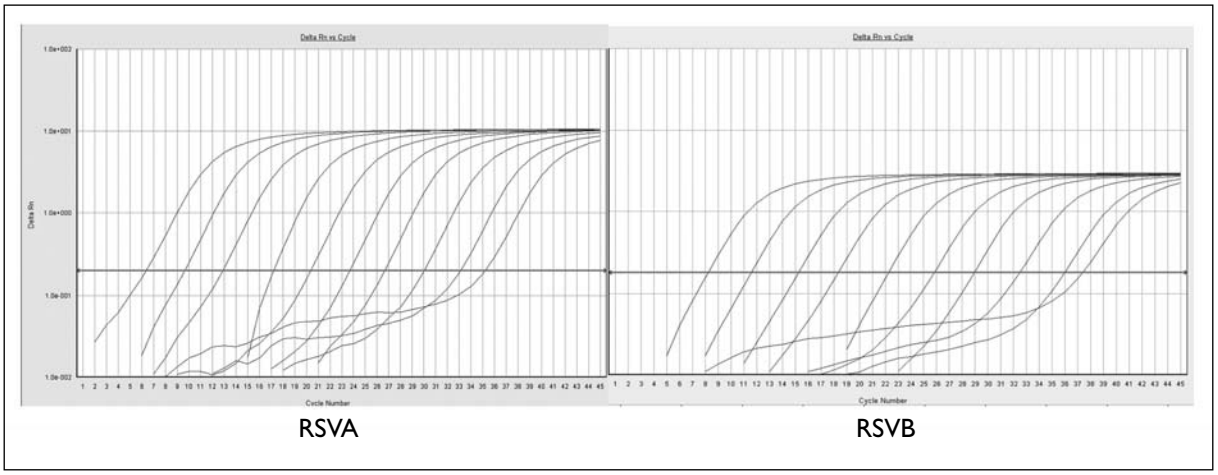

Figure 1. *Dynamic range of RSVA and RSVB genome quantification with real time RT-PCR assays. The genome equivalents (GEq) from RSVA and RSVB quantification standards are obtained using 10-fold dilutions from 10^{10} to 1 copies.*

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and detection limit were evaluated and confirmed by using different statistical tests like Dixon's test and Shapiro-Wilk test.

RESULTS

cRNA standard curves showed a dynamic range from 10^{10} to 10 copies and a sensitivity of 10 copies for both RSVA and RSVB (Figure I). Limit of detection was 1 copy and 10 copies for RSVA and RSVB, respectively with an acceptable values of intra- and inter-test variability. The cRNA curve showed a slope and an intercept of 3.2283 and 39.975 for RSVA and 3.3665 and 42.092 for RSVB.

CONCLUSIONS

Real Time RT-PCR assays using cRNA standards resulted sensitive, specific and more suitable for quantification of RSVA and RSVB. In conclusion, these molecular assays could represent an useful tool for rapid detection of RSVA and RSVB.

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