Rapid Detection of Respiratory Syncytial Virus Using RT-SIBA®

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Introduction

Rapid diagnosis of respiratory viruses improves patient management, minimizes unnecessary prescriptions of antibiotics, and prevents the spread of infections in environments which are prone to outbreaks, such as garrisons and day-care centres. Hence, we evaluated a rapid, portable molecular test system for near patient diagnostics with nasopharyngeal swab (NP) specimens obtained from patients with signs and symptoms of respiratory tract infection. We compared the performance of the novel, isothermal Reverse Transcription Strand Invasion Based Amplification (RT-SIBA®) assay with the conventional Reverse Transcription Real-Time PCR (RT-PCR) assay for detection of respiratory syncytial virus (RSV).

Materials and Methods

A total of 120 coded NP specimens (60 RSV positives and 60 influenza positives) obtained from Finnish garrisons and health centres during the years 2011-2013 were retrospectively and anonymously analysed with the RT-SIBA RSV assay (Orion Diagnostica) and the RealStar® RSV RT-PCR assay (Altona Diagnostics).

The RT-SIBA assay targeted the nucleoprotein region of RSV, utilizing the recombinase-dependent insertion of a specific invasion oligonucleotide and primers after reverse transcription [1]. The RT-SIBA reactions were run in the portable, fluorometric Orion GenRead® instrument (Figure 1) for 35 minutes at a constant temperature of 41°C. The results were reported as positive, negative, or invalid by the automated software. The positive reactions were reported as soon as the reaction reached the level of positivity. In case of an invalid result, a new analysis was conducted.

Figure 1. Orion GenRead® instrument (Orion Diagnostica)

Table 1. Sensitivity and specificity of the RT-SIBA-based RSV assay, relative to the RealStar® RSV RT-PCR assay.

	RT-SIBA RSV Positive	RT-SIBA RSV Negative	
RealStar® RSV RT-PCR	55	6	Sensitivity
Positive	True positive	False negative	90 % 95% CI 80-95%
RealStar® RSV RT-PCR	0	48	Specificity
Negative	False positive	True negative	100 % 95% CI 94-100%

CI - Confidence interval

Results and Conclusions

The sensitivity and specificity of the RT-SIBA RSV assay, relative to the RealStar® RSV RT-PCR assay, were 90.2% (CI95 80.2–95.4) and 100% (CI95 93.8-100), respectively (Table 1). 11 (9 %) samples were reported as invalids and they were excluded from the performance characteristics calculations. The RT-SIBA RSV results were available in less than 20 min after starting the analysis run in the Orion GenRead® instrument. The sample preparation took 7 min per sample, including 2 min hands-on time and 5 min incubation time at 95°C.

The RT-SIBA RSV assay was found to be specific, sensitive, and easy to use. The assay combined a simple and effective sample preparation with ready-to-use, freeze-dried reagents with a stand-alone instrument. Therefore, it can be applied in decentralized settings, contributing to faster near-patient diagnostics.

[1] Hoser MJ, et al. Strand Invasion Based Amplification (SIBA®): a novel isothermal DNA amplification technology demonstrating high specificity and sensitivity for a single molecule of target analyte. PLoS One. 2014 Nov 24;9(11):e112656

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