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# Evaluation of the reverse transcription strand invasion based amplification (RT-SIBA) RSV assay, a rapid molecular assay for the detection of Respiratory Syncytial Virus

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## Abstract

Respiratory Syncytial Virus (RSV) causes acute respiratory infections. Rapid RSV diagnosis has an impact on patient management. In a newly developed molecular assay, named reverse transcription strand invasion based amplification (RT-SIBA) RSV assay, RSV RNA is reverse transcribed to cDNA and amplified and detected under isothermal reaction conditions. The performance of this assay was evaluated.

Respiratory samples that tested positive (n=81) or negative (n=61) for RSV with the multiplex RT-PCR Anyplex II RV16 Detection Kit (Anyplex) were analysed with the RT-SIBA assay. Discordant samples were tested with the GeneXpert Flu/RSV XC assay. Consistent results in at least two of the three methods were defined as reference standard.

The RT-SIBA assay yielded a negative result for the 61 negative samples and a positive result in 71/81 (85.5%) of the Anyplex positive samples. After a resolution of discordant samples the positive and negative percent agreement of the RT-SIBA assay were 92% and 100%, respectively.

26 The RT-SIBA assay is a rapid molecular assay for the detection of RSV with good  
27 performance in clinical specimens.

28

29 **Keywords:**

30 SIBA; PCR; Respiratory syncytial virus; molecular diagnostics; comparison;  
31 sensitivity; specificity.

32

## Introduction

Respiratory Syncytial Virus (RSV) currently belongs to the *Pneumoviridae* family (Rima et al. 2017). RSV is a leading cause of acute respiratory infections (ARI) in children aged less than 5 years (Hall et al. 2009; Nair et al. 2010; Scheltema et al. 2017) and is associated with significant morbidity and mortality rates in adults, especially in elderly and immunosuppressed patients (Falsey 2005).

The rapid diagnosis of RSV infection is useful because: (i) it prevents empirical therapy with antibiotics, promotes antimicrobial stewardship and reduces healthcare associated costs. (ii) it helps to avoid nosocomial transmission and outbreaks in healthcare settings because appropriate infection control measures can be implemented (Mills et al. 2011; Ferronato et al. 2012; Thevenin et al. 2012).

Laboratory diagnosis potentially includes the detection of viral infection markers, e.g. myxovirus resistance protein A (Engelmann et al. 2015) and the detection of RSV, either based on antigen detection or the detection of viral nucleic acids (molecular assays). Antigen detection techniques include direct immunofluorescence assays and rapid antigen detection tests (RADTs). RADTs can be performed in laboratories or as point-of-care testing. These tests are easy to perform and provide results within 10-20 minutes. However, assays based on RSV antigen detection have a lower sensitivity than molecular assays (Chartrand et al. 2015; A Hogan et al. 2018). Molecular techniques for the detection of RSV show higher sensitivity, but traditional multiplex respiratory RT PCRs that include a separate extraction step are more time consuming (A Hogan et al. 2018). Lately, several rapid molecular assays have been developed with the aim to provide more rapid and sensitive RSV diagnostics (A Hogan et al. 2018).

Recently, a new assay based on an isothermal reverse transcription strand invasion based amplification (RT-SIBA) was developed for the rapid detection of RSV and showed excellent sensitivity and specificity (Eboigbodin et al. 2017). In brief, the method includes a reverse transcription of RSV RNA to cDNA and an immediate amplification and detection under

isothermal reaction conditions. A single-stranded invasion oligonucleotide separates the double-stranded target DNA leading to the generation of a single-stranded target template followed by polymerase-dependent extension of the target (Hoser et al. 2014; Eboigbodin et al. 2017).

The aim of this study was to perform an independent field evaluation of the performance of the RT-SIBA RSV assay for the detection of RSV in clinical samples.

## **Materials and methods**

### **Patients and samples**

Nasal and nasopharyngeal swabs were obtained at the university hospital of Lille, from outpatients or hospitalized patients with symptoms of acute respiratory tract infection. Specimens submitted to the laboratory for routine testing during the 2014-2016 period were used for this study. Swabs were collected in 1 mL of transport medium and were stored at -80°C until routine testing (performed 3 times a week outside the epidemic season and every working day during the winter season) by using the Anyplex II RV16 Detection Kit (Seegene, Korea). The clinical evaluation of the RT-SIBA RSV assay was performed on the remaining specimen volume. Demographic data were collected retrospectively. The study was carried out in accordance with the Declaration of Helsinki and was approved by the institutional review board with waiver of informed consent (Approval number: HP16/79).

### **Respiratory viruses testing with Anyplex II RV16 Detection Kit**

This multiplex RT PCR assay simultaneously detects most prevalent respiratory viruses that are clinically relevant, including RSV-A and RSV-B. Nucleic acids were extracted from 200µL of each respiratory specimen by using the Magtration System 12GC with the MagDEA Viral DNA/RNA 200 (GC) kit (Precision System Science Co., Ltd.; Japan) according to the manufacturer's instructions. The multiplex real-time RT-PCR Anyplex II RV16 (Seegene, Korea) (Anyplex) was run on the CFX96™ (Bio-Rad, Marnes-la-Coquette, France) platform, and data were analyzed by Seegene's Viewer software.

### **RSV testing with the RT-SIBA® RSV assay**

The RT-SIBA RSV assay (Orion Diagnostica, Finland) was developed to detect RSV-A and RSV-B and was performed by strictly following the manufacturer's instructions. Briefly, 50µL of each sample were collected from the transport medium tube and transferred into the heating tube that was subsequently maintained in a heating block at 95°C for 5 min. Then 40µL of the treated sample were transferred into the respiratory buffer tube and the mixture was vortexed for 3-5 seconds. Then 40µL of the mixture was transferred into the reagent tube. Up to 12 specimens were processed simultaneously. The reagent tubes were vigorously vortexed for at least 5 seconds, and inserted into the Orion GenRead® instrument. The run was performed at 41 °C for 45 min.

### **RSV testing with Xpert® Flu/RSV XC assay**

Specimens with discordant results in the RT-SIBA RSV versus the Anyplex assays were tested by using GeneXpert Flu/RSV XC (Cepheid). The assay is fully automated and integrates nucleic acid extraction, target amplification and detection from 300 µl of sample in the same cartridge.

### **Data analysis**

Descriptive statistics were performed using SPSS (IBM SPSS statistic 22). For the assessment of the RT-SIBA RSV assay performance the results found consistent by two different methods were used as reference standard. Because the reference standard was based on other laboratory assays and not on clinical outcomes (imperfect reference standard), the terms “positive percent agreement (PPA)” and “negative percent agreement (NPA)” were used, rather than the terms “sensitivity” and “specificity” (the formulas used to calculate these are the same). PPA, NPA and confidence interval (CI) values were calculated according to the Clinical and Laboratory Standards Institute's guidelines (Clinical and Laboratory Standards Institute 2002; Biswas 2016). However, in published literature, the terms “sensitivity” and “specificity” are commonly used for these kinds of performance evaluation studies. Therefore we use these terms in the discussion part.

## Results

A total of 142 samples previously tested with the Anyplex assay, comprising 81 RSV positive and 61 RSV negative samples, were included in this study. Among the positive samples RSV-A and RSV-B were detected in 47 and 36 samples, respectively. Both RSV-A and RSV-B were detected in two samples. 54.2% of the patients were male and the median age was 9 years (range 0 to 91 years). The patients with a RSV positive sample were younger than those with a negative result (median age, 5 versus 15 years,  $p=0.03$ ).

By using the RT-SIBA RSV assay a negative result was found for all the 61 negative samples tested. For the initially positive samples, the RT-SIBA RSV assay yielded a positive result in 71 out of 81 (87.7%) samples. As described in the study flow chart (Figure 1), the 10 samples with a positive result in the Anyplex assay (comprising 7 Anyplex RSV-A positive and 3 Anyplex RSV-B positive samples) and a negative result in the RT-SIBA RSV assay were tested with the Xpert® Flu/RSV XC assay. The Xpert® Flu/RSV XC assay showed a positive result in 6 samples (out of 10) (namely 4 Anyplex RSV-A positive and 2 Anyplex RSV-B positive samples). The results found consistent by two different methods were used as reference standard for the assessment of the RT-SIBA RSV assay performance (Table 1). The positive percent agreement (PPA) (corresponds to sensitivity) of the RT-SIBA RSV assay was 92% (95% CI, 84 - 96%) and the negative percent agreement (corresponds to specificity) was 100% (95% CI, 94 - 100%).

The run duration for RSV-negative samples is 45 min, but positive samples are usually detected much earlier, because the RT-SIBA RSV assay identifies positive test results as soon as the amplification threshold has been reached. In this study the median time to positivity was 11 min (range 7 to 35 min). 33.8% of positive samples were detected in less than 10 min and 94.4% were detected in less than 20 min.

## Discussion

Rapid molecular tests are of great interest for the management of patients with respiratory tract infection, especially in pediatric wards during seasonal outbreaks (Mills et al. 2011). To date the available low-complexity and rapid (result within an hour) molecular RSV tests include the Alere® i RSV assay (Alere Inc.), the BioFire FilmArray® Respiratory Panel (bioMérieux), the Xpert® Flu/RSV XC and Xpert® Xpress Flu/RSV assays (Cepheid Inc.), the cobas® Liat® Influenza A/B & RSV (Roche Diagnostics), and the Simplexa® Flu A/B & RSV kit (Focus Diagnostics). The sensitivities reported for rapid molecular assays range from 87 to 100% and the specificities from 94 to 100% (A Hogan et al. 2018; Banerjee et al. 2018; Huang et al. 2018). In this study, the novel RT-SIBA RSV assay was compared to the Anyplex assay, and the Xpert® Flu/RSV XC assay was used to resolve discordant samples. We found that the RT-SIBA RSV assay can detect RSV in nasal and nasopharyngeal swabs with a sensitivity of 92% and a specificity of 100%. These results were obtained with frozen samples obtained from various hospital units and during different seasons. RADTs, however, showed pooled sensitivities and specificities compared to RT-PCR of 74 to 81% and 97 to 99%, respectively (Chartrand et al. 2015; A Hogan et al. 2018). Thus the sensitivity of the RT-SIBA RSV assay is in the same range as reported for other rapid molecular tests and is superior to the sensitivity of RADTs.

Practical considerations, e.g. the turnaround time, the possibility of running single or multiple specimens at the same time and whether the assay is easy to perform can also be of importance. The RT-SIBA RSV results can be delivered within an hour after sample reception, but most of the positive samples can already be detected within 30 min including sample preparation. In our experience the assay was user-friendly, and a one hour-training was sufficient. Of the other rapid molecular assays the Alere® i RSV assay is the most rapid



one with a turnaround time of 13 min (Peters et al. 2017), but the other assays have the advantage to detect at least two viruses (RSV and Influenza virus) (A Hogan et al. 2018). In most of the above mentioned assays, except the Simplexa® Flu A/B & RSV kit , the Xpert® Flu/RSV XC and Xpert® Xpress Flu/RSV assays, only one sample can be processed on a single instrument at the same time (A Hogan et al. 2018). The RT-SIBA RSV assay is designed to process up to 12 samples simultaneously in a 45 min-run and is well suitable for the organization of rapid RSV testing of single specimens or small series of specimens depending on its use during or outside the epidemic seasons.

In conclusion, the RT-SIBA RSV assay showed a good performance in this evaluation. The turnaround time is nearly as rapid as RADTs but the sensitivity is that of a molecular assay. Due to the flexibility of the number of specimens processed simultaneously the assay is practical for the rapid molecular detection of RSV in routine diagnostics. A slight improvement of the sensitivity and a combination with the detection of Influenza virus could make it even more attractive.

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## **Conflict of interest:**

Funding for the study was provided by Orion Diagnostica, Finland.

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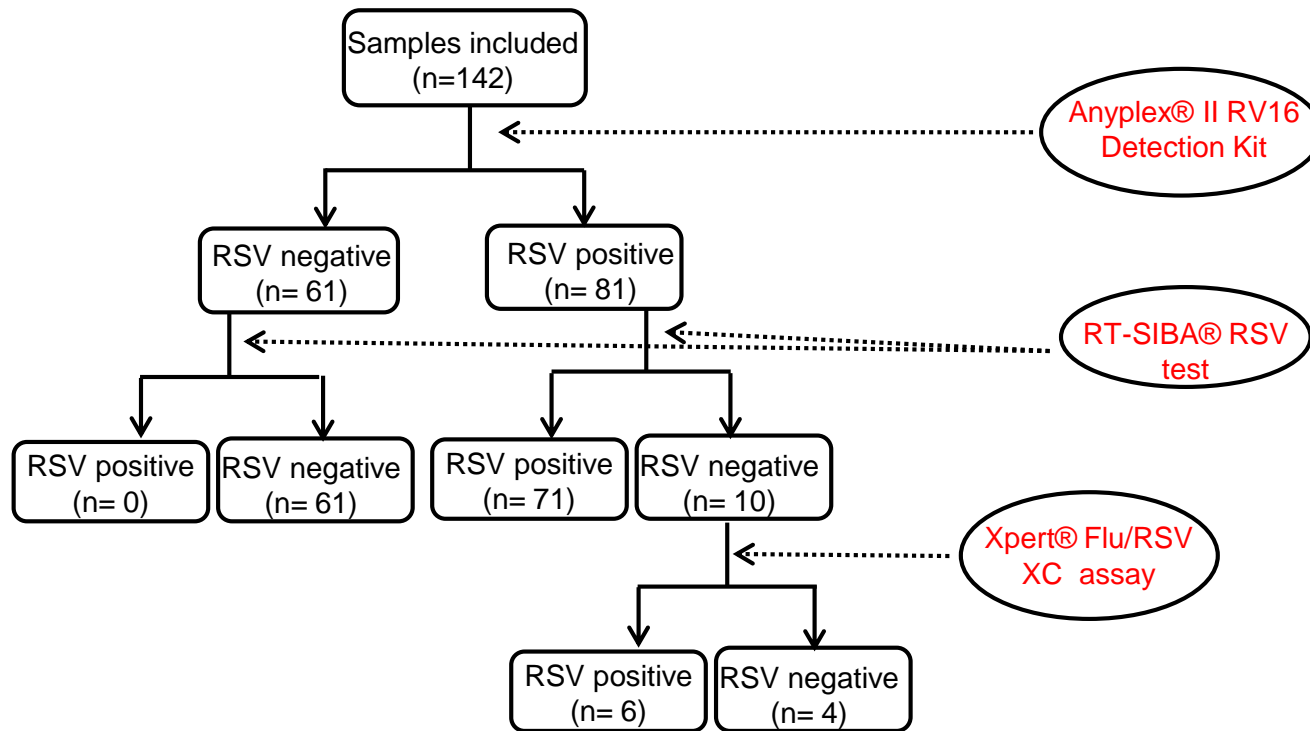
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**Figure 1.** Study flow chart

**Table 1.** Performance of the RT-SIBA® RSV test

<b>RT-SIBA® RSV test result</b>	<b>Reference result*</b>	
	Positive (n)	Negative (n)
Positive (n)	71	0
Negative (n)	6	65
SIBA® RSV test positive percent agreement (95% CI)	92% (84 - 96%)	
SIBA® RSV test negative percent agreement (95% CI)	100% (94 - 100%)	

\*A result consistently found by two different methods was considered as reference standard

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