Diagnostic Microbiology and Infectious Disease 95 (2019) 55–58

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Evaluation of the reverse transcription strand invasion based amplification (RT-SIBA) RSV assay, a rapid molecular assay for the detection of respiratory syncytial virus

Enagnon Kazali Alidjinou [a], Nicolas Lefebvre [a], Anny Dewilde [a], Minna Mäki [b], Didier Hober [a], Ilka Engelmann [a,*]

[a] Univ Lille, Faculté de Médecine, CHU Lille, Laboratoire de Virologie EA3610, F-59000 Lille, France
[b] Research and Development, Orion Diagnostica Oy, P. O. BOX 83, FI-02101, Espoo, Finland

ARTICLE INFO

Article history:
Received 6 February 2019
Received in revised form 17 April 2019
Accepted 17 April 2019
Available online 26 April 2019

Keywords:
SIBA
PCR
Respiratory syncytial virus
Molecular diagnostics
Comparison
Sensitivity
Specificity

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 analyzed with the RT-SIBA assay. Discordant samples were tested with the GeneXpert Flu/RSV XC assay. Consistent results in at least 2 of the 3 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. The RT-SIBA assay is a rapid molecular assay for the detection of RSV with good performance in clinical specimens.

1. Introduction

Respiratory syncytial virus (RSV) currently belongs to the *Pneumoviridae* family (Rima et al., 2017). RSV is a leading cause of acute respiratory infections 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, and (ii) it helps to avoid nosocomial transmission and outbreaks in healthcare settings because appropriate infection control measures can be implemented (Ferronato et al., 2012; Mills et al., 2011; 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, based on either 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 min. 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

* Corresponding author. Tel.: +33620444880; fax: 33320444895.
E-mail address: ilka.engelmann@chru-lille.fr (I. Engelmann).

https://doi.org/10.1016/j.diagmicrobio.2019.04.006
0732-8893/

polymerase-dependent extension of the target (Eboigbodin et al., 2017; Hoser et al., 2014).

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.

2. Materials and methods

2.1. 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).

2.2. 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 200 μL 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.

2.3. 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, 50 μL of each sample was 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, 40 μL of the treated sample were transferred into the respiratory buffer tube, and the mixture was vortexed for 3–5 s. Then, 40 μL 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 s and inserted into the Orion GenRead® instrument. The run was performed at 41 °C for 45 min.

2.4. 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.

2.5. 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 2 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 (Biswas, 2016; Garrett et al., 2008). 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.

3. 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 2 samples. A total of 54.2% of the patients were male, and the median age was 9 years (range 0–91 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 flowchart (Fig. 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 2 different methods were used as reference standard for the assessment of the RT-SIBA RSV assay performance (Table 1). The PPA (corresponds to sensitivity) of the RT-SIBA RSV assay was 92% (95% CI, 84–96%), and the NPA (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 7–35 min). A total of 33.8% of positive samples were detected in less than 10 min, and 94.4% were detected in less than 20 min.

4. 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.

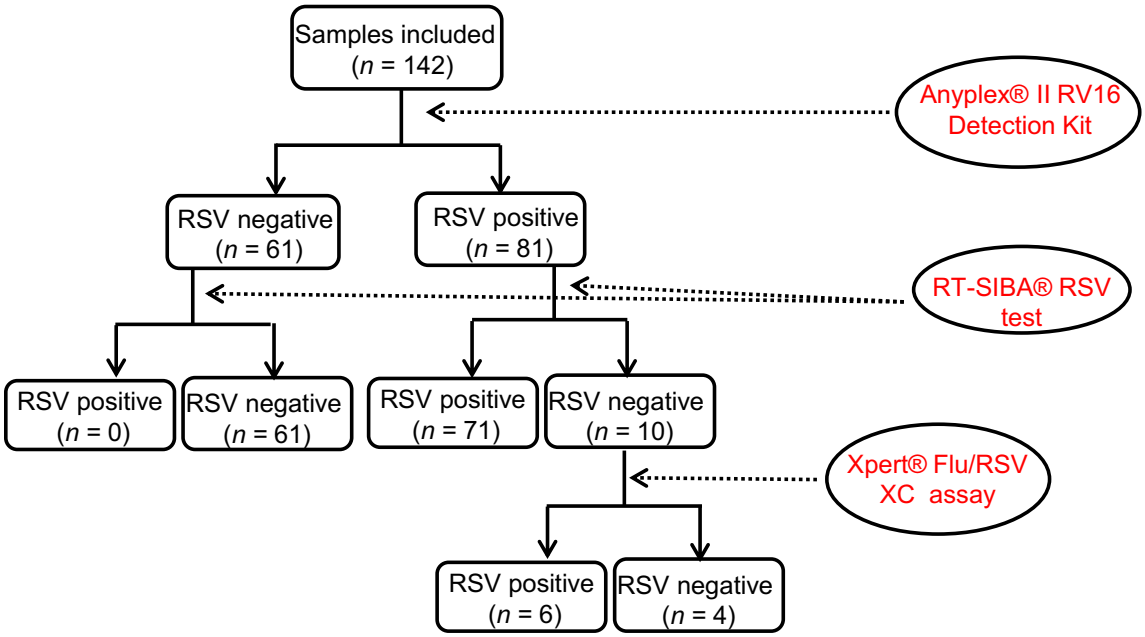

Fig. 1. Study flowchart.

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 1-h 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 2 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 1 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.

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

RT-SIBA® RSV test result	Reference result[a]	
	Positive (*n*)	Negative (*n*)
Positive (*n*)	71	0
Negative (*n*)	6	65
SIBA® RSV test PPA (95% CI)	92% (84–96%)	
SIBA® RSV test NPA (95% CI)	100% (94–100%)	

[a] A result consistently found by 2 different methods was considered as reference standard.

Funding information

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

Acknowledgments

The authors thank the technicians of the virology department for excellent technical assistance.

Conflict of interest

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

References

Banerjee D, Kanwar N, Hassan F, Essmyer C, Selvarangan R. Comparison of six sample-to-answer influenza A/B and respiratory syncytial virus nucleic acid amplification assays using respiratory specimens from children. J Clin Microbiol 2018 Nov;56(11).

Biswas B. Clinical performance evaluation of molecular diagnostic tests. J Mol Diagn JMD 2016;18(6):803–12.

Chartrand C, Tremblay N, Renaud C, Papenburg J. Diagnostic accuracy of rapid antigen detection tests for respiratory syncytial virus infection: systematic review and meta-analysis. J Clin Microbiol 2015;53(12):3738–49.

Eboigbodin KE, Moilanen K, Elf S, Hoser M. Rapid and sensitive real-time assay for the detection of respiratory syncytial virus using RT-SIBA®. BMC Infect Dis 2017;17(1):134.

Engelmann I, Dubos F, Lobert P-E, Houssin C, Degas V, Sardet A, et al. Diagnosis of viral infections using myxovirus resistance protein a (MxA). Pediatrics 2015;135(4):e985–93.

Falsey AR. Respiratory syncytial virus infection in elderly and high-risk adults. Exp Lung Res 2005;31(Suppl. 1):77.

Ferronato ÂE, Gilio AE, Ferraro AA, de Paulis M, Vieira SE. Etiological diagnosis reduces the use of antibiotics in infants with bronchiolitis. Clin Sao Paulo Braz 2012;67(9):1001–6.

Garrett Patricia E, Lasky Fred D, Meier Kristen L. User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline—Second Edition, CLSI document EP12-A2. Clinical and Laboratory Standards Institute (CLSI); 2008 (ISBN 1-56238-654-9).

Hall CB, Weinberg GA, Iwane MK, Blumkin AK, Edwards KM, Staat MA, et al. The burden of respiratory syncytial virus infection in young children. N Engl J Med 2009;360(6):588–98.

A Hogan C, Caya C, Papenburg J. Rapid and simple molecular tests for the detection of respiratory syncytial virus: a review. Expert Rev Mol Diagn 2018;18(7):617–29.

Hoser MJ, Mansukoski HK, Morrical SW, Eboigbodin KE. 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;9(11), e112656.

Huang H-S, Tsai C-L, Chang J, Hsu T-C, Lin S, Lee C-C. Multiplex PCR system for the rapid diagnosis of respiratory virus infection: systematic review and meta-analysis. Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis 2018;24(10):1055–63.

Mills JM, Harper J, Broomfield D, Templeton KE. Rapid testing for respiratory syncytial virus in a paediatric emergency department: benefits for infection control and bed management. J Hosp Infect 2011 Mar;77(3):248–51.

Nair H, Nokes DJ, Gessner BD, Dherani M, Madhi SA, Singleton RJ, et al. Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systematic review and meta-analysis. Lancet Lond Engl 2010;375(9725):1545–55.

Peters RM, Schnee SV, Tabatabai J, Schnitzler P, Pfeil J. Evaluation of Alere i RSV for rapid detection of respiratory syncytial virus in children hospitalized with acute respiratory tract infection. J Clin Microbiol 2017;55(4):1032–6.

Rima B, Collins P, Easton A, Fouchier R, Kurath G, Lamb RA, et al. ICTV virus taxonomy profile: Pneumoviridae. J Gen Virol 2017;98(12):2912–3.

Scheltema NM, Gentile A, Lucion F, Nokes DJ, Munywoki PK, Madhi SA, et al. Global respiratory syncytial virus-associated mortality in young children (RSV GOLD): a retrospective case series. Lancet Glob Health 2017;5(10):e984–91.

Thevenin T, Lobert PE, Dewilde A. Hober D. A respiratory syncytial virus isolate enables the testing of virucidal products. Int J Hyg Environ Health 2012;215(3):293–6.