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

4
5 Enagnon Kazali Alidjinou¹, Nicolas Lefebvre¹, Anny Dewilde¹, Minna Mäki², Didier Hober¹
6 and Ilka Engelmann¹

7
8 ¹Univ Lille, Faculté de Médecine, CHU Lille, Laboratoire de Virologie EA3610, F-59000 Lille,
9 France

10 ²Research and Development, Orion Diagnostica Oy, P. O. BOX 83, FI-02101, Espoo, Finland

11
12 **Abstract**

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

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

22 The RT-SIBA assay yielded a negative result for the 61 negative samples and a positive
23 result in 71/81 (85.5%) of the Anyplex positive samples. After a resolution of discordant
24 samples the positive and negative percent agreement of the RT-SIBA assay were 92% and
25 100%, 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

33 **Introduction**

34 Respiratory Syncytial Virus (RSV) currently belongs to the *Pneumoviridae* family (Rima et al.
35 2017). RSV is a leading cause of acute respiratory infections (ARI) in children aged less than
36 5 years (Hall et al. 2009; Nair et al. 2010; Scheltema et al. 2017) and is associated with
37 significant morbidity and mortality rates in adults, especially in elderly and
38 immunosuppressed patients (Falsey 2005).

39 The rapid diagnosis of RSV infection is useful because: (i) it prevents empirical therapy with
40 antibiotics, promotes antimicrobial stewardship and reduces healthcare associated costs. (ii)
41 it helps to avoid nosocomial transmission and outbreaks in healthcare settings because
42 appropriate infection control measures can be implemented (Mills et al. 2011; Ferronato et al.
43 2012; Thevenin et al. 2012).

44 Laboratory diagnosis potentially includes the detection of viral infection markers, e.g.
45 myxovirus resistance protein A (Engelmann et al. 2015) and the detection of RSV, either
46 based on antigen detection or the detection of viral nucleic acids (molecular assays). Antigen
47 detection techniques include direct immunofluorescence assays and rapid antigen detection
48 tests (RADTs). RADTs can be performed in laboratories or as point-of-care testing. These
49 tests are easy to perform and provide results within 10-20 minutes. However, assays based
50 on RSV antigen detection have a lower sensitivity than molecular assays (Chartrand et al.
51 2015; A Hogan et al. 2018). Molecular techniques for the detection of RSV show higher
52 sensitivity, but traditional multiplex respiratory RT PCRs that include a separate extraction
53 step are more time consuming (A Hogan et al. 2018). Lately, several rapid molecular assays
54 have been developed with the aim to provide more rapid and sensitive RSV diagnostics (A
55 Hogan et al. 2018).

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

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

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

66

67 **Materials and methods**

68 **Patients and samples**

69 Nasal and nasopharyngeal swabs were obtained at the university hospital of Lille, from
70 outpatients or hospitalized patients with symptoms of acute respiratory tract infection.
71 Specimens submitted to the laboratory for routine testing during the 2014-2016 period were
72 used for this study. Swabs were collected in 1 mL of transport medium and were stored at -
73 80°C until routine testing (performed 3 times a week outside the epidemic season and every
74 working day during the winter season) by using the Anyplex II RV16 Detection Kit (Seegene,
75 Korea). The clinical evaluation of the RT-SIBA RSV assay was performed on the remaining
76 specimen volume. Demographic data were collected retrospectively. The study was carried
77 out in accordance with the Declaration of Helsinki and was approved by the institutional
78 review board with waiver of informed consent (Approval number: HP16/79).

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

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

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

88 The RT-SIBA RSV assay (Orion Diagnostica, Finland) was developed to detect RSV-A and
89 RSV-B and was performed by strictly following the manufacturer's instructions. Briefly, 50µL
90 of each sample were collected from the transport medium tube and transferred into the
91 heating tube that was subsequently maintained in a heating block at 95°C for 5 min. Then
92 40µL of the treated sample were transferred into the respiratory buffer tube and the mixture
93 was vortexed for 3-5 seconds. Then 40µL of the mixture was transferred into the reagent
94 tube. Up to 12 specimens were processed simultaneously. The reagent tubes were
95 vigorously vortexed for at least 5 seconds, and inserted into the Orion GenRead® instrument.
96 The run was performed at 41 °C for 45 min.

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

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

102 **Data analysis**

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

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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.

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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

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

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

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183

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186

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190

191 **Conflict of interest:**

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

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196 **References**

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

200 Banerjee D, Kanwar N, Hassan F, Essmyer C, Selvarangan R. Comparison of Six Sample-
201 to-Answer Influenza A/B and Respiratory Syncytial Virus Nucleic Acid Amplification
202 Assays Using Respiratory Specimens from Children. *J Clin Microbiol.* 2018
203 Nov;56(11).

204 Biswas B. Clinical Performance Evaluation of Molecular Diagnostic Tests. *J Mol Diagn JMD.*
205 2016;18(6):803–12.

206 Chartrand C, Tremblay N, Renaud C, Papenburg J. Diagnostic Accuracy of Rapid Antigen
207 Detection Tests for Respiratory Syncytial Virus Infection: Systematic Review and
208 Meta-analysis. *J Clin Microbiol.* 2015 Dec;53(12):3738–49.

209 Clinical and Laboratory Standards Institute. User Protocol for Evaluation of Qualitative Test
210 Performance, EPI 12-A document. Wayne, PA; 2002.

211 Eboigbodin KE, Moilanen K, Elf S, Hoser M. Rapid and sensitive real-time assay for the
212 detection of respiratory syncytial virus using RT-SIBA®. *BMC Infect Dis.* 2017
213 10;17(1):134.

214 Engelmann I, Dubos F, Lobert P-E, Houssin C, Degas V, Sardet A, et al. Diagnosis of viral
215 infections using myxovirus resistance protein A (MxA). *Pediatrics.* 2015
216 Apr;135(4):e985-993.

217 Falsey AR. Respiratory syncytial virus infection in elderly and high-risk adults. *Exp Lung Res.*
218 2005 Sep;31 Suppl 1:77.

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

222 Hall CB, Weinberg GA, Iwane MK, Blumkin AK, Edwards KM, Staat MA, et al. The burden of
223 respiratory syncytial virus infection in young children. *N Engl J Med.* 2009 Feb
224 5;360(6):588–98.

225 Hoser MJ, Mansukoski HK, Morrical SW, Eboigbodin KE. Strand Invasion Based
226 Amplification (SIBA®): a novel isothermal DNA amplification technology
227 demonstrating high specificity and sensitivity for a single molecule of target analyte.
228 *PLoS One.* 2014;9(11):e112656.

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

- 232 Mills JM, Harper J, Broomfield D, Templeton KE. Rapid testing for respiratory syncytial virus
233 in a paediatric emergency department: benefits for infection control and bed
234 management. *J Hosp Infect.* 2011 Mar;77(3):248–51.
- 235 Nair H, Nokes DJ, Gessner BD, Dherani M, Madhi SA, Singleton RJ, et al. Global burden of
236 acute lower respiratory infections due to respiratory syncytial virus in young children:
237 a systematic review and meta-analysis. *Lancet Lond Engl.* 2010 May
238 1;375(9725):1545–55.
- 239 Peters RM, Schnee SV, Tabatabai J, Schnitzler P, Pfeil J. Evaluation of Alere i RSV for
240 Rapid Detection of Respiratory Syncytial Virus in Children Hospitalized with Acute
241 Respiratory Tract Infection. *J Clin Microbiol.* 2017;55(4):1032–6.
- 242 Rima B, Collins P, Easton A, Fouchier R, Kurath G, Lamb RA, et al. ICTV Virus Taxonomy
243 Profile: Pneumoviridae. *J Gen Virol.* 2017 Dec;98(12):2912–3.
- 244 Scheltema NM, Gentile A, Lucion F, Nokes DJ, Munywoki PK, Madhi SA, et al. Global
245 respiratory syncytial virus-associated mortality in young children (RSV GOLD): a
246 retrospective case series. *Lancet Glob Health.* 2017;5(10):e984–91.
- 247 Thevenin T, Lobert PE, Dewilde A, Hober D. A respiratory syncytial virus isolate enables the
248 testing of virucidal products. *Int J Hyg Environ Health.* 2012 Apr;215(3):293–6.
- 249

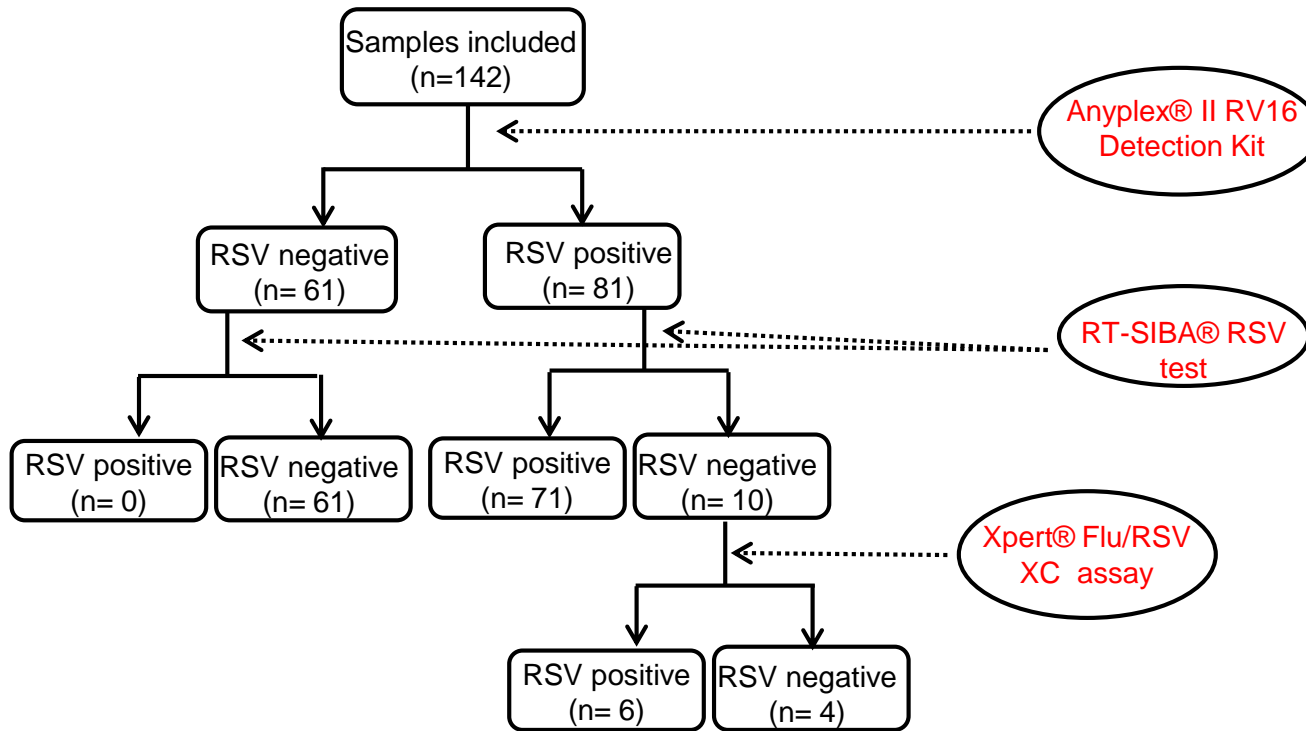


Figure 1. Study flow chart

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

RT-SIBA® RSV test result	Reference result*	
	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