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Multicenter evaluation of BD Veritor System and RSV K-SeT for rapid detection of respiratory syncytial virus in a diagnostic laboratory setting

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ABSTRACT

The recently introduced BD Veritor System RSV laboratory kit (Becton Dickinson, Sparks, MD, USA) with automatic reading was evaluated and compared with the RSV K-SeT (Coris BioConcept, Gembloux, Belgium) for the detection of respiratory syncytial virus (RSV) using 248 nasopharyngeal aspirates of children younger than 6 years old with respiratory tract infection. Compared to reverse transcriptase polymerase chain reaction as gold standard, both tests had an identical sensitivity of 78.1% and a specificity of 96.8% and 95.8% for the BD Veritor System and RSV K-SeT, respectively. Both antigen tests can be used to reliably confirm RSV in young children. However, a negative result does not definitively exclude the presence of RSV.

1. Introduction

Respiratory syncytial virus (RSV) is one of the most important pathogens causing severe lower respiratory tract infections in all age groups often requiring hospitalization (Popow-Kraupp and Aberle, 2011). Rapid laboratory diagnosis of RSV infection significantly decreases the use of antibiotics and additional laboratory testing. Also, it is associated with shorter hospitalization periods (Barenfanger et al., 2000; Byington et al., 2002). Reverse transcriptase polymerase chain reaction (RT-PCR) is accepted as gold standard for RSV detection due to its high sensitivity and specificity. However, it is a technically demanding, laborious, and expensive technique, limiting its usefulness in an outpatient setting or in a laboratory setting where molecular based techniques are not readily available. Therefore, antigen tests remain a useful tool for the rapid detection of RSV in clinical practice. The most common used antigen tests are based on lateral flow immunochromatography because of their simplicity and relatively low cost. Although their specificity is generally satisfying, these tests demonstrate lower sensitivities compared to PCR (Casiano-Colón et al., 2003; Jang et al., 2015). The BD Veritor System (Becton Dickinson, Sparks, MD, USA) is a recently introduced US Food and Drug Administration–cleared rapid RSV antigen assay that is interpreted with a portable automatic reader, which eliminates subjective interpretation. It has been evaluated in the United States with satisfactory results (Bell et al., 2014; Leonardi et al., 2014), but no European studies of the BD Veritor System have yet been published. The RSV Respi-Strip (Coris BioConcept, Gembloux, Belgium) has been commercialized for a longer period and has more recently become available as a cassette device called the RSV K-SeT. The RSV K-SeT uses the same antibodies and membrane as the RSV Respi-Strip. However, literature concerning the RSV Respi-Strip and the RSV K-SeT is scarce, and there are currently no published evaluations using RT-PCR as gold standard (Gregson et al., 2005). The aim of this study was to evaluate the performance of the BD Veritor System and the RSV K-SeT on nasopharyngeal aspirates (NPAs) using RSV RT-PCR as gold standard in a diagnostic laboratory setting.

2. Materials and methods

2.1. Participant enrollment and sample collection

This prospective, multicenter clinical trial was conducted from November 2014 to December 2014 at 2 study sites in Belgium (OLVZ Aalst and AZ Sint-Lucas Gent). The study consecutively included neonatal and pediatric patients younger than 6 years, both hospitalized and ambulatory, with symptoms of respiratory tract infection and who underwent a nasopharyngeal aspiration for respiratory viral testing. Samples were collected by nasopharyngeal wash using the syringe method with saline water to recover an NPA of approximately 1 mL. After collection, specimens were transported immediately to the laboratory at room temperature. Only remnant specimens were used for testing with the RSV K-SeT and BD Veritor System, and only 1 sample per subject was included in the study. Specimens were anonymized upon enrollment in the study. A total of 253 patients were included across

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Table 1
Enrollment statistics for evaluable specimens for both study sites (S).

	S1	S2	Total
Gender			
Male	84	59	143
Female	64	41	105
Age			
≤1 year	87	64	151
1–2 years	32	30	62
2–3 years	11	5	16
3–4 years	16	0	16
4–5 years	1	1	2
5–6 years	1	0	1
Inpatient status			
Hospitalized	122	87	209
Ambulatory	26	13	39
Total	148	100	248

S1, OLVZ Aalst; S2, AZ Sint-Lucas Gent.

both study sites. Enrollment statistics by site are presented in Table 1. If the specimen was not tested immediately, it was stored at 2–8 °C until analysis for a maximum of 72 hours (median: 4.9 hours, 2.5–97.5 percentile: 1.3–67.2 hours). The BD Veritor System and RSV K-SeT were performed at each site, while the RT-PCR was carried out at the principal site (OLVZ Aalst). Samples of AZ Sint-Lucas were stored at −80 °C until RT-PCR was performed.

2.2. BD Veritor System (Becton Dickinson)

The test was performed according to the manufacturer's instructions. Briefly, 300 μL of NPA was transferred into the prefilled RV Reagent C tube containing 100-μL detergent solution. After mixing, the tube was squeezed allowing 3 drops of the processed sample to be dispensed into the sample well. After 10 minutes of run time, the automatic reader was used for result interpretation: positive, negative, or invalid. The reader was checked each day of testing using the BD Veritor System Verification Cartridge. The Quality Control (QC)-positive and QC-negative control swabs (included in the kit) were performed once every package. Specimen testing only proceeded in the event of valid QC results for the reader and control swabs.

2.3. RSV K-SeT (Coris BioConcept)

The test was performed according to the manufacturer's instructions. Briefly, 100 μL of NPA was mixed with 4 drops of extraction buffer. After homogenizing, 100 μL of diluted sample was dispensed into the sample well of the cassette. The results were read visually after 15 minutes of incubation by 2 laboratory technicians independently and blinded for other test results. In case of discrepancy, the judgment of a third technician gave the final result. An RSV-positive control (C-1086; Coris BioConcept, not included in the kit) was performed once every package. Specimen testing only proceeded in the event of valid QC results.

2.4. Real-time RT-PCR testing

RT-PCR testing was based on a previously published method (Bonroy et al., 2007). Respiratory samples were liquefied with sputolysin and extracted on the NucliSens EasyMAG (bioMérieux, Marcy L'Etoile, France) using the generic protocol (200-μL input and 110-μL elution volume) and an RNA extraction and inhibition control (Diagenode Diagnostics, Liège, Belgium). One-step reverse transcription real-time PCR was performed on the ABI 7500 Fast (Life Technologies, Salt Lake City, UT, USA) with the TaqMan Fast Virus One step Master Mix. The PCR contains 2 targets for RSV: the nucleocapsid protein gene (300 nmol/L FP: 5′-GCTCTTAGCAAAGTCAAGTTRAATGATACA-3′, 300 nmol/L RP: 5′-GTTTTTGCACATCATAATTRGGAGT-3′, 100 nmol/L MGB-probe 5′-FAM-CTGTCATCYAGCAAAT-MGB-3′, 100 nmol/L 5′-FAM-CTGTCATCTAGTAAAT-MGB-3′) and the polymerase gene (300 nmol/L FP: 5′-AATACAGCCAAATCTAACCAACTTTACA-3′, 300 nmol/L RP: 5′-ATGCCAAGGAAGCATGCARTA-3′, 200 nmol/L RSV-A probe: 5′-FAM-GATGTGCTATTGTGCACTAA-MGB-3′, 200 nmol/L RSV-B probe: 5′-NED-CACTATTCCTTACTAAAGATGTC-MGB-3′). All primers bind RSV-A and RSV-B. The probes of the nucleocapsid protein gene cannot distinguish RSV-A from RSV-B; the probes of the polymerase gene distinguish RSV-A from RSV-B. The temperature profile used was 5 minutes 50 °C; 20 seconds 95 °C; 45 cycles composed of 3 seconds 95 °C and 30 seconds 60 °C. In the case of a PCR-negative result with both antigen tests positive, the PCR result was verified by a second RT-PCR method using a TaqMan array card (TAC, formerly TaqMan low-density array or TLDA) (Life Technologies, Carlsbad, CA, USA) (Driscoll et al., 2014).

2.5. Statistical analyses

The performance characteristics, such as sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the 2 antigen tests were calculated and expressed as a 95% confidence interval (CI) using RT-PCR as gold standard. The κ statistics (linear weights) were used to compare test agreement. The Student's *t* test was used to compare means, and Fischer's exact test was used to test the significance of contingency. $P < 0.05$ was considered statistically significant. Statistical analysis was performed with Microsoft Office Excel 2003 software (Microsoft Corporation, Redmond, WA, USA) and MedCalc Version 12.3.0.0 (MedCalc Software bvba, Ostend, Belgium).

3. Results

A total of 253 NPA samples from children younger than 6 years were included into the study of which 248 were eligible for study inclusion. Five samples (2.0%) were excluded because the sample was too viscous to pass the filter of the RV Reagent C tube or to migrate to the control line on the strip of the BD Veritor System resulting in an "invalid" interpretation by the reader. The remaining 248 evaluable specimens are listed by gender, age group, and inpatient status for both study sites in Table 1. Overall, 143 males (57.7%) and 105 (42.3%) females were included with a median (2.5–97.5 percentile) age of 10 months (1 month–3 years and 6 months). The overall performance characteristics of the BD Veritor System and the RSV K-SeT for detection of RSV compared to real-time RT-PCR as gold standard are presented in Table 2. For the BD Veritor System, the sensitivity and specificity were 79.1% and 96.8%, and for the RSV K-SeT, 79.1% and 95.8%, respectively. For both antigen tests, false-negative samples had a significantly higher cycle threshold (Ct) value compared to true-positive samples (see Fig. 1). Although the performance characteristics were very similar, there were 13 discrepant results between the BD Veritor System and the RSV K-SeT. Six samples were BD Veritor+/RSV K-SeT− of which 2 were RT-PCR negative and 4 RT-PCR positive (2 RSV-A and 2 RSV-B). These positive samples showed significantly higher Ct values compared to the antigen concordant positive samples (mean Ct value of 28.5 versus 21.4, $P < 0.001$). Seven samples were BD Veritor−/RSV K-SeT+ of which 3 were RT-PCR negative and 4 RT-PCR positive (all RSV-A). Again, the Ct values were significantly higher compared to the antigen concordant positive samples (mean Ct value of 26.7 versus 21.4, $P < 0.001$). One sample was judged positive by both antigen tests but repeatedly negative by the in-house RT-PCR and also by the TAC RT-PCR.

Considering RSV-A, the sensitivity for the BD Veritor System and RSV K-SeT was 80.9% (95% CI: 73.3–87.1%) and 82.4% (95% CI: 74.9–88.4%), respectively, while 64.7% (95% CI: 38.3–85.8%) and 52.9% (95% CI: 27.8–77.0%) for RSV-B. The Ct values of RSV-B–positive samples were not significantly higher compared to those of RSV-A–positive samples (mean Ct value of 24.2 [range: 17.2–33.6] versus 23.7 [range: 16.1–40.4], $P = 0.36$).

Although not statistically significant ($P = 0.62$), there was a trend toward a lower sensitivity in older patients. For children younger than

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Table 2
Comparison between BD Veritor System and the RSV K-SeT to RT-PCR as gold standard using NPAs of children <6 years (n = 248).

Assay and result	PCR result (no. of samples)		% Sensitivity (95% CI)	% Specificity (95% CI)	% PPV (95% CI)	% NPV (95% CI)	κ Value[a] (95% CI)
	Positive	Negative					
BD Veritor System							
Positive	121	3	79.1 (71.8–85.2)	96.8 (91.1–99.3)	97.6 (93.1–99.5)	74.2 (65.6–81.6)	0.72 (0.63–0.80)
Negative	32	92					
RSV K-SeT							
Positive	121	4	79.1 (71.8–85.2)	95.8 (89.6–98.8)	96.8 (92.0–99.1)	74.0 (65.3–81.5)	0.71 (0.62–0.80)
Negative	32	91					

[a] Weighted κ (linear weights).

1 year, the BD Veritor and the RSV K-SeT showed a sensitivity of 83.0% (95% CI: 73.8–90.0%), whereas for children older than 1 year, the sensitivity was 72.9% (95% CI: 59.7–83.6%).

4. Discussion

In this study, the BD Veritor System and RSV K-SeT antigen tests for the detection of RSV were evaluated on NPA of children younger than 6 years. Although there were 13 (5.2%) discrepant results between the 2 antigen tests, both exhibited an identical sensitivity of 79.1% and a comparable specificity around 96%. These numbers indicate that the BD Veritor System and RSV K-SeT performed equally for rapid detection of RSV in NPA in comparison to real-time RT-PCR as gold standard. It should be noted, however, that 5 samples (2%) could not be processed by the BD Veritor System because of the high viscosity of the NPA. These were not included in the calculations. The results for the BD Veritor System are similar to those previously published for NPA (Bell et al., 2014). As expected, the sensitivity found in our study was higher compared to the evaluations that used nasopharyngeal swabs as input samples (Bell et al., 2014; Leonardi et al., 2014). The sensitivity of the RSV K-SeT was lower than previously published (Gregson et al., 2005). This could be explained by the higher sensitivity of RT-PCR, used as the gold standard in this study, compared to direct fluorescent-antigen detection. It is unclear why a lower sensitivity for detection of RSV-B compared to RSV-A was observed for both antigen tests in our study. As Ct values were not different between RSV-A– and RSV-B–positive samples, there is no evidence for a difference in viral load. A difference in antibody affinity for RSV-A and RSV-B may have contributed, although analytic sensitivity studies on the BD Veritor System did not reveal a higher limit of detection for RSV-B strains compared to RSV-A strains (Package insert BD Veritor System, 2012). However, since different strains circulate each season, a lower affinity for RSV-B strains in our study cannot be excluded.

As previously reported by other groups (Bell et al., 2014; Miernyk et al., 2011), we observed a lower sensitivity in older patients. Moreover, there was a positive correlation between age and Ct value of positive samples in our study, although this association was not statistically significant. The reason for the lower viral load in older patients might be that older patients are more likely to be reinfected rather than suffering from a primary infection. Indeed, almost all children have serologic

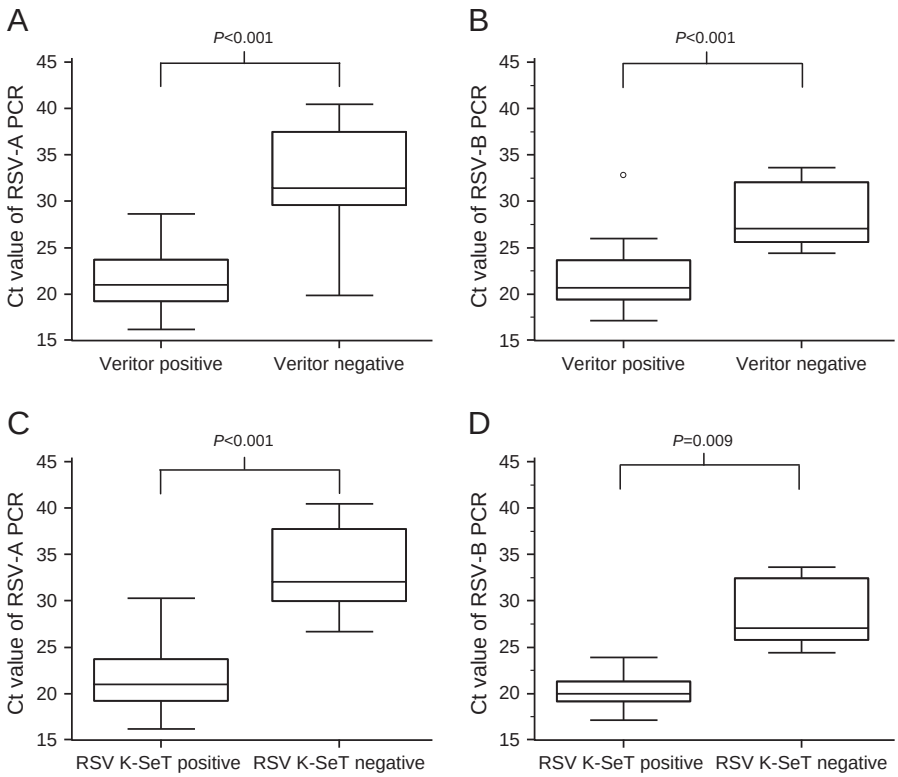

Fig. 1. Comparison of PCR Ct values of BD Veritor System RSV–positive and BD Veritor System RSV–negative samples for RSV-A (A, n = 136) and RSV-B (B, n = 17) and of RSV K-SeT–positive and RSV K-SeT–negative samples for RSV-A (C, n = 136) and RSV-B (D, n = 17). For both antigen tests, false-negative samples had a significantly higher Ct value (lower viral load) compared to true-positive samples.

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evidence of RSV infection at the age of 2 years (Popow-Kraupp and Aberle, 2011). Also, the difficulty in obtaining quality aspirate specimens or the lack of adequate nasal secretions in older patients might be a potential explanation.

The performance of both studied antigen tests is comparable to past literature demonstrating an inferior sensitivity of RSV antigen tests compared to molecular assays. This drawback has to be balanced with the benefits of ease of use, availability, and speed, applied to the specific situation of the laboratory. When there is a daily availability of molecular tests, there seems to be limited value of a rapid antigen test. However, in laboratories where molecular tests are not readily available, an antigen test can be of great value, despite its lower sensitivity.

A potential disadvantage of visually read rapid antigen tests is that some level of interpretation is required from the test operator and that classification of positive versus negative results can be quite subjective, especially for samples with a lower viral load where the test line can be very faint. The BD Veritor System eliminates this subjectivity by introducing the Veritor reader, which may potentially help to improve accuracy of test result. In our study, however, 2 test strips (0.8% of total) showed coloration of the membrane in the absence of a distinct line at the test position producing a false-positive result when reading with the Veritor reader after the prescribed runtime of 10 minutes. For both samples, the coloration disappeared after an additional incubation time of approximately 15 minutes, and when reading the strips again with the Veritor reader, the result was negative for both samples. This shows that even with the use of an automatic reader, a visual inspection of the device is still mandatory, not to judge the intensity of the test line but to verify the absence of abnormalities that hamper correct automatic reading. On the other hand, the RSV K-SeT device has to be interpreted by the operator. The recommendations for interpretation state that any reddish-purple line at the test line position, even weak, should be considered as a positive result. However, during the drying process of the device, a very faint shadow may appear at the test line position, which should not be regarded as a positive result. These interpretation rules are prone to interindividual variability and hence misdiagnosis. In this evaluation, using multiple experienced laboratory technicians to judge the result, the RSV K-SeT performed equally as the BD Veritor System. However, the RSV K-SeT might have a lower performance in a different setting such as the emergency department or the physician's office.

In conclusion, the new BD Veritor System and the RSV K-SeT showed a comparable performance. Both antigen tests can be used as a reliable test for rapid confirmation of RSV in NPA of young children. However, a negative result does not preclude RSV infection. The Veritor system offers the advantage of standardized, automatic result reading and faster time to result as compared to the RSV K-set.

Conflict of interest

None.

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