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Laboratory evaluation of rapid antigen detection tests for more-sensitive detection of Respiratory syncytial virus antigen

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SUMMARY

We evaluated two currently available rapid antigen detection tests (RADTs) for *Respiratory syncytial virus* (RSV), Sofia® RSV FIA and BinaxNOW RSV Card (BinaxNOW). Between November 2017 and February 2018, 395 nasopharyngeal swabs were collected from children diagnosed with acute respiratory infections, and were evaluated with these RADTs, reverse transcription–quantitative real-time PCR (RT–qPCR), and a direct immunofluorescence assay (DFA). The sensitivity of Sofia® RSV FIA (80.82%) was significantly higher than that of BinaxNOW (53.42%) when RT–qPCR was used as the standard. This was confirmed with DFA. The sensitivities of Sofia® RSV FIA (85.4% [41/48]) and BinaxNOW (58.3% [28/48]) were higher for RSV A than for RSV B (69.6% [16/23] and 43.5% [10/23], respectively). The optimal critical cycle threshold (Ct) values on RT–qPCR that correlated with Sofia® RSV FIA and BinaxNOW were 24 and 22, respectively. The kappa value for Sofia® RSV FIA and RT–qPCR in the patients aged \( \leq 2 \) years was 0.962, but 0.648 in those aged > 2 years. Thus, Sofia® RSV FIA is more sensitive than BinaxNOW; its results were affected by the RSV viral strain and load. It is more effective in children aged \( \leq 2 \) years than in those aged > 2 years.
1. INTRODUCTION

*Human respiratory syncytial virus* (RSV), renamed *Human orthopneumovirus* in 2017, in the family *Pneumoviridae* and the genus *Orthopneumovirus* (1), is divided into subtypes A and B, according to antigenicity differences in the G and F glycoproteins (2, 3). This classification was supported by the RSV genomic nucleotide sequence analysis (4). A retrospective study showed that RSV was associated with 48,000–74,500 in-hospital deaths in infants and children younger than 5 years in 2015, with 99% of these deaths occurring in developing countries (5). Therefore, RSV is regarded as an important cause of pneumonia-induced mortality in infants and young children (5, 6). However, it is difficult to distinguish acute RSV respiratory infections from acute respiratory infections with other etiologies based on clinical manifestations alone. Therefore, accurate and timely laboratory testing is urgently required to improve the clinical management of this disease (7).

A variety of RSV detection methods are available, including viral culture, serology, reverse transcription–real-time quantitative polymerase chain reaction (RT–qPCR), direct and indirect immunofluorescence assays (DFA and IFA), rapid antigen testing, and rapid molecular assays (8, 9). Nucleic acid detection methods require professional expertise and specialized equipment, which limits their use in point-of-care testing (POCT). This is especially true of RT–qPCR, which is a widely used method of detecting RSV because its sensitivity and specificity are high. POCT has been shown to reduce healthcare costs and permit informed decisions to be made
about treatment (10). Several rapid antigen detection tests (RADTs) are currently available for the detection of RSV with POCT. However, the low sensitivity of RADTs limits their use in POCT. Optimal RADTs for use in clinical diagnosis must be sensitive, simple to perform, and provide rapid results (10, 11).

In a previous study, we demonstrated that nasopharyngeal aspirates (NPAs) were better than throat swabs (TS) for detecting RSV with RADTs because RSV is likely to move into the lower respiratory tract (12). To identify the most sensitive RADTs, we evaluated the clinical performance of two RADTs in samples from Chinese pediatric patients: the BinaxNOW RSV Card (Alere Scarborough, Inc., Scarborough, ME, USA), based on an immunochromatographic membrane assay, and the Sofia® Fluorescent Immunoassay for RSV (Sofia® RSV FIA, Quidel Corp., San Diego, CA, USA), a recently developed second-generation, automated RADT that uses a europium dye to enhance its overall sensitivity and a portable automatic reader to interpret the assays (13, 14). We compared the results with those of RT–qPCR and DFA.

2. MATERIALS AND METHODS

2.1 Patients and specimens

Nasopharyngeal swabs (NPSs) were obtained with FLOQSwabs™ (Copan Flock Technologies, Brescia, Italy) from pediatric patients aged < 16 years who attended the Children’s Hospital Affiliated with the Capital Institute of Pediatrics (Beijing, China) with acute respiratory infections (ARIs), including acute upper-respiratory-tract
infections, bronchitis, bronchiolitis, and pneumonia, between November 2017 and February 2018. The diagnosis of ARI in this study was according to the Zhu Futang Textbook of Pediatrics (7th Edition) (15).

Upon arrival at the laboratory, the NPS specimens were processed immediately in 2.5 ml of viral transport medium (VTM) (Yocon Biotechnology Co., Ltd, Beijing, China). An aliquot (~310 µl) of each NPS specimen was used for the RADTs, and each remaining sample was centrifuged at 500 × g for 10 min to obtain the supernatant for use in RT–qPCR. The cell pellet was used for DFA.

2.2 RSV detection with the two RADTs

Both RADTs were performed according to the manufacturer’s protocols.

For Sofia® RSV FIA (14), 260 µl of each NPS specimen in VTM was combined in a test tube with the same volume of the reagent provided. The total volume of 120 µl of this reaction mixture was then added to the test kit. After approximately 15 min was allowed for the lateral flow of the reaction mixture, the test cassette was inserted into the portable fluorescence analyzer, Sofia (Quidel Corp.), and the results were automatically printed within 1 min as positive, negative, or invalid.

For the BinaxNOW RSV Card (16), 50 µl of each NPS specimen in VTM was mixed with the same volume of reagent solution. The total volume (100 µl) of the reaction mixture was added to the cassette, and the test result was examined visually after approximately 15 min and interpreted as positive, negative, or invalid according to the manufacturer’s protocol.

2.3 Direct immunofluorescence assay (DFA)

For the DFA, the cell pellets from the NPS samples were suspended in several
drops of sterile phosphate-buffered saline, and the resulting cell suspension was spotted onto an acetone-cleaned slide. An anti-RSV monoclonal antibody labeled with fluorescein isothiocyanate (FITC) from the D³ Ultra™ DFA Respiratory Virus Screening & ID Kit (Diagnostic Hybrids Inc., Athens, OH, USA) was used for RSV identification with DFA.

2.4 Nucleic acid extraction

Viral RNA was extracted from 140 µl of the NPS supernatant with the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany).

2.5 RT–qPCR

An in-house RSV RT–qPCR method was used, as described previously by Sun et al. (17). Briefly, each tube contained 25 µl of reaction mixture that contained 2.5 µl of isolated viral RNA, 0.1 µM forward and reverse primers, and 0.1 µM probe. TaqMan amplification and detection were performed with a real-time thermocycler, the ABI Prism 7500 Sequence Detection System (Applied Biosystems, Carlsbad, USA). The thermocycling conditions were: reverse transcription at 50 °C for 20 min and then initial denaturation at 95 °C for 10 min, followed by 45 cycles of 95 °C for 15 s and 60 °C for 60 s. The samples were considered to be positive if the amplification plot showed a definite exponential increase in the fluorescent signal.

The four laboratory technicians who performed the RADTs, DFAs, and RT–qPCRs were blinded to the results of the other tests and to the clinical presentation of the patients.
The original study was approved by the Ethics Committee of the Capital Institute of Pediatrics.

2.6 Statistical analysis

Excel (Microsoft Corporation, USA) was used for data collection and input, and IBM SPSS Statistics version 22.0 (IBM Corporation, USA) was used for all statistical analyses. The $\chi^2$ test and analysis of variance (ANOVA) were used for statistical comparisons. The sensitivities, specificities, positive and negative predictive values, and positive and negative likelihood ratios were calculated with 95% confidence intervals (CIs), using RT–qPCR as the standard, or the DFA as a comparison method. The validity and reliability of Sofia® RSV FIA and the BinaxNOW RSV Card were determined based on Youden’s index, the agreement rate, and the kappa value. A receiver operator characteristic (ROC) curve was used to evaluate the true positive rates (sensitivity on the y-axis) and the false positive rates ($1 - specificity$ on the x-axis) of the rapid tests performed with Sofia® RSV FIA and the BinaxNOW RSV Card compared with the rates determined with RT–qPCR, using the cycle threshold (Ct) as an indicator.

3 RESULTS

3.5 Clinical specimens included in the statistical analysis

A total of 398 specimens were collected and tested with the two RADTs for RSV, Sofia® RSV FIA and the BinaxNOW RSV Card. Three of these samples were later found to be deficient in clinical information, so they were excluded from the analyses. Therefore, 395 of the specimens tested for RSV were included in the analysis. These samples were obtained from 224 male and 171 female patients, with an average age
of 1.48 years (95% CI, 1.24–1.72 years). Among these patients, 310 (78.5%, 310/395) were ≤ 2 years old and 85 (21.5%, 85/395) were young children aged > 2 years.

3.6 Performance characteristics of the two tested RADTs in detecting RSV

Among the 395 samples analyzed, the 40 (10.1%, 40/395) that were positive for RSV according to the BinaxNOW RSV Card were also positive according to Sofia® RSV FIA. An additional 23 samples that were negative for RSV according to the BinaxNOW RSV Card were deemed positive for RSV with Sofia® RSV FIA. Therefore, the RSV-positive rate for Sofia® RSV FIA (16.0%, 63/395) was significantly higher than that for the BinaxNOW RSV Card (10.1%, 40/395; χ² = 5.9056, p = 0.015).

Based on results of RT–qPCR, 73 of the 395 specimens (18.5%, 73/395) were RSV-positive. Using the RT–qPCR results as the standard, the sensitivity of Sofia® RSV FIA (80.82%) was significantly higher than that of the BinaxNOW RSV Card (53.42%), whereas the specificities of Sofia® RSV FIA and the BinaxNOW RSV Card were both nearly 100% (Table 1). When we compared Sofia® RSV FIA with RT–qPCR, the kappa value was 0.840.

Among the 52 specimens (13.2%, 52/395) that were positive for RSV according to DFA, 47 were identified as positive for RSV with Sofia® RSV FIA and 35 with the BinaxNOW RSV Card. Among the 343 specimens (86.8%, 343/395) that were negative for RSV on DFA, 16 and five were deemed to be positive for RSV with Sofia® RSV FIA and the BinaxNOW RSV Card, respectively. When we used DFA as a method of comparison, the sensitivity of Sofia® RSV FIA (90.38%) was significantly higher than that of the BinaxNOW RSV Card (67.31%), whereas the specificities of Sofia® RSV FIA and the BinaxNOW RSV Card were both nearly 100% (Table 2).
3.3 Evaluation of the factors affecting the sensitivity of RADTs

Among the 73 specimens identified as positive for RSV with RT–qPCR, 48 were positive for RSV A, 23 were positive for RSV B, and two were positive for RSV A+B. Using the RT–qPCR results as the standard, the sensitivity of Sofia® RSV FIA was 85.4% (41/48) for RSV A detection and 69.6% (16/23) for RSV B detection ($\chi^2 = 1.568, p = 0.21$), whereas the sensitivity of the BinaxNOW RSV Card was 58.3% (28/48) for RSV A detection and 43.5% (10/23) for RSV B ($\chi^2 = 0.847, p = 0.357$).

Among these 73 specimens, the 22 specimens with Ct values lower than 24 were identified as RSV-positive by both RADTs, Sofia® RSV FIA and the BinaxNOW RSV Card. The lowest Ct value for the specimens identified as RSV-negative by the BinaxNOW RSV Card was 24, whereas the lowest Ct value for the specimens identified as RSV-negative by Sofia® RSV FIA was 29.

The optimal critical Ct value is the point with the lowest false-positive and false-negative rates and therefore the most accurate diagnosis of RSV. Using Ct as the indicator, the results of the ROC curve generated from our analysis revealed that the optimal Ct was 24 for Sofia® RSV FIA (Fig. 1) and 22 for the BinaxNOW RSV Card (Fig. 2).

The kappa value for the comparison of Sofia® RSV FIA and RT–qPCR was 0.962 in the group of patients aged ≤ 2 years. In contrast, this value was only 0.648 in the group of patients aged > 2 years (Table 3).

4 DISCUSSION

RADTs for POCT must be easy to perform by clinical staff with limited laboratory experience and provide results in less than 30 min, and they should be
useful for health-care professionals who have limited time to examine patients (7, 9, 16, 18). However, the low sensitivity of RADTs has limited their use in POCT, and many factors can affect the performance of these tests. Here, the clinical value in POCT of the Sofia® RSV FIA and BinaxNOW RSV Card tests, which detect RSV with different methods, were evaluated in a prospective laboratory study using samples from Chinese pediatric patients.

It has been reported that the accuracy of viral detection depends on the prevalence of the virus in the population at the time of testing. False-negative test results are more likely to occur when the disease prevalence is low (18). In the present study, the clinical specimens assessed with Sofia® RSV FIA and the BinaxNOW RSV Card were collected during the winter months in Beijing, a temperate region in the northern hemisphere, between November 2017 and February 2018, which is the season of high RSV prevalence (19).

The introduction of a europium dye and more sophisticated assays with digital analyzers, such as Sofia® RSV FIA, may enhance the overall sensitivity of RSV detection (14). In this study, we compared Sofia® RSV FIA with the BinaxNOW RSV Card, a common immune-antigenic RADT that lacks sufficient sensitivity to be used as the sole method of RSV diagnosis (16). The RSV-positive rate with Sofia® RSV FIA (16.0%) was higher than that with the BinaxNOW RSV Card (10.1%) ($p < 0.05$). Moreover, the sensitivity of Sofia® RSV FIA (80.82%) was significantly higher than that of the BinaxNOW RSV Card (53.42%) when RT–qPCR was used as the standard, and this result was supported when the results of the two rapid tests were compared with those of DFA.

Both the viral load in the specimen and the viral strain tested also affect the test results (18). It has been reported that RSV is likely to move into the lower respiratory
tract and to infect ciliated cells before nonciliated cells in infants and young children (21). In a previous study, we reported that the RSV-positive rate of rapid antigen detection in throat swabs (21.7%) from pediatric patients with acute lower-respiratory-tract infection was lower than that in nasopharyngeal aspirates (78.3%) from the same patients (12). Therefore, we collected NPS specimens in the present study. When we investigated the effect of the viral subtype on the test results, using RT–qPCR as the standard, the sensitivity of Sofia® RSV FIA for RSV A (85.4%, 41/48) was higher than that for RSV B (69.6%, 16/23), and the sensitivity of the BinaxNOW RSV Card for RSV A (58.3%, 28/48) was also higher than that for RSV B (43.5%, 10/23). However, more data are required to confirm the effect of the viral strain on the sensitivity of these two RADTs, because the differences detected in this study were not significant.

To obtain direct evidence of a correlation between the viral load within the specimens and the test results, we used the RT–qPCR Ct value of the specimens as an indicator of their viral loads. When the Ct values of the specimens were lower than 24, both rapid tests showed positive results with 100% sensitivity. Some specimens with Ct values > 24 (indicating a lower viral load) were assessed as negative by the BinaxNOW RSV Card, and some specimens with Ct values > 29 were assessed as negative by Sofia® RSV FIA. A ROC curve analysis revealed a correlation between the true-positive rate (sensitivity) and the false-positive rate (1 – specificity). The optimal critical Ct values were 24 for the Sofia® RSV FIA and 22 for the BinaxNOW RSV Card, which indicates the higher sensitivity of Sofia® RSV FIA. These results imply that the sensitivity of Sofia® RSV FIA was higher than that of the BinaxNOW RSV Card.
The kappa value for the comparison of Sofia® RSV FIA and RT–qPCR (0.962) was higher for the group of patients aged \( \leq 2 \) years than for the group of patients aged \( > 2 \) years (0.648). This finding could be attributed to the facts that the viral replication rate is higher during an individual’s first RSV infection than during subsequent RSV infections because of the lack of prior immunity, and that the children aged \( \leq 2 \) years are more likely to be experiencing their first RSV infection (10). However, in this study, we had fewer data on children aged \( > 2 \) years than on children aged \( \leq 2 \) years, so additional data on children aged \( > 2 \) years should be included in future studies.

In conclusion, we confirmed the high clinical sensitivity of Sofia® RSV FIA, which suggests that it is a more suitable RADT for RSV diagnosis than the BinaxNOW RSV Card, at least in Chinese pediatric patients. The performance of Sofia® RSV FIA as a rapid RSV test for the diagnosis of RSV infections in POCT correlated with the viral type and RSV load, and was more accurate in children aged \( \leq 2 \) years than in those aged \( > 2 \) years.

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COMPETING INTERESTS

The authors declare that they have no conflicts of interest.

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Table 1. Performance characteristics of two rapid tests for RSV detection using RT-qPCR as the standard

| Rapid test used  | Sensitivity (%) (no./total no.) | Specificity (%) (no./total no.) | Positive predictive value (%) (no./total no.) | Negative predictive value (%) (no./total no.) | Positive likelihood ratio | Negative likelihood ratio | Youden's index | agreement rate | Kappa value |
|------------------|---------------------------------|---------------------------------|-----------------------------------------------|-----------------------------------------------|---------------------------|--------------------------|----------------|----------------|------------|
| Sofia® RSV       | 80.82 (59/73)                   | 98.76 (318/322)                 | 93.65 (59/63)                                 | 95.78 (318/332)                               | 65.18                     | 0.19                     | 0.796          | 0.94           | 0.840      |
| Binax NOW        | 53.42 (39/73)                   | 99.69 (321/332)                 | 97.50 (39/40)                                 | 90.42 (321/355)                               | 172.32                    | 0.47                     | 0.531          | 0.91           | 0.644      |

Table 2. Performance characteristics of two rapid tests for RSV detection using DFA as the standard

| Rapid test used  | Sensitivity (%) (no./total no.) | Specificity (%) (no./total no.) | Positive predictive value (%) (no./total no.) | Negative predictive value (%) (no./total no.) | Positive likelihood ratio | Negative likelihood ratio | Youden's index | agreement rate | Kappa value |
|------------------|---------------------------------|---------------------------------|-----------------------------------------------|-----------------------------------------------|---------------------------|--------------------------|----------------|----------------|------------|
| Sofia® RSV       | 90.38 (47/52)                   | 95.33 (327/343)                 | 74.60 (47/63)                                 | 98.49 (327/332)                               | 19.35                     | 0.10                     | 0.86           | 94.68          | 0.75       |
| Binax NOW        | 67.31 (35/52)                   | 98.54 (338/343)                 | 87.53 (35/40)                                 | 96.57 (338/350)                               | 46.10                     | 0.33                     | 0.669          | 94.43          | 0.79       |

Table 3. Performance characteristics of Sofia® RSV for RSV detection in different age groups using RT-qPCR as the standard

| Rapid test used  | Age groups | Sensitivity (%) (no./total no.) | Specificity (%) (no./total no.) | Positive predictive value (%) (no./total no.) | Negative predictive value (%) (no./total no.) | Positive likelihood ratio | Negative likelihood ratio | Youden's index | agreement rate | Kappa value |
|------------------|------------|---------------------------------|---------------------------------|-----------------------------------------------|-----------------------------------------------|---------------------------|--------------------------|----------------|----------------|------------|
| Sofia® RSV       | <2y        | 68.12 (47/69)                   | 99.17 (239/241)                 | 93.44 (57/61)                                 | 95.22 (239/251)                               | 92.07                     | 0.32                     | 0.673          | 0.955          | 0.962      |
|                  | >2y        | 75.00 (3/4)                     | 97.51 (79/81)                  | 60.00 (3/5)                                  | 98.75 (79/80)                                | 30.36                     | 25.63                    | 0.725          | 0.964          | 0.648      |
Figure 1. The sensitivity (true-positive rate) and $1 - \text{specificity}$ (false-positive rate) of the rapid test Sofia® RSV FIA compared with RT–qPCR, using the cycle threshold (Ct) as the indicator.

Figure 2. The sensitivity (true-positive rate) and $1 - \text{specificity}$ (false-positive rate) of the rapid test BinaxNOW RSV Card compared with RT–qPCR, using the cycle threshold (Ct) as the indicator.