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Comparison of Cepheid's Analyte-Specific Reagents with BD Directigen for Detection of Respiratory Syncytial Virus▽

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For detection of respiratory syncytial virus (RSV), the BD Directigen RSV rapid antigen assay was compared to Cepheid's real-time reverse transcriptase PCR RSV analyte-specific reagents. The Directigen RSV assay resulted in a 23% false-negative rate, using PCR and chart review as the gold standard, indicating that rapid RSV PCR results would be advantageous.

Rapid detection of respiratory viruses is important for many reasons, including prevention of unnecessary antibiotic use, identification of individuals who can benefit from antivirals, and improvement of infection control by grouping individuals with the same viral infection. Respiratory syncytial virus (RSV) is the most common cause of bronchiolitis in young children (17). Three percent of all children are hospitalized with bronchiolitis in the first year of life, and RSV is the most common cause of this hospitalization (2). An estimated 50% of all children are infected with RSV during the first year of life, and by 3 years of age, 100% have experienced at least one infection (14). Since RSV infection does not induce protective immunity, reinfection is common.

Diagnosis of RSV infection can be made by observation of clinical signs and symptoms, characteristic chest radiographs, rapid antigen detection, viral culture, or reverse transcriptase PCR (RT-PCR) of nasopharyngeal (NP) specimens (2). The most common laboratory method for rapid detection of RSV includes rapid antigen (enzyme immunoassay) assays produced by multiple manufacturers, with various sensitivities of 57 to 98% and specificities between 70 and 100% (1, 5, 10, 11, 13, 16, 20). Other diagnostic tests include culture (routine tissue culture and shell vial culture), with sensitivities between 70 and 80% and specificities of 100% (10, 11, 19), and direct fluorescent antigen detection, with sensitivities between 93 and 100% and specificities of 95 to 100% (1, 10, 16). More recently, real-time RT-PCR assays have been described as demonstrating sensitivities of 73 to 97% and specificities of 64 to 99% (7, 9, 11). In this study, we compared assays using Cepheid's RSV analyte-specific reagents (ASR) to the BD Directigen RSV rapid antigen assay (BD Diagnostics, Sparks, MD).

Respiratory specimens ($n = 142$) consisting of NP aspirates ($n = 140$) and NP swabs ($n = 2$) from the 2005-2006 peak season were initially tested for RSV by the rapid antigen assay, following the manufacturer's protocol. The samples were put into 2 ml of viral transport media prior to being tested; for NP aspirates, the entire volume received was inoculated. These specimens were from patients ranging from 3 days to 74 years of age. Sixty-eight percent ($n = 96$) of the specimens were from infants (less than 1 year of age), 21% ($n = 30$) from individuals between 1 and 5 years of age, and 11% ($n = 16$) from individuals who were greater than 5 years of age. By the rapid antigen assay, 34% ($n = 48$) of the specimens were RSV positive. Of the positives, 81% ($n = 39$) were from individuals less than 1 year of age, 13% ($n = 6$) from individuals 1 to 5 years of age, and 6% ($n = 3$) from individuals greater than 5 years of age.

Retrospectively, the same specimens were tested using the Cepheid RSV ASR, which detect the nucleocapsid protein (N) gene of RSV types A and B by real-time RT-PCR on a SmartCycler (Cepheid, Sunnyvale, CA). After the rapid antigen assay was performed, the samples in viral transport media were frozen at −70°C until the nucleic acids from the samples were extracted, but no more than 2 months after sample collection. The external lysis protocol of a Roche total-nucleic-acid isolation kit on a MagNAPure LC (Roche Applied Science, Indianapolis, IN) was used, with 200 μl of each sample eluted into 50 μl. The sample preparation control bead included in the ASR was added to the lysis buffer prior to extraction, allowing for detection of PCR inhibition. QIAGEN's One-Step RT-PCR kit (QIAGEN, Valencia, CA) was used for the reverse transcriptase reaction, and Cepheid's RSV ASR were used for the real-time PCR. Each reaction mixture included 5 μl of extracted nucleic acid and 20 μl of the following mixture: 1× QIAGEN One-Step RT-PCR buffer, 2 μl of One-Step RT-PCR enzyme mixture, 400 μM of each deoxynucleoside triphosphate, 20 units of RNase inhibitor (New England Biolabs, Ipswich, MA), a volume of nuclease-free water sufficient to reach a total volume of 40 μl, and one RSV ASR bead. The ASR assay was performed on a SmartCycler, using the following cycling parameters: reverse transcription for one cycle at 48°C for 900 s and one cycle at 95°C for 900 s and amplification for 50 cycles at 94°C for 15 s, 60°C for 30 s, and 72°C for 15 s.

The analytical lower limit of detection for the RSV ASR was determined to be 12 copies/reaction (~586 copies/ml) by using diluted nucleic acid extracted from purified quantified RSV (Advanced Biotechnologies, Columbia, MD). For the precision studies, the nucleic acid was extracted daily and diluted with nuclease-free water to 586 copies/ml. Intrarun precision was determined by testing the lower limit of detection for five

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TABLE 1. Microorganisms which do not show cross-reactivity in the Cepheid RSV assay

Viruses
Adenovirus[a]
Parainfluenza viruses I/II/III[a]
Echovirus[a]
Coxsackie B virus[a]
Parvovirus B19[b]
Human metapneumovirus[a]
Influenza virus A[c]
Influenza virus B[c]
Herpes simplex virus I/II[a]
Cytomegalovirus[a]
Epstein-Barr virus[a]
Human herpesvirus 6[c]
Human herpesvirus 7[c]
Human herpesvirus 8[c]
Hepatitis C virus[e]
Human immunodeficiency virus[e]
Bacterial spp. and strains
Streptococcus pyogenes[e]
Streptococcus pneumoniae ATCC 49619[a,d]
Haemophilus influenzae[e]
Moraxella catarrhalis[e]
Staphylococcus aureus ATCC 29213[d,e]
Legionella pneumophila ATCC 33152[a,d]
Mycobacterium abscessus[a]
Mycobacterium chelonae ATCC 35752[d]
Mycobacterium fortuitum ATCC 6841[d]
Mycobacterium avium ATCC 25291[d]
Other
Cryptococcus neoformans[e]
Oral-pharyngeal flora[c]

[a] Isolate obtained from clinical material.
[b] Reference material obtained from BBI Diagnostics (West Bridgewater, MA).
[c] Reference material obtained from Advanced Biotechnologies (Columbia, MD).
[d] Reference material obtained from ATCC (Manassas, VA).
[e] Multiple strains obtained from clinical specimens were tested.

consecutive days, and interrun precision was determined by assaying five replicates on two consecutive days. Statistical analysis was performed using CLSI guidelines (4). The average cycle threshold for intrarun variability was 0.81, with an average coefficient of variation of 2.0%, and the cycle threshold for interrun variability was 0.85, with a coefficient of variation of 2.1%. These data indicate that results obtained using the RSV ASR have excellent reproducibility. In addition, the ASR showed no cross-reactivity when tested against the microorganisms listed in Table 1.

The ASR identified 56% ($n = 79$) of the specimens as positive. This is a significant increase, with 22% ($n = 31$) more samples being RSV positive by RT-PCR. While no published studies evaluate Cepheid's RSV ASR, the observed increase is consistent with the few published studies that have compared RSV rapid antigen detection to molecular detection (12, 15, 18). Of the ASR-positive samples, 75% ($n = 59$) were from individuals less than 1 year of age, 19% ($n = 15$) from individuals 1 to 5 years of age, and 6% ($n = 5$) from individuals greater than 5 years of age. Since it has been reported that older individuals tend to have lower viral burdens during RSV infection, one theory is that older patients would be more likely to have false-negative rapid antigen tests (3, 6, 8). However, only 13% ($n = 2$) of patients greater than 5 years of age and 33% ($n = 10$) of patients 1 to 5 years of age were rapid antigen assay negative and PCR positive. For patients less than 1 year of age, 11% ($n = 20$) were positive by PCR only. Though the sample size is limiting, there is not a striking association between increased age and false-negative rapid antigen assay results. The breakdown of ASR results compared to rapid antigen assay results is shown in Table 2. All discrepant results were confirmed by chart review. Patients positive by PCR only were clinically diagnosed with bronchiolitis ($n = 32$). There was one PCR-negative/rapid antigen assay-positive patient with no respiratory symptoms; upon repeat of the rapid antigen assay, this sample was negative. However, it should be noted that the sample was frozen for 2 months prior to retesting. In our hands, the sensitivity and specificity of the BD Directigen RSV assay were 59% and 98%, respectively, using a combination of PCR and chart review as the gold standard.

TABLE 2. Sensitivity and specificity of the Directigen RSV assay, using the Cepheid RSV ASR and chart review as the gold standard

Result for Directigen RSV assay[a]	No. of results of indicated type for Cepheid RSV ASR	
	Positive	Negative
Positive	47	1
Negative	32	62

[a] The sensitivity and specificity of the Directigen RSV assay were 59% and 98%, respectively.

Since the number of false negatives (23%, $n = 32$) is substantial for the Directigen RSV rapid antigen assay, it is recommended that physicians order RSV RT-PCR on specimens with negative rapid antigen tests for the most accurate laboratory diagnoses. Ideally, if an appropriate turnaround time can be offered, molecular detection should be the gold standard offered at all times for RSV diagnosis. While PCR is more expensive and requires more technologist time and skill, the increased accuracy of the results justifies the extra resources required, particularly for inpatients. In the inpatient setting, where the time required for the result is less critical than test accuracy, the use of PCR for RSV detection will potentially eliminate the need for unnecessary additional testing and antimicrobial therapy, benefiting both the patient and the institution. However, if the time required for the result is critical, as in the outpatient setting, the rapid antigen assay is more appropriate, providing results in about 15 min. Although the RT-PCR assay requires as little as 4 h, many laboratories would need to employ batch processing to maintain cost-effectiveness, thus lengthening the achievable turnaround time to beyond what is effective for the outpatient setting. However, the ASR are contained in a lyophilized bead that decreases the amount of skill and time required to set up the PCR while permitting the most accurate results.

(A preliminary report of this work has been presented previously [9a].)

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