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Influenza and respiratory syncytial virus detection in clinical specimens without nucleic acid extraction using FOCUS direct disc assay is substantially equivalent to the traditional methods and the FOCUS nucleic acid extraction–dependent RT-PCR assay

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ABSTRACT

In this study, we evaluated FOCUS diagnostic's Flu A/B & RSV direct kit (Direct Disc assay), designed to detect influenza (FLU) and respiratory syncytial viruses (RSV) directly in clinical specimens without nucleic acid extraction. This novel 'sample-to-answer', nucleic acid extraction–independent assay uses a unique disc to process, amplify, and detect viral targets in up to 8 specimens at a time. The performance of this assay for detecting FLU and RSV viruses was compared to the traditional methods (culture and/or direct florescent antibody testing) using 945 nasopharyngeal swab specimens. In addition, a total of 150 consecutive clinical specimens positive for FLU (FLU A = 50, FLU B = 50) or RSV (n = 50) were tested in parallel using the novel Direct Disc assay and FOCUS diagnostic's nucleic acid extraction–dependent assay to assess their relative performance. Compared to the traditional methods, the overall (prospective + retrospective) positive/negative percent agreement was determined to be 96.6%/98.1% for FLU A, 98.4%/99.9% for FLU B, and 99.3%/98.8% for RSV. Compared to the nucleic acid extraction–dependent assay, the positive percent agreement was 90% (n = 45/50) for FLU A, 92% (n = 46/50) for FLU B, and 98% (n = 49/50) for RSV. Overall, the Direct Disc assay showed good agreement with both traditional methods and nucleic acid extraction–dependent assay. Although we encountered some failures compared to the nucleic acid extraction–dependent assay, these limitations must be balanced against the substantial advantages of the extraction-free nature of this assay and rapid turnaround time.

1. Introduction

Influenza (FLU) and respiratory syncytial (RSV) viruses are important viral causes of respiratory infections. In the United States, it is estimated that FLU results in an average of 200,000 hospitalizations and 28,000 deaths annually (CDC, 2012). RSV is the leading cause of lower respiratory tract infections in infants and children, and it also causes severe respiratory disease in the elderly and high-risk adults, resulting in estimated 75,000–125,000 hospital admissions per year in the USA (CDC, 2011; Falsey and Walsh, 2005; Iwane et al., 2004).

In clinical laboratories, rapid antigen detection tests (RADTs) and direct florescent antibody testing (DFA) are often used for detecting FLU and RSV infections because they are simple to perform. However, both RADT and DFA were reported to have low sensitivity in the range of 38–69% and 47–93%, respectively (CDC, 2009; Ginocchio et al., 2009). In response to the low sensitivity of rapid tests, many laboratories perform virus culture or PCR on RADT- and DFA-negative specimens (Cruz et al., 2006; Ohm-Smith et al., 2004). Nucleic acid amplification testing is increasingly chosen as an alternative to antigen detection and virus culture because it is more sensitive, accurate, and rapid for detection of viral respiratory infections (Beck et al., 2010; Hymes et al., 2010; Novak-Weekley et al., 2012; Selvaraju and Selvarangan, 2010). Despite their overall excellent sensitivity and specificity, these assays are still categorized as high complexity and thus require highly trained personnel skilled in performing multiple steps including nucleic acid preparation. Further, these assays sometimes have long turnaround times because of batching of test specimens and other workflow constraints. Simplified real-time PCR assays with a shorter turnaround time could potentially improve the patient care by providing timely diagnosis, appropriate antiviral

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therapy, reducing unnecessary antibiotic use, and overall initiating appropriate clinical patient management practices.

Recently, FOCUS diagnostics developed a moderately complex nucleic acid extraction–independent real-time PCR assay (Direct Disc assay; Simplexa™ Influenza A/B & RSV Direct kit; Cypress, CA, USA) for rapid diagnosis of FLU A, FLU B, and RSV directly from nasopharyngeal swab specimens. In this study, the performance of the Direct Disc assay and the traditional methods were compared using a large number of clinical specimens. In addition, we evaluated the relative performance of the Direct Disc assay and the FOCUS diagnostic's nucleic acid extraction–dependent assay (Simplexa™ Influenza A/B & RSV assay; Cypress, CA, USA) by testing 150 consecutive FLU (FLU A = 50; FLU B = 50) and RSV (n = 50) positives specimens determined by Sofia Influenza A + B Fluorescence Immunoassay and the FOCUS nucleic acid extraction–dependent assay.

2. Materials and methods

2.1. Clinical specimens and study sites

A total of 945 nasopharyngeal swab specimens were collected prospectively (n = 722; 2010 and 2011) and retrospectively (n = 223; 2004 through 2010) from different age groups (<5 years through >60 years) from the USA (4 sites) and Australia (3 sites). For prospective specimen collection, male and female subjects from the general population exhibiting clinical symptoms of an upper respiratory infection were eligible for inclusion in this study. The subjects had at least 2 of the following symptoms: chills/sweats, cough, dyspnea (labored, difficult breathing), fatigue, headache, myalgia, nasopharyngeal congestion, runny nose, sore throat, and wheezing. Of 945 specimens, 305 were determined to be positive for any one of the FLU A (n = 147) or FLU B (n = 62) or RSV (n = 96) virus, and 640 specimens were determined to be negative for all the above-mentioned viruses using traditional testing methods (culture and/or DFA) that were performed at the respective specimen collection site. Then, the remaining specimens were collected and tested only by the Direct Disc assay at 4 US clinical trial sites, and the results were compared to the original traditional test result obtained at the specimen collection site.

2.2. Traditional testing methods

In accordance with Food and Drug Administration (FDA) guidelines, the traditional testing methods, i.e., virus culture (shell vial or tube) or DFA, were used as the comparator assays. Traditional testing methods differed among the participating laboratories. R-Mix or R-Mix Too shell vial culture (Diagnostic Hybrids, Athens, OH, USA) was performed as per the manufacturer instructions. Tube virus cultures were performed as described earlier (Newton et al., 2002). FLU and RSV were detected by using FDA-cleared fluorescent antibody kits, LIGHT DIAGNOSTICS™ SimulFluor® Flu A/Flu B kit or LIGHT DIAGNOSTICS™ SimulFluor® Respiratory Screen kit or LIGHT DIAGNOSTICS™ SimulFluor® Respiratory Panel I Viral Screening & Identification kit (EMD Millipore Corp. Billerica, MA, USA) or D^3 Ultra8 kit (Quidel Corp. San Diego, CA, USA). Specimens tested by RADT were not included in this study.

2.3. Real-time RT-PCR assays

The Direct Disc assay was performed on the 3M™ Integrated Cycler platform with an 8-specimen Direct Amplification Disc (DAD). Clinical specimen and Direct Disc assay reagent were loaded into the disc at 50-μL volume into respective wells (Fig. 1). The RT-PCR reaction was performed, and the results were interpreted according to the package insert of the Direct Disc assay.

For the FOCUS nucleic acid extraction–dependent assay, nucleic acid was extracted in a 50-μL elution volume from 200 μL of clinical specimen using Roche's RNA isolation kit on MagNA Pure Compact automated nucleic acid extraction system (Roche, Indianapolis, IN, USA). The RT-PCR assay was performed with 5 μL of nucleic acid template in a 96-well Universal disc on the 3M™ Integrated Cycler according to the package insert.

2.4. Discrepant resolution

In accordance with FDA recommendation, the discrepant analysis was performed only on RSV discrepant specimens because of lower sensitivity of traditional methods compared to PCR. All discrepant specimens (prospective and retrospective) were tested at 1 site by the FOCUS nucleic acid extraction–dependent assay. If sufficient volume of specimen was not available for testing with FOCUS nucleic acid extraction-dependent assay, then the specimens were tested by DFA (EMD Millipore Corp.) and/or RADT (Quidel Corp.). Discrepancy was resolved when the Direct Disc assay result and any one of the FOCUS nucleic acid extraction–dependent assay or DFA or RADT result correlated.

2.5. Comparison of Direct Disc and FOCUS nucleic acid extraction–dependent assays

The performance of the Direct Disc assay was compared to the FOCUS nucleic acid extraction–dependent assay, which uses the extracted nucleic acid template. Both the Direct Disc and FOCUS nucleic acid extraction–dependent assays were performed on the same day and on the same freeze/thaw cycle. A total of 150 consecutive positive specimens (50 each for FLU A, FLU B, and RSV) either by the Sofia Influenza A + B Fluorescence Immunoassay (Sofia; Quidel, San Diego, CA, USA) or by the FOCUS nucleic acid extraction–dependent assay during 2012–2013 respiratory season were tested. The FOCUS nucleic acid extraction–dependent assay was performed on all Sofia positive specimens to determine the true positivity and to obtain the cycle threshold (Ct) values. The overall percent positive agreement and agreement at different Ct ranges between Direct Disc and FOCUS nucleic acid extraction–dependent assays were analyzed.

2.6. Direct Disc assay performance between minimally and highly trained end users

To determine the agreement between minimally (minimal or no molecular diagnostic testing experience) and highly trained (molecular diagnostic testing technologists or PhD level personnel) end users, a subset of the total number of 300 clinical specimens (100/site) were tested with the Direct Disc assay. At each site, 1 minimally and 1 highly trained personnel performed this testing. The results from the designated minimally trained user at each site were compared with the results from the highly trained user. Discrepant analysis was not performed on any of these discordant specimens.

2.7. Reusability of DAD

To establish the reusability of DAD, a total of 30 samples negative for respiratory viruses and 31 positive samples each contrived with FLU A, FLU B, and RSV were tested by placing them at alternate positions (e.g., positions 1, 3, 5, and 7 for positive samples and positions 2, 4, 6, and 8 for negative samples) on the disc. The first run was performed using the first half of the DAD (e.g., positions 1–4), and after completion of first run, the second run was performed with the same disc, and samples were added at the positions, which were not used in the first run (e.g., positions 5–8). The test results from reused discs were compared to the results from