Clinical Evaluation of the ID-Tag™ RVP Test for Detection of Respiratory Viruses

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INTRODUCTION

Virology laboratories have historically used methods such as DFA and culture to diagnose six or seven respiratory virus infections, including Influenza A and B, Rhinovirus/Enterovirus, Parainfluenza types 1 to 3 Respiratory Syncytial Virus (RSV), and Adenovirus. With traditional methods such as DFA and culture that use microscopy, result turn around times are restricted especially in laboratories handling large volumes of respiratory specimens. In recent times, molecular testing including nucleic acid amplification tests (NAAT) have been developed for a number of respiratory viruses (1). Results of carefully controlled laboratory studies employing DFA, culture and PCR have indicated that traditional methods of diagnosis by DFA and culture have suboptimal sensitivity (2). The emergence of these new respiratory viruses since the year 2001, including human Metapneumovirus, SARS Coronavirus, Avian Influenza H5N1 and two new Coronaviruses HKU1 and NL63 has presented new challenges for the clinical laboratory. The absence of commercially available tests for these viruses leaves laboratories without tests to diagnose these emerging important respiratory infections. Therefore there is a need for new and improved diagnostic tests with improved sensitivity to diagnose both traditional and emerging respiratory virus infections. We have developed a multiple PCR test called the Respiratory Viral Panel (RVP) Test that can detect 20 different respiratory viruses in a single test (2,3). The test has been characterized and is currently being validated as an IVD test by Luminex Molecular Diagnostics (formerly TmBioscience Corporation) as the ID-Tag™ RVP Test. The RVP test is a qualitative assay designed for the simultaneous detection of a range of respiratory viruses in symptomatic adult and pediatric patients. In pre-clinical evaluations of the test we demonstrated that this test identified up to 10% more patients that were infected with respiratory viruses (4). In addition the test detected dual respiratory virus infections in 5% of symptomatic adult and pediatric patients. The objective of this study was to evaluate the ID-Tag™ RVP Test and compare its performance to the conventional DFA and culture tests for detecting respiratory viruses. This evaluation was part of a multi-center evaluation for a submission to the FDA for clearance as an in vitro diagnostic.

METHODS

1. Specimens – Nasopharyngeal (NP) specimens were collected under an ERBP approval (St Joseph’s Healthcare) from 227 patients in Hamilton Ontario region in the winter of 2006. Specimens were divided into aliquots. One aliquot was processed in the routine virologic laboratory for testing by DFA and shell vial culture (SVC) and a second aliquot was processed for ID-Tag™RVP testing.

2. DFA – Direct fluorescent antibody staining of respiratory specimens was performed using standard methods. Briefly, cells were spun, washed, spot amplified in replicate and fixed onto microscope slides. Antigens were visualized using FITCylated monoclonal antibodies. (Diagnostic Hybrids Inc.) To the following viruses: Influenza A and B, Parainfluenza types 1 to 3, RSV, Metapneumovirus, and Adenovirus.

3. Shell Vial Culture – DFA negative specimens were tested by Shell Vial culture using commercially available RSV, Rhinovirus and Influenza monoclonal antibodies (Diagnostic Hybrids Inc.).

4. Nucleic acid extraction: Total nucleic acid (DNA/RNA) was extracted from an aliquot of cells (5.0 mL) using the BioRobot M96 extraction kit and eluted in 50 uL sterile elution buffer.

5. ID-Tag™RVP Testing – The ID-Tag™RVP Test incorporates multiple Reverse Transcription (RT) PCR reactions with a proprietary Universal Tag sorting system (Luminex Molecular Diagnostics, formerly TmBioscience, Toronto) on the Luminex xMAP platform. The ID-Tag™RVP Test was performed according to the manufacturer’s protocol. Extracted nucleic acid (DNA-L) was amplified by multiple (15-20) RT-PCR producing amplicons for each of the virus targets/pseudotypes present in the specimen. The reaction products were treated with Stripping Alcohol Phosphate and Ethanol (to inactivate remaining nucleotides and degrade all primer). Multiple TSPE were performed to detect viral DNA by hybridization of hybridized primers and incorporation of biotin-ICTP. TSPE primers were designed by databased and contained a "tag" oligo sequence that hybridizes to a complimentary anti-tag oligo bound to 21 spectrally interrogated microspheres (see figure). Following TSPE, the reaction was added to microtiter plate containing biotin-labeled complete to the DFA panel in the present (see figure). A fluorescent reporter molecule (oligosulfophenacyramidines) is then bound to biotin-labeled TSPE products. Each labeled primer hybridizes only to its unique anti-tag complement. Therefore, each colored bead represents a specific virus by virtue of the bead/anti-tag/tagged primer association. The data are then analyzed on the Luminex. There are two laser beads: one laser identifies the color coded bead, and the other identifies the virus specific oligo added to the reaction. The result is identified in a single detection step and a summary of the report indicates which viruses are present in the sample. The entire assay takes about 5 hours to perform and can generate 20 virus results for each of 96 specimens in the plate.

RESULTS

6. Discordant Testing: All discordant DFA/Culture and RVP specimens or RVP positives for targets not detected by DFA/Culture, were tested by a second independent PCR with unique primers and the resulting amplicons were sequenced. The data from the second PCR was used to establish the true status of the specimen.

A total of 227 NP specimens were tested by DFA plus culture and the ID-Tag™ RVP Test. The ID-Tag™ RVP Test is used for the simultaneous detection of 20 respiratory viruses and the positive signals for the MS 2 control indicate a failure at either the extraction step or the reverse transcription step and may indicate the presence of amplification inhibitors which could lead to a false negative result. A separate Bacteriophage Lambda DNA positive run control serves as a positive control for the PCR and TSPE steps.

Two-twenty of the 227 specimens either failed to give a signal for the internal control indicating extraction failure or were called equivocal for one or more targets and were therefore excluded from the analysis. The final analysis included 205 results. The kit protocol was first evaluated for the 8 viruses detected by both DFA/Culture and RVP. Of the 205 specimens analyzed, 179 gave concordant results: 113 were positive by both assays and 66 were negative by both assays. There were 26 discordant specimens including 22 RVP positive/negative DFA/Culture and 4 RVP positive/negative DFA/Culture positive. After resolution of the discordants using a second PCR, and using positive analysis as the reference standard, DFA/Culture had a sensitivity of 95.4% (113/125) and a specificity of 86.8% (70/79) while the ID-Tag™ RVP Test had a sensitivity of 100% (125/125) and specificity of 99.3% (70/79).

DFA/Culture

RVP

Reference Std

+       + 125       9

+       - 0       70

-       + 151      10

-       - 1       42

Sensitivity 96.6% (113/117)

Specificity 75.0% (66/88)

Sensitivity 99.3% (113/113)

Specificity 80.8% (70/87)

70/79

SENSITIVITY

Discordant DFA/Culture and RVP specimens or RVP positives for targets not detected by DFA/Culture had a sensitivity of 90.4% (113/125) and a specificity of 96.6% (113/117). Sensitivity of 90.4% (113/125) and specificity of 96.6% (113/117) were calculated for one or more targets and were therefore excluded from the analysis. The results of the second PCR were used to establish the true status of the specimen.

The ID-Tag™ RVP Test detected an additional 13 virus types or subtypes not detected by DFA/culture including human Metapneumovirus, Parainfluenza type 4, Rhinovirus/Enterovirus, and Coronavirus OC43, NL63, HKU1 and identified Influenza A virus subtypes H1, H3 and H5. The ID-Tag™ RVP Test detected an additional 26 respiratory virus infections that were not detected by DFA/culture. The additional infections included 22 Rhinovirus/Enteroviruses, 3 HKU1 Coronaviruses, and 1 NL63 Coronavirus. All of these additional positives were confirmed by a second PCR and sequencing. Analysing the data for all types of respiratory infections, including Influenza A and B, Parainfluenza types 1 to 3, Respiratory Syncytial Virus (RSV), and Adenovirus. With Influenza A virus subtypes H1, H3, and H5. The ID-Tag™ RVP Test detected an additional 26 respiratory virus infections that were not detected by DFA/culture. The ID-Tag™ RVP Test detected an additional 13 virus types or subtypes not detected by DFA/culture including human Metapneumovirus, Parainfluenza type 4, Rhinovirus/Enterovirus, and Coronavirus OC43, NL63, HKU1 and identified Influenza A virus subtypes H1, H3 and H5.

7. CONCLUSIONS

In a clinical evaluation of 227 nasopharyngeal specimens, the ID-Tag™ RVP Test was more sensitive than DFA plus culture for detecting eight important respiratory viruses (100% vs. 93.4%). The ID-Tag™ RVP Test detects six dual respiratory virus infections that were not detected by DFA and culture. The clinical relevance of multiple respiratory tract infections in unknown and presently being evaluated.

REFERENCES


