Introduction

Current Respiratory Syncytial Virus (RSV) rapid tests are visual, subjective tests which claim sensitivity of 83%-95% versus culture. A new rapid RSV test is under development for use with the Sofia® Fluorescent Immunoassay Analyzer (FIA). The instrument-mediated interpretation of the test result eliminates the subjectivity and difficulties encountered with traditional visually-interpreted devices when the test signal, produced by some specimens, is close to the visual threshold.

Methods and Materials

The prototype Sofia RSV FIA employs three (3) simple steps to run the assay: (1) Rehydrating the extraction reagent, (2) placing the nasopharyngeal swab into the extraction reagent and transferring an aliquot of the extracted specimen onto the test cassette, then (3) placing the cassette into the Analyzer. All other steps are performed by the instrument.

The analytical sensitivity of the Sofia RS FIA was compared to that of Quidel’s visually-interpreted QuickVue® RSV 10 dipstick test. Five different strains of RSV (Zeptometrix Corp.) two type A (A2 & Long) and three type B (9320, WV/14617/85 and Wash/18537/62) were diluted in M5 transport media (Remel) to concentrations that yielded a Test Line optical density on the QuickVue visual test that non-technical readers have been demonstrated to interpret as positive 95% of the time. These dilutions were considered to correspond to the Limits of Detection (C95) for the respective strains on the QuickVue RSV 10 test.

From these concentrations, additional dilutions of 1:100, 1:150, 1:200, 1:300, and 1:400 were made in M5 media for each RSV strain and tested on the Sofia RS FIA. Five replicates were tested at each virus concentration on two different lots of Sofia RSV FIA devices. For each viral strain, the highest dilution from the C95 was considered to be the comparative LoD for the Sofia RS FIA.

The clinical specificity of the Sofia RS FIA was evaluated using 50 nasopharyngeal swab samples that were collected from 50 different asymptomatic donors. To be eligible for the study, donors were required to be free of the common symptoms of RSV infection, including runny nose, congestion, cough, and fever. Swabs were placed directly into the rehydrated extraction reagent within one hour of collection and tested in the Sofia RS FIA according to standard package insert directions.

Results

In the analytical sensitivity study, the dilutions of each viral strain from the C95 of QuickVue RSV10 that yielded 100% positive results on the Sofia RSV FIA were 1:200 for strain A2 (TCID50/ml: 1.14x10^10); 1:200 for strain A Long (TCID50/ml: 2.87x10^10); 1:300 for strain B 9320 (TCID50/ml: 3.3x10^10); 1:100 for strain B WV/14617/85 (TCID50/ml: 1.91x10^10); and 1:400 for strain B Wash/18537/62 (TCID50/ml: 1.1x10^9).

In the clinical specificity study, all 50 of the samples collected from asymptomatic donors exhibited negative results on the Sofia RS FIA.

Test Overview

Sample Preparation and Test Procedure

The prototype Sofia RSV Assay is designed as a fully integrated system with simple-to-use components that can provide a test result in approximately 15 minutes and can be used in any clinical setting, even those distant from traditional laboratory settings. The three system components are briefly described in the following figures:

Figure 1. The specimen is collected following the same protocol used in the Sofia Influenza A+B FIA. The test was designed to be compatible with nasopharyngeal swabs, nasal washes and aspirates. The specimen can either be added to the extraction tube and tested right after collection, or placed into viral transport media (such as M4, M5, or UTM) and tested at a later time. Saline is added to an extraction tube, followed by placing a swab specimen into the mixture, or adding a liquid sample. 120 μl of the extracted sample liquid is added to the sample port of the cassette with a dropper included in the test kit.

Figure 2. The cassette houses the lateral flow strip and has a laser printed 2D barcode label containing lot and method-specific information that is transferred to the Analyzer. The information includes assay type, cassette lot number, serial number, and expiration date. The system has a Read-now or “Batch” mode in which the device is allowed to develop on the bench top and at the end of the incubation period placed in the Analyzer to be scanned (requires 1 minute to scan and report result). The system also has a Walk-Assay mode in which the cassette can be placed into the Analyzer as soon as the specimen is added, and the reader automatically allows the device to develop for the appropriate time, then scans and reports the result.

Figure 3. The Sofia Analyzer is a fluorescence detection instrument designed to be portable and simple to use. It has a graphical user interface and is powered by either electrical outlet or battery. The interface is designed to reduce user interaction to a few simple steps: use barcode to input user and patient information, press key to open drawer, insert test cassette and close the drawer to start the test. The Analyzer will read the barcode on the cassette, uploading all pertinent information. At the appropriate time the Analyzer will scan the Cassette and subsequently display and print the test result.

Summary and Conclusion

Quidel’s prototype Sofia RSV FIA demonstrated 100 to 400 times higher analytical sensitivity compared to the visually-interpreted dipstick test. Although the competitive study using RSV B strain 9320 was done independently of the Sofia RSV FIA comparison to QuickVue RSV 10, it can be inferred that Sofia RSV is more sensitive than visual rapid diagnostic assays similar to QuickVue RSV 10.

A limited in-house clinical study of 50 freshly-collected nasopharyngeal swab specimens demonstrated specificity of 100%.

Acknowledgments

Partial funding received through a research grant from National Institute of Allergy & Infectious Diseases, through Medical College of Wisconsin, Dr. Kelly Hendrickson and Dr. Richard Egan Co-Principal Investigators.

Note

This poster presents information about a prototype RSV test that is in development. It is not available for sale in the U.S.