COMPARISON OF A RAPID TEST METHOD WITH REAL TIME RT-PCR FOR DETECTING INFLUENZA A H1N1 (2009) USING NASAL SWABS IN A CLINICAL STUDY

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Abstract

A clinical study was conducted at 3 general practitioner (GP) offices in the greater Sydney, Australia area to evaluate the performance of the Simplexa™ Influenza A H1N1 (2009) Real-time RT-PCR Assay (Focus Diagnostics Inc., Cypress, California) using nasal swab specimens. The clinical protocol was pre-approved by the National Research and Evaluation Ethics Committee (NREEC) of the Royal Australian College of General Practitioners. Informed consent was obtained from all subjects prior to specimen collection. All participating site personnel were trained in the proper collection of specimens and in the performance of the rapid test prior to initiation of the study.

Two nasal swab specimens were collected from each patient. One was frozen at -80 C° and shipped to Focus Diagnostic Inc. These specimens were subsequently tested at 3 clinical laboratory sites utilizing the Simplexa assay. The other swab was tested in the QuickVue® Influenza A + B test (Quidel Corporation, San Diego, California) within one hour of collection in accordance with the package insert instructions. Rapid test results were recorded at 10 minutes.

Two hundred fifty-one (251) evaluable specimens were received. Sixty-five (65) were found to be H1N1 positive and fifteen (15) were found to be non-H1N1 positive by the Simplexa assay. Of these positive specimens, the QuickVue test found 31 H1N1 positive (48% agreement) and 7 non-H1N1 positive (47% agreement). One hundred seventy-one (171) specimens were determined to be negative for influenza A by the Simplexa assay. Of these negative specimens, the QuickVue test found one positive (99% agreement).

Positive test results determined by the rapid test were also found to be positive by the RT-PCR test for both H1N1 and non-H1N1 influenza A. However, over half of the positive results were not detected using the rapid test. Negative rapid test results are not reliable and should be tested by a more sensitive test method such as RT-PCR.

Introduction

The 2009 influenza A (H1N1) virus caused the first global influenza pandemic in more than 40 years. It first emerged in March and April, 2009.

The QuickVue® Influenza A + B test is an immunochromatographic assay that tests for influenza A and B but does not distinguish between H1N1 and non-H1N1 influenza A . The Focus Diagnostics Simplexa™ Influenza A H1N1 (2009) assay is a multiplex real-time RT-PCR for the in vitro qualitative detection and differentiation of seasonal influenza A and 2009 H1N1 influenza viral RNA.

While it is important that results of these tests be obtained quickly, the accuracy of these assays is essential. The objective of this study was to compare the clinical performance of the Simplexa Influenza A H1N1 2009 test with the QuickVue Influenza A+B Test in detecting influenza A when using nasal swabs.

Study Design

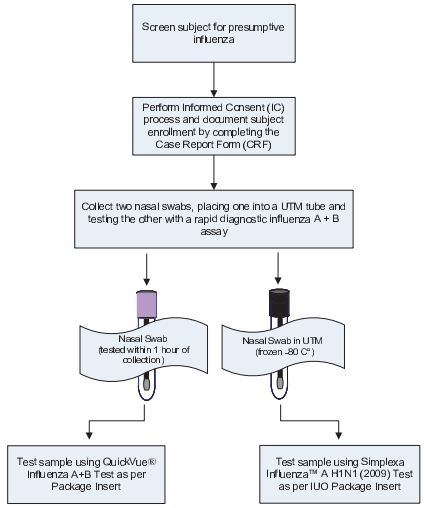

Figure 1: Schematic of Study Design

Methods

Patient Population

Inclusion criteria included male and female subjects of any age from the general population who exhibited clinical symptoms of a viral respiratory tract infection that might have been caused by influenza virus. The subjects had to have had a temperature of ≥38 °C at the time of visit or within the previous 48 hours, and at least two of the following symptoms: chills/sweats, cough, dyspnea (labored/difficult breathing), fatigue, headache, myalgia (deep muscle aches), nasal congestion, runny nose, or sore throat. Samples were collected prospectively during the months of July through September 2009. Patients were excluded if they or their legal guardians could not understand or consent to participation in the study.

Clinical Study Sites

Clinical sites in Mascot, Castle Hill, and Merrylands, Australia, participated in the procurement of samples and performance of the rapid test. The clinical protocol was approved by the National Research and Evaluation Ethics Committee (NREEC) of the Royal Australian College of General Practitioners (RACGP). Informed consent was obtained from all subjects, or their legal guardians, prior to specimen collection. All participating site personnel were experienced in the proper collection of specimens and in the performance of the QuickVue Influenza A+B rapid test.
Three clinical sites within the United States performed the Simplexa RT-PCR test.

Methods (cont.)

Rapid Test

The QuickVue Influenza A+B Test is an immunoassay that uses monoclonal antibodies specific for influenza A and B antigens. The Australian clinical sites described above performed the rapid QuickVue test in accordance with the package insert.

Simplexa Influenza A H1N1 2009 Test

The Simplexa Influenza A H1N1 2009 test is a real-time PCR amplification and detection system that utilizes a bi-functional fluorescent primer-probe to detect human influenza A virus RNA and for differential detection of 2009 influenza H1N1 virus. The clinical laboratory sites within the United States performed the Simplexa test in accordance with the package insert.

Specimen Collection

The QuickVue specimens were collected using the swabs provided in the kit. The specimens for testing in the Simplexa assay were collected using Copan Regular Flocked Sterile Swabs and Copan Universal Transport Medium (UTM: Copan Diagnostics, Murrieta California).

PCR Method

RNA extracted from clinical specimens were assayed using the IUO Simplexa Influenza A H1N1 (2009) kit on the 3M Integrated Cycler.

Results

Specimens were collected from a population with ages ranging from 6 months to 82 years. The highest numbers of samples were from young adults (ages 19 to 39; Figure 2). There were very few samples from patients older than 65 years.

Two hundred fifty-one (251) evaluable specimens were received. The Simplexa assay showed presence of influenza A in 80 (32%) samples: 65 were positive for H1N1 and 15 were positive for non-H1N1 influenza A. The QuickVue rapid test was positive in 38 of the 80 Simplexa-positive specimens (48% sensitivity), including 31 (48%) of specimens positive for H1N1 and 7 (47%) of those positive for non-H1N1 influenza A (Table 2).

One hundred seventy-one (171) specimens were determined to be negative for influenza A by the Simplexa assay, only one of which was positive on the QuickVue rapid test (Table 1).

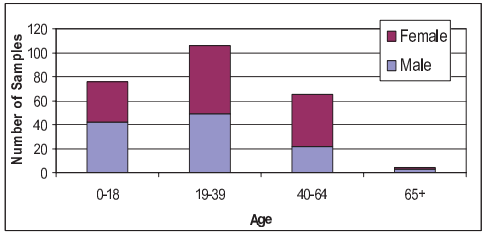

Figure 2: Age and Gender Distribution of Sample Population

Results (cont.)

Table 1: Overall results of Simplexa versus QuickVue

		QuickVue Influenza A+B Test Results			% Agreement
		Flu A Positive	Flu A Negative	Total	
Simplexa Influenza A H1N1 (2009) Real-Time RT-PCR	H1N1 + FluA Positive	38	42	80	Positive Agreement: 48% (38/80)
	Flu Negative	1	170	171	Negative Agreement: 99% (170/171)
	Total	39	212	251	

Table 2: Subtyped results of Simplexa real-time RT-PCR assay versus QuickVue Influenza A + B rapid test

		QuickVue Influenza A+B Test Results			% Agreement
		Flu A Positive	Flu A Negative	Total	
Simplexa Influenza A H1N1 (2009) Real-Time RT-PCR	H1N1 Positive	31	34	65	Positive Agreement: 48% (31/65)
	FluA Positive	7	8	15	Positive Agreement: 47% (7/15)
	Negative	1	170	171	Negative Agreement: 99% (170/171)
	Total	39	212	251	

Conclusions

More than half of the specimens positive by the Simplexa assay were negative using the rapid test. Because negative results on the rapid test may not rule out influenza A infection, follow-up testing using a more sensitive test method, such as RT-PCR, should be considered.

Acknowledgements

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FOCUS Diagnostics