RAPID, MULTIPLEXED SAMPLE-TO-ANSWER DETECTION OF INFLUENZA A, B, AND RSV USING THE 3M INTEGRATED CYCLER

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Revised Abstract

Background: The 2009 H1N1 pandemic demonstrated the need for simple and rapid molecular tests.* This study compared the sample-to-answer feature of the 3M Integrated Cycler versus RT-PCR using traditional extraction. The Simplexa™ Influenza A/B & RSV assay, a multiplex molecular assay that is in development and is designed to detect and discriminate influenza A, influenza B, and RSV, was used for the comparison.

Methods: Clinical nasopharyngeal swabs and aspirate specimens (negative, or containing influenza A, influenza B, or RSV) were obtained and were tested using a reference method requiring separate extraction and PCR-based amplification (using the Roche MagNA Pure LC instrument and 3M Integrated Cycler, respectively). These samples were also tested in parallel using the integrated cycler and its sample-to-answer disc. For samples run using the reference method, 200 µL of each specimen was extracted using the MagNA Pure LC instrument, and 5 µL of purified nucleic acid was amplified on the integrated cycler. Samples were run on the integrated cycler with the sample-to-answer disc by directly loading the specimen onto the microfluidic disc without prior extraction; both nucleic acid extraction and reverse transcription amplification were run to completion on the disc without any additional operator intervention. Concordance and run times for each system were compared, and the reproducibility of the Simplexa assay was measured.

Results: The Simplexa assay with the sample-to-answer feature provided results quickly, with few operator steps, and with performance comparable to RT-PCR using traditional extraction. There was nearly complete qualitative concordance between methods for both positive and negative samples for each virus tested. Reproducibility testing using the integrated cycler showed CV values (based on Ct values) of less than <5% for each target (influenza A, influenza B, and the internal control)The sample-to-answer system provided results in less than 45 minutes, whereas the RT-PCR using a traditional extraction method required 4 hours to complete (including multiple operator steps and instrument setup steps for nucleic acid extraction).

Conclusions: The Simplexa assay on the integrated cycler is a simple and rapid molecular test. The sample-to-answer feature provided results comparable to those obtained from a system requiring separate extraction and PCR amplification instruments. The sample-to-answer feature combined with speed at which results can be obtained make it a good choice as a simple and rapid molecular test.

Methods

Clinical Specimens: Nasal swabs, nasopharyngeal swabs, nasal aspirates, and swabs with unspecified respiratory sources were collected in Universal Transport Media (UTM; Copan Diagnostics Inc, Murrieta, CA). An aliquot of these samples had been previously tested using a reference method (using nucleic acid extraction on a MagNA Pure LC instrument, followed by amplification on the integrated cycler) as benchmark for comparison.

Sample-to-Answer Assay: A master reaction mix was prepared, and contained all necessary reaction components to lyse and stabilize viral RNA. In addition, the mix contained all reagents required to perform reverse transcription and real time-PCR amplification. Labeled primers contained in the mix targeted conserved regions of the Influenza A matrix segment, Influenza B matrix segment and RSV M gene. An additional set of primers was included to detect an RNA internal control which was also included in the reaction mix. Each primer was labeled with a different fluorescent dye with a distinct emission profile to distinguish the fluorescent signal from each target or internal control.

Sample-to Answer Reaction Setup: The assay was performed using a prototype 3M Sample-to-Answer Disc that was capable of running 4 specimens at a time. The disc contains chambers to which the reaction mix and specimen are added. Assays were run by adding 40 µL of the reaction mix and 10 µL of patient sample (directly from the specimen tube) to the disc. The disc was then immediately put into the 3M Integrated Cycler microfluidic platform (3M, St. Paul, MN), which was programmed with the following run parameters: 1 cycle of 50°C for 10 min; 1 cycle of 97°C for 2 min; 40 cycles of 102°C for 1 sec and 58°C for 10 sec for amplification and detection.

Reproducibility: Intra assay reproducibility was assessed by running samples from a 3M influenza A and B positive control swab that was placed in UTM. Four replicate samples were run and mean cycle threshold (Ct) values, standard deviations, and %CV were calculated.

Results

Assay Run Time Comparison: Assay run times, calculated based on required instrument setup time and instrument run-times, were determined. The sample-to-answer system required approximately 3 minutes to load samples and reagents, followed by 42 min for PCR (total of 45 min). This is approximately 25% of the time required to run the reference method (using automated nucleic acid extraction followed by amplification on the integrated cycler), which required approximately 90 min for nucleic acid preparation, 10 min for reagent setup, and 75 min for RT-PCR, for a total of 175 min.

Reproducibility: Intra assay reproducibility was assessed by running samples from a 3M influenza A and B positive control swab that was placed in UTM. Four replicate samples were run and mean Ct values, standard deviations, and %CV were calculated. Figure 1 shows amplification curve traces for influenza A, which demonstrate good reproducibility. The Ct values that were determined for influenza A, influenza B, and the internal control (Table 1) also show excellent reproducibility, with CV values <1.5%.

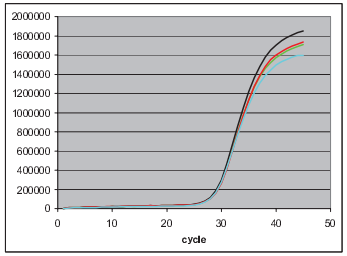

Figure 1. Well-to-well reproducibility for 4 influenza A samples amplified in the same disc using the sample-to-answer integrated cycler system.

Results (Cont.)

Method Comparison Studies: The sample-to-answer influenza A/B RSV assay provided complete concordance for influenza A and RSV clinical samples (Tables 2 and 4). Testing of the limited number of influenza B clinical samples available showed concordance in 5 of 6 samples tested (Table 3). The discrepant influenza B result is being evaluated.

Table 1. Intra assay reproducibility of Ct values for influenza A, influenza B, and internal control amplifications.

	Flu A	Flu B	IC
Well 1	26.6	29.9	33.8
Well 2	26.4	29.7	34.6
Well 3	26.6	30.5	34.3
Well 4	27.0	30.4	33.7
mean	26.7	30.1	34.1
stdev	0.3	0.4	0.4
CV	0.9	1.3	1.2

Table 2. Influenza A detection; concordance between reference method and sample-to-answer method.

		Reference Method			% Agreement
		Flu A Positive	Flu A Negative	Total	
Sample-to-Answer Flu A/B & RSV Real-Time RT-PCR	Flu A Positive	15	0	15	% Positive Agreement 100% (15/15)
	Flu A Negative	0	26	26	% Negative Agreement 100.0% (26/26)
	Total	15	26	41	

Table 3. Influenza B detection; concordance between reference method and sample-to-answer method.

		Reference Method			% Agreement
		Flu B Positive	Flu B Negative	Total	
Sample-to-Answer Flu A/B & RSV Real-Time RT-PCR	Flu B Positive	5	0	5	% Positive Agreement 83.3% (5/6)
	Flu B Negative	1	35	36	% Negative Agreement 97.2% (35/36)
	Total	6	35	41	

Table 4. RSV detection; concordance between reference method and sample-to-answer method.

		Reference Method			% Agreement
		RSV Positive	RSV Negative	Total	
Sample-to-Answer Flu A/B & RSV Real-Time RT-PCR	RSV Positive	8	0	8	% Positive Agreement 100% (8/8)
	RSV Negative	0	33	33	% Negative Agreement 100% (33/33)
	Total	8	33	41	

Results (Cont.)

Method Comparison (Cont.): In addition to qualitative concordance measurements, a semi-quantitative concordance determination was made by comparing Ct values obtained from the reference assay and from the sample-to-answer assay (for detection of influenza A viruses). Although some outliers were observed, correlation was generally good, with an R2 value of 0.89 (Figure 2).

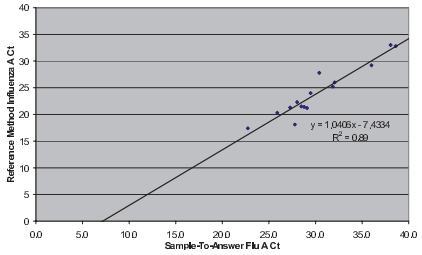

Figure 2. Comparison of Ct values from reference method for the detection of influenza A viruses.

Conclusions

- The Simplexa Flu A/B & RSV sample-to-answer assay on the integrated cycler is a simple and rapid molecular test. The assay provides answers in 45 minutes without a separate extraction system.
- The sample-to-answer assay produces results comparable to those produced by a reference assay that utilizes a traditional extraction system.
- A reaction mix has been designed that will lyse viruses, stabilize RNA, and be compatible with components in specimen collection media.
- The Simplexa Flu A/B & RSV assays are in development, they are not currently available for sale, and are not FDA cleared.

*(http://www.cdc.gov/flu/professionals/diagnosis/rapidlab.htm)

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