Rapid Sample-to-Answer Molecular Detection Using the 3M Integrated Cycler: Comparison to a Conventional Extraction and Amplification Method

Michelle Tabb*, Lucien Jacky, Huong Mai, Yuan Xie, Jules Chen, Michael Aye & Maurice Exner

Focus Diagnostics Inc., Cypress, CA

Revised Abstract

Introduction: The 3M integrated Cycler is a flexible platform that is capable of providing molecular diagnostic results in a sample-to-answer disc. This is achieved through unique chemistry and a uniquely designed microfluidic reaction vessel- the Direct Amplification Disc. We compared the assay performance of this system to that of a conventional system (with separate extraction and amplification instruments). In addition, we compared the hands-on time, assay run times, and overall assay throughput of both systems.

Materials & Methods: The analysis was performed using assays in development for both RNA targets (influenza A and B, and RSV) and DNA targets (HSV-1 and 2). Panels of clinical specimens with concentrations spanning the clinically observed ranges of each assay and for each target were analyzed in parallel using the 3M Integrated Cycler and a conventional system (utilizing the Roche MagNA Pure for nucleic acid extraction, and the Applied Biosystems 7500 Real-Time PCR System or the 3M Integrated Cycler for amplification and detection). Concordance between methods was determined.

Results: The results showed complete qualitative concordance between systems for all analytes tested. The sample-to-answer system required only 5 minutes of hands-on-time for assay setup, and the time from setup to run completion was approximately 35 minutes for DNA targets (which included setup and run time) and 1 hour for RNA targets; high-positive samples were detectable in as little as 20-30 minutes. The conventional system required considerable hands-on time (30-40 min), and took 3 to 4.5 hours to produce results (depending on the number of specimens being tested, and whether the target was DNA or RNA). The throughput of the Sample-to-Answer method and the conventional method was 120 samples/8 hour shift, and 192 samples/8 hour shift respectively.

Conclusion: While the overall throughput of the conventional system was greater than the 3M Integrated Cycler system, the benefits of minimal hands-on time, ease of use, small instrument footprint, and all-in-one nature of the Sample-to-Answer system provide an attractive alternative, particularly when rapid results are required.

Methods

Clinical specimens. A panel of Flu A, Flu B, RSV and negative nasal swab patient samples in universal transport media was obtained from Focus Diagnostics reference laboratory. Panels of HSV-1, HSV-2 and negative samples consisting of genital swab samples in universal transport media or cerebral spinal fluid were also obtained from the Focus Diagnostics reference laboratory. Samples were de-identified by the reference lab.

Sample-to-Answer Reactions. Master reaction mixes for Flu A/B & RSV or HSV 1/2 sample-to-answer assays were prepared with components necessary to release and stabilize viral nucleic acids and perform real-time PCR amplification. For Flu A/B RSV, the mix contains reagents required to perform reverse transcription as well as labeled primers targeting 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 a RNA Internal Control. For HSV 1/2, the mix contained labeled primers targeting conserved regions of the DNA polymerase gene for HSV-1 or HSV-2, together with an additional set of primers to detect a DNA Internal Control. Primers were labeled with different fluorescent dyes to distinguish the fluorescent signals from each target or internal control.

Methods (Cont.)

Sample-to-Answer Workflow. 50 µl of patient specimen and 50 µl of reaction mix for either Flu A/B RSV or HSV 1/2 were added to sample or reaction mix ports on the Direct Amplification Disc. Ports were covered and the disc was run in the 3M Integrated Cycler.

Conventional System Reactions. For Simplexa Flu A/B RSV[1]; 200 µl patient sample was extracted using Roche MagNA Pure LC and eluted in 50 µl. 5 µl extracted sample plus 5 µl Simplexa Flu A/B & RSV reaction mix were loaded onto the Universal Disc and run in the 3M Integrated Cycler. Run parameters: 10 min 47˚C, 2 min 97˚C followed by 40 cycles of 5 sec 97˚C, 30 sec 58˚C. For HSV 1/2 reference assay; 200 µl patient sample was extracted using Roche MagNA Pure LC and eluted in 50 µl. 10 µl extracted sample plus 15 µl HSV 1/2 reaction mix was loaded onto a 96-well plate and run on Applied Biosystems 7500 Real-Time PCR System. Run parameters: 10 min 95˚C followed by 50 cycles of 15 sec 95˚C, 35 sec 60˚C.

[1] CE Marked, 510(k) submitted

Results

Table 1. HSV 1 Concordance for Sample-to-Answer HSV 1/2.

		HSV 1/2 Reference Method			
		HSV-1 Positive	HSV-1 Negative	Total	% Agreement
Sample-to-Answer HSV 1/2	HSV-1 Positive	12	0	12	% Positive Agreement 100% (12/12)
	HSV-1 Negative	0	18	18	% Negative Agreement 100% (18/18)
	Total	12	18	30	

Table 2. HSV 2 Concordance for Sample-to-Answer HSV 1/2.

		HSV 1/2 Reference Method			
		HSV-2 Positive	HSV-2 Negative	Total	% Agreement
Sample-to-Answer HSV 1/2	HSV-2 Positive	12	0	12	% Positive Agreement 100% (12/12)
	HSV-2 Negative	0	18	18	% Negative Agreement 100% (18/18)
	Total	12	18	30	

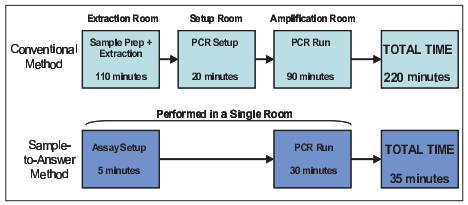

Figure 1. Workflow and time requirements to perform HSV 1/2 assays using Conventional versus Sample-to-Answer methods.

Results (Cont.)

Table 3. Influenza A Concordance for Sample-to-Answer Flu A/B & RSV.

		Simplexa Flu A/B & RSV			
		Flu A Positive	Flu A Negative	Total	% Agreement
Sample-to-Answer Flu A/B RSV	Flu A Positive	10	0	10	% Positive Agreement 100% (10/10)
	Flu A Negative	0	36	36	% Negative Agreement 100% (36/36)
	Total	10	36	46	

Table 4. Influenza B Concordance for Sample-to-Answer Flu A/B& RSV.

		Simplexa Flu A/B & RSV			
		Flu B Positive	Flu B Negative	Total	% Agreement
Sample-to-Answer Flu A/B RSV	Flu B Positive	5	0	10	% Positive Agreement 100% (5/5)
	Flu B Negative	0	41	41	% Negative Agreement 100% (41/41)
	Total	5	41	46	

Table 5. RSV Concordance for Sample-to-Answer Flu A/B & RSV.

		Simplexa Flu A/B & RSV			
		RSV Positive	RSV Negative	Total	% Agreement
Sample-to-Answer Flu A/B RSV	RSV Positive	10	0	10	% Positive Agreement 100% (10/10)
	RSV Negative	0	36	36	% Negative Agreement 100% (36/36)
	Total	10	36	46	

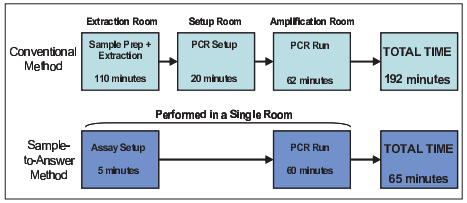

Figure 2. Workflow and time requirements to perform Flu A/B RSV assays using Conventional versus Sample-to-Answer methods.

Table 6. Throughput per 8 hour shift for Conventional versus Sample-to-Answer methods.

		Samples processed per 8 hour shift	Equipment needed
DNA	Conventional Method	192	2- Roche MagNA Pure LC 1- AB 7500 Prism
	Sample-to-Answer Method	120	1- 3M Integrated Cycler
RNA	Conventional Method	192	2- Roche MagNA Pure LC 1- 3M Integrated Cycler
	Sample-to-Answer Method	120	2 - 3M Integrated Cycler

Conventional method uses 96-well plates or discs. Sample-to-Answer method uses 8-sample Direct Amplification Discs.

Results (Cont.)

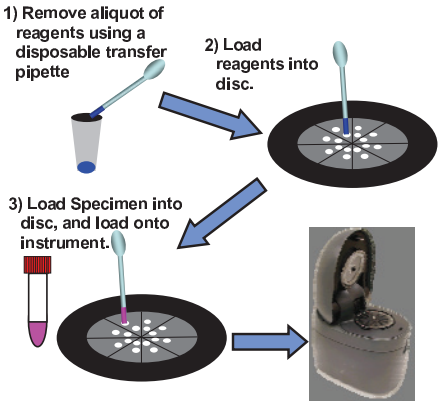

Figure 3. Workflow to perform Focus Sample-to-Answer assays. Up to 8 patient specimens can be processed in 1 run.

Conclusions

- The Simplexa Flu A/B & RSV Sample-to-Answer and Simplexa HSV 1/2 Sample-to-Answer Assays run on the 3M Integrated Cycler are simple and rapid molecular tests providing results directly from patient specimens in about an hour without a separate extraction system.
- The Sample-to-Answer assays produce results comparable to those produced by conventional high-complexity assays that utilize a separate extraction system.
- A reaction mix has been designed that will lyse viruses, stabilize RNA and DNA, and is compatible with components in clinical specimens or collection media.

Note: The Simplexa Flu A/B & RSV Sample-to-Answer and Simplexa HSV 1/2 Sample-to-Answer assays are in development, are not currently available for sale, and are not FDA cleared.

* Corresponding Author: mtabb@focusdx.com