M-6

MOLECULAR SAMPLE-TO-ANSWER DETECTION AND DISCRIMINATION OF HSV-1 AND HSV-2 IN 30 MINUTES USING THE INTEGRATED CYCLER

Yin-Peng Chen, Lucien Jacky, Pete Ludowise , Scott Lecy, Jeff Smith, Cindy Cheng, Brent Han, David Whitman, and Maurice Exner
Focus Diagnostics Inc., Cypress, CA, and 3M Infection Prevention Division, St. Paul, MN

Abstract (Revised)

Background: Molecular detection techniques have been shown to be more sensitive than conventional techniques for detection of viruses such as herpes simplex virus (HSV). Despite the advantages of using molecular assays, considerable expertise is required to run a molecular test. Furthermore, laboratories require specific designs to accommodate molecular testing. The 3M Integrated Cycler provides the capability to do sample-to-answer testing that minimizes the skill required to perform testing and eliminates the need for specialized extraction systems, or for separate extraction and detection areas. We tested the ability of the Integrated Cycler, and its accompanying microfluidic disc system, to detect and differentiate HSV-1 and HSV-2 in clinical specimens.
Methods: A panel of de-identified clinical specimens (20 cerebral spinal fluid and 20 swabs in viral transport media) containing quantified amounts of HSV-1 (n=20) and HSV-2 (n=20) virus was obtained. Virus concentrations in the specimens ranged from 0 copies to 50,000,000 copies/mL for HSV-1, and 0 copies to 170,000,000 copies/mL for HSV-2. Samples were assayed using the 3M Integrated Cycler sample-to-answer system and with a traditional real-time PCR amplification-based assay. For the Integrated Cycler system, raw specimens were loaded directly onto the microfluidic disc, and nucleic acid extraction and amplification were completed on-board the disc without any sample manipulation or pre-preparation. For the traditional real-time PCR method, 200 µL of each specimen was extracted using the Roche MagNA Pure system, and 10 µL of purified nucleic acid was then amplified on an AB 7500 real-time PCR system. Run times, detection sensitivity, and reproducibility were compared between systems.
Results: The 2 methods demonstrated 100% concordance. A regression analysis of the cycle threshold (Ct) values obtained using each method provided an R2 value of 0.86 for HSV-1 specimens and 0.96 for HSV-2 specimens. Inter and intra-assay precision studies showed that well-to-well and run-to-run reproducibility using the integrated cycler were excellent, with CV values (based on Ct values) of <5% for HSV-1 and HSV-2 targets, as well as for the internal extraction and amplification control. Total assay run time was 3.5 to 4 hours for the MagNA Pure and AB 7500 system, and 30 minutes for the Integrated Cycler system (with high-positive sample results available in as little as 15 min).
Conclusions: The sample-to-answer capability of the 3M Integrated Cycler was very effective. Results can be obtained much faster than when using separate extraction and amplification systems, and use of the Integrated Cycler minimizes labor and requires less expertise. The HSV assay was sensitive and reproducible, and the rapid, simple nature of the test could prove useful by providing more rapid results to assist in the evaluation of patients with suspected HSV infection.

Methods

Specimens: A panel of 40 de-identified CSF and swab samples submitted for quantitative HSV PCR testing was obtained from the Focus Diagnostics reference laboratory.
Traditional HSV-1 and 2 Real-Time PCR: The traditional method was run by the Focus Diagnostics reference laboratory, and involved nucleic acid extraction and purification using the Roche MagNA Pure instrument (Roche, Indianapolis, IN), followed by amplification and detection using the Applied Biosystems 7500 real-time PCR instrument (Applied Biosystems, Foster City, CA). The Scorpion primers of HSV-1, HSV-2, and internal control were labeled with CFR610, FAM, and Q670, respectively. For the traditional method, 200 µL of patient specimen was extracted, and the purified nucleic acids were eluted in 50 µL, of which 10 µL was used in the PCR. The PCR was carried out for 50 cycles (Table 1).

Methods (Cont.)

Sample-to-Answer HSV-1 and 2 Real-Time PCR: The sample-to-answer real-time PCR was run on the 3M Integrated Cycler with a novel microfluidic disc. The same HSV-1 and HSV-2 primers and internal control primers used in the traditional method were also used in this assay. For the sample-to-answer system, CSF or swab sample (10 µL) and 40 µL of real-time PCR mastermix were applied directly to the wells of the 3M microfluidic disc. The PCR was then carried out for 50 cycles (Table 1).

Table 1. Comparison of cycling conditions using Integrated Cycler and AB 7500

Step	Integrated Cycler	AB 7500
Denaturation	97°C, 2 min	95°C, 10 min
Cycling	102°C, 1 Sec	95°C, 15 Sec
	60°C, 10 Sec	60°C, 35 Sec

Results

Time to Result Comparison: Traditional real-time PCR methods require sample preparation and extraction processes. As outlined in Figure 1, total run time for these assays is approximately 220 minutes. In contrast, the sample-to-answer RT-PCR platform does not require a separate nucleic acid extraction step, sample preparation, or reagent preparation. In addition, samples do not need to be transferred between instruments (or between rooms) after each assay step. Rapid thermocycling was employed (Table 1) and was able to complete a run in approximately 30 minutes. For samples containing high concentrations of virus, definitive results (discernable positive amplification curves) could be observed after only 18 cycles (Figure 2A and B), which took only 15 minutes
Method Comparison Between the Sample-to-Answer Platform and the Traditional Real-Time PCR Method: A parallel study was performed using a reference method (MagNA Pure extraction followed by amplification on AB 7500) and the sample-to-answer test method performed using the 3M Integrated Cycler. The study used a panel of 40 CSF and swab samples that contained HSV-1 or HSV-2 with concentrations ranging from 0 – 170,000,000 copies/mL. The results showed 100% concordance between methods for both HSV-1 (Table 2) and HSV-2 (Table 3). In addition, the regression analyses of Ct values showed good correlation between the 2 methods, with correlation coefficients of 0.86 for HSV-1 (Figure 3) and 0.96 for HSV-2 (Figure 4).

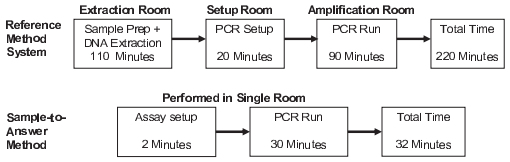

Figure 1. Schematic diagram showing workflow and time requirements to perform assays using the reference method system and the sample-to-answer system.

Results (Cont.)

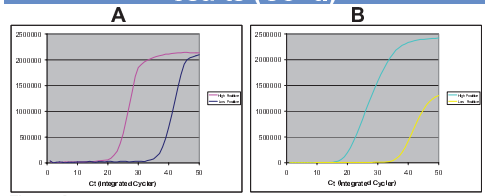

Figure 2. Sample amplification curves of high-positive and low-positive patient samples from the sample-to-answer system. Specimens positive for HSV-1 are shown in panel A, and specimens positive for HSV-2 are shown in panel B.

Table 2. Results from parallel studies detecting quantified amounts HSV-1 using the reference traditional method and the sample-to-answer method

Sample #	Specimen Type	Reference Method HSV-1 (Copies/ml)	Reference Method HSV-2 (Copies/ml)	Sample-to-Answer HSV-1 (Ct Value)	Sample-to-Answer HSV-2 (Ct Value)
1	CSF	47,600,000	Not Detected	22,8	Not Detected
2	Swab	35,700,000	Not Detected	17,9	Not Detected
3	Swab	5,020,000	Not Detected	20,1	Not Detected
4	Swab	152,000	Not Detected	28,5	Not Detected
5	Swab	105,000	Not Detected	25,2	Not Detected
6	CSF	64,800	Not Detected	32,0	Not Detected
7	Swab	30,600	Not Detected	30,8	Not Detected
8	CSF	11,000	Not Detected	30,1	Not Detected
9	CSF	9,860	Not Detected	34,6	Not Detected
10	Swab	7,200	Not Detected	32,2	Not Detected
11	CSF	4,900	Not Detected	32,0	Not Detected
12	CSF	2,070	Not Detected	35,5	Not Detected
13	CSF	1,690	Not Detected	34,0	Not Detected
14	Swab	722	Not Detected	34,4	Not Detected
15	CSF	613	Not Detected	33,8	Not Detected
16	Swab	84	Not Detected	43,8	Not Detected
17	Swab	19	Not Detected	37,6	Not Detected
18	CSF	Not Detected	Not Detected	Not Detected	Not Detected
19	CSF	Not Detected	Not Detected	Not Detected	Not Detected
20	Swab	Not Detected	Not Detected	Not Detected	Not Detected

Table 3. Results from parallel studies detecting quantified amounts HSV-2 using the reference traditional method and the sample-to-answer method

Sample #	Specimen Type	Reference Method HSV-1 (Copies/ml)	Reference Method HSV-2 (Copies/ml)	Sample-to-Answer HSV-1 (Ct Value)	Sample-to-Answer HSV-2 (Ct Value)
1	Swab	Not Detected	170,000,000	Not Detected	18,2
2	Swab	Not Detected	16,500,000	Not Detected	25,0
3	Swab	Not Detected	2,360,000	Not Detected	29,1
4	CSF	Not Detected	297,000	Not Detected	30,6
5	Swab	Not Detected	177,000	Not Detected	29,2
6	Swab	Not Detected	55,800	Not Detected	32,0
7	Swab	Not Detected	21,900	Not Detected	32,8
8	CSF	Not Detected	13,200	Not Detected	33,6
9	CSF	Not Detected	9,210	Not Detected	34,1
10	Swab	Not Detected	6,540	Not Detected	35,8
11	CSF	Not Detected	3,990	Not Detected	35,1
12	Swab	Not Detected	3,140	Not Detected	36,5
13	CSF	Not Detected	651	Not Detected	39,4
14	CSF	Not Detected	481	Not Detected	40,0
15	CSF	Not Detected	467	Not Detected	40,1
16	CSF	Not Detected	420	Not Detected	39,0
17	CSF	Not Detected	359	Not Detected	38,4
18	Swab	Not Detected	314	Not Detected	24,1
19	CSF	Not Detected	3	Not Detected	40,5
20	Swab	Not Detected	Not Detected	Not Detected	Not Detected

Results (Cont.)

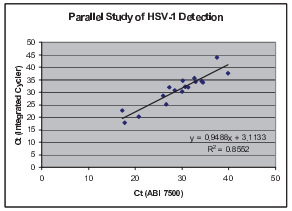

Figure 3. Correlation of Ct values from the reference method (extraction using MagNA Pure and amplification on AB 7500) and the sample-to-answer test method run on the 3M Integrated Cycler.

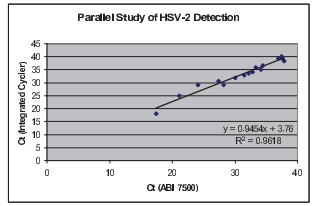

Figure 4. Correlation of Ct values from the reference method (extraction using MagNA Pure and amplification on AB 7500) and the sample-to-answer test method run on the 3M Integrated Cycler.

Conclusions

Conclusions

- Performance of the HSV-1 and HSV-2 sample-to-answer system using the 3M Integrated Cycler was comparable to that of the traditional method using automated extraction followed by real-time PCR.
- Both CSF and swab samples are suitable for the HSV-1 and 2 sample-to-answer system; positive samples from either specimen matrix were detected.
- The sample-to-answer platform has faster assay run times than the traditional PCR method used, with total run times of approximately 30 min (with positive samples being detected in as little as 15 min).
- The HSV-1 and HSV-2 sample-to-answer assay is in development, it is not currently available for sale, and is not FDA cleared.

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