T-19

COMPARISON OF 5 REAL-TIME PCR PLATFORMS USED FOR THE DETECTION OF CMV AND HSV VIRUSES.

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Abstract (Revised)

Background: A number of different real-time PCR instruments are currently available for use with laboratory developed tests. Each instrument has unique characteristics, including sample and volume capacities, cycling speeds, and fluorescence detection mechanisms. The capabilities and advantages of each instrument must be weighed against their detection sensitivity, which is one of the most critical parameters considered for use in a clinical laboratory setting. We evaluated five different platforms to compare detection sensitivity, PCR efficiency, and linear range of detection for two laboratory developed assays. One of the assays used in the study targeted human cytomegalovirus, while the other was a multiplex assay that detected and discriminated herpes simplex virus types 1 and 2. Instruments tested included the Roche LightCycler 480, the Cepheid Smart Cycler, and the Applied Biosystems 7900HT, 7500, and 7500 Fast sequence detection systems.
Methods: Analytical studies involved testing dilutions of purified DNA from each virus. Target DNA concentrations ranged from 200,000 to 2 copies per reaction. The final reaction volume was 25 uL, which included 10 uL of diluted DNA plus 15 uL of PCR mastermix. DNA from positive clinical specimens (extracted using the Roche MagNA Pure instrument) were also tested for each analyte.
Results: The analytical studies showed that sensitivities were roughly equivalent for each instrument, and PCR efficiencies were at least 90% in all instances. Instruments did vary with respect to the amplification cycle at which target DNA was detected. Results from clinical studies showed complete concordance for all instruments (all samples were detected with all instruments).
Conclusions: Despite the difference in instrument formats and capabilities, all instruments provided equivalent sensitivities; however, assays were not specifically optimized for each system, so it is possible that further instrument-specific chemistry optimizations could improve the performance of a specific instrument. Nevertheless, the observed efficacy of all instruments means that when choosing an instrument for a laboratory developed test, instruments can be selected based on specific desirable instrument features without concern for sacrificing assay performance and sensitivity.

Methods

Real-time PCR Instruments: Instruments evaluated included the Applied Biosystems 7900HT, 7500, and 7500 Fast Real-Time PCR Systems, the Cepheid SmartCycler®, and the Roche LightCycler 480. Product specifications relevant to thermocycling and optic systems of the instruments are listed in Table 1.

Sample Preparation: Aliquots (200 uL) of CMV, HSV-1, and HSV-2 purified virus stocks (Advanced Biotechnologies, Inc.) and 200 uL of each clinical specimen were extracted using a Roche MagNA Pure LC instrument, and its corresponding Total Nucleic Acid Isolation Kit. Purified nucleic acids from viral stocks were serial diluted with T.E. to a range of 2 to 200,000 copies/10uL. For clinical specimens, 4 uL of a 20,000 copies/uL internal positive control template solution was added to 200 uL of specimen to control for extraction and amplification processes.

Methods (Cont.)

Real Time PCR: The PCR reaction mixes used 300 nM of each target analyte primer, and 100 nM of each internal control primer. A standard 2x PCR mastermix plus primers was brought up to 15 uL with water, and 10 uL of DNA was added for reactions performed on the 7500, 7900HT, SmartCycler, and LC 480 (25 uL total reaction volume). For the 7500 Fast instrument, the final reaction volume was 10 uL using 5 uL of 5x PCR mix plus primers and water, and 5 uL of sample.
PCR was carried out in two stages. The first stage was a 10 minute incubation at 95°C to activate the polymerase, and the second stage consisted of a 2 step cycling protocol (Table 2).

Table 1. Instrument Specifications

Instrument	Dynamic Range (logs)	Thermal Cycling	Excitation	Detection	Operating Spectrum
7900HT	9	Peltier-based	Argon-ion Laser	CCD	FAM (450-527 nm)-Texas Red (590-610 nm)
7500	9	Peltier-based	Halogen Lamp	CCD	FAM (450-527 nm)-Cy5 (630-750 nm)
7500 Fast	9	Peltier-based	Halogen Lamp	CCD	FAM (450-527 nm)-Cy5 (630-750 nm)
SmartCycler	NS*	Ceramic plate	LED	LED	FAM (450-527 nm)-Cy5 (630-750 nm)
LC480	9	Peltier-based	Xenon Lamp	CCD	FAM (450-527 nm)-Cy5 (630-750 nm)

NS= Not Specified

Table 2. Cycling Conditions

Instrument	7500		7500 Fast		7900HT		SmartCycler		LC 480	
Temp(°C)	95	15 sec	95	3 sec	95	15 sec	95	15 sec	95	15 sec
Time	60	35 sec	60	30 sec	60	35 sec	60	60 sec	60	35 sec
Cycles	50		50		50		45		50	

Results

PCR Efficiency: A curve slope value was obtained after plotting Ct values vs. log concentration values for a series of CMV DNA dilutions that was run on each instrument, and the slopes ranged from -3.4 to -3.6, indicating 90% amplification efficiency or greater for all instruments (Fig. 1) . Although a noticeable trend of higher Ct values was observed with the 7900HT and LC 480 instruments, it did not appear to influence assay performance.

Viral DNA Detection by PCR: All instruments detected all replicates of HSV-1 DNA dilutions with concentrations of 40 copies/rx or above. At least 4/5 replicates of samples with target DNA between 2 and 20 copies/rx were detected in all other instruments except the SmartCycler, which showed a lower level of detection at 10 copies/rx or below (Table 3a). At least 4/5 HSV-2 replicates were detected at all concentrations above 4 copies/rx (Table 3b). At least 4/5 replicates of CMV DNA were detected by all instruments at all concentrations (Table 3c).

Clinical Sample Virus Detection – A series of CMV, HSV-1, and HSV-2 clinical samples with viral concentrations ranging from ~ 200 to >2,000,000 copies/mL were examined in parallel using all instruments. All the CMV (30), HSV-1 (9), and HSV-2 (21) positive samples were detected by all 5 instruments (Figures 2-4). As with the results from the analytical studies, Ct values did vary between instruments, although this did not appear to affect assay sensitivity.

Results (Cont.)

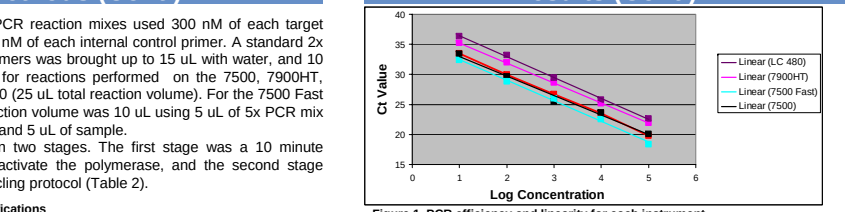

Figure 1. PCR efficiency and linearity for each instrument.

Table 3a. Detection of HSV-1 using different instruments

Copies/rx	7900HT # Detected	7500 # Detected	7500 Fast # Detected	LC 480 # Detected	SmartCycler # Detected
200,000	3/3	3/3	3/3	3/3	3/3
20,000	3/3	3/3	3/3	3/3	3/3
2,000	3/3	3/3	3/3	3/3	3/3
200	3/3	3/3	3/3	3/3	3/3
40	5/5	5/5	5/5	5/5	5/5
20	4/5	5/5	5/5	5/5	4/5
10	5/5	5/5	5/5	5/5	3/5
4	5/5	5/5	5/5	5/5	1/5
2	5/5	4/5	5/5	5/5	3/5
0	0/3	0/3	0/3	0/3	0/3

Table 3b. Detection of HSV-2 using different instruments

Copies/rx	7900HT # Detected	7500 # Detected	7500 Fast # Detected	LC 480 # Detected	SmartCycler # Detected
200,000	3/3	3/3	3/3	3/3	3/3
20,000	3/3	3/3	3/3	3/3	3/3
2,000	3/3	3/3	3/3	3/3	3/3
200	3/3	3/3	3/3	3/3	3/3
40	5/5	5/5	5/5	5/5	5/5
20	4/5	5/5	5/5	5/5	4/5
10	5/5	5/5	5/5	5/5	5/5
4	3/5	5/5	5/5	5/5	5/5
2	1/5	4/5	3/5	5/5	5/5
0	0/3	0/3	0/3	0/3	0/3

Table 3c. Detection of CMV using different instruments

Copies/rx	7900HT # Detected	7500 # Detected	7500 Fast # Detected	LC 480 # Detected	SmartCycler # Detected
200,000	3/3	3/3	3/3	3/3	3/3
20,000	3/3	3/3	3/3	3/3	3/3
2,000	3/3	3/3	3/3	3/3	3/3
200	3/3	3/3	3/3	3/3	3/3
40	5/5	5/5	5/5	5/5	5/5
20	5/5	5/5	5/5	5/5	5/5
10	5/5	5/5	5/5	5/5	5/5
4	5/5	5/5	5/5	5/5	4/5
2	5/5	4/5	4/5	5/5	5/5
0	0/3	0/3	0/3	0/3	0/3

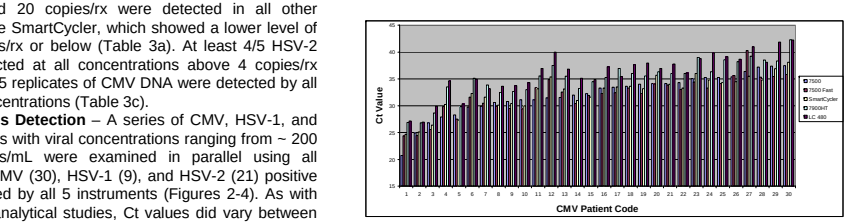

Figure 2. Amplification of CMV patient specimens using different instruments

Results (Cont.)

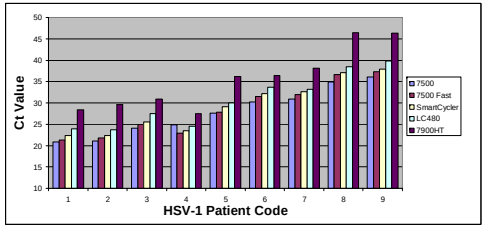

Figure 3. Amplification of HSV-1 patient specimens using different instruments

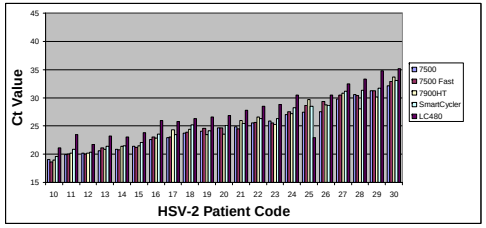

Figure 4. Amplification of HSV-2 patient specimens using different instruments

Conclusions

- Analysis of viral DNA from clinical specimens showed equivalent sensitivity between instruments (all samples were detected with all instruments). This held true for the 7500 Fast instrument which used only half of the input DNA volume compared to the other instruments.
- Studies with purified viral DNA indicated that instruments may vary with respect to analytical sensitivity, although more specimens would need to be run to ensure that differences were not solely due to sampling error. In addition, PCR mixes and cycling conditions were not optimized for each instrument, and this may have disadvantaged performance.
- Given the similar performances observed, it would appear that despite the differences in instrument hardware and software characteristics, all platforms performed well and provided excellent detection sensitivity.

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