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Abstract

Objectives: The Spartan DX™ (Spartan Bioscience Inc., Ottawa ON) is a compact, inexpensive, 4-well real-time PCR (RT-PCR) instrument. It uses a 2-temperature reaction cycle that promises more rapid results than conventional RT-PCR. The diagnosis of HSV infections of the CNS is a logical application for this instrument: on-demand, non-batched analysis where speed and convenience are critical. We evaluate the performance of a TaqMan®-based HSV assay adapted for the Spartan DX.

Methods: The RT-PCR HSV assay used at CHEO (reference-HSV) was modified for the Spartan DX (Spartan-HSV). Replicate testing of CSF spiked with HSV-1 DNA and with HSV-2 DNA (180 copies and 160 copies, respectively; ABI Inc., Columbia, MD) was performed. HSV-1 stock (3.2 TCID₅₀, 1 log > the lower limit of detection of reference-HSV) was added to blank CSF, extracted, and tested in same- and separate-day runs. Finally, archived CSF specimens (23 PCR+) 10 PCR- were tested. Extractions were performed by QIAamp DNA Mini Kit. Specimens were tested in parallel by reference-HSV (ABI 7500, BioRad iCycler). Spartan- and reference-HSV results (PCR [+/- C_t]) were compared.

Results: Spartan-HSV detected both HSV-1 and HSV-2 DNA: mean C_t values of samples spiked with 180 copies HSV-1/reaction (n=8) and with 160 copies HSV-2/reaction (n=8), were 35 (range: 34-36). For CSF samples spiked with 3.2 TCID₅₀ HSV-1 (n=6), the mean C_t was 35.6 (range: 35-36). For all study samples, Spartan and reference test results were in agreement. For clinical specimens, there was no meaningful C_t difference between methods (Spartan C_t - reference = 2 C_t).

Conclusions: The Spartan-HSV assay is reliable and reproducible, with performance equaling conventional RT HSV PCR. The Spartan DX is an excellent choice for laboratories needing an accurate, affordable, convenient platform to accommodate non-batched "stat" testing, or for smaller laboratories seeking to implement real-time molecular diagnostics in their facilities.

Introduction

The Spartan DX™ is a new generation of inexpensive real-time PCR instruments, capable of performing efficient, rapid analysis of a maximum of 4 samples in a single run. The efficiency of this instrument is achieved through the use of a novel heating system that moves 2 fixed temperature heat sources against a fixed reaction tube position, in a 2-temperature thermal cycle. The need for simple bi-phasic cycling is accomplished thanks to the kinetics of this heat transfer system, allowing annealing and extension to be performed at the same temperature. The combination of rapid cycling and small sample capacity make the Spartan DX™ ideally suited for non-batched testing, "stat" testing, or for low-throughput laboratories wanting to implement molecular diagnostic testing. Laboratory diagnosis of Herpes simplex (HSV) infections of the central nervous system (CNS) relies on the molecular detection of HSV DNA in the cerebrospinal fluid (CSF); rapid diagnosis is essential for guiding appropriate clinical intervention. Here, we examine the performance characteristics of a Spartan DX™ - based assay for the detection of HSV in CSF, and compare its performance to a conventional real-time PCR assay in use in our laboratory.

Objective

To evaluate the performance of a TaqMan®-based HSV assay adapted for the Spartan DX™ real-time PCR instrument.

Methods

Control and virus stocks

- HSV-1 and HSV-2 control nucleic acids (ABI Inc., Columbia, MD) were added to pooled blank CSF, to 180 copies/reaction and 160 copies/reaction, respectively.
- Titrated laboratory isolate HSV-1 was added to pooled blank CSF (final: 3.2 TCID₅₀ per reaction).

Clinical specimens

- Thirty-three archived clinical specimens [23 HSV-1 (+), 10 HSV (-)] previously tested using our in-house validated real time assay [based on (1)] were re-analyzed in parallel using this reference HSV assay and the Spartan - HSV assay.

Nucleic acid extraction

- DNA was manually extracted from each sample using the QIAAGEN QIAamp® DNA Mini Kit (QIAAGEN Inc., Mississauga, ON).

Real-time PCR

Reference-HSV assay

- HSV DNA was detected using an in-house validated real-time assay based on (1).
- Target gene: polymerase.
- 6 FAM / BHQ Probe (IDT Inc., Coralville IA).
- GAPDH probe Texas Red / BHQ (IDT Inc., Coralville IA).
- Quantitect Multiplex PCR no ROX Kit (QIAAGEN Inc., Mississauga, ON).
- Bio-Rad Cycler (Bio-Rad Laboratories Ltd., Mississauga, ON), ABI 7500 (Applied Biosystems, Foster City, CA).
- Total run time 110 minutes.

Methods



Spartan DX

- Recommended setup:

- Tube 1: sample to test, with HSV primers / probe
- Tube 2: sample to test, with GAPDH primers / probe
- Tube 3: negative control (no DNA)
- Tube 4: positive HSV control

Spartan - HSV assay

- Modified reference - HSV assay (2).
- GAPDH probe 6 FAM / BHQ (IDT Inc., Coralville IA).
- 78 minute total run time:
 - 10 minute instrument warm-up followed by

Step	Temperature	Time	Cycles
Initial denaturation	92.7°C	15 min	1
Denaturation	92.7°C	40 sec	45
Annealing + extension	57.2°C	40 sec	45

Results

Detection of control HSV-1 and HSV-2 DNA in CSF matrix

Virus	No. copies	Tests	C _t values		
			Mean	Median	Range
HSV-1	180	8	35	35	34-36
HSV-2	160	8	35.3	35	34-36

Detection of titrated HSV-1 stock in CSF matrix

Virus	Quantity*	Day	Tests	C _t values		
				Mean	Median	Range
HSV-1	3.2 TCID ₅₀	1	8	35.9	36	35-37
		2	8	36.8	37	36-38
		3	18	36.7	37	36-38
		4	12	36.8	37	35-39

* 1 log above the established lod of the reference-HSV assay

Detection of HSV in archived clinical specimens

HSV-negative CSF (n=23)

	Spartan-HSV	Reference-HSV*
Result	23/23 negative	23/23 negative

* 4 iCycler, 19 ABI 7500

HSV-positive CSF (n=10)

	Spartan-HSV	Reference-HSV*
Result	10/10 positive	10/10 positive

* 3 iCycler, 7 ABI 7500

Results

HSV-positive CSF (n=10)

Specimen	Spartan-HSV	Reference-HSV		Spartan Δ Reference
		ABI 7500	iCycler	
		C _t value *		
1	36	nt	34.1	+ 1.9
2	31	nt	31.1	- 0.1
3	31	nt	31.2	- 0.2
4	32	33.1	nt	- 1.1
5	28	30.6	nt	- 1.4
6	25	25.6	nt	- 0.6
7	30	29.9	nt	+ 0.1
8	33	32.1	nt	+ 0.9
9	34.2	35	nt	- 0.8
10	38	37.4	nt	- 0.6

* nt: not tested

Summary / Conclusion

- The real-time HSV assay modified for use on the Spartan DX™ reliably and reproducibly detected both HSV-1 and HSV-2 DNA.
- For all study specimens, Spartan-HSV and conventional real-time reference-HSV assay results were in agreement.
- Performance of the Spartan-HSV assay was equal to that of the reference-HSV assay, with results within 2 C_t.
- The Spartan-HSV assay requires 78 minutes to complete, versus 110 minutes for the reference-HSV assay.
- The Spartan DX™ instrument is robust, easy to operate, and features intuitive menu selections and software.
- The Spartan DX™ is an excellent choice of real-time PCR instrument for non-batched or "stat" testing. It is also well suited for smaller laboratories seeking an affordable instrument with which to implement low-throughput real-time molecular diagnostic testing.

References

1. Jerome KR, Huang ML, Wald A, Selke S, Corey L. 2002. Quantitative stability of DNA after extended storage of clinical specimens as determined by real-time PCR. J. Clin. Microbiol. 40 (7): 2609-11.
2. Spartan DX™ Protocol: Real-time PCR for Herpes Simplex Virus (HSV). Available at www.spartanbio.com/protocols.asp.