Comparison of ARIES® HSV 1&2 Assay, Simplexa™ HSV 1 & 2 Direct and a Real-Time PCR Lab Developed Test for the Detection of HSV from Lesions

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ASM 33 CLINICAL VIROLOGY SYMPOSIUM
MAY 7—10, 2017 | SAVANNAH, GEORGIA

ABSTRACT

Introduction: Nucleic acid amplification technologies (NAAT) are well-characterized methods for the rapid and sensitive diagnosis of a wide variety of infectious diseases. Herpes Simplex Virus (HSV) amplification is no exception, demonstrating as much as a nine-fold enhancement in sensitivity over viral culture in some settings. Certainly, HSV NAAT is the standard of care for diagnosis of HSV encephalitis and is becoming the method of choice for the detection from lesions.

Materials and Methods: We evaluated the performance of two commercial real-time PCR systems, ARIES® HSV 1&2 Assay (Luminex) and Simplexa™ HSV 1 & 2 Direct (DiaSorin Molecular), with a Lab Developed real-time PCR Test (LDT) for the Detection of HSV from Lesions. Limit of detection analyses (LOD) were performed using serial dilutions of viral stocks of both HSV1 and HSV2 obtained from the New York State Department of Health, quantified against the MGB HSV positive control (ELITech) using MGB Alert® HSV ASR Primers and Probe (MGB). The evaluation of clinical performance involved a prospective analysis of 62 lesion swabs in viral transport, received in the laboratory for HSV testing. Samples were divided into 4 aliquots and frozen at -80ºC prior to testing by the 3 investigational systems and for clinical purposes using our laboratory's test of record (TOR). The TOR for lesion specimens is a conventional PCR with an enzyme-labeled oligo sorbent assay (ELOSA) for amplicon detection. In addition, 38 retrospective specimens stored at -80ºC and previously determined to be positive for HSV by the TOR, were included.

Results: The analytical sensitivity studies demonstrated that the LODs (log10 copies/ml of specimen) for Aries were ~3.6 for both viruses, while the LODs for Simplexa were 3.6 for HSV1 and 3.1 for HSV2. The LODs for direct testing with the LDT were 4.8 and 4.0, respectively. Of the 100 clinical specimens, 52 were positive by all 4 methods, 5 were positive by Aries, Simplexa and TOR, 2 were positive by Aries and Simplexa only, 1 was positive by TOR only and 40 specimens were negative by all methods. One specimen positive by all four methods was positive for both HSV1 and HSV2 by Aries only. Specimens were considered to be true positive if positive by 2 or more assays. From these analyses, the sensitivities of both Aries and Simplexa were 100%, while the sensitivities of LDT and TOR were 88 and 96%, respectively.

Conclusions: In summary, commercial assays demonstrated excellent performance and differentiated HSV1 & HSV2, which some physicians consider important. In addition, these systems are simple and easy to run.

INTRODUCTION

Herpes Simplex Viruses, types 1 and 2 (HSV 1 & 2) also known as human herpesvirus 1 and 2, are two members of the herpesvirus family that infect humans. Although not absolute, the primary difference between the two viral types is where they usually establish latency in the body. The "site of preference" for HSV 1 is the trigeminal ganglion, a collection of nerves near the ear. From there the virus travels toward the mucosal cells of the lips, mouth or face. For HSV 2 this preferred site is the sacral ganglion found at the base of the spine and from there to the genital area of the body. Both viral types are most contagious during active outbreaks, are easily transmitted to their sites of preference and can be spread to other sites.

Many patients with genital herpes don't know they are infected. Symptomology of the infection is often atypical making clinical diagnosis difficult. Diagnosis relies on direct detection of the virus with culture as the gold standard. Culture is limited to laboratories that have adequate facilities, is labor intensive and lacks adequate sensitivity. Culture failures can occur due to the stage of the lesion at sampling, inadequate sampling, specimen degradation in transit or contamination of the culture with bacteria. Serology testing can be helpful but is not truly type specific therefore requiring further confirmatory testing. In addition, seroconversion may take up to six months and seropositivity is definitive of a past infection but does not prove the etiology of a specific genital ulcer.

Numerous studies have demonstrated improvements in the diagnosis of HSV infection with the use of nucleic acid amplification technology (NAAT) including improved sensitivities and time to result. Initial FDA approved molecular tests had test results available within 4 to 8 hours, such as Luminex MultiCode-RTx Herpes Simplex Virus 1 & 2 Kit and BD ProbeTec Herpes Simplex Viruses (HSV I & 2) QX Amplified DNA Assays. However, these systems are labor intensive. More recently, less complex sample-to-result molecular platforms such as Luminex ARIES® HSV 1&2 Assay and Diasorin Simplexa™ HSV 1 & 2 Direct Assay have been introduced into the marketplace.

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Poster Number: Monday 41
Abstract Control Number:
2017-A-330-ASM-CVS

RESULTS

ARIES® Cassette & Instrument

1. Assay type	7. Side cassette
2. Cassette barcode (top)	8. Cassette expiration date
3. Cassette barcode (side)	9. Cassette lot number
4. PCR tube	10. Cassette part number
5. Cassette serial number	11. Back seal
6. Sample chamber	12. Cassette cap

Table 1. LDT and TOR Primers and Probes

Assay	Target	Sequence (5'-3')
TOR	gB (HSV-1/2)	
	HSV gB1	GCA TCG TCG AGG AGG TGG AC
	HSV gB2-b	TTG AAG CGG TCG GCG GCG TA
	Probe (HPR)	CGA CGA GTT TGT GCT GGC GA
LDT	Glycoprotein C (HSV1)	
	SN-HSV1-F	GATGCCGGTTTCGGAATTC
	SN-HSV1-R	CCCATGGAGTAACGCCATATCT
	SN-HSV1-GC-P	ACCCGCATGGAGTTCCGCCTC
	UL3 Gene (HSV2)	
	SN-HSV2-F	TTGCACCCCAGAACCATGA
	SN-HSV2-R	TCGACTCTATGGGCGTCGTA
	SN-HSV2-UL3-P	CCTGGCGCGTCGCCGAA

Table 2. Sample Population

Specimen Type	Prospective (n=62) HSV1/HSV2/Neg	Retrospective (n=38) HSV1/HSV2/Neg
Oral	5/1/11	4/1/0
Genital	5/2/17	6/12/0
Other (skin, eye)	3/3/13	3/7/0
Not specified	2/0/0	3/2/0

Table 3. Analytical Sensitivity

Virus	LOD (Log copies/ml) Simplexa	Aries	LDT	TOR
HSV-1 (NYS PT #1113)	3.57	3.66	4.81	3.90
HSV-2 (NYS PT #1186)	3.07	3.63	3.99	3.90

Table 4. Reproducibility using Lab made Positive control

	FOCUS		ARIES		LDT	
	HSV1	HSV2	HSV1	HSV2	HSV1	HSV2
Mean	32.2	33.8	10/10	9/10 Pos,	37.64	36.14
STD	0.79	0.37	Pos	1 Invalid	1.08	0.92
CV	2.46%	1.10%			2.87%	2.56%

Simplexa™ Disc, Reagents, & Instrument

Table 5. HSV 1+2 Assay performance with clinical samples

N	TRUE	ARIES	Simplexa	LDT	ELOSA
27	HSV-1	+	+	+	+
3	HSV-1	+	+	-	+
1	HSV-1	+	+	-	-
1	HSV-2	HSV1 & HSV2	- HSV-2	- HSV-2	+
24	HSV-2	+	+	+	+
2	HSV-2	+	+	-	+
1	HSV-2	+	+	-	-
1	NEG	-	-	-	+
40	NEG	-	-	-	-

Table 6. Sensitivity, Specificity, Discordance and Accuracy

Virus		N	Discordant	Sensitivity	Specificity	Kappa	Accuracy
HSV (any)	ARIES	100	0	100%	100%	100%	100%
	Simplexa	100	0	100%	100%	100%	100%
	LDT	100	7	88%	100%	86%	93%
	ELOSA	100	2	97%	98%	94%	97%
HSV-1	ARIES	100	1	100%	99%	98%	99%
	Simplexa	100	0	100%	100%	100%	100%
	LDT	100	4	87%	100%	90%	96%
HSV-2	ARIES	100	0	100%	100%	100%	100%
	Simplexa	100	0	100%	100%	100%	100%
	LDT	100	3	89%	100%	92%	97%

Table 7. Workflow Comparison of Aries & Simplexa HSV 1 & 2 Assays

Description	ARIES®	Simplexa™
Tech hands on time per specimen	< 5 mins	< 5 mins
Amount of sample required	200 ul	50 ul
Reagent prep/ mix	none/ reagents incorporated in cartridge	reaction mix requires thawing before loading in the disc
Maximum capacity load	12 samples	8 samples
Walkaway capacity/run time	115 mins	65 mins
Priority processing	none	none
Operating system (os)	windows	windows
Set up on system	easy	very easy
Approximate Maximum throughput in 8.5 hr shift	~4 runs of 12 = 48 samples	~7 runs of 8 = 56 samples

MATERIALS & METHODS

Clinical samples: Prospectively analyzed lesion swabs in viral transport (n=62), sent to the laboratory for HSV detection, were maintained at -80°C after receipt in the laboratory and analyzed within 48 hr by TOR. Retrospectively analyzed specimens (n=38), previously determined to be positive for HSV by TOR, were analyzed alongside prospective samples. Specimens were defined as true positive if they were positive for HSV by two or more PCR assays.

Viral Isolates: Viral stocks HSV1 and HSV2 were obtained from the New York State Department of Health. DNA from these stocks was isolated by extraction on a MagNA Pure Compact and quantified against ELITech's MGB HSV positive control using MGB Alert® HSV ASR Primers and Probe (MGB). Tenfold serial dilutions of the viral stocks were used for testing limit of detection analyses (LOD).

ARIES® HSV 1&2: Specimens in viral transport medium (200ul) are added to the sample chamber of an ARIES® HSV 1&2 Assay cassette. The cassette containing lyophilized Master Mix reagents for HSV 1&2 and specimen processing control (SPC) is then placed into an ARIES® System magazine. The magazine is inserted into an ARIES® System and once a run is started, the SPC is automatically added to the sample chamber as well as isolation and purification of nucleic acids processes are automated within the ARIES® System.

Simplexa™ HSV 1 & 2 Direct: The Simplexa™ HSV 1 & 2 Direct assay system is a real-time PCR that enables the direct amplification, detection and differentiation of HSV-1 and/or HSV-2 DNA without nucleic acid extraction. Specimens from unprocessed CSF or genital swab in viral transport medium (50ul) are added to the Simplexa system. The system consists of the Simplexa™ HSV 1 & 2 Direct assay, the Integrated Cycler (with Integrated Cycler Studio Software), the Direct Amplification Disc and associated accessories. In the Simplexa™ HSV 1 & 2 Direct assay, bi-functional fluorescent probe-primers are used together with corresponding reverse primers to amplify HSV-1, HSV-2 and internal control targets. Well conserved regions of the HSV-1 and HSV-2 DNA polymerase genes are targeted to identify HSV-1 and HSV-2 DNA respectively in the specimen. An internal control is used to detect PCR failure and/or inhibition.

HSV Conventional PCR-ELOSA LDA (TOR): Approximately 50 to 100 µl of specimen is placed in a boiling water bath. HSV DNA was amplified from 5 µl of boiled sample in a 9600 thermal cycler with biotin-labeled primers targeting gB (Table 1). Amplicons were captured in a streptavidin coated microtiter plate and detected with a horseradish peroxidase-labeled probe. Substrate development was measured in a plate reader.

Real-Time PCR Lab Developed Test for the Detection of HSV from Lesions: Specimens in viral transport medium (5ul) are directly added to a SmartCycler reaction tube containing internal control plasmid (ICP), HSV 1 & 2 and ICP primers & probes (table 1) along with PerfeCTa qPCR ToughMix (Cat. 95112-012, Quanta Biosciences) and amplified on the Cepheid SmartCyler instrument.

Statistical Analysis: Probit analyses were performed using SPSS version 8.0.

RESULTS & DISCUSSION

- Clinical specimens included 22 oral, 42 genital, 29 other, and 7 not specified. No Invalid results were obtained out of 100 specimens tested with all three methods. The Aries & Simplexa was positive for 59 of 59 true positive samples (31 (53%) HSV1 positive) and negative for all 41 true negative specimens, while the TOR and LDT missed 2 and 7 of the true positives, respectively (Table 6). Based on these findings, the sensitivity and specificity were 100% and 100% for any HSV detected for Simplexa and Aries. For one sample Aries detected HSV1 in addition to the true positive HSV2, having specificity at 98.6% for HSV1. The sensitivity and specificity for any HSV detected for direct testing with the LDT were 88% and 100%.
- The LOD was determined by endpoint analysis of 10 fold serial dilutions of DNA extracted from 2 viral stocks (HSV1 & HSV2). From this analysis it was evident that the LOD of Simplexa and Aries for HSV1 were 3.57 and 3.60 log copies/ml, respectively. For HSV2, Simplexa and Aries LODs were 3.07 and 3.63 log copies/ml, respectively. While the LODs for HSV1 & HSV2 for direct testing with the LDT were 4.8 and 4.0 log copies/ml, respectively (Table 3).
- Direct testing with the LDT appears to be problematic in part due to the low sample volume being tested. Studies are ongoing to improve the assay; and preliminary data shows improvements in LOD and sensitivity of clinical specimens.
- Analysis of HSV1 & HSV2 positive controls from each assay demonstrated excellent precision (Table 4).
- Commercial Real-time PCR systems offer enhanced workflow and significantly reduced time to results as compared with the TOR and other commercial methods. Furthermore, the workflow is optimal for semi on demand testing and or batch testing.