

# Detection of Herpes Simplex Virus Type 1 by Strand Displacement Amplification on the BD ProbeTec™ ET System

D. WOLFE, T. BRINK, L. KOHLER, AND D. SHANK

BD Diagnostic Systems • 7 Loveton Circle • Sparks, MD 21152, USA

## ABSTRACT

Encephalitis due to herpes simplex virus (HSV) is the most commonly fatal of all encephalitides. Untreated, the mortality rate is 70%, compared with as low as 19% among those who receive treatment with 38% of treated patients returning to normal function. Most cases of herpes simplex encephalitis (HSE) are caused by HSV-1 although a significant portion is due to HSV-2, which predominates in cases of HSV aseptic meningitis. There is, therefore, a clinical need for rapid and sensitive tools to aid in the diagnosis of HSE. To that end, we have developed a novel Strand Displacement Amplification-based method for the real-time detection of HSV-1 using a universal fluorescent energy transfer probe on the BD ProbeTec™ ET System. We report that this system has an analytical sensitivity of < 100 genomic equivalents of HSV-1 per reaction. Preliminary data obtained with a panel of isolates from diverse locales demonstrates that the assay is specific for HSV-1 and does not exhibit cross-reactivity with HSV-2 or any of 26 bacteria, fungi, or other viruses that may be found in the cerebrospinal fluid. The HSV-1 assay has potential as a rapid diagnostic and useful menu addition to the BD ProbeTec™ ET System.

## INTRODUCTION

Encephalitis caused by the herpes simplex virus (HSV) has the highest fatality rate of all the encephalitides with an annual incidence of 1 to 4 per million.<sup>1</sup> Herpes simplex encephalitis (HSE) affects people of all ages at any time of year and in adults is thought to be due to a reactivation of a latent virus. Symptoms may include fever, headaches, seizures, an altered level of consciousness and personality change. The similarity of these symptoms to other maladies makes clinical diagnosis difficult. Untreated, the mortality rate for HSE is 70%, compared with as low as 19% among those who receive treatment. Of the treated patients, however, only 38% return to normal function.<sup>2,3</sup>

The virus is rarely detected in cerebrospinal fluid using cell culture, with only 4% of the cases being culture-positive. The "gold standard" method of diagnosis involving brain biopsies is invasive and controversial with significant risk of long-term morbidity. Serological methods are also inadequate for diagnosis of HSE due to a 2–3 week delay in appearance of antibody response after initial infection. Alternate techniques such as Computer-Assisted Tomography and Magnetic Resonance Imaging are not specific and lack sensitivity as diagnostic tools. There is therefore, a clinical need to develop a rapid and sensitive tool to aid in the diagnosis of HSE. To that end, we report on a novel Strand Displacement Amplification (SDA)-based real-time method for the detection of HSV-1 using a universal fluorescent energy transfer probe on the BD ProbeTec™ ET System.

## REFERENCES

1. Markoulatos et al. 2001. Laboratory diagnosis of common herpes virus infections of the central nervous system by a multiplex PCR assay. *J Clin Micro.* 39(12): 4426-4432.
2. Smalling et al. 2002. Minireview Molecular approaches to detecting herpes simplex virus and enterovirus in the central nervous system. *J Clin Micro.* 40(7): 2317-2322.
3. Whitley et al. 1999. Viral encephalitis. *Pediatrics in Review.* 20(6): 192-198

## METHODS

**DNA TARGET.** A fragment of the HSV-1 genome containing the SDA target region was cloned into the *Escherichia coli* plasmid vector, pUC19, and used for assay development. Analytical quantification of the plasmid stock was performed using the PicoGreen® dsDNA Quantitation assay (Molecular Probes, Inc.).

**DATA ANALYSIS.** All experiments were performed on the BD ProbeTec™ ET System (Figure 1). Data were analyzed using a novel Passes After Threshold (PAT) algorithm developed for this instrument (Figure 2). Negative samples never achieve the minimum threshold of fluorescence and are assigned a PAT value of zero. Positive samples have PAT values of between 1 and 60.

**ANALYTICAL SENSITIVITY.** To determine the limit of detection of the HSV-1 assay, SDA reactions were performed on dilutions of cloned target nucleic acid. Serial dilutions of viral particles from an HSV-1 strain were also analyzed. The stock of viral particles was enumerated by electron microscopy (Electron Microscopy Bioservices). Sixteen replicates were tested at each target level (Figure 3).

Twenty-three strains of HSV-1 from various geographical locations were tested at a 1:10, 1:1,000 and 1:100,000 dilution of the organism stock. The concentrations of the samples from Ohio State University (OSU) and Quest Diagnostics, Inc. (DGX) were not determined. However, the 2 stocks of HSV-1 from the American Type Culture Collection (ATCC) VR-260 and VR-539, were  $\sim 1.50 \times 10^4$  TCID<sub>50</sub>/μL and  $\sim 2.0 \times 10^5$  TCID<sub>50</sub>/μL respectively (Table 1).

Table 1. Testing of HSV-1 Stock Vials

Sample #	Dilution of Stock		
	1:10	1:1,000	1:100,000
OSU 0-2021			
OSU 0-450			
OSU 0-1010			
OSU 0-2526			
OSU 0-1753			
OSU D-8-1973			
OSU 7-370			
OSU 0116-3			
OSU 1136			
OSU A.P.			
ATCC VR-260			
ATCC VR-539			
DGX Clin1			
DGX Clin2			
DGX Clin3			
DGX Clin4			
DGX Clin5			
DGX Clin6			
DGX Clin7			
DGX Clin8			
DGX Clin9			
DGX Clin10			
DGX Clin19			
Positive	OSU: Ohio State University		
Negative	ATCC: American Type Culture Collection		
Suspected laboratory contaminant	DGX: Quest Diagnostics, Inc.		

- All strains were positive at 1:10 dilution of stock, except O-2526
- 20 of 23 strains were positive at 1:1,000 dilution of the stock
- 15 of 23 strains were positive at 1:100,000 dilution of the stock

**SPECIFICITY/CROSS-REACTIVITY.** Assay specificity was evaluated using 15 strains of HSV-2 and cell lysates from 26 other bacterial, viral and fungal pathogens that have the potential to be found in CSF (Tables 2 and 3). HSV-2 stocks received from OSU and DGX were not quantified and a 1:10 dilution of the parental suspension was tested. In contrast, the two stocks of HSV-2 from the ATCC were of known infective titers: VR734 at  $1.58 \times 10^9$  TCID<sub>50</sub>/μL and VR540 at  $1.58 \times 10^4$  TCID<sub>50</sub>/μL.

Table 2. Strains of HSV-2 Used in the HSV-1 Assay Cross-Reactivity Panel

Strain Number
OSU 0-2053
OSU D-8575
OSU C5
OSU 7-2667
ATCC VR-734
ATCC VR-540
DGX Clin11
DGX Clin12
DGX Clin13
DGX Clin14
DGX Clin15
DGX Clin16
DGX Clin17
DGX Clin18
DGX Clin20

OSU: Ohio State University  
 ATCC: American Type Culture Collection  
 DGX: Quest Diagnostics, Inc.

- None of the 15 HSV-2 strains cross-reacted in the BD ProbeTec™ ET HSV-1 Assay

Table 3. HSV-1 Assay Specificity/Cross-Reactivity Panel

#	Organism	Strain #	Organisms/reaction
1	Adenovirus-5	ABi 74-070	$6.6 \times 10^4$
2	<i>Blastomyces dermatitidis</i>	ATCC 4292	$4.4 \times 10^5$
3	<i>Candida albicans</i>	ATCC 44808	$1.7 \times 10^5$
4	<i>Cryptococcus neoformans</i>	ATCC 36556	$8.9 \times 10^4$
5	Cytomegalovirus (AD-169)	ABi 68-125	$6.6 \times 10^4$
6	Enterovirus (Echovirus-11)	ABi 74-084	$6.6 \times 10^4$
7	Epstein-Barr virus	SIGMA 104H0854	$6.7 \times 10^5$
8	<i>Escherichia coli</i>	ATCC 11775	$4.4 \times 10^6$
9	<i>Fusobacterium nucleatum</i>	ATCC 25586	$5.4 \times 10^5$
10	<i>Haemophilus influenzae</i>	ATCC 33533	$5.0 \times 10^6$
11	<i>Histoplasma capsulatum</i>	ATCC 12700	$4.4 \times 10^5$
12	HIV-1 (IIIB)	ABi 4314-042198	$6.6 \times 10^4$
13	<i>Listeria monocytogenes</i>	ATCC 7644	$8.2 \times 10^6$
14	<i>Mycoplasma pneumoniae</i>	ATCC 63-030	$6.7 \times 10^5$
15	<i>Neisseria meningitidis</i>	ATCC 13077	$3.6 \times 10^6$
16	<i>Propionibacterium acnes</i>	ATCC 6919	$2.7 \times 10^6$
17	<i>Pseudomonas aeruginosa</i>	ATCC 27853	$7.4 \times 10^6$
18	Respiratory Syncytial virus (Long strain)	ABi 74-093	$6.6 \times 10^4$
19	Rhinovirus	UCHSC Clin74	$1.2 \times 10^5$
20	<i>Staphylococcus aureus</i> , non-protein A-producing	ATCC 25923	$4.8 \times 10^6$
21	<i>Staphylococcus epidermidis</i>	ATCC E155	$1.8 \times 10^6$
22	<i>Streptococcus agalactiae</i>	ATCC 12386	$5.2 \times 10^6$
23	<i>Streptococcus mitis</i>	ATCC 6249	$3.2 \times 10^6$
24	<i>Streptococcus mutans</i>	ATCC 25175	$2.0 \times 10^6$
25	<i>Streptococcus pneumoniae</i>	ATCC 6303	$3.5 \times 10^5$
26	<i>Streptococcus pyogenes</i>	ATCC 19615	$3.5 \times 10^5$

ATCC = American Type Culture Collection ABi = Advanced Biotechnologies, Inc.  
 UCHSC = University of Colorado Health Science Center

- None of the 26 bacteria, viruses or fungi tested cross-reacted in the BD ProbeTec™ ET HSV-1 Assay

## RESULTS

Figure 1. BD ProbeTec™ ET System HSV-1 Workflow

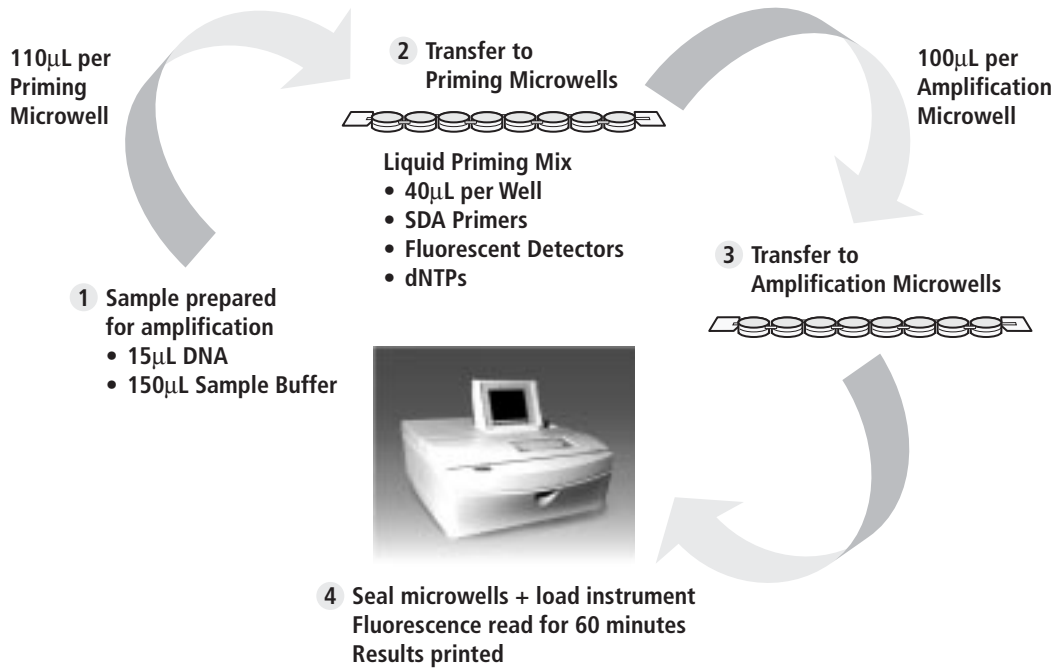


Figure 2. BD ProbeTec™ ET System PAT Algorithm

- T3 is the time at which the background corrected signal crosses a pre-determined threshold
- T3 = Time-To-Threshold
- The same threshold is used for every sample
- Passes After Threshold score = 60 - T3
- Lower T3 scores and corresponding higher PAT values correlate with more efficient SDA
- Negative samples have PAT = 0
- Positive samples have PAT < 60 (typically 40–55 depending on the assay and target level)

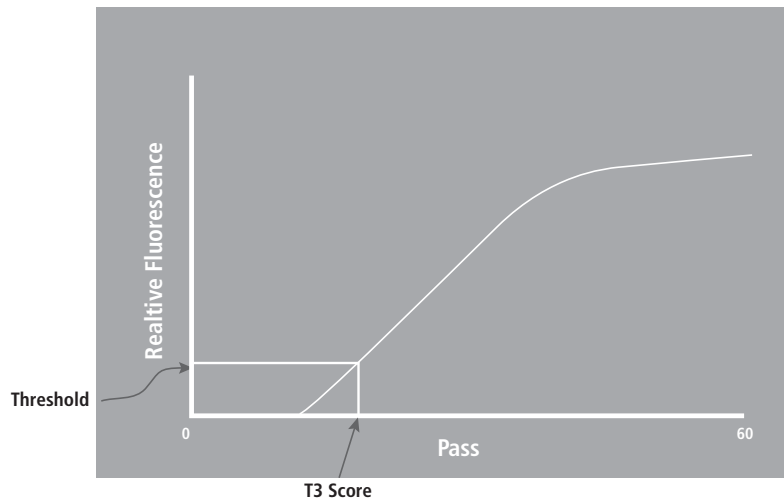
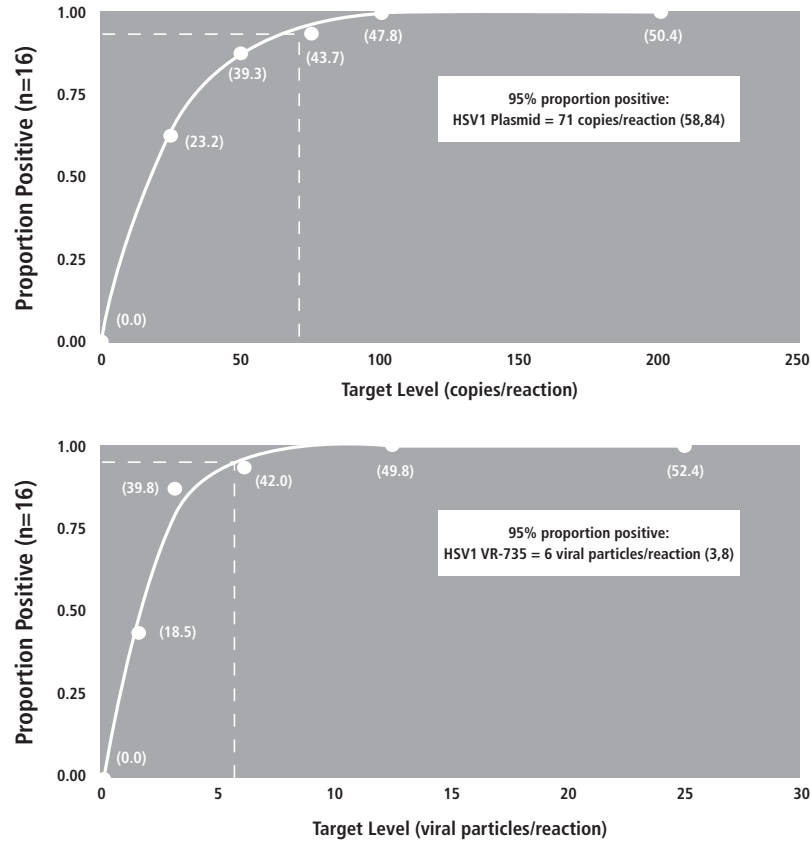


Figure 3. Limit of Detection for Cloned DNA and Viral Particles



Numbers in parenthesis represent the mean PAT scores of n = 16

### DISCUSSION AND CONCLUSION

- We have developed a novel assay for the detection of HSV-1 using Strand Displacement Amplification on the BD ProbeTec™ ET System.
  - Analytical sensitivity:
 

Plasmid DNA	71 (95% CI: 58,84)	genomic equivalents per reaction
VR735	6 (95% CI: 3,8)	viral particles per reaction
  - The HSV-1 assay is specific and does not cross-react with HSV-2 or other bacteria, viruses, or fungi likely to be encountered in CSF samples.
  - Twenty-two of twenty-three HSV-1 strains were detected by this assay at a 1:10 dilution of the HSV-1 stocks.
    - O-2526 was not detected at 1:10 and 1:1,000. This may be due to sequence variations, low titer, or assay inhibition.
- In progress:
  - Further expansion of HSV-1 sequence database.
  - An internal amplification control is being developed to verify negative results and monitor potentially inhibitory specimens.
  - Conversion of the HSV-1 assay to the user-friendly dried microwell format employed in the BD ProbeTec™ ET CT/GC Amplified DNA Assay.
  - Specimen processing format to maximize sample volume per assay and clinical sensitivity.
  - Development of a test for HSV-2 to complement the HSV-1 assay on the BD ProbeTec™ ET System.