Herpes Simplex Virus (HSV) Detection Protocol

A. Introduction

This assay tests for the presence of Herpes Simplex Virus (HSV) Types I and II using end point PCR† with a 6-FAM/BHQ-1 TaqMan® probe. A positive control (human *Glyceraldehyde 3-phosphate dehydrogenase* gene; *GAPDH*) is used to ensure that the purified DNA sample is free of PCR inhibitors.

PCR run time is about 45 min.

B. Equipment

- Spartan DX™ instrument
- Microcentrifuge
- Vortex
- Ice bucket or cold block
- Pipettes

C. Materials

- Filtered pipette tips
- 0.2 ml flat-cap PCR tubes (VWR, Cat. No. 53550-106)
- QIAamp DNA Mini Kit (QIAGEN, Cat. No. 51306)
- PCR-grade Mineral Oil (Biotools, Cat. No. 20.032)
- Primers which recognize HSV and *GAPDH* (Biosearch Technologies)(Table 1)
- TaqMan probes for HSV and *GAPDH* (Biosearch Technologies)(Table 1)
- QuantiTect Multiplex PCR NoROX Kit (QIAGEN, Cat. No. 204743)
- Sterile water (DNase- and RNase-free)
- Known HSV-positive DNA sample

Primer/Probe	Forward (5'-3')	Reverse (5'-3')	Amplicon size (bp)
HSV primers	CCg TCA gCA CCT TCA TCg A	CgC Tgg ACC TCC gTg TAg TC	124
HSV probe	6-FAM-CCA CgA gAT CAA ggA CAg Cgg CC-BHQ1		
GAPDH primers	gAA ggT gAA ggT Cgg AgT	CAT ggg Tgg AAT CAT ATT ggA A	150
GAPDH probe	6-FAM-CAA Cgg ATT Tgg TCg TAT Tgg gCg C-BHQ1		

6-FAM = 6-carboxy-fluorescein, BHQ1 = Black Hole Quencher 1

Table 1. Primer/probe sequences and amplicon sizes.

D. Preparation

- Purify DNA sample with QIAamp DNA Mini Kit as per manufacturer's instructions in the Blood and Body Fluid Spin Protocol (page 27)(http://www1.qiagen.com/HB/QIAampDNAMiniAndDNABloodMiniKit_EN)
  Minor modifications to QIAamp protocol:
  Step 6: Add 230 µl of ethanol to the sample.
  Vortex for 15 s.
  Incubate at room temperature for 10 min.
  Step 10: Elute with 100 µl of Buffer AE. Then re-apply filtrate back to column.
  Repeat incubation and centrifugation steps.
- Turn on Spartan DX instrument and let it warm up for a minimum of 10 min
- Set up thermal cycling program as per Table 2

Step	Temperature	Time	Cycles
Initial denaturation	95°C	210 s	1
Denaturation	95°C	25 s	45
Annealing/extension	47°C	30 s	45

Table 2. Cycling parameters.

E. Protocol

1. Prepare two seperate HSV and *GAPDH* master mixes, as per Table 3
2. In a seperate lab area, add the following to each tube:
    - Tube 1: 15 µl of HSV master mix
    - Tube 2: 15 µl of *GAPDH* master mix
    - Tube 3: 15 µl of HSV master mix
    - Tube 4: 15 µl of HSV master mix
3. In a separate lab area, add the following:
    - Tube 1: 5 µl of purified DNA in question
    - Tube 2: 5 µl of purified DNA in question
    - Tube 3: 5 µl of sterile water (negative control)
    - Tube 4: 5 µl of known HSV-positive DNA (positive control)
4. Mix and spin down reaction tubes
5. Overlay reaction mixtures with 15 µl of mineral oil
6. Spin down reaction tubes
7. Insert tubes into Spartan DX instrument and start your run
8. End point PCR is determined to be positive if the last cycle reading has a fluorescence value greater that 5
9. Representative real-time PCR results are shown in Figure1

Reagent	HSV Master Mix		*GAPDH* Master Mix	
	Reaction Formulation	Volume	Reaction Formulation	Volume
QuantiTect Multiplex PCR NoROX Reaction Mix (2X)	10 µl x (_____+0.5*) Sample #		10 µl x (_____+0.5*) Sample #	
Forward primer (10 µM)	0.4 µl x (_____+0.5*) Sample #		0.4 µl x (_____+0.5*) Sample #	
Reverse primer (10 µM)	0.4 µl x (_____+0.5*) Sample #		0.4 µl x (_____+0.5*) Sample #	
Probe (1 µM)	1.25 µl x (_____+0.5*) Sample #		1.25 µl x (_____+0.5*) Sample #	
Sterile water	2.95 µl x (_____+0.5*) Sample #		2.95 µl x (_____+0.5*) Sample #	
Total volume of master mix	**15 µl/reaction**	**____ µl**	**15 µl/reaction**	**____ µl**

* Recommended volume correction factor for pipetting error.

Table 3. Components of PCR master mixes for HSV and *GAPDH*.

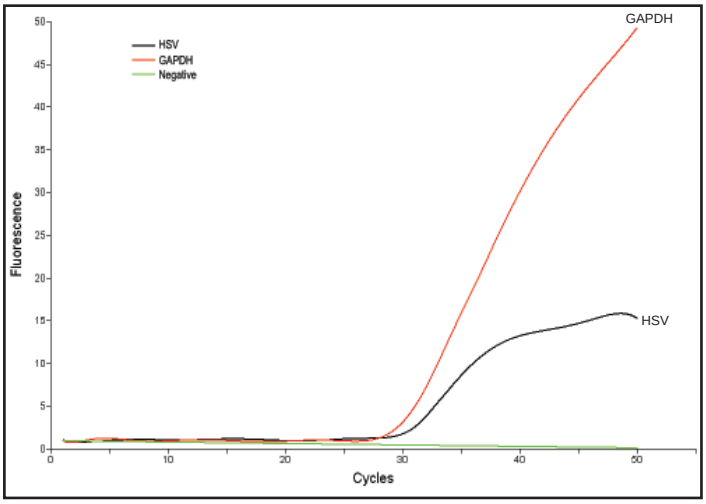

Figure 1. Real-time graph of HSV and GAPDH results.

References

1. Jerome KR et al. (2002). Quantitative stability of DNA after extended storage of clinical specimens as determined by real-time PCR. *J Clin Micro.* 40:2609-2611.

2. Scacco S et al. (2003). Pathological mutations of the human *NDUFS4* gene of the 18-kDa (AQDQ) subunit of Complex I affect the expression of the protein and the assembly and function of the Complex. *J Bio Chem*. 278:44161-44167.

† - An end point assay is described as an assay that uses data from images collected at the first and last cycles of a PCR run to determine the success or failure of the reaction. End point analysis mode is selected in the options menu of the SpartanDX™.