



UK Clinical Virology Network



Performance of in-house real-time PCR assays for the detection of herpes simplex virus types 1 and 2

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August 2006

Summary

Laboratories across the UK are using a range of different in-house assays for the detection of viral targets. A small-scale assessment of ten such assays in use for the detection for HSV-1 and -2 infections indicated that the majority of these assays perform at an equivalent level of sensitivity and with 100% specificity.

Introduction

Although UK laboratories are using different in-house assays for the detection of viral targets, their performance has not been compared. To address this, the Clinical Virology Network (CVN) set up an assessment of in-house real-time PCR assays, and in collaboration with the Evaluations Unit (EU) of the HPA developed a panel of specimens to gain some insight into how the different assays compare to enable adoption of best practice.

Materials and Methods

The CVN invited laboratories to submit Standard Operating Procedures of in-house real-time assays that are in regular diagnostic use to the study. A summary of these protocols is given in Appendix 1.

A specimen panel consisting of 22µl aliquots of extracted DNA generated from both clinical HSV cases (swab) and tissue-culture grown material was prepared by HPA-MEC. The panel incorporated specimens prepared by two different extraction methods to avoid any bias associated with this process: manual (QIAamp DNA Blood Mini Kit, Qiagen) and automated (Magna Pure LC Total Nucleic Acid kit; Roche). The panel composition is given in Table 1.

Table 1: Specimen panel composition (for HSV-1 and HSV-2)

Sample type	Extraction method	No. of tests	Details
Virus-positive tissue culture fluid	Automated	9	Extract diluted to limit of originating lab's assay \pm four fivefold dilution steps
Virus-positive tissue culture fluid	Manual	9	Extract diluted to limit of originating lab's assay \pm four fivefold dilution steps
Clinical sample (swab)	Automated	7	Clinical material from six known HSV-positive and one negative
TOTAL		50 (25 HSV-1; 25 HSV-2)	

Appropriate dilutions of the extracted material (prepared using the appropriate elution buffer) were established by HPA-EU using the routine diagnostic assay of the HPA Sexually Transmitted Bacteria Reference Laboratory (STBRL) (Appendix 1). The panel numbers were randomised and the panel distributed on dry ice to the participant laboratories, to be stored at -70°C and tested within 10 working days of receipt.

Results

Nine laboratories submitted SOPs for inclusion in the study, all of which returned results. Respondents provided information on Ct (cycle at which the signal crosses the threshold signifying positivity), subtype (where available), run validation, and interpretation of results. One laboratory (L8) received the panel at room temperature, but completed testing following storage at 4°C for three days. One laboratory (L4) sent independent result sets from two different assays and real-time machines (Stratagene MX3000 bplex assay used in their laboratory for genital specimens or confirmatory testing, and Roche LightCycler assay for all other specimen types).

Of the SOPs submitted, all but two determined HSV type in the detection reaction with a single amplification; Laboratories L6 and L8 performed type-specific assays. One laboratory (L3) diluted the panel prior to use, resulting in a requirement for retesting and specimen volume issues.

Laboratories L7 and L8 commented that they would normally have co-extracted an internal control in order to validate their results.

All laboratories correctly identified HSV types 1 and 2 with two exceptions. One HSV-2 positive clinical specimen (panel number HSV29) was untypable by three laboratories using typing by melting point analysis (Labs L4 (LC), L5 and L9; LightCycler, Roche). However, it was correctly identified by Lab L6, which appears to use the same method as L4, L5 and L9. Lab L4 noted that they use a second assay (Mx3000p, Stratagene) to investigate such anomalies, and this resulted in the correct assignation of HSV-2. Lab L9 provisionally misidentified two specimens from HSV-1 dilutions as HSV-2 positive (due to dual peaks in the melting point analysis). These are considered as untypable, and would normally be retested using a confirmatory typing assay (Artus; Qiagen); this was not possible in this study due to volume constraints.

Inter-laboratory Ct values are given in Appendix 2 (together with details of the panel composition), and are represented graphically in Figures 1a (dilutions of tissue-culture material) and 1b (Clinical specimens). Table 2 summarises the number of positives obtained by each laboratory for the HSV-1 and HSV-2 dilutions; the majority of labs had similar levels of detection, with the exception of Lab L3 which is an assay undergoing evaluation and not in routine diagnostic use. There is a suggestion that Lab L6 might be slightly less sensitive for the detection of HSV-2: 8 positives from the dilution series were detected compared to an average of 10.6 and with higher Ct values, plus a failure to detect one of the HSV-2 positive specimens derived from a clinical specimen (panel no. HSV19). The final results for each panel member are represented in Figure 2.

Table 2: Number of positive reactions from each laboratory

Lab Identification ¹	HSV-1 dilutions (n=18)	HSV-2 dilutions (n=18)	HSV-1 positive clinical specimens (n=6)	HSV-2 positive clinical specimens (n=6)
HPA	12	13	6	6
L1	10	12	6	6
L2	11	12	6	6
L3	4	8	5	6
L4 (LC)	10	11	6	6
L4 (S)	9	9	6	6
L5	10	11	6	6
L6	9	8	6	5
L7	9	12	6	6
L8	10	10	6	6
L9	9+2 ²	11	6	6 ³
Average	9.4	10.6	5.9	5.9

1: HPA: source laboratory; the assay from Lab L3 is undergoing evaluation and is not in routine diagnostic use. Lab L8 received the panel at room temperature

2: 9 dilutions correctly identified as HSV-1; 2 members initially identified as HSV-2 positive, but subsequently recorded as non-typable; these would normally have be subjected to a second typing assay for confirmation prior to reporting

3: includes one specimen where no Ct was given, but written information suggests a non-typable positive obtained.

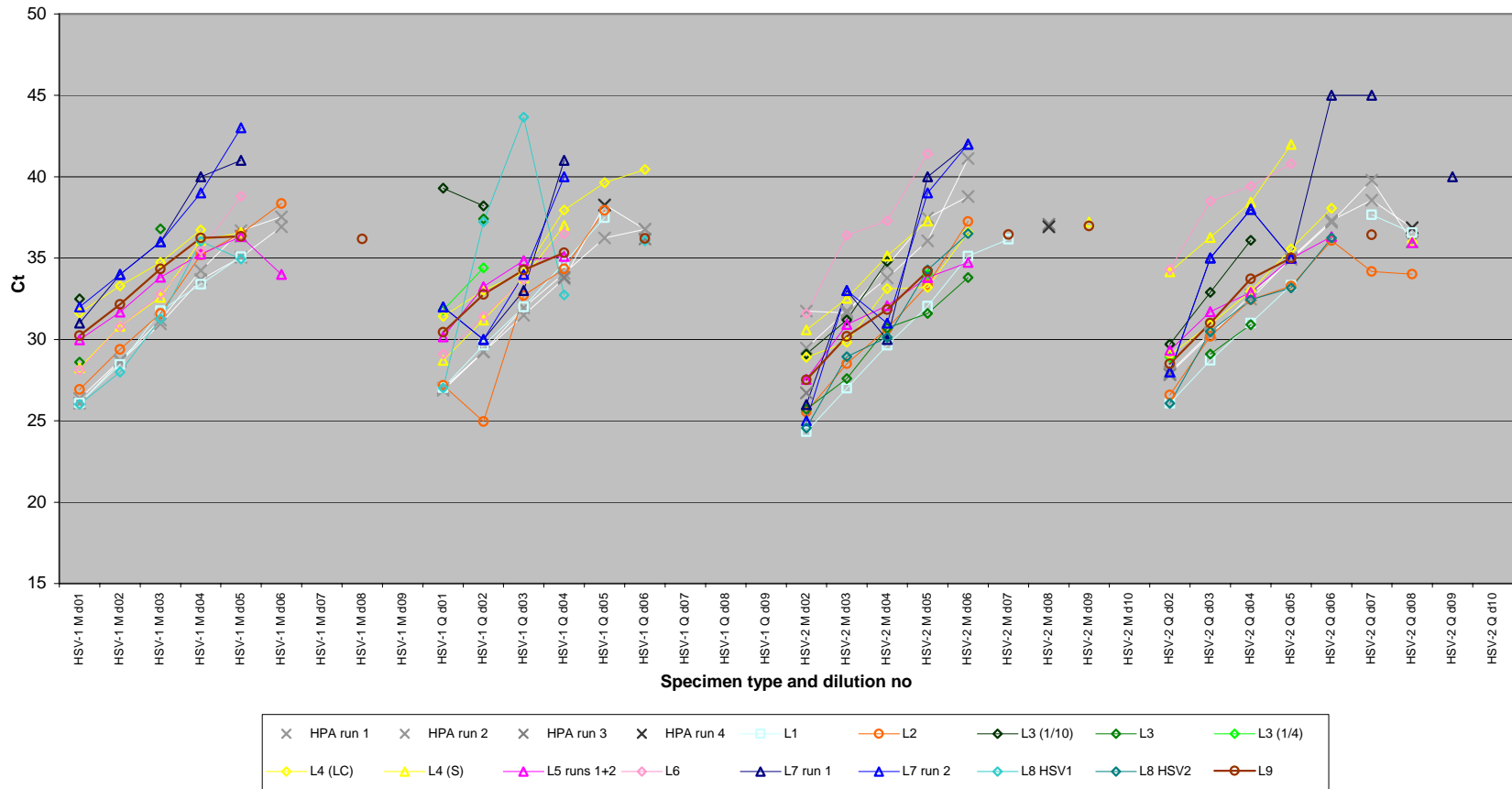


Figure 1a: Graphical representation of Ct results from dilutions of tissue-culture grown material

Key: (LC): LightCycler; (S): Stratagene, numbers in parentheses indicate dilution of input material, Q: QIAamp (manual) and M: MagNA Pure (automated) extraction.

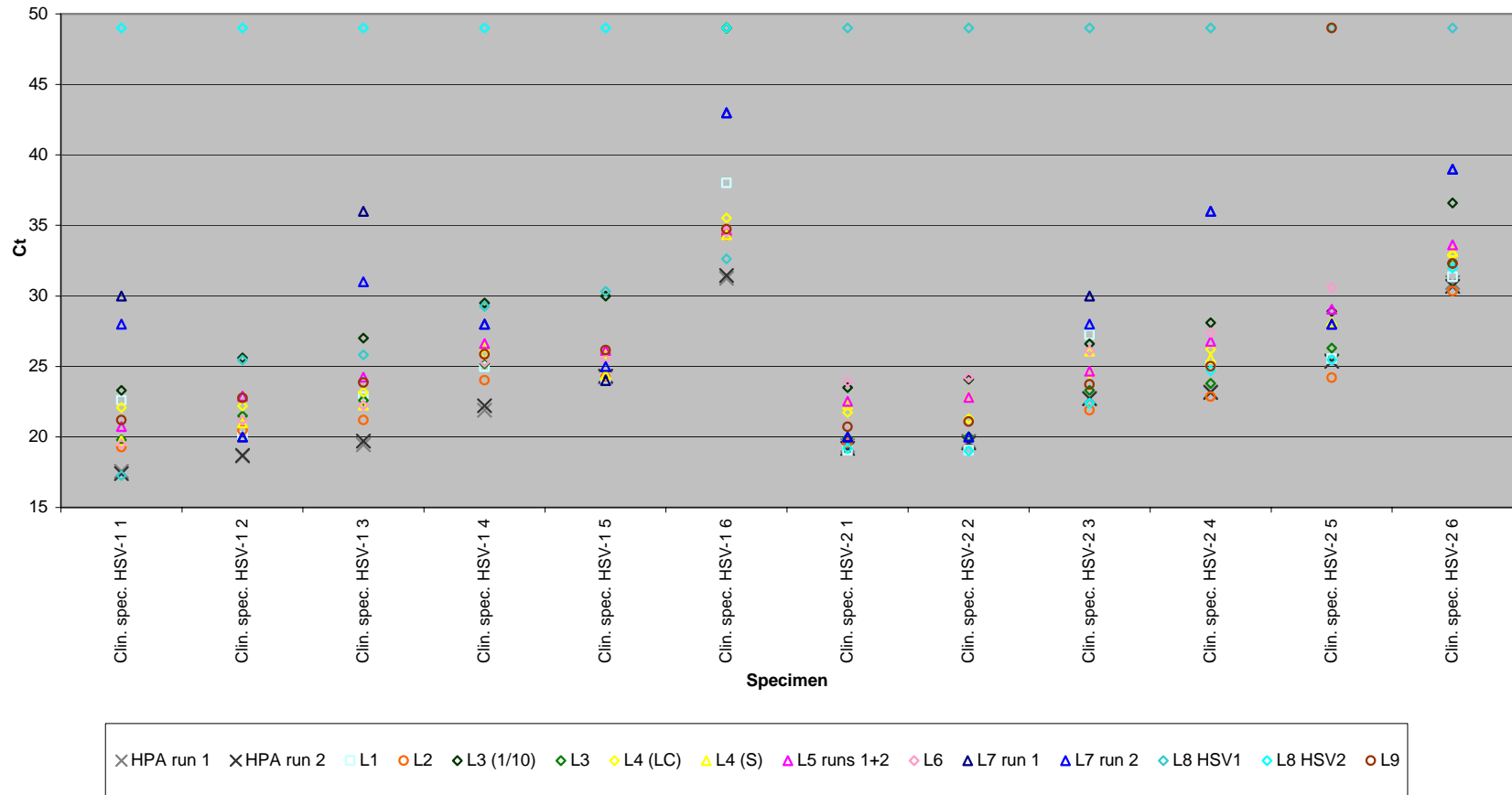


Figure 1b: Graphical representation of Ct results from clinical specimens

Key: (LC): LightCycler; (S): Stratagene, numbers in parentheses indicate dilution of input material. Negatives were assigned a Ct of 49 for the purposes of graphical representation in this figure.

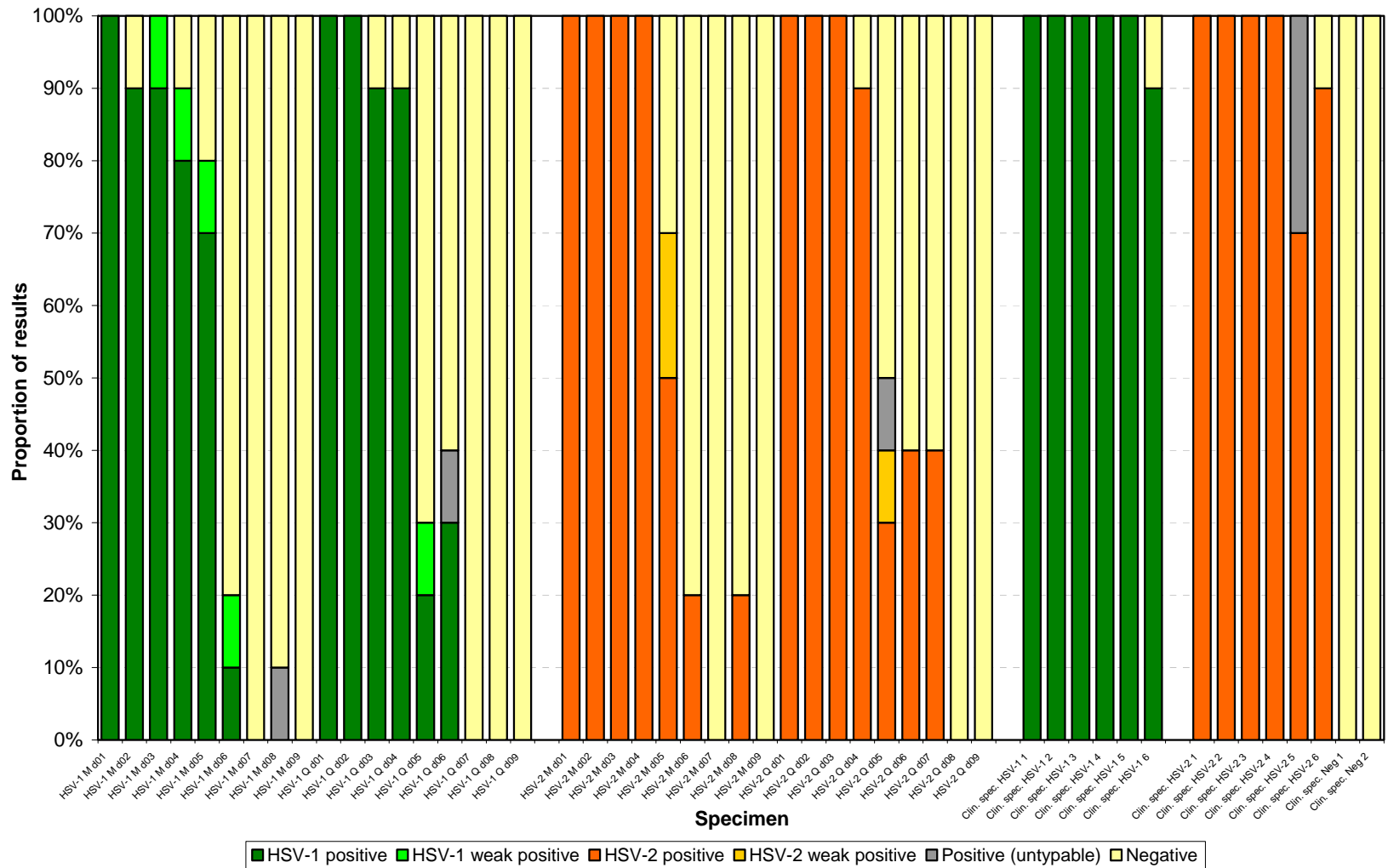


Figure 2: Distribution of final results from the panel specimens
M: MagNA Pure extracted; Q: QIAamp extracted; dX: dilution number

Conclusion

There are a range of in-house real-time PCR assays in use across the UK, which use different chemistries and platforms (Appendix 1). In general, the results were comparable from all of these assays and there was no difference between the results obtained from the two extraction methods. Lab L8 received the panel at room temperature and stored it at 4 °C, but there is no indication that this affected the results obtained. Lab L3 performed less well than the others, but the assay used is undergoing evaluation rather than being in routine diagnostic use. There is a suggestion that Lab L6 may have a reduced sensitivity for HSV-2. Three laboratories apparently using the same method were unable to subtype one extract from an HSV-2 positive swab specimen (typing by melting point analysis), and one of these laboratories was also unable to subtype two HSV-1 dilution specimens. One lab failed to amplify one of the clinical specimens.

In conclusion, the majority of in-house real-time PCRs for the detection of HSV-1 and HSV-2 performed at a more or less equivalent level of sensitivity in this small-scale assessment.

Acknowledgements

We would like to thank members of STBRL (especially Michelle Cole and Sarah Alexander) for providing the clinical specimens used in this assessment, and for access to their laboratory, equipment and reagents.

Appendices

Appendix 1: Assay summary from SOP's provided

Lab ID	Platform	Input vol	Detection method ¹	Gene target ²	Comments	Reference
HPA	Rotor-Gene 2000 (Corbett)	10µl		gD (HSV), gG (typing)	Originating laboratory assay. Multiplex for GUD (HSV, <i>Treponema pallidum</i> and <i>Haemophilus ducreyi</i>)	CDC (Atlanta); unpublished
L1	ABI 7500 (Applied BioSystems)	10µl	DLP	gG/gD	Specimens tested neat and at 1:10 dilution	None given
L2	ABI 7500 (Applied BioSystems) or Rotor-Gene 3000 (Corbett)	10µl	DLP	gG/gD		Scoular A, Gillespie G, Carman W. (2002). <i>Sex Transm Inf</i> 78 21-25.
L3	ABI 7500 (Applied BioSystems)	10µl (neat plus 1:10)	DLP	gG/gD	Assay under development and not in routine use.	Scoular A, Gillespie G, Carman W. (2002). <i>Sex Transm Inf</i> 78 21-25.
L4 (S)	Mx3000p (Stratagene)	5µl	DLP	gG (HSV2) /gD (HSV1)		Weidmann M, Meyer-Konig U & Hufert FT. (2003). <i>J Clin Micro</i> 41 1565-1568.
L4 (R)	LightCycler (Roche)	5µl	FRET	Pol	HSV-1 and -2 distinguished by melting point analysis	Burrows J <i>et al.</i> (2002) <i>BMC Microbiol</i> 2 2-12. (method based on Espy MJ <i>et al.</i> (2000). <i>J Clin Micro</i> 38 795-799 modified by TIB Molbiol)
L5	LightCycler (Roche)	?	FRET	Pol	HSV-1 and -2 distinguished by melting point analysis	Espy MJ, Uhk JR <i>et al.</i> (2000). <i>J Clin Micro</i> 38 795-799.
L7	ABI 7700/ 7900 (Applied BioSystems)	5µl	DLP	Not specified	Internal amplification control used.	None given
L6	ABI 7000 (Applied BioSystems)	5µl	DLP	UL42/gpG	HSV-1/-2 detected in separate reactions	Aberle SW & Puchammer-Stocke E. (2002). <i>J Clin Virol</i> 25 79-85.
L8	ABI 7000 (Applied BioSystems)	10µl	DLP	gG (HSV2) /gD (HSV1)	Internal control added prior to extraction	None given
L9	LightCycler (Roche)	5µl	FRET	Pol	HSV-1 and -2 distinguished by melting point analysis from a single reaction	Espy MJ, Uhk JR <i>et al.</i> (2000). <i>J Clin Micro</i> 38 795-799.

1. FRET: Fluorescence resonance energy transfer; DLP: Dual-labelled probes (also known as TaqMan or hydrolysis probes). 2. gG: glycoprotein G; gD: glycoprotein D; SOP: standard operating procedure.

Appendix 2a: Panel details and Ct results by laboratory for diluted tissue culture material

Panel no	Extn method	Specimen Details	Dilution	Ct results (by laboratory)																		
				HPA1	HPA2	HPA3	HPA4	L1	L2	L3 (1/10)	L3	L3 (1/4)	L4 (LC)	L4 (S)	L5 (1)	L5 (2)	L6	L7 (1)	L7 (2)	L8 HSV1	L8 HSV2	L9
HSV33	M	Tissue culture (HSV-1) d1	1:160	26.08	26.37	nt	nt	26.09	26.92	32.5	28.6	nt	31.62	28.25	29.98	nt	28.1	31	32	26.01	neg	30.24
HSV39	M	Tissue culture (HSV-1) d2	1:800	28.64	28.72	nt	nt	28.46	29.38	>40	>40	nt	33.3	30.81	31.67	nt	30.8	34	34	28	neg	32.15
HSV26	M	Tissue culture (HSV-1) d3	1:4000	30.95	31.19	nt	nt	31.75	31.59	>40	36.8	>40	34.73	32.59	33.82	nt	32.8	36	36	31.26	neg	34.34
HSV45	M	Tissue culture (HSV-1) d4	1:20,000	33.67	34.24	nt	nt	33.39	35.2	>40	>40	nt	36.73	36.1	35.24	nt	35.5	40	39	36.03	neg	36.23
HSV38	M	Tissue culture (HSV-1) d5	1:100,000	35.05	36.68	36.35	36.35	35.09	36.51	>40	>40	nt	neg	36.57	36.31	nt	38.8	41	43	34.94	neg	36.33
HSV24	M	Tissue culture (HSV-1) d6	1:500,000	36.92	37.54	neg	neg	neg	38.35	>40	>40	nt	neg	neg	34	nt	neg	neg	neg	neg	neg	neg
HSV10	M	Tissue culture (HSV-1) d7	1:2,500,000	neg	neg	neg	neg	neg	neg	>40	>40	nt	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
HSV20	M	Tissue culture (HSV-1) d8	1:12,500,000	neg	neg	nt	nt	neg	neg	>40	>40	nt	neg	neg	neg	neg	neg	neg	neg	neg	neg	36.18
HSV12	M	Tissue culture (HSV-1) d9	1:62,500,000	neg	neg	nt	nt	neg	neg	>40	>40	nt	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
HSV35	Q	Tissue culture (HSV-1) d1	1:160	27.01	26.87	nt	nt	27.03	27.18	39.3	nt	31.8	31.42	28.71	30.15	nt	29.1	32	32	27.02	neg	30.45
HSV06	Q	Tissue culture (HSV-1) d2	1:800	29.22	29.25	nt	nt	29.65	24.96	38.2	37.4	34.4	33.02	31.2	33.24	nt	31.4	30	30	37.23	neg	32.76
HSV13	Q	Tissue culture (HSV-1) d3	1:4000	31.48	31.95	nt	nt	31.99	32.7	>40	>40	nt	34.11	33.78	34.85	nt	33.7	33	34	43.66	neg	34.27
HSV28	Q	Tissue culture (HSV-1) d4	1:20,000	33.74	34.06	33.82	33.82	34.71	34.33	>40	>40	nt	37.95	37	35.1	nt	36.5	41	40	32.74	neg	35.32
HSV09	Q	Tissue culture (HSV-1) d5	1:100,000	38.29	36.24	neg	38.25	37.49	37.94	>40	>40	nt	39.63	neg	neg	neg	neg	neg	neg	neg	neg	neg
HSV30	Q	Tissue culture (HSV-1) d6	1:500,000	36.77	36.77	36.17	nt	neg	neg	>40	>40	nt	40.44	neg	neg	neg	neg	neg	neg	36.06	neg	36.21
HSV25	Q	Tissue culture (HSV-1) d7	1:2,500,000	neg	neg	neg	neg	neg	neg	>40	>40	nt	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
HSV31	Q	Tissue culture (HSV-1) d8	1:12,500,000	neg	neg	nt	nt	neg	neg	>40	>40	nt	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
HSV01	Q	Tissue culture (HSV-1) d9	1:62,500,000	neg	neg	nt	nt	neg	neg	>40	>40	nt	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
HSV34	M	Tissue culture (HSV-2) d1	1:800	31.74	29.46	26.71	26.71	24.33	25.54	29.1	25.7	nt	28.9	30.58	27.55	nt	31.6	26	25	neg	24.55	27.52
HSV08	M	Tissue culture (HSV-2) d2	1:4000	31.63	31.75	nt	nt	27.01	28.51	31.2	27.6	nt	29.82	32.52	30.92	nt	36.4	33	33	neg	28.94	30.18
HSV27	M	Tissue culture (HSV-2) d3	1:20,000	34.53	33.78	nt	nt	29.64	30.66	34.8	30.7	nt	33.12	35.14	32.08	nt	37.3	30	31	neg	30.12	31.83
HSV21	M	Tissue culture (HSV-2) d4	1:100,000	37.47	36.06	nt	nt	32.05	33.34	>40	31.6	34	33.2	37.28	33.8	nt	41.4	40	39	neg	34.27	34.19
HSV17	M	Tissue culture (HSV-2) d5	1:500,000	38.78	41.11	nt	nt	35.11	37.25	>40	33.8	>40	36.59	neg	34.74	nt	neg	42	42	neg	36.51	neg
HSV03	M	Tissue culture (HSV-2) d6	1:2,500,000	neg	neg	nt	nt	36.16	neg	>40	>40	nt	neg	neg	neg	neg	neg	neg	neg	neg	neg	36.45
HSV50	M	Tissue culture (HSV-2) d7	1:12,500,000	37.07	36.92	37.07	nt	neg	neg	nt	>40	>40	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
HSV22	M	Tissue culture (HSV-2) d8	1:62,500,000	nt	nt	neg	neg	neg	neg	>40	>40	nt	neg	37.22	neg	neg	neg	neg	neg	neg	neg	36.98
HSV36	M	Tissue culture (HSV-2) d9	1:125,000,000	nt	nt	neg	neg	neg	neg	>40	>40	nt	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
HSV43	Q	Tissue culture (HSV-2) d1	1:800	27.9	28	27.83	27.83	26.08	26.59	29.7	nt	28.9	29.05	34.16	29.32	nt	34.3	28	28	neg	26.07	28.5
HSV02	Q	Tissue culture (HSV-2) d2	1:4000	30.29	30.41	nt	nt	28.71	30.18	32.9	29.1	nt	30.91	36.27	31.7	nt	38.5	35	35	neg	30.48	31.01
HSV14	Q	Tissue culture (HSV-2) d3	1:20,000	32.73	32.48	nt	nt	31.01	32.45	36.1	30.9	nt	32.97	38.43	32.89	nt	39.4	38	38	neg	32.44	33.72
HSV44	Q	Tissue culture (HSV-2) d4	1:100,000	34.92	35	nt	nt	33.32	33.28	>40	>40	nt	35.56	41.99	34.97	nt	40.8	35	35	neg	33.16	35
HSV23	Q	Tissue culture (HSV-2) d5	1:500,000	37.2	37.31	nt	nt	neg	36.07	>40	>40	nt	38.06	neg	neg	36.31	neg	45	neg	neg	36.23	neg
HSV11	Q	Tissue culture (HSV-2) d6	1:2,500,000	39.79	38.55	nt	nt	37.66	34.18	>40	>40	nt	neg	neg	neg	neg	neg	45	neg	neg	neg	36.43
HSV46	Q	Tissue culture (HSV-2) d7	1:12,500,000	35.99	36.86	35.99	nt	36.56	34.01	nt	>40	>40	35.98	neg	35.95	nt	neg	neg	neg	neg	neg	neg
HSV42	Q	Tissue culture (HSV-2) d8	1:62,500,000	nt	nt	neg	neg	neg	neg	>40	>40	nt	neg	neg	neg	neg	neg	40	neg	neg	neg	neg
HSV48	Q	Tissue culture (HSV-2) d9	1:125,000,000	nt	nt	neg	neg	neg	neg	>40	>40	nt	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg

HSV-1 specimens are shown in green, HSV-2 in orange, negatives in blue, untypables in yellow. dX represents the dilution number. neg: not detected; nt: not tested. Extraction method: M=MagNA Pure; Q=QIAamp. Ct results key: (LC): LightCycler; (S): Stratagene, fractions in parentheses indicate dilution of input material.

Appendix 2b: Panel details and Ct results by laboratory for clinical specimens

Panel no	Extn method	Specimen Details	Dilution	Ct results (by laboratory)																
				HPA1	HPA2	L1	L2	L3 (1/10)	L3	L3 (1/4)	L4 (LC)	L4 (S)	L5 (1)	L5 (2)	L6	L7 (1)	L7 (2)	L8HSV1	L8 HSV2	L9
HSV32	M	Clin. Spec. (HSV-1)	1:2	17.56	17.39	22.6	19.28	23.3	19.8	nt	22.08	19.74	20.74	nt	19.5	30	28	17.25	neg	21.2
HSV15	M	Clin. Spec. (HSV-1)	1:4	18.63	18.68	20.01	20.48	25.6	21.5	nt	22.2	21	22.89	nt	21.2	20	20	25.46	neg	22.77
HSV04	M	Clin. Spec. (HSV-1)	1:2	19.45	19.71	22.86	21.2	27	22.6	nt	23.17	22.27	24.23	nt	22.25	36	31	25.82	neg	23.85
HSV07	M	Clin. Spec. (HSV-1)	1:2	21.89	22.21	24.98	24.01	29.5	25.2	nt	25.89	26.39	26.62	nt	25.3	28	28	29.26	neg	25.87
HSV18	M	Clin. Spec. (HSV-1)	1:10	24.24	24.32	24.2	24.27	30	25.9	nt	25.88	24.4	26.13	nt	25.5	24	25	30.33	neg	26.15
HSV47	M	Clin. Spec. (HSV-1)	1:2	31.24	31.44	38.01	34.28	nt	>40	>40	35.53	34.35	34.7	nt	34.6	43	43	32.63	neg	34.73
HSV40	M	Clin. Spec. (HSV-2)	1:8	19.39	19.19	19.05	19.58	23.5	19.9	nt	21.73	22.32	22.53	nt	23.9	20	20	neg	19.18	20.72
HSV49	M	Clin. Spec. (HSV-2)	1:5	19.71	19.58	19.05	14.41	nt	20	24.1	21.29	22.85	22.8	nt	24.2	20	20	neg	19.01	21.08
HSV41	M	Clin. Spec. (HSV-2)	1:2	22.74	22.7	27.26	21.9	26.6	23.3	nt	24.5	26.08	24.66	nt	26.1	30	28	neg	22.39	23.73
HSV05	M	Clin. Spec. (HSV-2)	1:2	23.21	23.15	25.06	22.85	28.1	23.8	nt	26.21	25.54	26.78	nt	27.4	36	36	neg	24.72	25.01
HSV29	M	Clin. Spec. (HSV-2)	1:2	25.4	25.37	25.54	24.2	28.9	26.3	nt	27.98	28.15	29.05	nt	30.6	28	28	neg	25.48	Equiv
HSV19	M	Clin. Spec. (HSV-2)	1:4	30.96	30.67	31.35	30.34	36.6	32.3	nt	32.9	33.13	33.63	nt	neg	39	39	neg	31.98	32.29
HSV37	M	Clin. Spec. (neg)	1:3	neg	neg	neg	neg	>40	>40	nt	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
HSV16	M	Clin. Spec. (neg)	1:3	neg	neg	neg	neg	>40	>40	nt	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg

HSV-1 specimens are shown in green, HSV-2 in orange, negatives in blue, untypables in yellow. neg: not detected; nt: not tested; equiv: equivocal. Extraction method: M=MagNA Pure; Q=QIAamp
 Ct results key: (LC): LightCycler; (S): Stratagene, fractions in parentheses indicate dilution of input material.