

Validation of Alternate Sample Collection Devices for Testing Genital Samples in the MultiCode-RTx[®] HSV PCR Assay

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T-2



Abstract

The purpose of this study was to validate the suitability of an expanded array of specimen transport media, namely, APTIMA[®] (GenProbe Inc.), E-Swab (COPAN Diagnostics Inc.), ThinPrep[®] (Hologic Inc.), and SurePath[™] (Becton Dickinson Inc.) for use in the detection of Herpes Simplex Virus (HSV) DNA using a previously validated real-time PCR assay. The following four parameters were assessed: 1) Confirmation of substantial equivalence of the limit of detection (LOD) for the novel sample types with the currently validated sample type (Universal Transport Media; UTM). 2) Generation of clinical validation data supporting the acceptability and comparability of APTIMA, E-Swab, ThinPrep and SurePath for use in the MultiCode-RTx (EraGen Biosciences Inc.) HSV-RTx PCR assay (VML-HSV-RTx). 3) Determination of specimen stability (nucleic acid integrity) at relevant storage temperatures and times for APTIMA, E-Swabs, ThinPrep and SurePath collection devices. 4) Comparability of assay performance between the current user-developed MultiCode-RTx HSV assay, MultiCode-RTx using in-vitro diagnostic (IVD) cleared reagents on an alternate instrument platform, and the IVD-cleared MultiCode-RTx assay.

The results of these experiments indicate the analytical LOD values for both HSV-1 and HSV-2 were comparable amongst the sample types evaluated and substantially equivalent to the currently claimed value for UTM. Positive and negative concordance, assay accuracy and stability were acceptable for all sample types. Crossover experiments demonstrated that comparable assay performance to that obtained using the VML-HSV-RTx version of the assay could be expected using the IVD cleared MultiCode-RTx kit with either the cleared instrument (LightCycler v1.2 - Roche) and IVD data reduction software, or the Rotor-Gene Q (QIAGEN) and the RUO data reduction software.

Methods

In brief, the VML-HSV-RTx PCR assay was performed as follows: nucleic acid was recovered from Gen-Probe APTIMA swabs, Copan Diagnostics E-Swabs, Hologic ThinPrep cytology vials and BD Diagnostic SurePath cytology vials using the MagNA Pure LC Total Nucleic Acid Kit (Roche) – followed by amplification and detection in the Rotor-Gene Q (QIAGEN).

The multiplex design allowed for differentiation of HSV-1 and HSV-2 by melt peaks, 84.0°C to 86.0°C and 87.0°C to 89.0°C respectively.

Crossover experiments were conducted using the IVD cleared MultiCode-RTx kit with the cleared instrument (LightCycler v1.2) and IVD data reduction software, as well as with the Rotor-Gene Q and the RUO data reduction software.

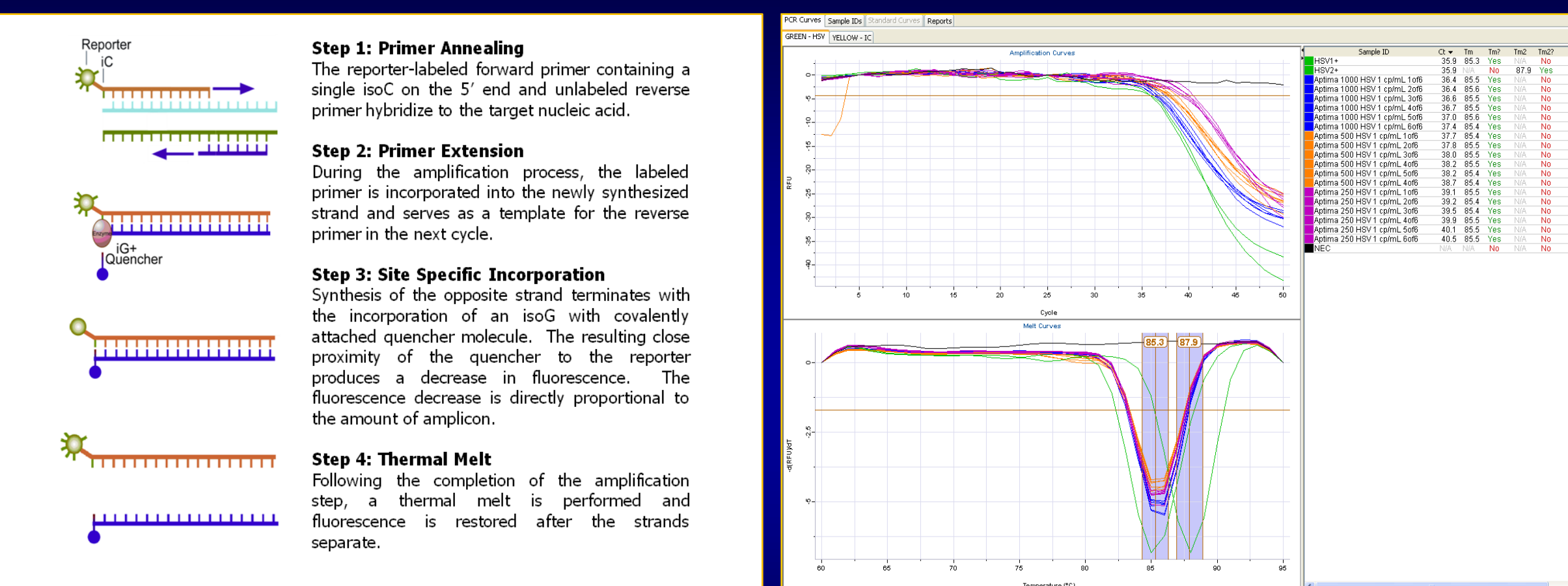


Figure 1. Schematic illustration of MultiCode[®]-RTx system and instrument readout from HSV RTx.

Results

Sample Matrix	Nominal conc. (HSV-1 cp/mL)	PCR Results (Pos/Total) ^a	Mean Ct value (95% CI)	Derived LOD ^b (95% CI)
APTIMA	1000	24/24 (100%)	36.7 (35.8 - 37.6)	269 HSV-1 cp/mL (147 - 323 cp/mL)
	500	24/24 (100%)	35.4 (34.4 - 36.4)	
	250	21/24 (87.5%)	39.0 (37.2 - 40.8)	
E-Swab	1000	24/24 (100%)	35.8 (34.5 - 37.1)	225 HSV-1 cp/mL (165 - 312 cp/mL)
	500	24/24 (100%)	37.6 (36.1 - 39.1)	
	250	23/24 (95.8%)	38.4 (36.6 - 40.2)	
ThinPrep	1000	24/24 (100%)	36.9 (35.3 - 38.5)	550 HSV-1 cp/mL (368 - 741 cp/mL)
	500	23/24 (95.8%)	38.1 (35.3 - 40.9)	
	250	15/24 (62.5%)	39.9 (37.3 - 42.5)	
SurePath	1000	24/24 (100%)	36.7 (35.5 - 37.9)	269 HSV-1 cp/mL (130 - 373 cp/mL)
	500	24/24 (100%)	37.4 (35.7 - 39.1)	
	250	22/24 (91.7%)	38.3 (35.9 - 40.7)	

Table 1. Confirmation of HSV-1 LOD for APTIMA, E-Swab, ThinPrep and SurePath collection devices in the VML-HSV-RTx PCR assay. (a) Positive indicates that a Ct value of ≤ 40 with an appropriate peak T_m value was obtained. (b) Probit analysis on data shown indicated HSV-1 LOD values for endocervical and vaginal samples of between 225 cp/mL and 550 cp/mL, based on a 95% probability of a positive result.

Sample Matrix	Nominal conc. (HSV-2 cp/mL)	PCR Results (Pos/Total) ^a	Mean Ct value (95% CI)	Derived LOD ^b (95% CI)
APTIMA	1000	24/24 (100%)	35.2 (34.4 - 36.0)	≤ 250 HSV-1 cp/mL (nd) ^c
	500	24/24 (100%)	37.0 (35.9 - 38.1)	
	250	24/24 (100%)	38.0 (36.5 - 39.5)	
E-Swab	1000	24/24 (100%)	34.8 (34.0 - 35.6)	≤ 250 HSV-1 cp/mL (nd) ^c
	500	24/24 (100%)	36.3 (35.2 - 37.4)	
	250	24/24 (100%)	35.7 (34.1 - 37.3)	
ThinPrep	1000	24/24 (100%)	35.9 (34.5 - 37.3)	700 HSV-2 cp/mL (575 - 1020 cp/mL)
	500	20/24 (83.3%)	38.7 (36.1 - 41.3)	
	250	16/24 (66.7%)	39.7 (38.5 - 40.9)	
SurePath	1000	24/24 (100%)	37.1 (35.7 - 38.5)	273 HSV-2 cp/mL (222 - 475 cp/mL)
	500	24/24 (100%)	38.0 (36.1 - 39.9)	
	250	17/24 (70.8%)	39.7 (38.5 - 40.9)	

Table 2. Confirmation of HSV-2 LOD for APTIMA, E-Swab, ThinPrep and SurePath collection devices in the VML-HSV-RTx PCR assay. (a) Positive indicates that a Ct value of ≤ 40 with an appropriate peak T_m value was obtained. (b) Probit analysis on data shown indicated HSV-2 LOD values for endocervical and vaginal samples of between ≤ 250 cp/mL and 700 cp/mL, based on a 95% probability of a positive result. (c) LOD values could not be determined for these sample types because all replicates were positive at all concentrations tested.

Sample Type	HSV-1 PCR Result	Expected HSV-1 Result	
		Positive	Negative
APTIMA	Positive	30	0
	Negative	0	50
E-Swab	Positive	30	0
	Negative	0	50
ThinPrep	Positive	30	0
	Negative	0	50
SurePath	Positive	30	0
	Negative	0	50

Sample Type	HSV-2 PCR Result	Expected HSV-2 Result	
		Positive	Negative
APTIMA	Positive	30	0
	Negative	0	50
E-Swab	Positive	30	0
	Negative	0	50
ThinPrep	Positive	30	0
	Negative	0	50
SurePath	Positive	30	0
	Negative	0	50

Tables 3 and 4. Concordance of observed with expected results for HSV-1 and HSV-2. Blinded challenge panels were constructed and tested using each collection device. Positive samples were generated by spiking confirmed HSV-PCR negative residual samples with either HSV-1 or HSV-2 viral culture supernatants to mimic low, medium and high positive samples.

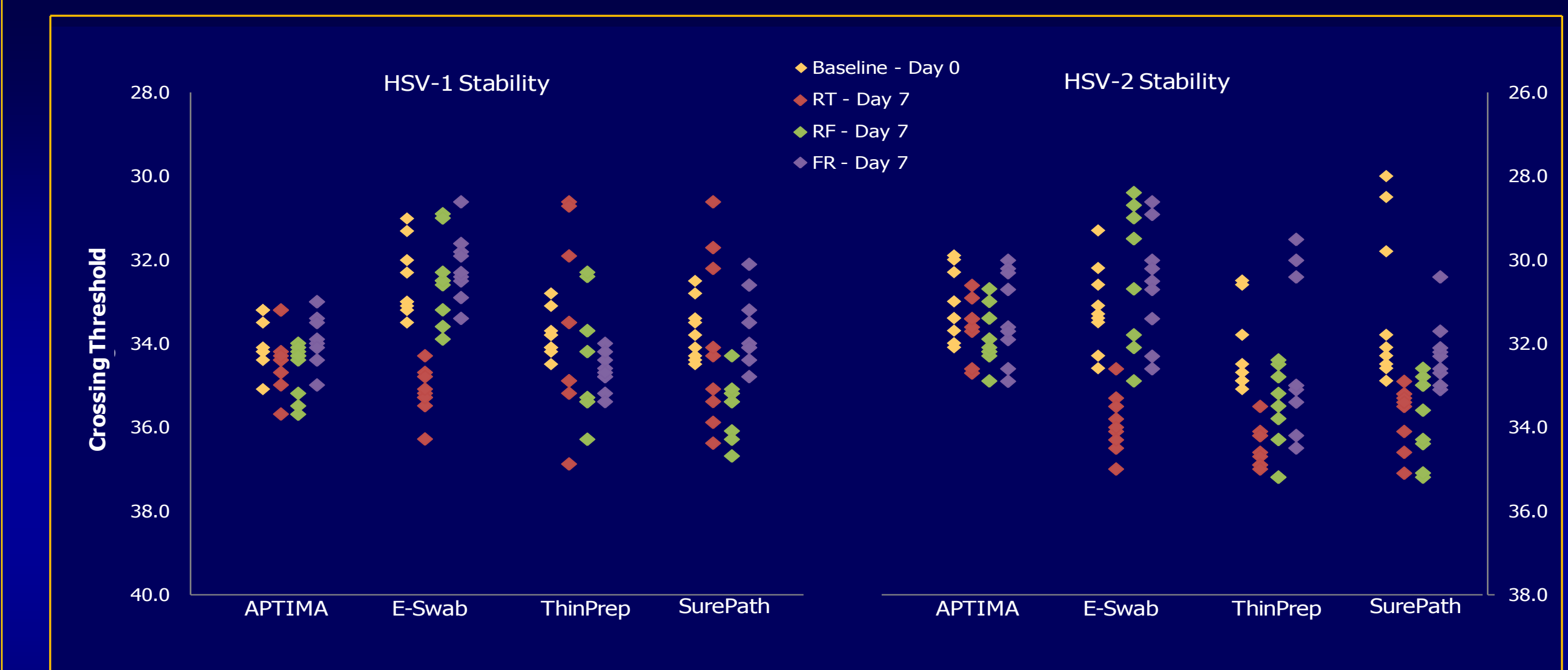


Figure 2. Results of HSV-1 and HSV-2 PCR sample stability studies. All samples were tested in triplicate under each storage condition (room temperature, refrigerated and frozen) examined. A storage condition was considered acceptable only if all replicates tested generated a positive result in the HSV-RTx PCR assay and if the mean Δ Ct value of replicates tested under that condition differed from the mean baseline value by ≤ 3.0 cycles (representing approximately 1.0 log₁₀ as an appropriate guardband).

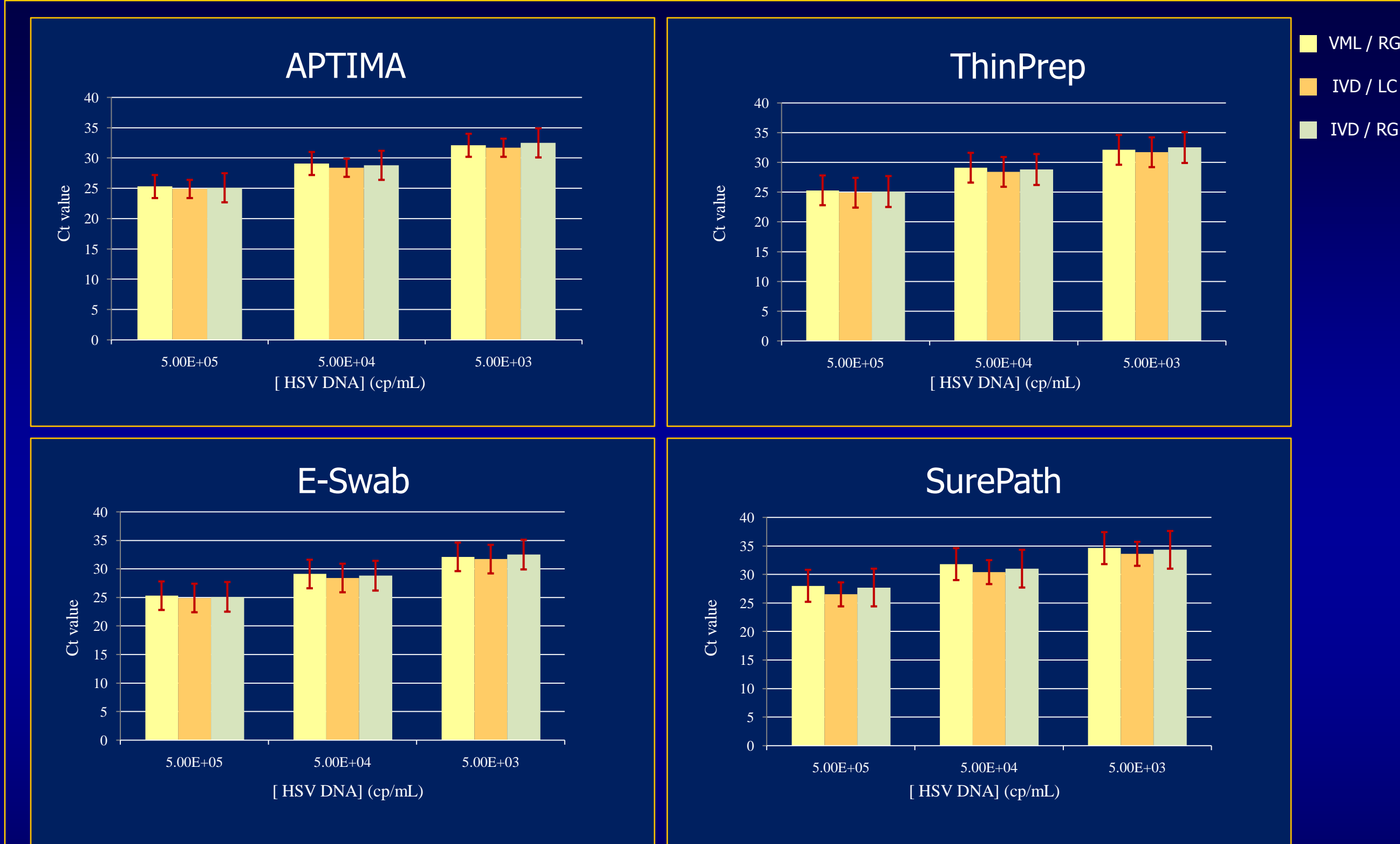


Figure 3. Average Ct values of HSV-1 and HSV-2 positive samples combined (n=20) obtained during clinical concordance testing by viral concentration. Categorical results for all samples tested using the IVD kit components were entirely congruent with expected results, and with those obtained using VML-HSV-RTx assay reagents, irrespective of the instrument or data reduction software utilized.

Conclusions

- Analytical LOD values for both HSV-1 and HSV-2 were comparable amongst the sample types (APTIMA, E-Swab, ThinPrep and SurePath) evaluated and substantially equivalent to the currently claimed value for UTM, namely, 500 cp HSV DNA/mL.
- Positive and negative concordance and assay accuracy were acceptable for all sample types. Analysis of Ct values across sample types demonstrated consistency of assay performance for all matrices.
- HSV-1 and HSV-2 DNA was stable in unprocessed APTIMA, E-Swab, ThinPrep and SurePath collections for up to 7 days under room temperature, refrigerated and frozen storage conditions.
- Crossover experiments demonstrated comparable assay performance to that obtained using the VML-HSV-RTx version of the assay could be expected using the IVD cleared MultiCode-RTx kit with either the cleared instrument (LightCycler v1.2) and IVD data reduction software, or the Rotor-Gene Q and the RUO data reduction software.