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Short communication

Comparison of two automated methods for detection and differentiation of herpes simplex virus in clinical specimens

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ABSTRACT

Background: The Aptima Herpes Simplex Virus (HSV) 1&2 Assay recently received Health Canada approved for detection and differentiation of HSV-1 and HSV-2 from anogenital sites. This assay uses target capture, transcription mediated amplification, and real-time detection of messenger RNA (mRNA) produced in host cells during active HSV infection. To evaluate its performance, the Aptima assay was compared to another Health Canada approved assay, the BD ProbeTec Herpes Simplex Viruses HSV 1&2 Qx Amplified DNA Assay, which uses strand displacement amplification technology.

Methods: As recommended by the manufacturers, the Aptima and ProbeTec assays were performed on the Panther and Viper instruments, respectively. Analytical sensitivity and specificity were assessed using 10-fold serial dilution of viruses in viral universal transport media (UTM), and nucleic acids extracted and concentrated from other viruses including all members of the Herpesviridae family. The clinical sensitivity and specificity were assessed retrospectively using 60 archived specimens, and prospectively using 158 swabs in UTM. Discrepant results were resolved with real-time PCR using the Altona Diagnostics RealStar alpha Herpes assay.

Results: Both the Aptima and ProbeTec assays showed excellent analytical and clinical specificity. However, the Aptima HSV assay failed to detect HSV in specimens with low viral loads, resulting in reduced sensitivity for HSV-2 during the retrospective evaluation at 85.0%, and for HSV-1 at 85.0% during the prospective evaluation.

Conclusions: This study compared the Aptima and ProbeTec HSV assays and demonstrated that detection of HSV mRNA using the Aptima HSV assay was less sensitive in both retrospective and prospective analyses.

1. Background

Herpes simplex virus types 1 and 2 (HSV-1 and HSV-2) cause a spectrum of disease, but most frequently present as dermal and mucous membrane lesions at oral or anogenital sites [1,2]. Clinical manifestations can occur with primary infections or reactivation from latency in neural ganglia [1,2]. HSV type differentiation is important for patient management, as HSV-2 tends to recur more frequently than HSV-1 [3]. Nucleic acid amplification assays (NAATs) have become the reference standard in clinical laboratories for HSV detection and differentiation due to their speed and accuracy, and various NAATs are now commercially available [4–10]. Recently, the Aptima HSV 1&2 assay (A-HSV) on the Panther system (Hologic Inc., San Diego, CA) received Health Canada approval for this purpose for anogenital specimens. When compared to the Health Canada approved BD ProbeTec HSV 1&2 Qx Amplified DNA assay (HSV-Qx) method on the Viper platform (Becton Dickinson Ltd., Oxford UK), A-HSV showed reduced sensitivity [10]. With archived specimens used during their evaluation, it was postulated that higher sensitivity might be achieved for A-HSV if specimens were tested prospectively [10].

Abbreviations: A-HSV, Aptima HSV assay; HSV-Qx, BD ProbeTec HSV assay; CI, confidence intervals; HSV-1 and -2, herpes simplex virus 1 and 2; LoD, limit of detection; MaxRFU, maximum relative fluorescence unit; mRNA, messenger RNA; NAATs, nucleic acid amplification assays; HV-PCR, RealStar alpha Herpesvirus PCR; STM, specimen transport medium; SDA, strand displacement amplification; Ct, threshold cycles; TNA, total nucleic acid; TMA, transcription mediated amplification; UTM, viral universal transport media

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2. Objectives

The current study aimed to compare the ability of A-HSV and HSV-Qx to detect and differentiate HSV using retrospectively and prospectively collected specimens.

3. Study design

In this study, 216 flocked polyester swabs were collected prospectively in 3 ml of viral universal transport media (UTM) (Copan Diagnostics Inc., Murrieta CA) from lesions at mucocutaneous or dermal sites (Tables S1 and S2). For retrospective analysis, 20 negative and 20 positive specimens each for HSV-1 and HSV-2 were chosen randomly from specimens collected within 30 days that were stored at −80 °C, which were previously tested using the HSV-Qx assay (Table S2). For prospective analysis, 156 consecutive swabs submitted to Queen Elizabeth II Health Sciences Centre (Halifax, NS) were tested, and held at 4 °C for no more than 24 h were used (Table S1). In both analyses, specimens were processed according to manufacturer recommendations and tested in parallel using A-HSV on the Panther or HSV-Qx assay on the Viper instrument. Preprocessing for A-HSV required placing 500 µl of specimen into 2.9 ml of Aptima specimen transport medium (STM). The Panther instrument used 400 µl of this mixture for target capture, transcription mediated amplification (TMA), and real-time fluorescence detection of the amplified HSV UL42 gene RNA. For HSV-Qx, 500 µl of specimen was diluted into 2 ml of ProbeTec Qx diluent, heated at 114 °C for 15 min, and cooled to room temperature prior to use. The Viper instrument used 1.0 ml of the mixture for nucleic acid extraction, and strand displacement amplification (SDA) of the HSV DNA targeting the glycoprotein G gene.

Results of A-HSV and HSV-Qx were compared, and discrepant results were resolved by real-time PCR using a RealStar alpha Herpesvirus PCR kit (αHV) (Altona Diagnostics, Hamburg, Germany) following a NucliSENS easyMAG (bioMérieux) nucleic acid extraction from 500 µl of primary specimen [7]. Following discrepant analysis, 2-by-2 contingency tables were used to compare each method against a consensus reference standard defined as concordant results (positive or negative) between two methods. Sensitivity and specificity were calculated with 95% confidence intervals (CI). Fisher's exact (two-tailed) with P-value < 0.05 being defined as statistically significant.

Analytical specificity was assessed using high titer suspensions of bacteria or non-HSV viruses that included all members of the *Herpesviridae* family (Table S3). For analytical sensitivity, 10-fold dilutions in UTM of HSV-1 (ATCC VR-733) and HSV-2 (ATCC VR-734) cultured stocks were performed, and subjected to the A-HSV and HSV-Qx methods. The limit of detection (LoD) was estimated using a Probit analysis, based on triplicate values obtained from three independent experiments (Figure S1). Quantification of HSV-1 and HSV-2 virus stocks was estimated following total nucleic acid (TNA) extraction of 200 µl of specimen on a MagNA Pure LC instrument and real-time PCR amplification using the LightCycler HSV 1/2 Detection kit on a LightCycler 1.0 platform (Roche Diagnostics, Mannheim, Germany) [5]. The resulting threshold cycle (Ct) values were compared to standard curves generated with plasmids containing the HSV target [6].

4. Results

In the retrospective, prospective, or both analyses combined, both assays showed excellent specificity for A-HSV and HSV-Qx (Tables 1 and S4). However, A-HSV failed to detect low viral concentrations of HSV-2 in the retrospective analysis (P = 0.026) (Tables 2). Similarly, reduced sensitivity for HSV-1 (P = 0.006) was noted during the prospective analysis (Tables 1). Analytical sensitivity analyses was consistent with lower sensitivity of A-HSV for both HSV-1 and HSV-2 (Figure S1), and all discrepant results except one fell below the LoD for A-HSV (Figure S1 and Table 2). For A-HSV, the LoDs for HSV-1 and

Table 1
Summary of the method performance characteristics for the detection and differentiation of HSV during the retrospective and prospective analyses.

Test period	Specimen type (n)	Aptima HSV assay				BD ProbeTec HSV Qx			
		HSV-1		HSV-2		HSV-1		HSV-2	
		Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Retrospective*	Overall* (n = 60)	95.0 (80.8–95.0)	100.0 (92.9–100.0)	85.0** (69.8–85.0)	100.0 (92.4–100.0)	100.0 (86.8–100.0)	100.0 (93.4–100.0)	100.0 (86.8–100.0)	100.0 (93.4–100.0)
	Anogenital* (n = 39)	91.7** (69.0–91.7)	100.0 (94.3–100.0)	86.7** (67.4–86.7)	100.0 (93.6–100.0)	100.0 (78.7–100.0)	100.0 (94.7–100.0)	100.0 (82.7–100.0)	100.0 (94.2–100.0)
	Non-anogenital* (n = 21)	100.0 (69.5–100.0)	100.0 (95.3–100.0)	80.0** (36.2–80.0)	100.0 (95.1–100.0)	100.0 (69.5–100.0)	100.0 (95.3–100.0)	100.0 (55.4–100.0)	100.0 (95.0–100.0)
Prospective*	Overall* (n = 156)	85.0** (76.3–85.0)	100.0 (97.1–100.0)	95.5 (82.2–95.5)	100.0 (97.3–100.0)	100.0 (93.1–100.0)	100.0 (97.7–100.0)	100.0 (87.8–100.0)	100.0 (98.0–100.0)
	Anogenital* (n = 79)	84.6** (62.5–84.6)	100.0 (98.0–100.0)	94.7 (79.6–94.7)	100.0 (97.9–100.0)	100.0 (80.0–100.0)	100.0 (98.2–100.0)	100.0 (86.0–100.0)	100.0 (98.1–100.0)
	Non-anogenital* (n = 77)	85.2** (73.1–85.2)	100.0 (97.5–100.0)	100.0 (36.8–100.0)	100.0 (98.8–100.0)	100.0 (90.0–100.0)	100.0 (97.9–100.0)	100.0 (36.8–100.0)	100.0 (98.8–100.0)

* A breakdown of specimen types is provided in supplementary Table S1 and S2 for the retrospective and prospective analyses, respectively.
** P-value < 0.05 compared to the consensus reference standard.

Table 2
Summary of the discrepant results between the Aptima and ProbeTec HSV assays.

Test period	Site	A-HSV		HSV-Qx		αHV-PCR		Final Results*	Estimated viral concentration (copies/mL)**
		Ttime	Result	MaxRFU	Result	Ct	Result		
Retrospective	Genital	ND	NEG	1866	HSV-1	32.82	HSV-1	HSV-1	699
	Genital	ND	NEG	2015	HSV-2	31.94	HSV-2	HSV-2	1185
	Vaginal	ND	NEG	1509	HSV-2	31.81	HSV-2	HSV-2	1287
	Buttock	ND	NEG	1967	HSV-2	31.87	HSV-2	HSV-2	1239
Prospective	Genital	ND	NEG	1260	HSV-1	32.11	HSV-1	HSV-1	1139
	Genital	ND	NEG	578	HSV-2	37.42	HSV-2	HSV-2	35
	Vaginal	ND	NEG	1462	HSV-1	34.06	HSV-1	HSV-1	299
	Chin	ND	NEG	1163	HSV-1	34.43	HSV-1	HSV-1	232
	Mouth	ND	NEG	643	HSV-1	37.69	HSV-1	HSV-1	25
	Mouth	ND	NEG	1519	HSV-1	29.70	HSV-1	HSV-1	5956

* A case was defined by concordant results (positive or negative) between at least two assays. Discrepant results between A-HSV and HSV-Qx were resolved by RealStar alpha Herpesvirus PCR (αHV-PCR).

** HSV viral loads were estimated by total nucleic acid followed by real-time PCR using the Roche HSV-1/2 detection kit, and comparing the resulting Ct values against a standard curve previously generated with plasmid DNA controls [6]. The equations for HSV-1 and HSV-2 were $y = -3.354 \times -1.917$ ($R^2 = 0.9971$) and $y = -3.597 \times -1.974$ ($R^2 = 0.9949$), respectively. Abbreviations: Aptima HSV assay (A-HSV), BD ProbeTec HSV assay (HSV-Qx), herpes simplex virus 1 and 2 (HSV-1 and -2), maximum relative fluorescence unit (MaxRFU), negative (NEG), not detected (ND), RealStar alpha Herpesvirus PCR (αHV-PCR), and threshold cycle (Ct).

HSV-2 were 2818 and 1584 copies/mL, whereas for HSV-Qx, LoDs were 5 and 14 copies/mL, respectively (Figure S1). Overall, when retrospective and prospective analyses were combined, the overall sensitivity of A-HSV for detection of HSV-1 was 90.0% and for 90.5% for HSV-2 (Figure S4). When broken down by genital and non-genital specimens, the sensitivity was 88.0% and 91.4% for HSV-1, and 91.2% and 87.5% for HSV-2, respectively (Figure S4).

5. Discussion

Prompt and accurate diagnosis of HSV is important for therapeutic and preventative interventions. Previous studies compared A-HSV to viral culture and immunofluorescence typing, and demonstrated sensitivities of A-HSV for HSV-1 of 98.2% and 99.4% for HSV-2 [11]. When compared to other NAATs, A-HSV had sensitivities of approximately 90% for HSV-1 and HSV-2 [8–10]. This study adds to the literature by comparing A-HSV to HSV-Qx prospectively and retrospectively, and both were performed on fully automated instruments that are Health Canada approved for anogenital specimens. While excellent specificity was noted with both assays, A-HSV failed to detect HSV at low viral concentrations, resulting in lower sensitivities of HSV-1 and HSV-2 of approximately 90% compared to the reference standard.

May and Tang [10] noted a similar reduced sensitivity of A-HSV compared to HSV-Qx; however, their evaluation was limited to retrospectively collected specimens. Compared to DNA targets for PCR- or SDA-based NAATs, freeze-thaw cycles incurred during processing of archive specimens could potentially have hampered the detection of UL42 messenger RNA (mRNA) produced in host cells during active HSV infection, which is the target of TMA amplification in A-HSV [12,13]. This study compared A-HSV to HSV-Qx using both retrospectively and prospectively collected specimens. Both showed a lack of sensitivity of A-HSV due to failure to detect HSV at low viral loads (Tables 1 and 2). Since A-HSV is only licensed for anogenital specimens, these specimen types were analyzed independently. Discrepant results were observed with both anogenital and non-anogenital specimens (Table 2), and both specimen types showed reduced sensitivity for the detection of HSV-1 during the prospective evaluation (Table 2). Only three HSV-2 samples were observed in non-anogenital specimens (Table S2), and no such conclusions could be inferred from the prospective analysis. When both retrospective and prospective results were combined, both HSV-1 and HSV-2 showed sensitivities of approximately 90%, regardless of anatomical site (figure S4).

Quantification of HSV in viral stocks or discrepant results were derived from standard curves generated with plasmid DNA. As such, only the quantity of HSV DNA in specimens could be assessed, whereas A-HSV relies on the detection of HSV mRNA [12,13]. Regardless, this approach did demonstrate that all discrepant results were due to specimens with low HSV viral loads. The reasons for the failure of A-HSV to detect low concentrations of HSV is likely multifactorial, including differences in NAAT methodologies, reagents, or even viral targets. In the prospective evaluation, low HSV-1 and HSV-2 viral loads were seen in 12.0 and 8.8% of HSV-positive anogenital specimens, and in 8.6% and 12.5% of HSV-positive non-anogenital specimens, respectively. Whether the generalization of these proportions of specimens with low HSV viral loads is valid to all patient populations is uncertain given the ability to detect RNA (or DNA) would be influenced by a number of factors including the timing of illness, specimen collection, antibiotic exposure, and host factors. Further studies could investigate the influence of these factors on viral shedding more in depth, but these do not deter the conclusions that the Health Canada approved methods for A-HSV is less sensitive than HSV-Qx due to failure to detect low HSV viral loads.

It should be noted that specimen collection with UTM may also play a role, given the required preprocessing step (1:7 dilution into STM buffer for A-HSV vs. 1:5 into BD buffer for HSV-Qx), and the lower amount of this mixture used by the instruments (400 µl with Panther compared to 1.0 ml with Viper). Increasing the quantity of nucleic acids processed may be an opportunity for improvement, but this would require A-HSV kit reformulation, re-validation, and resubmission to regulatory authority for approval. Alternatively, Sam et al. [9] demonstrated that higher sensitivity could be achieved with A-HSV by placing swabs directly into STM buffer. The use of Aptima STM solely for HSV testing in our laboratory poses practical challenges and was not evaluated.

Overall, the inability of A-HSV to detect low viral loads of HSV mRNA in clinical specimens positive for HSV DNA explains the lower sensitivity of A-HSV compared to HSV-Qx. Laboratories evaluating A-HSV should consider method comparisons with specimens collected in both STM and UTM, in attempts to increase its sensitivity.

Credit author statement

All authors have made a substantial contribution to the study conception and design, data acquisition, analysis, and interpretation. Each drafted or revised the article for intellectual content, and agree to be accountable for all aspects of the work related to the accuracy or integrity. Each author approved the final version.

Transparency document

The Transparency document associated with this article can be found in the online version.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jcv.2019.04.010.

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