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Evaluation of a transcription mediated amplification assay for detection of herpes simplex virus types 1 and 2 mRNA in clinical specimens☆

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ABSTRACT

Background: Herpes simplex viruses (HSV) are double-stranded DNA human herpesviruses (HHVs) that have the capacity to cause significant morbidity and mortality in humans. Like HHV5 (Cytomegalovirus) and HHV8 (Kaposi's sarcoma virus), HSV type 1 (HSV-1), and HSV type 2 (HSV-2) (HHV1, HHV2) selectively package certain viral messenger RNAs inside mature virions, as well as expressing those mRNAs in infected cells.
Objectives: To evaluate the clinical and analytical performance of Aptima HSV 1&2 assay (AHSV), a newly developed automated real time transcription-mediated amplification (TMA) nucleic acid amplification test (NAAT) for HSV-1 and 2 UL42 mRNAs, compared to viral culture and HSV DNA NAAT.
Study design: Cutaneous and mucocutaneous lesion swab specimens from a population of symptomatic female and male subjects attending a U.S. public health clinic (n = 758) were evaluated by shell vial culture with fluorescent antibody staining for HSV-1 and 2. Specimens were then tested with AHSV for HSV-1 and 2 on the Panther instrument. Specimens from subjects with discordant culture—TMA paired results were tested using an FDA-cleared test for HSV-1 and 2 viral DNA. Analytical performance of AHSV was evaluated using test panels consisting of laboratory strains of HSV-1 and 2 and a variety of non-target human DNA viruses.
Results: Compared to culture, AHSV was sensitive and specific for detection of HSV-1 and 2 in patient lesion swab specimens, exhibiting clinical sensitivities of 98.2% (95% CI: 92.9–99.7) and 99.4% (95% CI: 96.0–99.9), respectively. Addition of HSV DNA NAAT discordant resolution testing results to culture results improved AHSV sensitivity for HSV-1 and 2–99.2% (95% CI: 94.7–99.9) and 100% (95% CI: 97.5–100), respectively. Clinical specificity of AHSV for HSV-1 and 2 detection was 97.8% (95% CI: 96.3–98.8) and 94.5% (95% CI: 92.2–96.1), respectively, compared to culture; and 99.5% (95% CI: 98.5–99.9) and 99.5% (95% CI: 98.3–99.7), respectively, compared to culture with discordant resolution. Analytical sensitivity (95% limit of detection) of AHSV for HSV-1 (McIntyre strain) was 28.9 $TCID_{50}$/mL (95% FL: 23.4–37.9), and 0.54 $TCID_{50}$/mL (95% FL: 0.42–0.75) for HSV-2 (MS strain). AHSV did not cross-react with laboratory strains of HHV-3, HHV-4, HHV-5, HHV-6, and four other non-HHV human DNA viruses.
Conclusions: Real time transcription-mediated amplification NAAT for HSV viral mRNA is a sensitive and specific method for detection of herpes simplex virus infection in symptomatic patients.

1. Background

The Herpesviridae family of enveloped double-stranded DNA viruses are important human pathogens that have the capacity for imposing significant morbidity and mortality following infection, and have been shown to increase risk for infection and transmission of other viruses, including human immunodeficiency virus and human papillomavirus [1,2]. Herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) are two of the eight known human herpes

☆ This study evaluated the analytical and clinical performance of a novel molecular test method for herpes simplex viruses.

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viruses (HHV), and are a common cause of oral and anogenital lesions. HSV-1 typically causes oral lesions and HSV-2 typically causes genital lesions, although oral lesions due to HSV-2 occur occasionally, and genital lesions due to HSV-1 infection are increasing [3,4].

For many years, cell culture was the gold standard method for detection of HSV in lesion specimens. Nucleic acid amplification tests (NAATs) for detection of HSV genomic DNA have been developed that are faster and more sensitive than culture [5–8]. However, the early DNA-based NAATs were laboratory-developed PCR assays that are not practical for many clinical laboratories. More recently, FDA-cleared NAATs for HSV DNA detection have become available which are more extensively validated and less labor intensive than laboratory-developed PCR assays [9–12].

HSV-1 and 2 share a unique feature with HHV DNA viruses HHV-5 (Cytomegalovirus) and HHV-8 (Kaposi's Sarcoma virus), in that certain viral mRNAs are selectively packaged inside the virion particles as well as expressed inside infected cells [13–15]. This is thought to provide these viruses with a pre-fabricated mRNA complement that facilitates rapid expression of viral proteins soon after viral entry into the host cell [13].

In this study, we evaluated a new approach for detecting HSV infection by use of a real-time transcription-mediated amplification NAAT for the qualitative detection and differentiation of viral mRNAs from HSV-1 and HSV-2.

2. Objectives

This study evaluated the analytical and clinical performance of the transcription mediated amplification (TMA)-based Aptima HSV 1&2 (AHSV) assay compared to HSV shell vial culture for detection of HSV-1 and HSV-2 in cutaneous and mucocutaneous lesion specimens obtained from symptomatic patients attending a U.S. public health clinic.

3. Study design

3.1. Analytical specimen testing

For analytical sensitivity testing, laboratory strains of HSV-1 (McIntyre Strain, ATCC VR-539, American Type Culture Collection, Manassas, USA) and HSV-2 (MS strain, ATCC VR-540) were grown in monolayer tissue culture and the viral titer (50% tissue culture infectious dose/mL [$TCID_{50}$/mL]) determined by end point dilution. HSV-1 and HSV-2 viral stocks were then diluted into Aptima sample transport medium (STM; Hologic, Inc, San Diego, USA) to yield eight panels ranging from 30 $TCID_{50}$/mL to 0.003 $TCID_{50}$/mL. For each virus-positive panel and a negative STM panel, 60 replicates were tested using combinations of two Panther instruments and two separate AHSV reagent lots. The analytical sensitivity of AHSV for detecting HSV-1 and 2 nucleic acids was determined by synthesizing in vitro RNA transcripts from plasmid DNA vectors containing HSV-1 or 2 UL42 gene sequences, followed by serial dilution of RNAs in Aptima STM to create eight RNA-positive test panels for each viral type.

Analytical specificity of AHSV was determined by performing cross reactivity and interference testing with 46 closely related viruses, common flora and opportunistic organisms (34 bacteria, 10 viruses, 1 protozoa, 1 yeast) that may be found in skin and anogenital specimens. Organisms were combined into 12 different pools based on microorganism type and panels were prepared by spiking into Aptima STM to create a bulk stock, with three aliquots made from each stock. One aliquot was left negative to assess cross-reactivity, the second aliquot was spiked with HSV-1 UL42 RNA at 100 copies per reaction to assess interference with detection of HSV-1 and the third aliquot was spiked with HSV-2 UL42 RNA at 100 copies per reaction to assess interference with detection of HSV-2. Ten replicates were tested from each panel member. Most organisms were spiked at 1×10^6 colony forming units (CFU)/reaction or cells/reaction. Two organisms were spiked below this concentration due to limited available initial concentrations. No organism was spiked at less than 4×10^4 CFU/reaction or cells/reaction. Purified and quantified DNA or RNA (4×10^4 copies per reaction) was used for five of the microorganisms because cultured material was not available.

3.2. Clinical specimens

A total of 758 lesion swab specimens collected from patients seen at Public Health-Seattle & King County clinics were included in the study, of which 663 were genital, 72 were oral/facial, 16 were skin and 7 were not otherwise specified. Lesion swab specimens were placed in Universal Transport Medium (UTM, Copan Diagnostics, Murrieta, CA) and stored at 4 °C for 1–3 days before testing by HSV shell vial culture. Specimens were stored at −70 °C for 1–16 months and tested retrospectively by AHSV on the Panther instrument.

3.3. Shell vial culture

Specimens were tested for HSV-1 and HSV-2 by MRC-5 shell vial centrifugation culture using the MicroTrak HSV 1/HSV 2Culture Identification/Typing Test (Trinity Biotech, Jamestown, NY) [16]. Briefly, 0.2 mL of specimen was inoculated into two MRC-5 shell vials, the shell vials were centrifuged at 700*g* for 1 h and incubated at 37 °C for 16–20 h, and the two shell vial coverslips were fixed and stained with fluorescein-labeled monoclonal antibodies to either HSV-1 or HSV-2.

3.4. HSV nucleic acid amplification testing

Clinical specimens and panels of laboratory-derived viral strains were tested with the Aptima HSV 1&2 assay (AHSV), a research use only real time transcription mediated amplification (TMA) assay that detects and differentiates HSV-1 and HSV-2 UL42 mRNAs expressed in infected cells and packaged in HSV-1 and HSV-2 virions (Fig. 1). For AHSV, 0.5 mL of each UTM specimen was added to an Aptima Specimen Transport Tube containing 2.9 mL of Aptima STM, and 0.4 mL of the diluted specimen was processed by magnetic bead-based RNA target capture followed by real time TMA for amplicon generation and detection on the Panther automated instrument (Hologic, Inc, San Diego CA). A relative fluorescence unit range cutoff of greater than 1000 was used to determine sample reactivity status. All AHSV testing was performed on a blinded basis at Hologic. Positive and negative run controls consisting of HSV-1 and 2 UL42 RNAs at 750 copies/mL each, or negative STM, respectively, were included in every run on the Panther instrument and were used to validate run performance.

3.5. Discrepant analysis

AHSV was compared to shell vial culture as the reference standard to calculate sensitivity and specificity, before and after discordant analysis using the AmpliVue HSV 1+2 Assay (Quidel, Athens, OH), a FDA-cleared NAAT. The AmpliVue Assay is a helicase dependent amplification assay that detects and differentiates HSV-1 and HSV-2 genomic DNA. For the AmpliVue assay, 20 μL of UTM specimen was added to a Dilution Tube containing 1.6 mL of Dilution Buffer, 50 μL of the diluted specimen was added to a Reaction Tube, and the Reaction Tube was incubated for 45 min at 64 °C