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Automated processing, extraction and detection of herpes simplex virus types 1 and 2: A comparative evaluation of three commercial platforms using clinical specimens

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ABSTRACT

Background: Recently, automated platforms have been developed that can perform processing, extraction and testing for herpes simplex virus (HSV) nucleic acid on a single instrument.
Objectives: In this study, we compared three commercially-available systems; Aptima® /Panther (Hologic, San Diego, CA), ARIES® (Luminex Corporation, Austin, TX), and cobas® 4800 (Roche Molecular Systems Inc, Pleasanton, CA) for the qualitative detection of HSV-1/2 in clinical samples.
Study design: Two-hundred seventy-seven specimens (genital [n = 193], dermal [n = 84]) were submitted for routine HSV-1/2 real-time PCR by a laboratory developed test. Following routine testing, samples were also tested by the Aptima, ARIES, and cobas HSV-1/2 assays per the manufacturer's recommendations. Results were compared to a "consensus standard" defined as the result obtained from ≥3 of the 4 assays.
Results: Following testing of 277 specimens, the cobas and ARIES assays demonstrated a sensitivity of 100% for HSV-1 (61/61) and HSV-2 (55/55). The Aptima assays showed a sensitivity of 91.8% (56/61) for HSV-1 and 90.9% (50/55) for HSV-2. Percent specificities for HSV-1 were 96.2% (202/210) by cobas, 99.5% (209/210) by ARIES and 100% (236/236) by Aptima. For HSV-2, the specificities were 98.1% (211/215) by cobas, 99.5% (215/216) by ARIES and 100% (216/216) by Aptima. The turnaround time for testing 24 samples was 2.5 h by the cobas 4800, 3.1 h by Aptima/Panther, and 3.9 h by ARIES.
Conclusions: The three commercial systems can perform all current functions on a single platform, thereby improving workflow and potentially reducing errors associated with manual processing of samples.

1. Background

Herpes simplex virus types 1 and 2 (HSV-1/2) are a significant cause of disease worldwide and an important public health concern. HSV is one of the most common causes of sexually transmitted disease, with HSV-2 accounting for the majority of genital herpes infections. However, the number of genital infections caused by HSV-1 is on the rise, especially among young adults [1]. These viruses are also associated with a variety of dermal and oral lesions, and may result in disseminated disease among immunocompromised hosts and infected neonates [2].

Establishing a laboratory diagnosis for HSV infections is important, due to the nonspecific clinical manifestations and the potential to initiate antiviral therapy. Historically, the laboratory methods used to diagnose HSV include viral cell culture (e.g., tube cell culture and/or rapid shell vial), serology, and molecular detection of viral nucleic acid in clinical specimens. Although cell culture is still used in some clinical laboratories to recover HSV-1/2 from genital and dermal lesions, the turnaround time is at least 24 h following receipt of the specimen in the clinical laboratory. Serologic testing for IgM- and IgG-class antibodies to HSV has limited diagnostic value, given that IgM antibodies often show cross-reactivity [3,4] and IgG antibodies may take up to 2 weeks following infection to become detectable [4]. Due to these limitations, molecular testing (e.g., nucleic acid amplification tests [NAATs]) has become a routine method employed in the diagnosis of HSV-1/2 infections [5–8]. Molecular testing has demonstrated increased sensitivity over cell culture, and has reduced the turnaround time to 4–6 h in most laboratories [9].

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Despite several advantages over conventional methods, molecular tests have, to date, required separate processes for nucleic acid extraction and amplification/detection. The necessity for manual intervention during the testing process increases the risk for errors, especially in high volume diagnostic laboratories. In 2015, >30,000 dermal and genital specimens were submitted to our reference laboratory for HSV-1/2 testing by real-time PCR.

2. Objectives

This high testing volume prompted us to evaluate the performance of three new, commercial assays/platforms (Aptima® HSV-1/2/Panther [Hologic, San Diego, CA], ARIES® HSV-1/2 [Luminex Corporation, Austin, TX], and cobas® HSV-1/2/cobas 4800 [Roche Diagnostics, Pleasanton, CA]) designed for automated processing and qualitative detection of HSV-1/2 nucleic acid. The goal of this study was to compare the performance of these automated systems and determine whether these platforms yielded advantages in terms of workflow and testing throughput.

3. Materials and methods

3.1. Study design

Two-hundred seventy-seven prospective clinical specimens (genital [n = 193], dermal [n = 84]) were included in this study. Samples were submitted for HSV-1/2 real-time PCR, and following routine testing, were stored at 4 °C. Within 24 h of routine testing, the samples were tested in a blinded manner by each of the following commercial assays: Aptima HSV-1/2, ARIES HSV-1/2, and cobas HSV-1/2. The results of each assay were compared to a consensus standard, defined as the result achieved by ≥3 of the 4 assays. If a consensus result was not achieved (i.e., two assays were negative, while the other two assays were positive), the result was not included in the final data analysis.

3.2. Routine testing by real-time PCR

For routine testing, prospective genital (n = 193) and dermal (n = 84) swabs in viral transport media (VTM) were processed by extracting 0.2 mL of each sample using the total nucleic acid extraction protocol on the MagNA Pure LC 2.0 (Roche Diagnostics, Indianapolis, IN). Subsequently, 5 μL of each extract was combined with 15 μL of HSV-1/2 Roche analyte specific reagents (ASR) in a LightCycler cuvette (Roche) and tested on a LightCycler 2.0 (Roche) as previously described [10].

3.3. Automated testing by three commercial systems

Within 24 h of routine testing, specimens were pulled from refrigerate (4 °C) storage and tested by the following assays/platforms: Aptima HSV-1/2 on the Panther instrument (Hologic), ARIES HSV-1/2 on the ARIES analyzer (Luminex), and cobas HSV-1/2 on the cobas 4800 (Roche). At the time of this evaluation, the Aptima HSV-1/2 assays were labeled for investigational use only for the qualitative detection and differentiation of HSV-1/2 mRNA using target capture, transcription mediated amplification (TMA). Prior to testing by Aptima HSV-1/2, 0.5 mL of each VTM specimen was pipetted into an Aptima specimen transfer tube (Hologic) containing 2.9 mL of Aptima specimen transport media (STM). Following mixing of the sample, the Panther instrument processed 0.4 mL of sample by the Aptima HSV-1/2 assays. Both the ARIES and cobas HSV-1/2 assays utilize real-time PCR for the detection and differentiation of HSV-1/2 DNA, and were labeled as Food and Drug Administration (FDA)-cleared at the time of this study. The ARIES HSV-1/2 assay requires a load volume of 0.2 mL of VTM, while the cobas HSV-1/2 test uses 0.4 mL of specimen. Testing by all three assays was performed according to the manufacturer's recommendations, with the following exceptions. First, the ARIES and cobas HSV-1/2 assays are FDA-cleared for specimens collected using UTM™ Viral Transport Media (Copan Diagnostics, Murrieta, CA) and the MSwab™ collection, transport and preservation system (Copan Diagnostics), respectively; however, in our study, specimens were collected using a variety of swab types and VTM. Second, the cobas HSV-1/2 assays are FDA-cleared for testing of anogenital lesions, but our study also assessed the performance of each assay using dermal specimens.

3.4. Statistics and data analysis

Overall agreement, percent sensitivity, specificity, predictive values, and 95% confidence intervals were calculated using www.graphpad.com/quickcalcs. The 95% confidence intervals were calculated using the modified Wald method. McNemar's statistical analysis was performed by comparing the performance of each test with a p-value of <0.05 being defined as statistically significant. Kappa (κ) values were calculated by comparing each individual assay to the consensus result, and levels of agreement (moderate [0.41–0.6], substantial [0.61–0.8], near perfect [0.81–1.0]) were assigned as previously described [11]. A consensus result was not achieved for six samples, and these results were not included in the calculations.

4. Results

Among 277 prospective clinical specimens tested in this study, 116 (41.9%) gave a consensus result of positive for either HSV-1 (n = 61; 52.6%) or HSV-2 (n = 55; 47.4%). Six (2.2%) samples did not yield a consensus result, due to 2 assays being positive and 2 assays being negative for HSV-1 or HSV-2 on the same sample. Interestingly, when the data were analyzed by specimen type, 22 (26.2%) of 84 dermal specimens were positive for either HSV-1 (n = 17; 77.3%) (Table 1) or HSV-2 (n = 5; 22.7%) (Table 2). Of 193 genital specimens tested, 94 (48.7%) were positive with 44 (46.8%) being identified as HSV-1 (Table 1) and 50 (53.2%) being identified as HSV-2 (Table 2) by at least 3 of the 4 molecular tests.

When the results of each automated platform were compared to those of the consensus standard, we observed a sensitivity and specificity of >90% by each assay. Specifically, the Aptima HSV-1 and HSV-2 assays showed a sensitivity of 91.8 (56/61) and 90.9% (50/55), respectively, with a percent specificity of 100% for both HSV-1 (210/210) and HSV-2 (216/216). The ARIES HSV-1 and HSV-2 assays each showed 100% sensitivity and 99.5% specificity. Finally, the cobas assays yielded a sensitivity of 100% for both HSV-1 (61/61) and HSV-2 (55/55); however, the specificity of the cobas HSV-1 and HSV-2 tests was determined to be 96.2 (202/210) and 98.1% (211/215), respectively (Tables 1 and 2). Overall agreement with the consensus result, as measured by kappa, ranged from 0.92 (95% CI, 0.86–0.97) by the cobas HSV-1 test to 0.99 (95% CI, 0.97–1.0) by the ARIES HSV-1 and HSV-2 assays, indicating near perfect agreement.

5. Discussion

In this study, we compared the performance of three commercial assays and testing platforms for the automated detection and differentiation of HSV-1/2 in genital and dermal lesion swab specimens. To our knowledge, this is the first study to evaluate the recently FDA-cleared cobas and ARIES HSV-1/2 assays, and the first report to directly compare the Aptima, ARIES, and cobas assays