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Comparative Evaluation of AmpliVue HSV 1+2 Assay with ELVIS Culture for Detecting Herpes Simplex Virus 1 (HSV-1) and HSV-2 in Clinical Specimens

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The AmpliVue HSV 1+2 assay was compared to the ELVIS HSV ID and D^3 Typing Culture System for the qualitative detection and differentiation of herpes simplex virus 1 (HSV-1) and HSV-2 DNA in 1,351 cutaneous and mucocutaneous specimens. Compared to ELVIS, AmpliVue had sensitivities of 95.7 and 97.6% for detecting HSV-1 and HSV-2, respectively. Following arbitration of discordant results by an independent molecular method, the AmpliVue assay had a resolved sensitivity and specificity of 99.2 and 99.7%, respectively, for both HSV-1 and HSV-2, whereas ELVIS had a resolved sensitivity of 87.1% for HSV-1 and 84.5% for HSV-2.

Herpes simplex virus 1 (HSV-1) and HSV-2 are responsible for a variety of human diseases, of which cutaneous and mucocutaneous infections are the most common (1–3). In the United States, the seroprevalences for HSV-1 and HSV-2 adult infections are 80 and 20%, respectively. Worldwide, these rates are much higher, particularly in underdeveloped countries (1, 4, 5). Though there is no cure for HSV infection, antiviral therapies are available that reduce the severity of symptoms, the duration of viral shedding, and the frequency of recurrence (6–9). However, the clinical diagnosis of cutaneous and mucocutaneous herpetic infections is problematic since it is neither sensitive nor specific (10). Therefore, timely and accurate diagnostic laboratory tests are necessary for instituting appropriate therapeutic management, counseling patients with primary infection, making decisions regarding intrapartum delivery, and justifying the use of long-term suppressive therapy.

A multicenter study was conducted to evaluate the performance of the AmpliVue HSV 1+2 assay (Quidel, San Diego, CA) compared to that of the ELVIS HSV ID and D^3 Typing System (Quidel DHI, Athens, OH). A total of 1,351 cutaneous (skin, n = 271; penile, n = 129) and mucocutaneous (vaginal/cervical, n = 699, oral, n = 165; anorectal, n = 35; urethral, n = 18; ocular, n = 18; nasal, n = 16) specimens were prospectively collected and evaluated in this comparative study. However, 15 of these 1,351 specimens were excluded from the study analysis because 8 specimens yielded invalid AmpliVue results, 3 specimens produced bacterial contamination in the ELVIS culture system, and 4 specimens were positive in the ELVIS culture system but could not be typed according to the manufacturer's protocol. These 15 specimens were removed from the study, leaving 1,336 specimens for comparative analyses. All specimens were collected on swabs, transported to the laboratory in viral transport medium (VTM), and stored at 4 to 8°C, and all testing was performed within 72 h of specimen receipt. The ELVIS and AmpliVue tests were performed at four different clinical laboratories that represented the investigative authors' various geographic locations in the United States.

ELVIS culture was performed according to the package insert (11). Shell vials were screened microscopically after 24 and 48 h of incubation for the appearance of an intracellular blue color that was indicative of HSV infection. HSV isolates were typed with fluorescein-labeled monoclonal antibodies specific for HSV-1 and HSV-2. The AmpliVue assay is an FDA-cleared assay that detects and differentiates HSV-1 and HSV-2 DNA by using a helicase-dependent amplification (HDA) reaction that simultaneously amplifies an HSV-1-specific sequence and an HSV-2-specific sequence in the presence of an internal control (IC) sequence. The HSV-1 target sequence is located at the 5′ end of the UL20 and UL19 genes, while the HSV-2 target sequence is located between the UL47 and UL48 genes. The nature of the positive IC is proprietary.

The AmpliVue assay consists of three major steps: (i) specimen preparation involving a one-step dilution, (ii) isothermal HDA of target sequences specific for HSV-1 and HSV-2, and (iii) detection of the DNA amplicons by target-specific hybridization probes via a colorimetric reaction on a lateral-flow strip that is embedded in a self-contained disposable cassette to prevent amplicon contamination. Specimen preparation involves one simple dilution of 20 μl of VTM to a dilution tube containing a buffer. After mixing by inversion, 50 μl of the diluted specimen is transferred into a 0.2-ml reaction tube that contains lyophilized HDA reagents, de-

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TABLE 1 Comparison of AmpliVue HSV 1+2 assay with ELVIS culture for detection of HSV-1 before and after discordant-result analysis[a]

AmpliVue HSV 1+2 assay result	No. of ELVIS culture results			% Sensitivity	% Specificity	% PPV[b]	% NPV[c]	% Prevalence	% Total agreement
	Positive	Negative	Total						
Before discordant-result analysis									
Positive	179	34	213						
Negative	8	1,115	1,123						
Total	187	1,149	1,336	95.7 (91.8–97.8)[d]	97.0 (95.9–97.9)	84.0 (78.5–88.3)	99.3 (98.6–99.6)	14.0	96.9
After discordant-result analysis[a]									
Positive	208	3	211						
Negative	2	1,121	1,123						
Total	210	1,124	1,334	99.0 (96.6–99.7)	99.7 (99.2–99.9)	98.6 (95.9–99.5)	99.8 (99.4–100.0)	15.7	99.6

[a] Two of the 42 discordant specimens could not be tested because of insufficient volume. Discordant-result analysis was performed by using alternative molecular methods (Lyra HSV 1+2/VZV) and bidirectional sequencing.
[b] PPV, positive predictive value.
[c] NPV, negative predictive value.
[d] Each value in parentheses is the 95% confidence interval.

oxynucleoside triphosphates, primers, and probes. Incubation at 64°C for 45 min results in the release of the HSV DNA and subsequent isothermal amplification of the target sequence by HSV-1- and HSV-2-specific primers. The amplified DNA is detected by a set of specific detection probes included in the reaction tube; the HSV-1 target hybridizes to two specific probes labeled with biotin (BioTEG) and digoxigenin (DIG), and the HSV-2 target hybridizes to two specific probes labeled with BioTEG and 6-carboxyfluorescein (FAM). A competitive IC is included in the reaction tube to monitor for inhibitory substances in the clinical specimens, reagent failure, or device failure. The IC target is amplified by HSV-2-specific primers and hybridizes to the biotin-labeled HSV-2 probe and the IC-specific probe labeled with 2,4-dinitrophenyl (DNP-TEG).

Detection of the amplified DNA with specific probes is achieved by using the AmpliVue cassette. The cross-contamination-proof AmpliVue cassette contains lateral-flow DNA detection strips coated with anti-DNP antibodies (C line), anti-DIG antibodies (T1 line), and anti-FAM antibodies (T2 line). HSV-1 amplicons with BioTEG- and DIG-labeled probes are captured by the anti-DIG antibodies at the T1 line, and HSV-2 amplicons with BioTEG- and FAM-labeled probes are captured by anti-FAM antibodies at the T2 line, while IC amplicons with BioTEG- and DNP-labeled probes are captured by anti-DNP antibodies at the C line. The biotin in the amplicon-probe complexes captures the streptavidin-conjugated colored particles for visualization, and a test result is a pink-to-red line that is read visually. A positive result for HSV-1 (detection of HSV-1 DNA) is reported when the T1 line is visible through the cassette detection window, while a positive result for HSV-2 (detection of HSV-2 DNA) is reported when the T2 line is visible through the detection window. A positive result for both HSV-1 and HSV-2 (detection of both HSV-1 and HSV-2 DNAs) is reported when the T1 and T2 lines are both visible through the cassette detection window. A negative result (no detection of HSV-1 or HSV-2 DNA) is reported only when the C line is displayed. The C line must always be visible to report a positive or negative HSV result. The assay result is invalid when the T1 line, T2 line, and C line are not visible. If repeat testing of an invalid specimen produced a second invalid result, the specimen was reported as invalid and no further testing was performed.

Specimens with discordant ELVIS and AmpliVue test results were sent to a reference laboratory (Quidel-DHI, Athens, OH) for arbitration analysis by alternative molecular assays, i.e., the Lyra Direct HSV 1+2/VZV assay (Quidel, San Diego, CA) and bidirectional sequence analysis (Beckman Coulter Genomics, Danvers, MA). For HSV-1, the Lyra assay targets the gene sequence responsible for glycoprotein G, while for HSV-2, the LYRA targets the gene sequence for glycoprotein G2. Any amplicons generated by the Lyra assay were analyzed by bidirectional sequencing, which provided perfect correlation with Lyra in detecting HSV-1 or HSV-2 DNA when it was present in the sample.

The performance of the AmpliVue HSV test compared to that of ELVIS culture for detecting HSV-1 in 1,351 specimens before and after discordant-result analysis is shown in Table 1. Fifteen specimens were removed from the analysis because of invalid AmpliVue results ($n = 8$), bacterial contamination in ELVIS culture ($n = 3$), or positive but untypeable HSV isolates according to the test protocol ($n = 4$). Thus, 1,336 specimens were available for comparative study. Compared to ELVIS as the reference method, the AmpliVue assay had a sensitivity of 95.7% and a specificity of 97.0%. Of the 42 discordant specimens, 40 were available for arbitration testing. Two specimens could not be tested because of insufficient sample volume and were removed from the analysis. As shown in Table 1, the sensitivity and specificity of the AmpliVue assay for detecting HSV-1 improved to 99.0 and 99.7%, respectively, after discordant-result resolution was obtained by the independent molecular methods. Importantly, 31 of the 34 "negative" ELVIS culture results were false negative after discordant-result resolution by molecular testing, producing a resolved ELVIS culture sensitivity of 87.1% for HSV-1.

Table 2 shows the performance of the AmpliVue HSV assay compared to that of ELVIS culture for the detection of HSV-2. With ELVIS as the reference method, the AmpliVue assay had a sensitivity of 97.6% and a specificity of 95.7% for detecting

TABLE 2 Comparison of Amplivue HSV 1+2 assay with ELVIS culture for detection of HSV-2 before and after discordant-result analysis[a]

AmpliVue HSV 1+2 assay result	No. of ELVIS culture results			% Sensitivity	% Specificity	% PPV[b]	% NPV[c]	% Prevalence	% Total agreement
	Positive	Negative	Total						
Before discordant-result analysis									
Positive	206	48	254						
Negative	5	1,077	1,082						
Total	211	1,125	1,336	97.6 (94.6–99.0)[d]	95.7 (94.4–96.8)	81.1 (75.8–85.4)	99.5 (98.9–99.8)	15.8	96.0
After discordant-result analysis[a]									
Positive	243	3	246						
Negative	2	1,080	1,082						
Total	245	1,083	1,328	99.2 (97.1–99.8)	99.7 (99.2–99.9)	98.8 (96.5–99.6)	99.8 (99.3–99.9)	18.4	99.6

[a] Eight of the 53 discordant specimens could not be tested because of insufficient volume. Discordant-result analysis was performed by using alternative molecular methods (Lyra HSV 1+2/VZV) and bidirectional sequencing.
[b] PPV, positive predictive value.
[c] NPV, negative predictive value.
[d] Each value in parentheses is the 95% confidence interval.

HSV-2. Discordant test results were encountered for 53 specimens. Eight of these specimens were removed from arbitration testing because of insufficient sample volume. Table 2 shows the results for the remaining HSV-2 specimens after discordant-result arbitration. It is noteworthy that of the 48 AmpliVue-positive, ELVIS-negative specimens, 45 were found to be true-positive AmpliVue and false-negative ELVIS culture results. Following arbitration, the resolved AmpliVue sensitivity and specificity for HSV-2 were 99.2 and 99.7%, respectively, while the resolved ELVIS sensitivity for HSV-2 was 84.5%.

Historically, culture has been the mainstay for establishing a laboratory diagnosis of HSV infection. ELVIS has been the preferred culture method because it reduces the time to detection from days to 24 h while maintaining comparable sensitivity and eliminating the need for subjective interpretation of a cytopathic effect (12–14). More recently, gene amplification assays have been developed that detect and type HSV in cutaneous and mucocutaneous specimens with a sensitivity greater than that of culture methods (12, 15, 16). However, a limitation of these molecular-analysis-based assays is that they require the use of highly trained personnel along with the use of specialized and relatively expensive equipment.

The results of this study show that the AmpliVue HSV 1+2 assay was more sensitive than ELVIS culture for detecting HSV-1 and HSV-2 in a wide variety of cutaneous and mucocutaneous specimens. After molecular arbitration of discordant results, the moderately complex AmpliVue test had a resolved sensitivity of >99% for both HSV-1 and HSV-2, whereas ELVIS culture had resolved sensitivities of 87.1 and 84.5% for HSV-1 and HSV-2, respectively.

Other advantages of the AmpliVue assay include the following. (i) The detection of amplicons is performed in a self-contained, disposable cartridge, thereby minimizing the risk of extraneous contamination. (ii) The test does not require the use of any specialized equipment other than a stationary 64°C heat block with a heated lid. (iii) The assay can be performed as an "on-demand" test with only 2 min of hands-on time and a reliable specimen time to result of approximately 60 min.

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