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AmpliVue® HSV 1+2 ASSAY

For the qualitative detection and differentiation of *Herpes simplex virus* 1 (HSV-1) and *Herpes simplex virus* 2 (HSV-2) nucleic acids isolated from cutaneous or mucocutaneous lesion of symptomatic patients.

FOR *IN VITRO* DIAGNOSTIC USE

℞ ONLY

Contents

INTENDED USE

The AmpliVue HSV 1+2 Assay is an *in vitro* diagnostic test for the direct, qualitative detection and differentiation of *Herpes simplex virus* 1 (HSV-1) and *Herpes simplex virus* 2 (HSV-2) DNA in cutaneous or mucocutaneous lesion specimens from symptomatic patients. The test is intended for use as an aid in diagnosis of HSV infection in symptomatic patients.

***Warning*: The AmpliVue HSV 1+2 Assay is not intended for use with cerebrospinal fluid (CSF). The assay is not intended for prenatal screening.**

SUMMARY AND EXPLANATION OF THE TEST

Infection with *herpes simplex virus* (HSV) is among the most ubiquitous of human infections.[1] HSV infects neonates, children and adults, and the virus is spread by direct contact with virus in secretions from either symptomatic or asymptomatic individuals.[1-4] Following primary infection, HSV establishes lifelong latent infections which periodically reactivate and may be associated with recurrent episodes of disease, with or without clinical symptoms, and as such, the infection may often be transmitted unknowingly.[5-8]

Herpes simplex viruses are categorized into two types: HSV-1 and HSV-2. HSV-1 causes oral and genital, and occasionally facial, lesions. Initial infection is the most severe with ulcerative, painful stomatitis that usually occurs in children.[9] Reactivation of HSV-1 in the mouth usually causes lesions on the lip ("fever blisters" or "cold sores").[10] Worldwide, approximately 90% of the population has antibodies to one or both HSV (HSV-1 and HSV-2).[11] HSV-2 is primarily associated with genital and neonatal infections, and at least 50 million persons in the United States are infected with HSV-2 genital herpes.[12] However, recent studies suggest that 20%-50% of incident episodes of genital herpes are caused by HSV-1 and the proportion of such incident cases due to HSV-1 may be increasing.[13-17]

Accurate identification of persons with HSV infection is necessary for optimal patient management and prevention of transmission. Because of inherent inaccuracies, clinical diagnosis of HSV infection should be confirmed by laboratory testing.[18]

Virus culture is the most definitive means to diagnose an HSV infection.[19] Culture involves inoculating a specimen onto a tissue culture cell monolayer followed by daily microscopic observation for cytopathic effects (CPE). The characteristic CPE of HSV generally appears within 24 to 72 hours, but may take up to 5 days. Cultures are typically maintained for at least 1 week before reported as negative if no CPE are observed. Virus culture is therefore slow and labor intensive.[18] A histochemical method using a genetically engineered cell line (the ELVIS® test: Enzyme-Linked Virus Inducible System) allows HSV-infected cells to undergo a change in color that can be visualized by light microscopy.[20] The ELVIS procedure takes a minimum of 16 hours to detect HSV. All these methods require a cell culture facility, and the timing of culture is critical for success.

Nucleic acid amplification tests for the detection of HSV DNA have been reported to be more sensitive and rapid than virus culture and antigen detection methods.[21, 22] However, they usually require DNA extraction prior to amplification and specialized instrumentation for the process. The IsoAmp® HSV Assay is based on Helicase Dependent Amplification (HDA) technology and a disposable lateral-flow detection device.[23-25] Although the IsoAmp HSV Assay is easy-to-use (moderate complexity) and does not require expensive instrument, it doesn't differentiate HSV-1 and HSV-2.

The AmpliVue HSV 1+2 Assay detects and differentiates HSV-1 and HSV-2 DNA by a HDA reaction which simultaneously amplifies a HSV-1 specific sequence and a HSV-2 specific sequence in the presence of an internal control sequence. The amplicons are subsequently detected by a three line DNA test strip embedded in the cross-contamination-proof Detection Chamber.[26-29] A clinical specimen is diluted in a Dilution Tube and added to the lyophilized amplification reagent in a Reaction Tube. After amplification for 45 minutes at a constant temperature, the Reaction Tube is inserted into the disposable Detection Chamber, where HSV-1 and HSV-2 DNA is captured by

specific detection probes, resulting in a colored line that is visually read. The results are available in about one hour of obtaining the specimens and do not require complex instrumentation.

ASSAY PRINCIPLE

The AmpliVue HSV 1+2 Assay consists of three major steps: (1) specimen preparation (one-step dilution), (2) isothermal Helicase Dependent Amplification (HDA) of target amplicons specific to HSV-1 and HSV-2, and (3) detection of the amplified DNA by target-specific hybridization probes via a colorimetric reaction on a lateral-flow strip which is embedded in a self-contained disposable Detection Chamber to prevent amplicon contamination.[26-29]

Specimen preparation involves one simple dilution step in which specimens in viral transport medium are diluted 80-fold in Dilution Tubes.

The diluted sample is transferred into a 0.2 mL Reaction Tube which contains lyophilized HDA reagents, dNTPs, primers and probes. Incubation at 64°C for 45 minutes 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: HSV-1 target hybridizes to two specific probes labeled with Biotin (BioTEG) and Digoxigenin (DIG) and HSV-2 target hybridizes to two specific probes labeled with Biotin (BioTEG) and 6-carboxyfluorescein (6-FAM). A competitive internal control (IC) is included in the Reaction Tube to monitor inhibitory substances in clinical samples, 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 a IC specific probe labeled with 2,4-dinitrophenyl (DNP-TEG).

Detection of the amplified DNA with specific probes is achieved by AmpliVue Detection Chambers. The cross-contamination-proof AmpliVue Detection Chambers carry 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 amplicon with BioTEG and DIG-labeled probes is captured by anti-DIG antibodies at the T1-Line and HSV-2 amplicon with BioTEG and FAM-labeled probes is captured by anti-FAM antibodies at the T2-Line, while the IC amplicon with BioTEG and DNP-labeled probes is captured by anti-DNP antibodies at the C-Line. The Biotin in the amplicon-probe complexes captures the streptavidin-conjugated color particles for visualization and the test result is shown as colored lines that are visually read.

A positive result for HSV-1 (detection of HSV-1 DNA) is reported when the T1 line is visible through the detection window of the Detection Chamber, while a positive result for HSV-2 (detection of HSV-2 DNA) is reported when the T2 line is visible through the detection window of the Detection Chamber. A positive result for both HSV-1 and HSV-2 (detection of both HSV-1 and HSV-2 DNA) is reported when both the T1 line and the T2 line are visible through the detection window of the Detection Chamber. A negative result (no detection of HSV-1 or HSV-2 DNA) is reported when only the C line is displayed. The assay result is regarded as invalid when the T1 line, T2 line and C line are not present and the assay should be repeated.

MATERIALS PROVIDED

Cat. #M210
16 Tests per Kit

Component	Quantity	Storage
Detection Chambers	16/kit	2°C to 30°C
Amplicon Cartridge	16/kit	2°C to 30°C
Dilution Tubes Part	16 tubes/kit, 1.6 mL	2°C to 8°C
Reaction Tubes Part	16 tubes/kit	2°C to 8°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Viral transport medium:
  The following transport media have been tested and had assay performance verified:
  Remel M4, Remel M5, Remel M4RT, BD Universal Viral Transport Media and Bartels VTM
- External positive controls for HSV-1 and HSV-2 (i.e., HSV-1 or HSV-2 positive patient specimens or NATrol *Herpes simplex virus* Type 1 Strain: MacIntyre and NATrol *Herpes simplex virus* Type 2 Strain: MS (Zeptometrix, Corporation), or Quidel Molecular HSV 1+2 Control Set #M109 which serves as an external assay control).
- Thermometer

- Sterile DNase-free filter-blocked or positive displacement pipette tips
- Appropriately sized pipettors
- Disposable gloves
- Stopwatch or timer
- Scissors or a blade
- Heat block with heated lid capable of 64°C ± 2°C temperature

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use.
- Do not use the reagents after their expiration date.
- Do not interchange caps among reagents as contamination may occur and compromise test results.
- Open the tubes only when adding aliquots into tubes or removing aliquots from tubes. Keep the tubes closed at any other time to avoid contamination.
- To avoid contamination of the environment with HSV amplicons, do not open the reaction tubes post-amplification.
- Avoid microbial and deoxyribonuclease (DNase) contamination of reagents when removing aliquots from tubes. The use of sterile DNase-free disposable filter-blocked or positive displacement pipettor tips is recommended.
- Use a new pipettor tip for each specimen or reagent.
- Performing the assay outside of the recommended time ranges can produce invalid results. Assays not completed within specified time ranges should be repeated.
- In cases where open-tube PCR tests are conducted in the same general area by the laboratory, separated or segregated working areas should be used for specimen preparation and amplification/detection activities. Supplies and equipment should be dedicated to each area and should not be moved from one area to another. Gloves must always be worn and must be changed before going from one area to another. Gloves must be changed before manipulating the reagents.
- Always handle specimens as if they are infectious and in accordance with safe laboratory procedures such as those described in CDC Biosafety in Microbiological and Biomedical Laboratories[32] and in CLSI Document M29 Protection of Laboratory Workers from Occupationally Acquired Infections.[33]
- Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
- Maintenance and decontamination of workspace and equipment should follow and be performed according to established laboratory protocols and schedules.
- Testing should be performed in an area with adequate ventilation.
- Dispose of containers and unused contents in accordance with Federal, State and Local regulatory requirements.
- Wear suitable protective clothing, gloves, and eye/face protection when handling the contents of this kit.
- Wash hands thoroughly after handling.
- For additional information on hazard symbols, safety, handling and disposal of the components within this kit, please refer to the Safety Data Sheet (SDS) located at quidel.com.

STORAGE, HANDLING AND STABILITY

HSV Specimens

- The following transport media have been tested and had assay performance verified: Remel M4, Remel M5, Remel M4RT, BD Universal Viral Transport Media/Copan UTM and Bartels VTM.
- Swabs collected from lesions can be stored in the viral transport medium at 2°C to 8°C for up to 5 days before being tested. Swabs can also be stored at –70°C or below for long term storage.
- Protect specimens against exposure to excessive heat.

Reagents

- Store assay reagents and Detection Chambers as indicated on their individual labels.

ASSAY PROCEDURE

Specimen Preparation

1. 15 minutes prior to the amplification step, warm a heating block with a heated lid to 64°C ± 2°C.

2. Place the required number of Dilution Tubes in a rack. Mark the Dilution Tubes on the cap and/or side of the Tube.
   Note: One (1) Dilution Tube is required for each specimen and control to be tested.
3. Elute specimen material present on a swab by vigorous agitation (i.e., vortexing) of the transport tube.
4. Transfer 20 µL of each clinical specimen from the original collection tube or control to be tested to an identified Dilution Tube (blue cap). Close the cap and mix the solution well by inverting the tubes for a minimum of 5 times.
   Note: Use a new pipette tip for each specimen.
   Note: The diluted specimen or control can be stored at room temperature (20°C to 25°C) for 1 hour, at 2°C to 8°C for 8 hours and for 1 month at –20°C to –70°C.

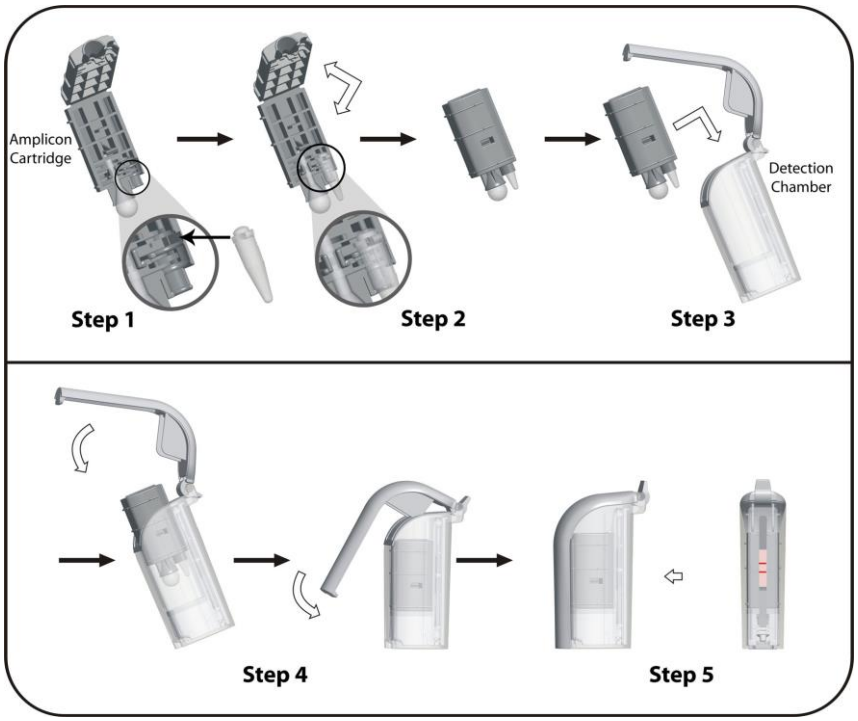
Amplification

1. Place the same number of Reaction Tubes in a rack. Mark the Reaction Tubes on the cap and/or side of the Tube.
   Note: Remove the required number of Reaction Tubes from the protective pouch, remove the excess air and reseal the bag.
   Note: The following steps (2-3) MUST be performed without stopping.
2. Transfer 50 µL of the diluted specimen or control to corresponding labeled Reaction Tubes, mix the solution by pipetting up and down 3 to 5 times and close the cap. The solution should be clear, free of solid material.
   Note: Use a new pipette tip for each diluted sample.
3. Incubate the Reaction Tubes at 64°C ± 2°C for 45 minutes ± 5 minutes in a heating block with a heated lid.
   Note: Be sure that all tubes are in tight contact with heat block.
   Note: To avoid laboratory contamination, once the tube has been closed and the amplification reaction started, **DO NOT** open the Reaction Tube.

Detection

1. Tear open a new Detection Chamber package. Label the Detection Chamber appropriately. Make sure the buffer bulb is attached in the Amplicon Cartridge.
2. Place the Reaction Tube into the Amplicon Cartridge (Figure 1, step 1). Be sure to place the HINGE of the Reaction Tube cap into the largest slot adjacent to the buffer bulb.
   Note: Make sure a buffer bulb is attached in the Amplicon Cartridge.
3. Close the Amplicon Cartridge (Figure 1, step 2) ensuring that it snaps shut.
   Note: If the cartridge does not snap shut, reposition the tube within the cartridge.
4. Insert the closed Amplicon Cartridge into the Detection Chamber (Figure 1, step 3). Make sure the arrow, located on the top of the Amplicon Cartridge, faces the detection strip (Reaction Tube should face the razor blade and the plastic bulb containing the running buffer should face the pin).
5. Keep the device upright and press the handle of the outer casing to close the device (Figure 1, step 4). The handle will lock into place when closed completely (Figure 1, step 5).
6. Read result after 15 minutes. Results are stable up to 60 minutes after the Detection Chamber has been snapped shut.
   Note: When there is no liquid flow on the detection strip, gently tap the Detection Chamber on the work surface to initiate the flow and read result after 15 minutes.
7. Discard the used Detection Chambers in sealed bags and as appropriate for your laboratory.
   Warning: **DO NOT** open the AmpliVue Detection Chamber after use. Opening the Detection Chamber after use may result in amplicon contamination of the test area.

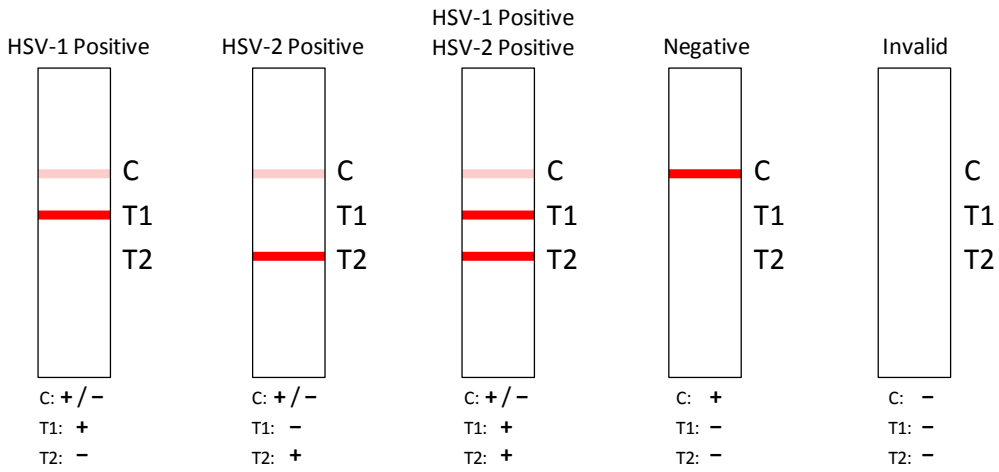
Figure 1. Chamber Detection



INTERPRETATION OF RESULTS

- **Positive:** Always read the Test lines (T1 and T2) first.
  - When T1 line is visible, report the assay result as "HSV-1 DNA detected."
  - When T2 line is visible, report the assay result as "HSV-2 DNA detected."
  - When T1 and T2 are visible, report the assay result as "HSV-1 and HSV-2 Positive: HSV-1 and HSV-2 DNA detected
- **Negative:** When no visible T line is present (T1–T2–), a visible C line indicates that the Internal Control DNA has been amplified and detected, eliminating the possibility of a false negative due to failure of amplification or device, and thus the assay result should be reported as negative – "no HSV DNA detected." The C line intensity may vary with each test. Any pink to red colored visible line in the control signifies a valid test.
- **Invalid:** If T and C lines are not present (T1–/T2–/C–), then the assay is invalid and the test needs to be repeated.

Figure 2. Interpretation of Results



The interpretation of the assay results is done according to the following criteria:

Test line 1 (T1) Reading	Test line 2 (T2) Reading	Control line (C) Reading	Interpretation of result
T1+	T2–	C+ or C–	**HSV-1 Positive**: HSV-1 DNA detected
T1–	T2+	C+ or C–	**HSV-2 Positive**: HSV-2 DNA detected
T1+	T2+	C+ or C–	**HSV-1 and HSV-2 Positive**: HSV-1 and HSV-2 DNA detected
T1–	T2–	C+	**Negative**: No HSV-1 or HSV-2 DNA detected
T1–	T2–	C–	**Invalid**: Failure due to inhibitory specimen, reagent failure, or device failure. Repeat test with original specimen.
Note: The absence of a C line (control) in conjunction with a positive test line (T1, T2 or T1 and T2) means that target material was successfully amplified. This occurs because of the overabundance of amplicons that generates competition with the internal control targets.			

QUALITY CONTROL

The AmpliVue HSV 1+2 Assay incorporates several controls to monitor assay performance.

1. A competitive internal control (IC) is included in the Reaction Tube to monitor inhibitory substances in clinical samples, reagent failure or device failure.
2. Commercially available external positive HSV-1 and HSV-2 controls should be used in accordance with your lab standards. Previously characterized positive HSV-1 and HSV-2 specimens may be used in lieu of commercial
   HSV-1 and HSV-2 controls.
3. Viral transport media or previously characterized negative specimen may be used as an external negative control. This must be treated as a patient specimen and should be performed in accordance with current lab standards.

Note: It is recommended that the reactivity of each new lot and each new shipment of the AmpliVue HSV 1+2 Assay be verified on receipt and before use. External control tests should be performed thereafter in accordance with appropriate Federal, State and Local guidelines. The AmpliVue HSV 1+2 Assay should not be used in patient testing if the external controls do not produce the correct results.

LIMITATIONS

- The AmpliVue HSV 1+2 Assay has been tested with male and female cutaneous and mucocutaneous lesion specimens only. Performance with other specimen types has not been assessed.
- The AmpliVue HSV 1+2 Assay is not intended for use with cerebrospinal fluid (CSF).
- The assay is not intended to be used for prenatal screening.
- This test may not detect a co-infection of HSV-1 and HSV-2 in cases where the two virus types are not equally represented in the swab specimen.
- As with many diagnostic tests, results from the AmpliVue HSV 1+2 Assay should be interpreted in conjunction with other laboratory and clinical data available to the physician.
- HSV viability and/or infectivity cannot be inferred from a positive test result since target DNA may persist in the absence of infectious virus
- A negative test does not exclude the possibility of infection because test results may be affected by improper specimen collection/transport/handling (inadequate specimen collection), presence of inhibitor(s), technical error, specimen mix-up, concurrent antiviral therapy, or the presence of insufficient DNA for detection.
- A trained health care professional should interpret assay results in conjunction with the patient's medical history, clinical signs and symptoms and the results of other diagnostic tests.

EXPECTED RESULTS

The prevalence of HSV-1 and HSV-2 with the AmpliVue HSV 1+2 Assay in cutaneous (skin lesion, genital – penis), or mucocutaneous (anorectal, genital – vaginal/cervical, nares, ocular, oral lesion and urethral) was estimated for the one thousand three hundred forty-three (1343) specimens with valid AmpliVue HSV 1+2 Assay results. Seven (7) of one thousand three hundred forty-three (1343) specimens were not included in the performance analysis due to contamination or invalid data by reference method.

The prevalence of HSV-1 and HSV-2 with the AmpliVue HSV 1+2 Assay was calculated for the combined sites based on the age of the patient and the specific source of specimen and are presented below.

Combined Study – Cutaneous Prevalence by Age						
Age	HSV- 1			HSV-2		
	Total #	Total Positive	Prevalence	Total #	Total Positive	Prevalence
≤ 5 years	37	2	5.4%	37	1	2.7%
6 to 21 years	68	13	19.1%	68	6	8.8%
22 to 59 years	225	20	8.9%	225	49	21.8%
≥ 60 years	70	5	7.1%	70	18	25.7%
	Percent	**95% CI**		**Percent**	**95% CI**	
Positive Predictive Value	76.9%	88.6%-100%		79.5%	68.8%-87.1%	
Negative Predictive Value	100%	94.6%-98.5%		99.7%	98.3%-99.9%	

Combined Study – Cutaneous Prevalence by Specific Source						
Source	HSV- 1			HSV-2		
	Total #	Total Positive	Prevalence	Total #	Total Positive	Prevalence
Skin lesion	271	27	10.0%	271	48	17.7%
Genital – penis	129	13	19.1%	129	26	20.2%

Combined Study – Mucocutaneous Prevalence by Age						
Age	HSV- 1			HSV-2		
	Total #	Total Positive	Prevalence	Total #	Total Positive	Prevalence
≤ 5 years	39	10	25.6%	39	1	2.6%
6 to 21 years	190	42	22.1%	190	34	17.9%
22 to 59 years	606	104	17.2%	606	132	21.8%
≥ 60 years	107	16	15.0%	107	17	15.9%
Not provided	1	1	100%	1	0	0%
	Percent	**95% CI**		**Percent**	**95% CI**	
Positive Predictive Value	87.1%	81.3%-91.3%		81.8%	75.5%-86.7%	
Negative Predictive Value	98.7%	97.5%-99.3%		99.5%	98.6%-99.8%	

Combined Study – Mucocutaneous Prevalence by Specific Source						
Source	HSV- 1			HSV-2		
	Total #	Total Positive	Prevalence	Total #	Total Positive	Prevalence
Anorectal	35	2	5.7%	35	8	22.9%
Genital – vaginal/cervical	691	109	15.9%	691	168	24.3%
Nasal	16	5	31.3%	16	2	12.5%
Ocular	18	3	16.7%	18	1	5.6%
Oral lesion	165	54	32.7%	165	2	1.2%
Urethral	18	0	N/A	18	3	16.7%

PERFORMANCE CHARACTERISTICS

Clinical Performance

The performance of the AmpliVue HSV 1+2 Assay was evaluated at five geographically diverse locations within the United States. A total of one thousand three hundred fifty-five (1355) specimens from symptomatic male and female patients were tested. Patient population ranged from ≤ 5 years to ≥ 60 years. The swab specimens have been categorized as cutaneous (skin lesion, genital – penis), or mucocutaneous (anorectal, genital – vaginal/cervical, nares, ocular, oral lesion and urethral). Nineteen (19) tests were considered invalid and were removed from the performance analysis.

The reference ELVIS viral culture used in this study was unable to detect co-infected specimens. Due to this, if a specimen was positive for HSV-2 it was removed from the calculation of the HSV-1 clinical performance. Two hundred eleven (211) specimens identified as HSV-2 positive by ELVIS have been removed from the initial one thousand three hundred thirty-six (1336) specimens. The data below is for the remaining one thousand one hundred twenty-five (1125) specimens.

Combined Sites – HSV-1 Cutaneous Lesions (N=340)								
Reference Method							95% CI	
AmpliVue HSV 1+2 Assay		POS	NEG	Total	**Sensitivity**	100%	88.6%	100%
	POS	30	9	39	**Specificity**	97.1%	94.6%	98.5%
	NEG	0	301	301				
	Total	30	310	340				

Combined Sites – HSV-1 Mucocutaneous Lesions (N=785)								
Reference Method							95% CI	
AmpliVue HSV 1+2 Assay		POS	NEG	Total	**Sensitivity**	94.9%	90.3%	97.4%
	POS	149	22	171	**Specificity**	96.5%	94.8%	97.7%
	NEG	8	606	614				
	Total	157	628	785				

The table below details the HSV-2 results for the one thousand three hundred thirty-six (1336) specimens.

Combined Sites – HSV-2 Cutaneous Lesions (N=399)								
Reference Method							95% CI	
AmpliVue HSV 1+2 Assay		POS	NEG	Total	Sensitivity	98.3%	91.0%	99.7%
	POS	58	15	73	Specificity	95.6%	92.8%	97.3%
	NEG	1	325	326				
	Total	59	340	399				

Combined Sites – HSV-2 Mucocutaneous Lesions (N=937)								
Reference Method							95% CI	
AmpliVue HSV 1+2 Assay		POS	NEG	Total	Sensitivity	97.4%	93.4%	99.0%
	POS	148	33	181	Specificity	95.8%	94.2%	97.0%
	NEG	4	752	756				
	Total	152	785	937				

ANALYTICAL PERFORMANCE

Precision – Repeatability

For the Precision/Within Laboratory Repeatability study, a blinded four-member panel consisting of medium positive and low positive, high negative 3x, 1x, ≤0.3x LOD, respectively) and negative HSV-1 and HSV-2 samples were tested in triplicate by two (2) operators, twice a day (2X) for twelve (12) days on all three instruments (triplicate testing x 2 operators x 12 days = 72 results per level for each virus). Positive and negative controls were run in triplicate with each test run. Results of the Precision/Within Laboratory Repeatability study for the AmpliVue HSV 1+2 Assay performed are presented in the tables below.

Repeatability Study Summary for HSV-1			
Category	**Rate of Detection**	**Overall Percent Agreement**	**95% Confidence Interval**
HSV 1+2 High Negative	35/72	51%	40% to 63%
HSV-1 Low Positive	72/72	100%	95% to 100%
HSV 1+2 Moderate Positive	72/72	100%	95% to 100%
HSV-1 Negative Sample	0/72	100%	95% to 100%
HSV-1+2 Positive Control	72/72	100%	95% to 100%
Assay Negative Control	0/72	100%	95% to 100%

Repeatability Study Summary for HSV-2			
Category	**Rate of Detection**	**Overall Percent Agreement**	**95% Confidence Interval**
HSV 1+2 High Negative	43/72	40%	30% to 52%
HSV-2 Low Positive	72/72	100%	95% to 100%
HSV 1+2 Moderate Positive	72/72	100%	95% to 100%
HSV-2 Negative Sample	0/72	100%	95% to 100%
HSV-1+2 Positive Control	72/72	100%	95% to 100%
Assay Negative Control	0/72	100%	95% to 100%

Precision/Reproducibility

The reproducibility of the AmpliVue HSV 1+2 Assay was evaluated at three test sites. Reproducibility was assessed using a panel consisting of four panel members: HSV 1+2 High Negative; HSV-1 Low Positive; HSV-2 Low Positive; and HSV 1+2 Moderate Positive members. The HSV-1 Low Positive member served as a HSV-2 Negative member and the HSV-2 Low Positive member served as a HSV-1 Negative member. The panel members were prepared in HSV Negative Matrix that consisted of a pool of HSV negative cheek swabs in M4 medium. HSV Negative Matrix was spiked with quantified HSV 1 and 2 viral stocks at pre-determined TCID50 concentrations. The HSV viral stock was diluted in the HSV Negative Matrix to three (3) different concentration levels, defined as High Negative member (0.3 x LOD), Low Positive member (1 x LOD) and Moderate Positive member (3 x LOD level).

Each run tested the four member panel of four members in triplicate and also included three each of HSV-1 + HSV-2 positive control, and negative control. Two (2) operators per test site each carried out one run of the four member panel plus controls per test day for five (5) days using one lot of the AmpliVue HSV 1+2 Assay. Results of the Reproducibility study for the AmpliVue HSV 1+2 Assay performed at three sites are presented in the tables below.

Reproducibility Study Summary for HSV-1									
Category	**Site**						**Rate of Detection**	**Overall Percent Agreement**	**95% Confidence Interval**
	Site #1		**Site #2**		**Site #3**				
	Rate of Detection	**Percent Agreement**	**Rate of Detection**	**Percent Agreement**	**Rate of Detection**	**Percent Agreement**			
HSV 1 +2 High Negative	16/30	47%	9/30	70%	20/30	33%	45/90	50%	40% to 60%
HSV-1 Low Positive	30/30	100%	29/30	97%	30/30	100%	89/90	99%	94% to 100%
HSV 1 +2 Moderate Positive	30/30	100%	30/30	100%	30/30	100%	90/90	100%	96% to 100%

Reproducibility Study Summary for HSV-1									
Category	Site #1		Site #2		Site #3		Rate of Detection	Overall Percent Agreement	95% Confidence Interval
	Rate of Detection	Percent Agreement	Rate of Detection	Percent Agreement	Rate of Detection	Percent Agreement			
HSV-2 Low Positive	0/30	100%	0/30	100%	0/30	100%	0/90	100%	96% to 100%
HSV-1+2 Positive Control	30/30	100%	30/30	100%	30/30	100%	90/90	100%	96%-100%
Negative Control	0/30	100%	0/30	100%	0/30	100%	0/90	100%	96%-100%

Reproducibility Study Summary for HSV-2									
Category	Site						Rate of Detection	Overall Percent Agreement	95% Confidence Interval
	Site #1		Site #2		Site #3				
	Rate of Detection	Percent Agreement	Rate of Detection	Percent Agreement	Rate of Detection	Percent Agreement			
HSV-1+2 High Negative	20/30	33%	17/30	43%	13/30	57%	50/90	44%	35% to 55%
HSV-2 Low Positive	30/30	100%	30/30	100%	30/30	100%	90/90	100%	96% to 100%
HSV-1+2 Moderate Positive	30/30	100%	30/30	100%	30/30	100%	90/90	100%	96% to 100%
HSV-1 Low Positive	0/30	100%	0/30	100%	0/30	100%	0/90	100%	96% to 100%
HSV-1+2 Positive Control	30/30	100%	30/30	100%	30/30	100%	90/90	100%	96% to 100%
Negative Control	0/30	100%	0/30	100%	0/30	100%	0/90	100%	96% to 100%

Analytical Specificity/Cross-Reactivity

A study was performed to evaluate the performance of the AmpliVue HSV 1+2 Assay in the presence of eighty-nine (89) microorganisms that might be found in lesion specimens. The panel members were obtained from suppliers as purified genomic DNA (GD) or quantified cultures (QC), or prepared in house (IHC) by growing each organism and quantifying the culture. Each potentially interfering or cross-reactive microorganism was tested in three (3) replicates the presence of negative matrix or 3x LOD HSV-1 and HSV-2. Clinically relevant levels of viruses and bacteria are typically 10^6cfu/mL or higher for bacteria and 10^5 pfu/mL or higher for viruses. Purified and quantified DNA or RNA was used for nine (9) of the microorganisms. For these microorganisms 10^6 copies per mL (cp/mL) or higher was used.

None of the eighty-nine (89) microorganisms that might be found in lesion specimens interfere or cross-react with the assay.

Cross Reactivity Panel		
Microorganism	**Member Type (GD, QC , IHC)**	**Test Concentration**
Bacteria (N=52)		
Acholeplasma laidlawi PG8	QC	7.1 x 10^6 cfu/mL
Acinetobacter calcoaceticus	QC	9.80 x 10^6 cfu/mL
Acinetobacter lwoffi	IHC	4.55 x 10^6 cfu/mL
Bacteroides fragilis Z029	QC	8.8 x 10^6 cfu/mL
Bordetella bronchiseptica	QC	1.17 x 10^6 cfu/mL
Bordetella pertussis E431	QC	1.73 x 10^6 cfu/mL
Chlamydia trachomatis VR-347	QC	3.00 x 10^6 cfu/mL
Chlamydia trachomatis D-UW3	QC	7.83 x 10^7 IFU/mL
Chlamydia trachomatis LGV-II 434 DNA	GD	1.5 x 10^7 cp/mL
Chlamydophila pneumoniae DNA	GD	1.6 x 10^6 cp/mL
Clostridium difficile NAP1	QC	6.77 x 10^6 cfu/mL
Clostridium perfringens Type A	QC	1.06 x 10^6 cfu/mL
Corynebacterium diphtheriae	QC	3.44 x 10^6 cfu/mL
Enterobacter cloacae Z101	QC	5.70 x 10^6 cfu/mL
Enterococcus faecalis VSE	QC	8.60 x 10^6 cfu/mL
Escherichia coli ATCC 43895	QC	1.13 x 10^6 cfu/mL
Fusobacterium nucleatum	IHC	8.05 x 10^6 cfu/mL
Gardnerella vaginalis	QC	1.20 x 10^6 cfu/mL
Haemophilis influenzae type A	QC	4.00 x 10^6 cfu/mL
Haemophilus ducreyi Class I DNA	GD	2.97 x 10^6 cp/mL
Klebsiella pneumoniae	QC	9.75 x 10^6 cfu/mL
Lactobacillus acidophilus Z048	QC	2.00 x 10^6 cfu/mL
Legionella pneumophila	QC	1.42 x 10^6 cfu/mL
Mobiluncus curtisii V125 [DSM 2711] ATCC 43063	QC	3.2 x 10^6 cfu/mL
Mobiluncus mulieris ATCC 35240	QC	1.76 x 10^6 cfu/mL
Moraxella cartarrhalis Ne11	QC	9.90 x 10^6 cfu/mL
Mycoplasma hominis LBD-4	QC	1.30 x 10^6 cfu/mL
Mycoplasma hyorhinis BTS-7	QC	6.6 x 10^6 cfu/mL
Mycoplasma orale CH 19299	QC	3.08 x 10^6 cfu/mL
Mycoplasma pneumoniae M129	QC	3.16 x 10^6 CCU/mL
Mycoplasma salivarium H110	QC	1.67 x 10^6 cfu/mL
Neisseria gonorrhoeae Z017	QC	5.73 x 10^6 cfu/mL
Neisseria meningitidis Serogroup A	QC	7.07 x 10^6 cfu/mL
Prevotella melaninogenica ATCC 25845	QC	7.3 x 10^6 cfu/mL
Proteus mirabilis	QC	1.19 x 10^6 cfu/mL
Pseudomonas aeruginosa	QC	1.32 x 10^6 cfu/mL
Salmonella enteriditis	QC	5.40 x 10^6 cfu/mL
Salmonella typhimurium	QC	4.60 x 10^6 cfu/mL
Staphylococcus aureus MRSA	IHC	7.52 x 10^6 cfu/mL
Staphylococcus aureus MSSA	IHC	7.02 x 10^6 cfu/mL
Staphylococcus epidermidis MRSE	IHC	1.75 x 10^6 cfu/mL
Staphylococcus saprophyticus	QC	3.00 x 10^6 cfu/mL

Cross Reactivity Panel		
Microorganism	**Member Type (GD, QC , IHC)**	**Test Concentration**
Streptococcus agalactiae	QC	2.20 x 10^6 cfu/mL
Streptococcus mitis	QC	2.43 x 10^6 cfu/mL
Streptococcus mutans Z072	QC	1.17 x 10^6 cfu/mL
Streptococcus pneumonia	QC	2.8 x 10^6 cfu/mL
Streptococcus pyogenes ATCC 9898	QC	6.38 x 10^6 cfu/mL
Streptococcus salivarius	IHC	2.75 x 10^6 cfu/mL
Toxoplasma gondii	QC	6.6 x 10^6 tachyzoties/mL
Treponema pallidum Nichols	QC	2.0 x 10^6 Tp/mL[2]
Trichomonas vaginalis Z070	QC	1.65 x 10^6 trophzoites/mL
Ureaplasma uralyticum NCTC 10177 DNA	GD	1.23 x 10^6 cp/mL
Yeast (N=7)		
Candida albicans	QC	2.00 x 10^6 cfu/mL
Candida glabrata Z007	QC	9.73 x 10^6 cfu/mL
Candida guilliermondii Z008	QC	9.96 x 10^6 cfu/mL
Candida krusei Z009	QC	5.33 x 10^6 cfu/mL
Candida lusitaniae Z010	QC	6.56 x 10^6 cfu/mL
Candida parapsilosis Z011	QC	1.24 x 10^6 cfu/mL
*Candida tropicalis Z*012	QC	1.0 x 10^6 cfu/mL
Virus (N=30)		
Influenza A/Mexico/4108/2009 H1N1	QC	4.08 x 10^6 $TCID_{50}$/mL
Adenovirus 2	QC	1.02 x 10^5 $TCID_{50}$/mL
Adenovirus 7 VR-7	QC	1.58 x 10^5 $TCID_{50}$/mL
Coronavirus OC43 VR-1558	QC	2.42 x 10^5 $TCID_{50}$/mL
Coxsackievirus B4	QC	1.08 x 10^5 $TCID_{50}$/mL
Cytomegalovirus AD-169	QC	9.55 x 10^5 $TCID_{50}$/mL
Echovirus 11 ODH-37285	QC	2.14 x 10^5 $TCID_{50}$/mL
Enterovirus Type 71	QC	1.00 x 10^5 $TCID_{50}$/mL
Epstein-Barr Virus B95-8	GD	2.22 x 10^5 cp/mL
Influenza B Hong Kong VR-791	QC	9.53 x 10^6 $TCID_{50}$/mL
Hepatitis B Virus	QC	3.44 x 10^5 IU/mL
Hepatitis C Virus	QC	7.58 x 10^5 IU/mL
HHV-8	QC	1.26 x 10^5 $TCID_{50}$/mL
HIV-1 Subtype B RNA	GD	1.14 x 10^5 cp/mL
hMPV (Italy) A1	QC	3.66 x 10^5 $TCID_{50}$/mL
Human Herpes 6 virus Z29 strain	QC	1.95 x 10^5 $TCID_{50}$/mL
Human Herpes 7 virus SB strain	QC	1.15 x 10^5 $TCID_{50}$/mL
Human papilloma virus 16 DNA	GD	4.3 x 10^5 cp/mL
Human papilloma virus 18 DNA	GD	1.8-3.6 x 10^5 cp/mL
Measles virus	QC	1.95 x 10^5 $TCID_{50}$/mL
Mumps virus	QC	5.89 x 10^5 $TCID_{50}$/mL
Parainfluenza Type 1 #2	QC	3.97 x 10^5 $TCID_{50}$/mL
Parainfluenza Type 2	QC	3.15 x 10^5 $TCID_{50}$/mL
Parainfluenza Type 3 NY14	QC	2.36 x 10^5 $TCID_{50}$/mL
Parainfluenza Type 4B VR-1377	QC	1.37 x 10^5 $TCID_{50}$/mL

Cross Reactivity Panel		
Microorganism	**Member Type (GD, QC , IHC)**	**Test Concentration**
RSV A Long VR-26	QC	4.36×10^4 $TCID_{50}$/mL
RSV B Washington VR-1401	QC	3.43×10^5 $TCID_{50}$/mL
Rubella virus	QC	4.17×10^5 $TCID_{50}$/mL
Simian Virus type 40 Pa-57 ATCC strain VR-239	QC	3.16×10^5 $TCID_{50}$/mL
VZV DNA	GD	1.5×10^5 cp/mL

Interference Studies

This study was performed to evaluate potential interference with AmpliVue HSV 1+2 Assay with a panel of thirty-three (33) substances, five (5) different viral transport media, and microorganisms from cross reactivity panel that may be present in clinical specimens. The study was carried out in the presence of HSV-1 and HSV-2 at 3 x LOD to evaluate potential interference to the detection of the HSV target. The study was also carried out in the absence of HSV to evaluate potential interference to the detection of internal control of AmpliVue HSV 1+2 Assay. Each potential interfering substance was tested in triplicate.

Interfering Substances

The analytical performance of AmpliVue HSV 1+2 Assay was characterized in the presence of interfering substances at potentially highest ("the worst case") concentrations to evaluate the susceptibility of the HSV assay to interference. By "worst case," each interfering substance was introduced into the assay by directly wetting a clean, dry Remel M4 kit swab with the substance and placing the swab directly in transport media. Calculated concentrations are based on an estimated volume of 200 µL of substance introduced by the swab. Each panel member was tested in triplicate spiked with HSV-1 HF and HSV-2 G strains separately at 3 x LOD. The panel was also tested in triplicate in the absence of HSV transport media to see if the potentially interfering substances interfere with the detection of the internal control. No interference was observed in the presence of the potential interfering substances tested.

Interfering Substance Panel	
Substance	**Test Concentration**
Seminal Fluid	7%
Cornstarch	1.25 mg/mL
Acetamidophenol	5 mg/mL
Feces	7%
Acetylsalicylic Acid	10 mg/mL
Chlorpheniramine	5 mg/mL
Dextromethorphan	10 mg/mL
Whole blood with EDTA	7%
Female Urine	7%
Male Urine	7%
Acyclovir (Acycloguanosine)	7 mg/mL
Albumin	3.3 mg/mL
Casein	7 mg/mL
K-Y Brand Jelly	7%
Douche	7%
Monistat 1	7%
Monistat 3	7%
Tioconazole 1	7%
Preparation H	7%
Lanacane	7%
Listerine	7%
Abreva	7%

Interfering Substance Panel	
Substance	**Test Concentration**
Carmex Cold Sore Lip Balm	7%
Releev cold sore treatment	7%
Crest	7%
Mucin (Bovine Submaxillary Gland, type I-S)	60 µg/mL
Buffy coat	7%
YeastGard	7%
Vagisil Crème	7%
Lip clear Lysine+	7%
Clotrimazole 3 Vaginal Cream	7%
Balneol Hygienic Cleansing Lotion	7%
Ortho Options Gynol II Extra Strength Vaginal Contraceptive Jelly	7%

Viral Transport Media

The performance of the AmpliVue HSV 1+2 Assay was assessed with Remel M5, Remel M4RT, Bartels VTM, and BD Universal Viral Transport (UVT)/ Copan UTM. (Remel M4 had previously been assessed and found to not interfere with the assay). Each medium was tested after spiking with HSV-1 HF and HSV-2 G strain to a final concentration of approximately 3 x LOD to determine if the viral transport media interferes with the detection of HSV targets in positive samples. The media were tested in the absence of HSV-1 and HSV-2 (medium only) to see if the viral transport media interfere with the detection of the internal control in negative samples.

There was no interference observed with the Remel M4RT, Remel M5, Bartels VTM, and BD UVT/Copan UTM media for the detection of HSV-1 and HSV-2 target or the internal control. Remel M4RT, Remel M5, Bartels VTM, and BD UVT/Copan UTM did not interfere with the detection of HSV-1 and HSV-2 target or the internal control.

Cross-Reactivity Panel Members

The performance of the AmpliVue HSV 1+2 Assay was characterized by testing the eighty-seven (87) microorganisms that were evaluated for analytical specificity and cross reactivity in the presence of HSV-1 HF and HSV-2 G at 3 x LOD separately to see if the presence of these organisms interferes with the detection of HSV targets. Each panel member was tested in triplicate. None of the cross reactivity panel members interfered with the detection of HSV-1 and HSV-2 target.

Specimen Stability

A study was performed to confirm the stability of HSV-1 and HSV-2 in viral transport media in accordance with recommended storage and handling specifications of each medium tested. The five (5) media described above were spiked with HSV-1 or HSV-2 at 3 x LOD and stored at 2°C to 8°C or –70°C. The media was tested by AmpliVue HSV 1+2 Assay at multiple time points. Based on this study at 3x LOD, HSV-1 and HSV-2 are stable in all five (5) media for 7 days at 2°C to 8°C, and for 34 days at –70°C.

Competitive Inhibition

The performance of the AmpliVue HSV 1+2 Assay was assessed for competitive interference using simulated samples in two studies mimicking co-infections. The first study used simulated samples with one target at a concentration near the LOD (3 x LOD) and the other target at higher concentrations (30 x LOD to 3000 x LOD). The second study used simulated samples that had equal concentrations of HSV-1 virus and HSV-2 virus (3 x LOD to 3000 x LOD).

In the first study competitive inhibition was not observed with simulated samples containing one target at a concentration near the LOD (3 x LOD) and the other target at up to 300 x LOD. However, competitive inhibition was observed for both HSV-1 and HSV-2 with simulated samples containing one target at a concentration near the LOD (3 x LOD) and the other target at 3000 x LOD.

In the second study competitive inhibition was not observed with simulated samples containing equal concentrations of HSV-1 virus and HSV-2 virus, from 3 x LOD to 3000 x LOD.

Limit of Detection

A Limit of Detection (LOD) study was performed to evaluate the analytical sensitivity of AmpliVue HSV 1+2 Assay using two representative viral strains of HSV-1 (McIntyre & HF) and two representative strains of HSV-2 (G & MS). Quantified ($TCID_{50}$/mL) cultures of the HSV-1 and HSV-2 strains were serially diluted to five (5) concentrations in HSV-negative matrix pools and tested in replicates of ten (10) on three reagent lots. The observed LOD of a HSV strain was determined as the lowest concentration level that had a positivity rate of ≥95%. The observed limit of detection of HSV-1 and HSV-2 were determined to be 1.1 x 10^5 $TCID_{50}$/mL and 1.1 x 10^4 $TCID_{50}$/mL, respectively.

Results obtained with each HSV LOD Panels					
Strain	Concentration $TCID_{50}$/mL	Positive/Total	Positivity rate	95% CI	
HSV-1 McIntyre	1.00 x10^6	30/30	100%	88.65%	100%
	3.33 x10^6	30/30	100%	88.65%	100%
	1.1 x10^5	30/30	100%	88.65%	100%
	3.70 x10^4	24/30	80.0%	62.69%	90.50%
	1.23 x10^4	8/30	26.7%	14.18%	44.45%
HSV-1 HF	1.00 x10^6	30/30	100%	88.65%	100%
	3.33 x10^5	30/30	100%	88.65%	100%
	1.11 x10^5	30/30	100%	88.65%	100%
	3.70 x10^4	23/30	76.7%	59.07%	88.21%
	1.23 x10^4	9/10	30.0%	16.66%	47.88%
HSV-2 G	1.00 x10^5	30/30	100%	88.65%	100%
	3.33 x10^4	30/30	100%	88.65%	100%
	1.11 x10^4	29/30	100%	88.30%	100%
	3.70 x10^3	28/30	93.3%	78.68%	98.15%
	1.23 x10^3	24/30	73.3%	55.55%	85.82%
HSV-2 MS	1.00 x10^5	30/30	100%	88.65%	100%
	3.33 x10^4	30/30	100%	88.65%	100%
	1.11 x10^4	30/30	100%	88.65%	100%
	3.70 x10^3	30/30	100%	88.65%	100%
	1.23 x10^3	27/30	90%	74.38%	96.54%

The LOD was confirmed with the same two (2) HSV-1 and two (2) HSV-2 reference strains diluted to the observed LOD and tested with twenty (20) replicates using three (3) lots of AmpliVue HSV 1+2 Assay. Since all HSV-1 and HSV-2 strains show positivity rates of ≥95% with all three (3) validation lots, the observed LOD is confirmed for both HSV-1 and HSV-2.

Clinical Isolates Testing: In addition, twenty (20) HSV-1 and twenty (20) HSV-2 clinical isolates were cultured and quantified in $TCID_{50}$/mL. Each isolate was diluted to the corresponding LOD in HSV-negative matrix and tested in triplicate. AmpliVue HSV 1+2 Assay was able to detect all 20 HSV-1 and 20 HSV-2 clinical isolates.

Assay LOD: The final assay LOD claim is 1.1 x $10^5$$TCID_{50}$/mL for HSV-1 and 1.1 x 10^4 $TCID_{50}$/mL for HSV-2.

Carry-over/Cross Contamination

Test results confirm that carry-over and cross contamination does not occur with AmpliVue HSV 1+2 Assay. High HSV-1 (HSV-2) positive samples were tested in series alternating with negative samples. In order to challenge the device, cultured and quantified viral stock served as high positive sample. HSV-1 HF (7.96 x 10^8 $TCID_{50}$/mL) and HSV-2 G (2.27 x 10^7 $TCID_{50}$/mL) viral stocks were used directly without dilution, for the highest concentration available. Remel M4 viral transport media was used as negative sample. Ten (10) replicates of negative sample together with assay controls were run by two (2) operators to confirm that negative samples (Remel M4 viral transport media) generate a negative result 100% of the time. Five (5) replicates of high-concentration positive and negative samples were tested

in a series, alternating sample types. All HSV-1 and HSV-2 high positive samples gave positive results and all the negative samples gave HSV negative results.

DISPOSAL

Dispose of hazardous or biologically contaminated materials according to the practices of your institution.

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GLOSSARY

REF Catalogue number	CE CE mark of conformity
EC REP Authorized Representative in the European Community	LOT Batch code
 Use by	 Manufacturer
 Temperature limitation	 Intended use
Rx ONLY Prescription use only	 Consult e-labeling instructions for use
IVD For *In Vitro* diagnostic use	 Contains sufficient for XX determinations
CONT Contents/Contains	