Aptima Herpes Simplex Viruses 1 & 2 Assay

For *in vitro* diagnostic use.

For U.S. Export only.

General Information

Intended Use

The Aptima Herpes Simplex Viruses 1 & 2 assay (Aptima HSV 1 & 2 assay) is an *in vitro* real time nucleic acid amplification test (NAAT) for the qualitative detection and differentiation of messenger RNA (mRNA) from herpes simplex virus (HSV) type 1 (HSV-1) and type 2 (HSV-2) on the Panther™ system.

The assay may be used to test clinician-collected swab specimens from skin lesions in the anogenital or oral region and placed in viral transport media (VTM) or Aptima specimen transport medium (STM). The assay will be used to aid in the diagnosis of HSV-1 and/or HSV-2 infections in symptomatic male and female patients.

The device is not intended for use with cerebrospinal fluid or for prenatal screening.

Summary and Explanation of the Test

Herpes simplex virus types 1 and 2 (HSV-1 and HSV-2) are double-stranded DNA viruses belonging to the alpha herpesviridae subfamily. Although HSV-1 and HSV-2 are closely related, they are genetically and serologically distinct (1). In the United States, during 2005-2010, HSV-1 seroprevalence was 53.9% and HSV-2 seroprevalence was 15.7% (2).

HSV-1 and HSV-2 usually infect abraded skin or oral or genital mucosae, causing painful lesions. Following an initial symptomatic phase, the viruses establish latent infections in the sensory nerve ganglia causing incurable lifelong infections in humans. Many events, such as physical or emotional stress, fever, ultraviolet light, and tissue damage, can cause viral reactivation leading to recurring lesions or asymptomatic shedding (1, 3).

Although both HSV-1 and HSV-2 can infect oral and genital mucosa, HSV-1 accounts for a majority of non-genital infections. Genital HSV infection is one of the most prevalent sexually transmitted infections in the United States. While HSV-2 is still the most common cause of genital herpes, recent studies suggest an increase in the incidence of HSV-1 induced genital herpes (4). Genital HSV infections can facilitate acquisition and transmission of HIV (5). In addition, pregnant women with late-term primary HSV genital infection have a 50% chance of passing the virus to the fetus and are at higher risk for spontaneous abortion and premature delivery (6).

A high percentage of asymptomatic HSV infections are unrecognized by the patient or physician (7). Accurate diagnosis of HSV infections improves counseling, leads to effective treatment, and reduces transmission (4).

Historically, HSV infections have been diagnosed using viral culture followed by HSV typing using immunofluorescence, which are time-consuming and labor-intensive procedures. Nucleic acid amplification tests (NAATs) have proven to be more sensitive than culture methods and provide a much shorter time-to-result (4).

The Aptima HSV 1 & 2 assay is a NAAT developed for use on the automated Panther system that utilizes target capture, transcription mediated amplification (TMA™), and real-time detection of HSV-1, HSV-2, and an internal control (IC). The Aptima HSV 1 & 2 assay amplifies and detects mRNAs for HSV-1 and HSV-2 (8). These RNAs are expressed from the viral genome during the infection cycle, and are packaged inside HSV-1 and HSV-2 viral particles prior to virus release from infected cells (9). The Aptima HSV 1 & 2 assay, therefore, detects virus-infected cells and the mature virus particles themselves.

Principles of the Procedure

The Aptima HSV 1 & 2 assay involves three main steps, which all take place in a single tube on the Panther system: target capture, target amplification by TMA, and detection of the amplification products (amplicon) by fluorescent labeled probes (torches). The assay incorporates an IC in every test to monitor targeted nucleic acid capture, amplification and detection.

Specimens are collected in or transferred to a tube containing STM that lyses the cells, releases the mRNA, and protects it from degradation during storage. When the Aptima HSV 1 & 2 assay is performed, the target mRNA is isolated from the specimen by use of capture oligomers that are linked to magnetic microparticles. The capture oligomers contain sequences complementary to specific regions of the HSV mRNA target

molecules as well as a string of deoxyadenosine residues. During the hybridization step, the sequence-specific regions of the capture oligomers bind to specific regions of the HSV mRNA target molecule. The capture oligomer:target complex is then captured out of solution by decreasing the temperature of the reaction to room temperature. This temperature reduction allows hybridization to occur between the deoxyadenosine region on the capture oligomer and the poly-deoxythymidine molecules that are covalently attached to the magnetic particles. The microparticles, including the captured HSV mRNA target molecules bound to them, are pulled to the side of the reaction tube using magnets and the supernatent is aspirated. The particles are washed to remove residual specimen matrix that may contain amplification inhibitors.

After target capture is complete, the HSV mRNA is amplified using TMA, which is a transcription-based nucleic acid amplification method that utilizes two enzymes, MMLV reverse transcriptase and T7 RNA polymerase. The reverse transcriptase is used to generate a DNA copy of the target mRNA sequence containing a promoter sequence for T7 RNA polymerase. T7 RNA polymerase produces multiple copies of RNA amplicon from the DNA copy template.

Detection is achieved using single-stranded nucleic acid torches that are present during the amplification of the target and hybridize specifically to the amplicon in real time. Each torch has a fluorophore and a quencher. The quencher suppresses the fluorescence of the fluorophore as it is designed to be in close proximity when not hybridized to the amplicon. When the torch binds to the amplicon, the quencher is moved farther away from the fluorophore and it will emit a signal at a specific wavelength when excited by a light source. More torch hybridizes when more amplicon is present. The increase in fluorescent signal from progressive amplification is detected by fluorometers within the Panther system. The Panther system can detect and discriminate between the three fluorescent signals corresponding to HSV-1, HSV-2 and IC amplification products. The fluorescence (measured in relative fluorescence units [RFU]) is monitored over time to produce a real-time fluorescence emergence curve for each reporter dye. The Panther system software compares the fluorescence emergence curves to fixed cut off times to report results (TTime) for HSV-1, HSV-2 and IC.

Warnings and Precautions

A. To reduce the risk of invalid results, carefully read the entire package insert and the *Panther System Operator's Manual* prior to performing this assay.

B. For professional use.

Laboratory Related

C. Use only supplied or specified disposable laboratory ware.

D. Use routine laboratory precautions. Do not pipet by mouth. Do not eat, drink or smoke in designated work areas. Wear disposable, powderless gloves, protective eye wear, and laboratory coats when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and kit reagents.

E. Work surfaces, pipettes, and other equipment must be regularly decontaminated with 2.5% to 3.5% (0.35 M to 0.5 M) sodium hypochlorite solution.

F. Dispose of all materials that have come in contact with specimens and reagents according to local, state, and federal regulations (10, 11, 12, 13). Thoroughly clean and disinfect all work surfaces.

Specimen Related

G. Expiration dates for the specimen transfer kits pertain to the collection/transfer of specimens and not to specimen testing. Specimens collected/transferred any time prior to these expiration dates are valid for testing provided they have been transported and stored in accordance with the package insert, even if the expiration date on the transfer tube has passed.

H. Specimens may be infectious. Use Universal Precautions (10, 11, 12) when performing this assay. Proper handling and disposal methods should be established according to local regulations (13). Only personnel adequately trained in the use of the Aptima HSV 1 & 2 assay and trained in handling infectious materials should perform this procedure.

I. Maintain proper storage conditions during specimen shipping to ensure the integrity of the specimen. Specimen stability under shipping conditions other than those recommended has not been evaluated.

J. Avoid cross-contamination during the specimen handling steps. Be especially careful to avoid contamination by the spread of aerosols when loosening or uncapping specimens. Specimens can contain extremely high levels of organisms. Ensure that specimen containers do not contact one another, and discard used materials without passing over open containers. Change gloves if they come in contact with specimen.

K. Upon piercing, liquid can discharge from Aptima transport tube caps under certain conditions. Refer to the appropriate *Test Procedure* for more information.

L. If the lab receives an Aptima swab specimen transport tube with no swab, two swabs or a swab not supplied by Hologic, the specimen must be rejected.

Assay Related

M. Do not interchange, mix, or combine assay reagents from kits with different master lot numbers. Controls and assay fluids may be interchanged.

N. Avoid microbial and nuclease contamination of reagents.

O. Cap and store all assay reagents at specified temperatures. The performance of the assay may be affected by use of improperly stored assay reagents. See "*Reagent Storage and Handling Requirements*" and "*Panther System Test Procedure*" for more information.

P. Do not combine any assay reagents or fluids without specific instruction. Do not top off reagents or fluids. The Panther system verifies reagent levels.

Reagent Storage and Handling Requirements

A. The following table shows the storage conditions and stability for reagents and controls.

Reagent	Unopened Storage	Open Kit (Reconstituted)	
		Storage	Stability
Amplification Reagent	2°C to 8°C		
Amplification Reconstitution Solution	15°C to 30°C	2°C to 8°C	30 days[1]
Enzyme Reagent	2°C to 8°C		
Enzyme Reconstitution Solution	15°C to 30°C	2°C to 8°C	30 days[1]
Promoter Reagent	2°C to 8°C		
Promoter Reconstitution Solution	15°C to 30°C	2°C to 8°C	30 days[1]
Target Capture Reagent	15°C to 30°C	15°C to 30°C[2]	30 days[1]
Negative Control	2°C to 8°C		Single use vial
Positive Control	2°C to 8°C		Single use vial
Internal Control	2°C to 8°C		Single use vial

[1] When reagents are removed from the Panther system, they should be immediately returned to their appropriate storage temperatures.

[2] Storage condition for the working Target Capture Reagent (Target Capture Reagent with Internal Control added).

B. Discard any unused reconstituted reagents and working Target Capture Reagent (wTCR) after 30 days or after the Master Lot expiration date, whichever comes first.

C. Reagents stored onboard the Panther system have 120 hours of onboard stability.

D.  The Promoter Reagent and reconstituted Promoter Reagent are photosensitive. Protect these reagents from light during storage and preparation for use.

E. Avoid cross-contamination during reagent handling and storage. Recap all reconstituted reagents with new reagent caps each time prior to storage.

F. Do not freeze reagents.

Specimen Collection and Storage

Note. *Handle all specimens as if they contain potentially infectious agents. Use Universal Precautions.*

Note. *Take care to avoid cross-contamination during sample handling steps. For example, discard used material without passing over open tubes.*

Clinician-collected swab specimens from anogenital and oral lesions placed in the STM or VTM can be used.

Lesion samples may be collected using either the:

- Aptima Multitest Swab Specimen Collection kit (for STM)
- Commercially available VTM collection kit

A. Instruction for collection

Refer to the appropriate specimen collection kit package insert for specific collection instructions.

B. Specimen transport and storage before testing

1. Swab specimens collected in Aptima Multitest Swab Specimen Collection kit
    a. Transport and store the specimen in the Aptima swab specimen transport tube at 2°C to 30°C for up to 60 days after collection.
    b. If longer storage is needed, store specimens at ≤ –20°C up to 90 days after collection.
2. Swab specimens collected in VTM collection kit
    a. Transport and store the specimen in the VTM tube at 2°C to 8°C for up to 3 days after collection.
    b. Prior to testing with the Aptima HSV 1 & 2 assay, specimens collected in VTM must be transferred into the transfer tube from the Aptima Specimen Transport kit that contains 2.9 mL of STM according to the instructions below.
    c. Preparation of the specimen transfer area
        i. Put on clean powderless gloves.
        ii. Wipe down work surfaces and pipettors with 2.5% to 3.5% (0.35 M to 0.5 M) sodium hypochlorite solution.
        iii. Allow the sodium hypochlorite solution to contact work surfaces and pipettors for at least 1 minute, then follow with a DI water rinse. Dry the surfaces with clean paper towels.
        iv. Cover the bench with clean, plastic-backed, absorbent laboratory bench covers.
        v. In the specimen transfer area, place a test tube rack containing a sufficient number of Aptima specimen transfer tubes corresponding to the number of VTM specimens being tested.
        vi. Label each Aptima specimen transfer tube with the accession number or specimen ID.
    d. Specimen Transfer Procedure

i. To reduce the risk of contaminating other specimens, work with one VTM specimen at a time.

ii. Put on clean powderless gloves and place specimens to be tested in the specimen transfer area.

iii. Obtain one VTM specimen. Uncap the corresponding Aptima specimen transfer tube, placing the cap on the bench with the threads facing up.

iv. Vortex the VTM specimen for 3 to 10 seconds. Uncap the tube, placing the cap on the bench with the threads facing up.

v. Within 1 minute of vortexing, pipet 0.5 mL of the VTM specimen into the Aptima specimen transfer tube from the Aptima Specimen Transport kit that contains 2.9 mL of STM.

vi. Dispose of the pipette tip in a container of 0.5% sodium hypochlorite solution.

vii. Recap the Aptima specimen transfer tube tightly. Gently invert the tube 2 to 3 times to ensure complete mixture of the specimen.

viii. Recap the tube containing the leftover VTM specimen for storage at ≤ –70°C if desired.

ix. Repeat steps iii to viii for the transfer of subsequent specimens. Change powderless gloves often and especially if they come in contact with specimen.

e. After transfer to an Aptima specimen transfer tube, specimens may be transported and stored at 2°C to 30°C for up to 30 days.

f. If longer storage is needed, freeze the VTM specimen in the Aptima specimen transfer tube at ≤ –20°C up to 90 days.

C. Specimen storage after testing:

1. Specimens that have been assayed must be stored upright in a rack.
2. The specimen tubes should be covered with a new, clean plastic film or foil barrier.
3. If assayed samples need to be frozen or shipped, remove penetrable cap and place new non-penetrable caps on the specimen transport tubes. If specimens need to be shipped for testing at another facility, recommended temperatures must be maintained.
4. Prior to uncapping previously tested and recapped specimens, specimen transport tubes must be centrifuged for 5 minutes at 420 RCF (relative centrifugal force) to bring all of the liquid down to the bottom of the tube. **Avoid splashing and cross-contamination.**

Note. *Specimens must be shipped in accordance with applicable national, international, and regional transportation regulations.*

Panther System

Reagents for the Aptima HSV 1 & 2 assay are listed below for the Panther system. Reagent Identification Symbols are also listed next to the reagent name.

Reagents and Materials Provided

Note: *For information on any hazard and precautionary statements that may be associated with reagents, refer to the Safety Data Sheet Library at www.hologic.com/sds.*

Aptima Herpes Simplex Viruses 1 & 2 Assay Kit

100 tests (2 assay boxes and 1 Controls kit), Cat. No. PRD-03568

Controls are available separately. See individual catalog number below.

Aptima Herpes Simplex Viruses 1 & 2 Assay Refrigerated Box

(store at 2°C to 8°C upon receipt)

Symbol	Component	Quantity
A	**Amplification Reagent** *Non-infectious nucleic acids dried in buffered solution.*	1 vial
E	**Enzyme Reagent** *Reverse transcriptase and RNA polymerase dried in HEPES buffered solution.*	1 vial
PRO	**Promoter Reagent** *Non-infectious nucleic acids dried in buffered solution.*	1 vial
IC	**Internal Control** *Non-infectious nucleic acids in buffered solution.*	1 x 0.3 mL

Aptima Herpes Simplex Viruses 1 & 2 Assay Room Temperature Box

(store at 15°C to 30°C upon receipt)

Symbol	Component	Quantity
AR	**Amplification Reconstitution Solution** *Aqueous solution containing glycerol and preservatives.*	1 x 7.2 mL
ER	**Enzyme Reconstitution Solution** *HEPES buffered solution containing a surfactant and glycerol.*	1 x 5.8 mL
PROR	**Promoter Reconstitution Solution** *Aqueous solution containing glycerol and preservatives.*	1 x 4.5 mL
TCR	**Target Capture Reagent** *Nucleic acids in a buffered salt solution containing solid phase and non-infectious nucleic acids.*	1 x 26.0 mL
	Reconstitution Collars	3
	Master Lot Barcode Sheet	1 sheet

Aptima Herpes Simplex Viruses 1 & 2 Controls Kit (Cat. No. PRD-03569)

(store at 2°C to 8°C upon receipt)

Symbol	Component	Quantity
CONTROL –	**Negative Control** *Buffered solution.*	5 x 2.7 mL
CONTROL +	**Positive Control** *Non-infectious nucleic acids in buffered solution.*	5 x 1.7 mL
	Control Barcode Sheet	1 sheet

Materials Required But Available Separately

Note. *Materials with catalog numbers listed are available from Hologic, unless otherwise specified.*

Material	Cat. No.
Panther System	—
Panther Run Kit for Real Time Assays (for real time assays only)	PRD-03455 (5000 tests)
Aptima Assay Fluids Kit (also known as Universal Fluids Kit) contains Aptima Wash Solution, Aptima Buffer for Deactivation Fluid, and Aptima Oil Reagent	303014 (1000 tests)
Multi-tube units (MTUs)	104772-02
Panther Waste Bag Kit	902731
Panther Waste Bin Cover	504405
Or, Panther System Run Kit *(when running non-real time-TMA assays in parallel with real time-TMA assays)* *Contains MTUs, waste bags, waste bin covers, auto detect, and assay fluids*	303096 (5000 tests)
Aptima Assay Fluids Kit *(contains Aptima Wash Solution, Aptima Buffer for Deactivation Fluid, and Aptima Oil Reagent)*	303014 (1000 tests)
Multi-tube units (MTUs)	104772-02
Tips, 1000 µL conductive, liquid sensing	10612513 (Tecan)
Aptima Specimen Transfer Kit *for use with specimens collected in VTM*	301154C
Aptima Specimen Transfer Kit — printable *for use with specimens collected in VTM*	PRD-05110
P1000 tips	—
Aptima Multitest Swab Specimen Collection Kit	PRD-03546

Bleach (minimum 5.0% or 0.7 M sodium hypochlorite solution)	—
Note: Mix one part Bleach with one part deionized water to make diluted working bleach solution [2.5% to 3.5% (0.35 M to 0.5 M) sodium hypochlorite solution].	
Disposable, powderless gloves	—
Aptima penetrable caps	105668
Replacement non-penetrable caps	103036A
Reagent Replacement Caps	
Amplification, Enzyme, and Promoter reagent reconstitution solutions	*CL0041(100 caps)*
TCR	*501604 (100 caps)*
Plastic-backed laboratory bench covers	—
Lint-free wipes	—
Pipettor	—
Tips	—
Vortex Mixer	—

Panther System Test Procedure

Note. *See the Panther System Operator's Manual for additional procedural information.*

A. Work Area Preparation

1. Clean work surfaces where reagents will be prepared. Wipe down work surfaces with 2.5% to 3.5% (0.35 M to 0.5 M) sodium hypochlorite solution. Allow the sodium hypochlorite solution to contact surfaces for at least 1 minute and then follow with a DI water rinse. Do not allow the sodium hypochlorite solution to dry.
2. Clean a separate work surface where samples will be prepared. Use the procedure described above (step A.1).
3. Cover the bench surfaces on which the reagents and samples will be prepared with clean, plastic-backed absorbent laboratory bench covers.
4. Wipe pipettors with 2.5% to 3.5% (0.35 M to 0.5 M) sodium hypochlorite solution. Allow the sodium hypochlorite solution to contact surfaces for at least 1 minute and then follow with a DI water rinse. Do not allow the sodium hypochlorite solution to dry.

B. Reagent Reconstitution/Preparation of a New Kit

Note. *Reagent reconstitution should be performed prior to beginning any work on the Panther system.*

1. Prior to testing, Amplification, Enzyme, and Promoter Reagents must be reconstituted by combining contents of the bottles of lyophilized reagent with the appropriate reconstitution solution.
   a. Allow the lyophilized reagents to reach room temperature (15°C to 30°C) before use.
   b. Pair each reconstitution solution with its lyophilized reagent. Before attaching the reconstitution collar, ensure that the reconstitution solution and reagent have matching label symbols.
   c. Check the lot numbers on the Master Lot Barcode Sheet to ensure that the appropriate reagents are paired.

d. Open the lyophilized reagent vial and firmly insert the notched end of the reconstitution collar into the vial opening (Figure 1, Step 1).

e. Open the matching reconstitution solution bottle, and set the cap on a clean, covered work surface.

f. While holding the reconstitution solution bottle on the bench, firmly insert the other end of the reconstitution collar into the bottle opening (Figure 1, Step 2).

g. Slowly invert the assembled bottles. Allow the solution to drain from the bottle into the glass vial (Figure 1, Step 3).

h. Gently swirl the solution in the bottle to mix. Avoid creating foam while swirling the bottle (Figure 1, Step 4).

i. Wait at least 15 minutes for the lyophilized reagent to go into solution, then invert the assembled bottles again, tilting at a 45° angle to minimize foaming (Figure 1, Step 5). Allow all of the liquid to drain back into the plastic bottle.

j. Remove the reconstitution collar and glass vial (Figure 1, Step 6).

k. Recap the plastic bottle. Record operator initials and reconstitution date on the label (Figure 1, Step 7).

l. Discard the reconstitution collar and glass vial (Figure 1, Step 8).

***Warning**. Avoid creating foam when reconstituting reagents. Foam compromises level-sensing in the Panther system.*

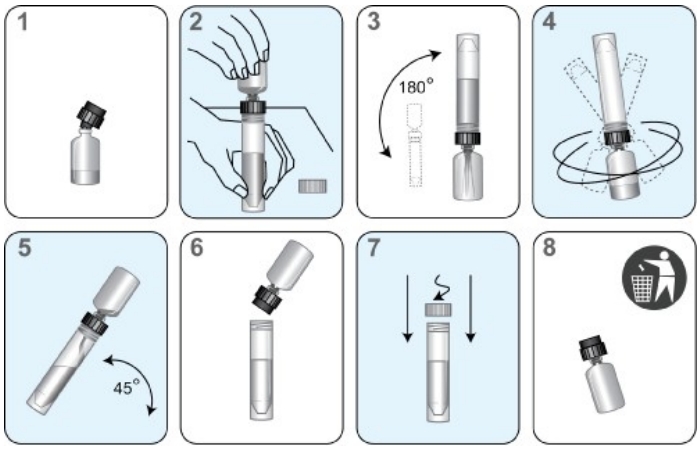

Figure 1. Reagent Reconstitution Process

2. Prepare Working Target Capture Reagent (wTCR)

a. Pair the appropriate bottles of TCR and IC.

b. Check the reagent lot numbers on the Master Lot Barcode Sheet to make sure that the appropriate reagents in the kit are paired.

c. Open the bottle of TCR, and set the cap on a clean, covered work surface.

d. Open the bottle of IC and pour the entire contents into the bottle of TCR. Expect a small amount of liquid to remain in the IC bottle.

e. Cap the bottle and gently swirl the solution to mix the contents. Avoid creating foam during this step.

f. Record operator initials and the current date on the label.

g. Discard the IC bottle and cap.

C. Reagent Preparation for Previously Prepared Reagents

1. Previously prepared Amplification, Enzyme, and Promoter reagents, must reach room temperature (15°C to 30°C) prior to the start of the assay.
2. If wTCR contains precipitate, warm wTCR at 42°C to 60°C for up to 90 minutes. Allow the wTCR to equilibrate to room temperature prior to use. Do not use if precipitate persists.
3. Verify that the reagents have not exceeded their storage stability times, including onboard stability.
4. Thoroughly mix each reagent by gently inverting prior to loading on the system. Avoid creating foam when inverting reagents.
5. Do not top off reagent bottles. The Panther system will recognize and reject bottles that have been topped off.

D. Specimen Handling

1. Allow the controls and specimens to reach room temperature prior to processing.
2. **Do not vortex samples.**
3. Visually confirm that each specimen tube meets one of the following criteria:
   a. The presence of a single pink Aptima collection swab in a swab specimen transport tube.
   b. The absence of a swab in the Aptima specimen transfer tube for VTM specimens.
4. Inspect specimen tubes before loading into rack:
   a. If a specimen tube contains bubbles in the space between the liquid and the cap, centrifuge the tube for 5 minutes at 420 RCF to eliminate the bubbles.
   b. If a specimen tube has a lower volume than typically observed when collection instructions have been followed, centrifuge the tube for 5 minutes at 420 RCF to ensure that no liquid is in the cap.

Note: *Failure to follow Steps 4a-4b may result in liquid discharge from the specimen tube cap.*

Note: *Up to 4 separate aliquots can be tested from each specimen tube. Attempts to pipette more than 4 aliquots from the specimen tube can lead to processing errors.*

E. System Preparation

1. Set up the system according to the instructions in the *Panther System Operator's Manual* and "*Procedural Notes"*. Make sure that the appropriately sized reagent racks and TCR adapters are used.

Procedural Notes

A. Controls

1. The positive control and negative control tubes can be loaded in any rack position or in any Sample Bay Lane on the Panther system. Specimen pipetting will begin when one of the following 2 conditions has been met:
   a. The controls are currently being processed by the system.
   b. Valid results for the controls are registered on the system.
2. Once the control tubes have been pipetted and are processing for a specific reagent kit, patient specimens can be tested with the associated kit up to 24 hours **unless:**
   a. Controls results are invalid.
   b. The associated assay reagent kit is removed from the system.
   c. The associated assay reagent kit has exceeded stability limits.
3. Each control tube can be tested once. Attempts to pipette more than once from the tube can lead to processing errors.

B. Temperature

Room temperature is defined as 15°C to 30°C.

C. Glove Powder

As in any reagent system, excess powder on some gloves may cause contamination of opened tubes. Powderless gloves are recommended.

Quality Control

A. Run Validity Criteria:

The software automatically determines run validity. The software will invalidate a run if either or both controls (negative and positive) have invalid results.

A run may be invalidated by an operator if technical, operator, or instrument difficulties are observed and documented while performing the assay.

An invalid run must be repeated.

B. Control Validity:

Table 1 defines the TTime validity criteria for the Negative and Positive Controls.

Table 1. TTime Validity Criteria

	IC TTime	HSV-1 TTime	HSV-2 TTime
Negative Control	≥7.0 and ≤40.0	-	-
Positive Control	≥7.0 and ≤53.0	≥3.0 and ≤35.0	≥3.0 and ≤35.0

***Note**: External quality control samples (not provided) should be tested in conformance with local, state, and/or federal regulation or accreditation requirements and each laboratory's standard Quality Control procedures.*

***Note**: For assistance with out-of-range controls, contact Hologic Technical Support.*

***Note**: When TTime cannot be calculated, a dash (-) will be displayed.*

Test Interpretation

Test results are automatically determined by the assay software. Results for HSV-1 and HSV-2 detection are reported separately. Table 2 shows the possible results reported in a valid run and result interpretations. Samples with invalid test results should be retested. Report the first valid result.

Table 2. Results Interpretation

HSV-1 Result	HSV-2 Result	Interpretation
HSV1 neg	HSV2 neg	Negative: No HSV-1 or HSV-2 mRNA detected
HSV1 neg	HSV2 POS	HSV-2 positive: HSV-2 mRNA detected
HSV1 POS	HSV2 neg	HSV-1 positive: HSV-1 mRNA detected
HSV1 POS	HSV2 POS	HSV-1 and HSV-2 positive: HSV-1 and HSV-2 mRNA detected
Invalid	Invalid	Invalid: There was an error in the generation of the result. Specimen should be retested.

Table 3 shows TTime criteria for determining the result for a particular specimen. A test may also be invalid due to other parameters being outside expected range.

Table 3. TTime Criteria

	IC TTime	HSV-1 TTime	HSV-2 TTime
Negative	≥7.0 and ≤45.0	-	-
HSV1 positive **HSV2 negative**	- or ≥7.0 and ≤53.0	≥3.0 and ≤53.0	-
HSV1 negative **HSV2 positive**	- or ≥7.0 and ≤53.0	-	≥3.0 and ≤53.0
HSV1 positive **HSV2 positive**	- or ≥7.0 and ≤53.0	≥3.0 and ≤53.0	≥3.0 and ≤53.0
Invalid	-	-	-

Note: *When TTime cannot be calculated, a dash (-) will be displayed.*

Limitations

A. Use of this assay is limited to personnel who have been trained in the procedure. Failure to follow the instructions given in this package insert may result in erroneous results.

B. Reliable results are dependent on adequate specimen collection, transport, storage, and processing.

C. The device is not intended for use with cerebrospinal fluid or for prenatal screening.

Panther System Analytical Assay Performance

Viral Transport Media (VTM)

The performance of the Aptima HSV 1 & 2 assay was assessed with commonly used types of VTM (BD Universal Viral Transport/Copan Universal Transport Media, Remel M4RT, Remel M4, and Remel M5). Each medium was spiked separately with HSV-1 MacIntyre strain or HSV-2 MS strain viral particles at ~3X the Limit of Detection (LoD). Each panel was then transferred according to the instructions in the STM package insert. To assess potential interference of different types of VTM, HSV-negative (un-spiked) panels were also diluted in STM and tested at forty replicates per panel. All negative panels were 100% valid and negative, and all HSV-1 or HSV-2 spiked panels were 100% positive for the appropriate HSV type.

Analytical Sensitivity

The analytical sensitivity/LoD of the Aptima HSV 1 & 2 Assay was determined by testing a series of panels consisting of HSV-1 or HSV-2 virus diluted in pooled negative clinical specimens in both STM and VTM diluted in STM-based matrices. For HSV-1, MacIntyre and HF viral strains were tested. For HSV-2, MS and G strains were tested. At least 60 replicates were tested at each concentration for each panel member for each matrix and virus strain across 3 reagents lots.

Probit regression analysis was preformed to provide the predicted 95% detection limit for each HSV strain in each matrix in each lot. LoD was determined to be the concentration at which ≥95% positivity of replicates tested is achieved based on the highest calculation among the three reagent lots.

Table 4. HSV 1 & 2 LoD in VTM and STM

HSV Type/Strain	Specimen Type	LoD TCID50/mL (95% Confidence)
HSV-1 MacIntyre	STM	60.6 (37.9 – 143.2)
	VTM	186.9 (148.1 – 266.5)
HSV-1 HF	STM	78.9 (47.7 – 195.3)
	VTM	159.3 (98.3 – 326.7)
HSV-2 MS	STM	18.2 (10.7 – 46.1)
	VTM	28.7 (15.6 – 105.6)
HSV-2 G	STM	18.8 (13.2 – 36.4)
	VTM	128.8 (57.8 – 584.2)

LoD Verification

LoD was verified using two clinical isolates of HSV-1 and two clinical isolates of HSV-2 that were isolated from HSV-positive clinical specimens and cultured and quantitated in-house. Each isolate was tested with the Aptima HSV 1 & 2 assay using 60 replicates each at 1X LoD, 3X LoD, and 10X LoD. Testing was completed in both STM and VTM matrix for all four clinical isolates and was conducted using 3 lots of reagents. All replicates for all clinical isolates at all three concentrations tested were detected by the Aptima HSV 1 & 2 assay, demonstrating that the assay can accurately detect a range of both HSV-1 and HSV-2 isolates at the determined LoD.

Co-Infection

Panels were built with HSV-1 viral particles at 3X LoD and HSV-2 virus at 1000X LoD, and with HSV-2 at 3X LoD and HSV-1 at 1000X LoD. Additional panels were built containing HSV-2 at 100X the concentration of HSV-1 at 3X LoD. All testing resulted in 100% detection for both HSV-1 and HSV-2.

Cross-Reactivity

To evaluate the analytical sensitivity and specificity of the Aptima HSV 1 & 2 assay in the presence of non-targeted microorganisms that could be present in clinical specimens, panels of non-targeted microorganisms were built in STM to a test concentration of 1 x 10^5 units/mL for viruses and 1 x 10^6 units/mL for all other organisms. Organisms were tested in the absence of HSV or in the presence of either HSV-1 or HSV-2 at 3X LoD. Forty-seven of the 48 microbes tested had no effect on assay performance at 1 x 10^6 units/mL; *Streptococcus pneumoniae* showed no interference at 1 x 10^5 units/mL (Table 5).

Table 5. Analytical Specificity

Microorganism	Concentration
Acinetobacter calcoaceticus	1x10^6 CFU/mL[1,2]
Acinetobacter lwoffii	1x10^6 CFU/mL[1,2]
Actinomyces israelii	1x10^6 RNA copies /mL[2]
Adenovirus type 1	1x10^5 TCID50/mL[3]
Alcaligenes faecalis	1x10^6 CFU/mL[1]
Atopobium vaginae	1x10^6 RNA copies /mL[2]
Bacteroides fragilis	1x10^6 CFU/mL[1,2]
Bifidobacterium adolescentis	1x10^6 CFU/mL[1,2]
BK virus	1x10^5 DNA copies/mL[3]
Bordetella bronchiseptica	1x10^6 CFU/mL[1,2]
Bordetella pertussis	1x10^6 CFU/mL[1,2]
Campylobacter jejuni	1x10^6 CFU/mL[1,2]
Candida glabrata	1x10^6 CFU/mL[1,2]
Clostridium difficile	1x10^6 CFU/mL[1,2]
Clostridium perfringens	1x10^6 CFU/mL[1,2]
Corynebacterium genitalium	1x10^6 CFU/mL[1,2]
Cryptococcus neoformans	1x10^6 CFU/mL[1,2]
Enterobacter aerogenes	1x10^6 CFU/mL[1,2]
Enterobacter cloacae	1x10^6 CFU/mL[1,2]
Enterococcus faecium	1x10^6 CFU/mL[1,2]
Enterococcus faecalis	1x10^6 CFU/mL[1,2]
Epstein-Barr virus	1x10^5 DNA copies/mL[3]
Escherichia coli	1x10^6 CFU/mL[1,2]
Fusobacterium nucleatum	1x10^6 CFU/mL[1,2]
Gardnerella vaginalis	1x10^6 CFU/mL[1,2]
Haemophilus ducreyi	1x10^6 CFU/mL[1,2]
Hepatitis B virus	1x10^5 IU/mL[4,3]

Table 5. Analytical Specificity

Microorganism	Concentration
Klebsiella pneumoniae	$1x10^6$ CFU/mL[1,2]
Lactobacillus crispatus	$1x10^6$ CFU/mL[1,2]
Moraxella catarrhalis	$1x10^6$ CFU/mL[1,2]
Mycoplasma hominis	$1x10^6$ RNA copies /mL[2]
Mycoplasma orale	$1x10^6$ RNA copies /mL[2]
Neisseria gonorrhoeae	$1x10^6$ CFU/mL[1,2]
Neisseria meningitidis	$1x10^6$ CFU/mL[1,2]
Parvovirus B19	$1x10^5$ TCID50/mL[3]
Prevotella bivia	$1x10^6$ CFU/mL[1,2]
Propionibacterium acnes	$1x10^6$ CFU/mL[1,2]
Proteus mirabilis	$1x10^6$ CFU/mL[1,2]
Proteus vulgaris	$1x10^6$ CFU/mL[1,2]
Pseudomonas aeruginosa	$1x10^6$ CFU/mL[1,2]
Staphylococcus aureus	$1x10^6$ CFU/mL[1,2]
Staphylococcus epidermidis	$1x10^6$ CFU/mL[1,2]
Staphylococcus saprophyticus	$1x10^6$ CFU/mL[1,2]
Streptococcus mitis	$1x10^6$ CFU/mL[1,2]
Streptococcus pneumoniae	100,000 CFU/mL[1,2]
Streptococcus pyogenes	$1x10^6$ CFU/mL[1,2]
Varicella-zoster virus	$1x10^5$ DNA copies/mL[3]
West Nile virus	$1x10^5$ TCID50/mL[3]

[1]CFU = Colony Forming Units, [2]Procured internally from Hologic, Inc.,[3]Obtained from ZeptoMetrix Corporation (Buffalo NY), [4]IU=International Units

Interference

Potentially interfering substances listed in Table 6 were tested in the Aptima HSV 1 & 2 assay at initial concentrations of 5% vol/vol (V/V), which is equivalent to 100% Swab Capacity (SC); or at concentrations of 0.03% or 5% wt/vol (W/V); or at 4 x 10^5 cells/mL for Leukocytes. Panels were built in STM and evaluated for potential effects on both assay sensitivity and specificity. Sensitivity performance was evaluated separately for both HSV-1 and HSV-2 by spiking viral particles into substance containing panels at 3X the LoD. HSV-negative panels containing each substance were also evaluated for specificity.

No effect on assay performance was observed in the presence of a representative brand of the following exogenous substances at 5%W/V or V/V (100% SC): vaginal lubricant; anti-fungal cream; douche; feminine spray; cold sore medication; lip balm; body lotion; body powder; glacial acetic acid wash solution; hemorrhoid cream; cough suppressant; toothpaste; and mouthwash. Spermicide/contraceptive jelly caused no interference at a concentration of 4% W/V or 80% of SC. No interference was observed in the presence of a representative brand of anti-viral medication at 5% W/V. No effect on assay performance was observed in the following endogenous substances tested at 5%V/V or W/V (100% SC): urine, mucus, and seminal fluid. No interference was observed in the following endogenous substances at the final concentrations stated: leukocytes ($4x10^5$ cells/mL); saliva (4% W/V / 80% SC); protein (4% W/V / 80% SC); whole bood (0.5% V/V / 10% SC); and feces (0.03% W/V / 0.6% SC).

Table 6: Interfering Substances

Substance	Brand/Source	Final Concentration*.
vaginal lubricant	KY Jelly	5% V/V
spermicide/contraceptive jelly	Options Gynol II	4% W/V
anti-fungal cream	Monistat 3	5% W/V
douche	Up & Up Feminine Wash	5% V/V
feminine spray	FDS Feminine Deodorant Spray	5% W/V
cold sore medication	Releev	5% W/V
lip balm	Carmex	5% W/V
body lotion	Vaseline Aloe Fresh	5% W/V
powder	Summer's Eve Powder	5% W/V
glacial acetic acid wash solution	glacial acetic acid wash solution	5% V/V
hemorrhoid cream	Preparation H	5% W/V
urine	In-house urine collection	5% V/V
whole blood	In-house whole blood collection	0.5% V/V
leukocytes	Biological Specialty Corporation Leukocytes	$4x10^5$ Cells/mL
saliva	In-house saliva collection	4% W/V
mucus	Sigma Aldrich Mucine	0.3% W/V
seminal fluid	seminal fluid	5% V/V
feces	feces	0.03% W/V
cough suppressant	Dayquil	5% V/V
toothpaste	Sensodyne	5% W/V
protein	Casein	4% W/V
antiviral drug	acyclovir	5% W/V
mouthwash	Listerene	5% V/V

*Final Concentrations represent final concentration (FC) in the sample when tested on the Panther instrument.
In terms of collection SC, 5% FC = 100% SC; 4% FC = 80% SC; 0.5% FC = 10% SC; 0.03% FC = 0.6% SC

HSV-2 Contrived Oral

Aptima HSV 1 & 2 assay testing was conducted using a contrived clinical specimen matrix to provide additional performance data for the detection of HSV-2 in oral samples. HSV-2 MS strain viral particles were spiked into HSV-negative VTM or STM oral clinical matrices at 3X LoD or 1000X LoD for each respective media. Fifteen replicates of HSV-negative samples, twenty-five replicates of HSV-2 at 3X LoD, and twenty-five replicates of HSV-2 at 1000X LoD for both VTM and STM matrices were tested by operators blinded to panel content. Results showed 100% detection of HSV-2-containing positive oral contrived panels and 0% detection in all negative samples in both STM and VTM clinical matrices.

Panther System Clinical Assay Performance

Reproducibility

Aptima HSV 1 & 2 assay reproducibility was evaluated at three external US sites. Testing was performed using three lots of assay reagents and six operators (two at each site). At each site, testing was performed for at least six days. Panel members were created by spiking HSV-1 and/or HSV-2 viral particles into STM. Final HSV-1 concentrations ranged from 0 $TCID_{50}$/mL to 86.96 $TCID_{50}$/mL and final HSV-2 concentrations ranged from 0 $TCID_{50}$/mL to 1.63 $TCID_{50}$/mL.

The robustness of the Aptima HSV 1 & 2 assay was assessed by testing HSV-negative panel members and panel members containing low and moderate levels of HSV-1 and HSV-2. Agreement with expected results was 100% for HSV-1 and HSV-2 in the negative and moderate positive panel members and ≤ 100% in panel members with concentrations near or below the 95% LoD of the assay in STM spiked with viral particles.

Table 7 shows the agreement of Aptima HSV 1 & 2 assay results with expected results for all panel members.

Table 7. Agreement of Aptima HSV 1 & 2 Assay Results With Expected Results

Conc		Target Conc ($TCID_{50}$/mL)		Expected Result		Agreed (n)			Agreement (%) (95% CI)	
HSV-1	**HSV-2**	**HSV-1**	**HSV-2**	**HSV-1**	**HSV-2**	**N**	**HSV-1**	**HSV-2**	**HSV-1**	**HSV-2**
Neg	Neg	0	0	Neg	Neg	108	108	108	100 (96.6-100)	100 (96.6-100)
LPos	Neg	28.90	0	Pos	Neg	108	103	108	95.4 (89.6-98.0)	100 (96.6-100)
Neg	LPos	0	0.54	Neg	Pos	108	108	105	100 (96.6-100)	97.2 (92.1-99.1)
LPos	MPos	28.90	1.63	Pos	Pos	108	97	108	89.8 (82.7-94.2)	100 (96.6-100)
MPos	LPos	86.96	0.54	Pos	Pos	108	108	108	100 (96.6-100)	100 (96.6-100)
HNeg	Neg	3.00	0	Pos	Neg	108	50	108	46.3 (37.2-55.7)	100 (96.6-100)
Neg	HNeg	0	0.20	Neg	Pos	108	108	86	100 (96.6-100)	79.6 (71.1-86.1)

CI = Score confidence interval, Conc = concentration, HNeg = high negative, LPos = low positive, MPos = moderate positive, Neg = negative, Pos = positive

Table 8 shows the HSV-1 and HSV-2 signal variability in low and moderate positive panel members between sites, between operators, between lots, between days, between runs, within runs, and overall in panel members with positive Aptima HSV 1 & 2 assay results.

Table 8. Signal Variability of the Aptima HSV 1 & 2 Assay in Low and Moderate Positive Panel Members

			Between Sites	Between Operators	Between Lots	Between Days	Between Runs	Within Runs	Total
Virus Conc	N	Mean TTime	SD (%CV)	SD (%CV)	SD (%CV)	SD (%CV)	SD (%CV)	SD (%CV)	SD (%CV)
HSV-1									
LPos	103	24.68	0	0.23	1.63	0.71	0.54	0.88	2.07
			(0)	(0.95)	(6.62)	(2.89)	(2.18)	(3.55)	(8.40)
LPos	97	23.91	0	0	2.18	0.86	0	1.60	2.84
			(0)	(0)	(9.11)	(3.58)	(0)	(6.71)	(11.87)
MPos	108	22.96	0	0.22	1.54	0.31	0.68	0.94	1.96
			(0)	(0.97)	(6.69)	(1.34)	(2.96)	(4.11)	(8.55)
HSV-2									
LPos	105	25.49	0	0.70	0.84	0	0	2.52	2.74
			(0)	(2.74)	(3.30)	(0)	(0)	(9.87)	(10.76)
LPos	108	25.34	0	0	1.54	0.86	0.59	2.67	3.26
			(0)	(0)	(6.08)	(3.41)	(2.34)	(10.53)	(12.85)
MPos	108	22.91	0	0	1.09	0.35	0.42	1.06	1.62
			(0)	(0)	(4.76)	(1.53)	(1.83)	(4.64)	(7.07)

Conc = concentration, CV = coefficient of variation, LPos = low positive, MPos = moderate positive, SD = standard deviation
Note: Variability from some factors may be numerically negative. This can occur if the variability due to those factors is very small. In these cases, SD and CV are shown as 0.

Clinical Performance

A prospective, multicenter clinical study was conducted to establish the performance characteristics of the Aptima HSV 1 & 2 assay. Male and female individuals (n = 839) with active skin lesions in the anogenital[1] or oral[2] regions were enrolled from 19 US clinical sites, including family planning, dermatology, pediatrics/adolescent, sexually transmitted infection, private practice, and public health clinics, hospitals, universities, and clinical research sites. Two (2) swab specimens were collected from a single lesion from each subject: one was collected with a swab from a commercially available VTM collection kit and one was collected with a swab from the Aptima Multitest swab collection kit. Specimens were processed in accordance with the appropriate package insert instructions and tested with the ELVIS HSV ID and D[3] Typing Test system viral culture and a validated bidirectional PCR/sequencing procedure to establish a composite reference method interpretation for HSV-1 and HSV-2. The composite reference method interpretation was considered: A) positive if either ELVIS HSV ID and D[3] Typing Test system viral culture or PCR/sequencing had a positive result for the HSV type (HSV-1 or HSV-2) and B) negative if PCR/sequencing had a negative result for one HSV type and ELVIS HSV ID and D[3] Typing Test system viral culture had a negative result (or a positive result for the other HSV type[3]). Specimens were tested with an FDA-cleared assay for HSV-1 and HSV-2 to clarify the HSV type when: A) PCR/sequencing detected both HSV-1 and HSV-2 and B) the combined results of the composite reference method tests were positive for both HSV types.

The clinical performance of the Aptima HSV 1 & 2 assay for detection of HSV-1 and HSV-2 was evaluated in specimens/samples collected from lesions in the anogenital and oral regions. Aptima HSV 1 & 2 assay testing was performed at 3 external laboratories. There were 108 Aptima HSV 1 & 2 assay runs generated; 107

[1] Includes abdomen, anus, buttocks, cervix, foreskin, glans penis/balanus, groin, mons pubis, penis (shaft), peri-anal area, perineum, rectum, scrotum, thigh, urethra/urethral orifice, vagina, vulvar area, and other.
[2] Includes gums, lips, mouth, tongue, and other.
[3] The ELVIS HSV ID and D[3] Typing Test system cannot detect co-infected specimens. Only HSV-2 negative specimens can be typed for HSV-1.

(99.1%) runs were valid and 1 run (0.9%) was invalid due to hardware error. There were 1629 samples processed in valid Aptima HSV 1 & 2 assay runs; 1628 (99.9%) had final valid results and 1 (0.1%) had a final invalid result due to a hardware error (this sample was not retested because it did not have sufficient volume). There were 7 samples (0.4%) that had initial invalid results; of these, 6 were retested and had valid results.

In total, 790 subjects (285 male and 505 female) were evaluable for inclusion in the performance analyses; 544 had lesions in the anogenital region and 246 had lesions in the oral region.

Overall, for detection of HSV-1 and HSV-2 in specimens/samples collected from lesions in the anogenital region, sensitivity ranged from 93.4% to 98.4% and specificity ranged from 92.8% to 99.8% (Tables 9 and 10).

Table 9 shows the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the Aptima HSV 1 & 2 assay for detection of HSV-1 and the prevalence of HSV-1 (based on the composite reference method) in anogenital lesions for each specimen type.

Table 9. Clinical Performance of the Aptima HSV 1 & 2 Assay for Detection of HSV-1 in Anogenital Lesions by Specimen Type

Specimen Type	Lesion Location	N	TP	FP	TN	FN	Prev (%)	Sensitivity % (95% CI)[3]	Specificity % (95% CI)[3]	PPV % (95% CI)[4]	NPV % (95% CI)[4]
VTM	Anogenital	528	71	1	451	5[1]	14.4	93.4 (85.5-97.2)	99.8 (98.8->99.9)	98.6 (93.0-100)	98.9 (97.6-99.6)
	Male anogenital	192	19	1	170	2	10.9	90.5 (71.1-97.3)	99.4 (96.8-99.9)	95.0 (78.6-99.8)	98.8 (96.4-99.9)
	Female anogenital	336	52	0	281	3	16.4	94.5 (85.1-98.1)	100 (98.7-100)	100 (93.7-100)	98.9 (97.1-99.8)
Aptima swab STM	Anogenital	531	71	2	454	4[2]	14.1	94.7 (87.1-97.9)	99.6 (98.4-99.9)	97.3 (91.1-99.6)	99.1 (97.9-99.8)
	Male anogenital	192	20	2	169	1	10.9	95.2 (77.3-99.2)	98.8 (95.8-99.7)	90.9 (74.5-98.7)	99.4 (97.2-100)
	Female anogenital	339	51	0	285	3	15.9	94.4 (84.9-98.1)	100 (98.7-100)	100 (93.6-100)	99.0 (97.2-99.8)

Aptima swab STM = Aptima Multitest swab specimen, Prev = prevalence, VTM = VTM sample

[1]Two samples had negative culture results and one had a non-typable HSV positive culture result.

[2]One specimen had a negative culture result and one had a non-typable HSV positive culture result.

[3]Score CI

[4]PPV 95% CI computed from the exact 95% CI for the positive likelihood ratio, NPV 95% CI computed from the exact 95% CI for the negative likelihood ratio

Table 10 shows the sensitivity, specificity, PPV, and NPV of the Aptima HSV 1 & 2 assay for detection of HSV-2 and the prevalence of HSV-2 (based on the composite reference method) in anogenital lesions for each specimen type.

Table 10. Clinical Performance of the Aptima HSV 1 & 2 Assay for Detection of HSV-2 in Anogenital Lesions by Specimen Type

Specimen Type	Lesion Location	N	TP	FP	TN	FN	Prev (%)	Sensitivity % (95% CI)[3]	Specificity % (95% CI)[3]	PPV % (95% CI)[4]	NPV % (95% CI)[4]
VTM	Anogenital	533	248	7	270	8[1]	48.0	96.9 (94.0-98.4)	97.5 (94.9-98.8)	97.3 (94.7-98.8)	97.1 (94.6-98.7)
	Male anogenital	194	79	2	110	3	42.3	96.3 (89.8-98.7)	98.2 (93.7-99.5)	97.5 (92.0-99.7)	97.3 (93.0-99.4)
	Female anogenital	339	169	5	160	5	51.3	97.1 (93.5-98.8)	97.0 (93.1-98.7)	97.1 (93.8-99.0)	97.0 (93.4-99.0)
Aptima swab STM	Anogenital	535	253	20	258	4[2]	48.0	98.4 (96.1-99.4)	92.8 (89.1-95.3)	92.7 (89.4-95.3)	98.5 (96.3-99.6)
	Male anogenital	194	79	6	106	3	42.3	96.3 (89.8-98.7)	94.6 (88.8-97.5)	92.9 (86.5-97.1)	97.2 (92.8-99.4)
	Female anogenital	341	174	14	152	1	51.3	99.4 (96.8-99.9)	91.6 (86.3-94.9)	92.6 (88.5-95.7)	99.3 (96.6-100)

Aptima swab STM = Aptima Multitest swab specimen, Prev = prevalence, VTM = VTM sample

[1]All eight samples had negative culture results.

[2]All four specimens had negative culture results.

[3]Score CI

[4]PPV 95% CI computed from the exact 95% CI for the positive likelihood ratio, NPV 95% CI computed from the exact 95% CI for the negative likelihood ratio

Sensitivity for detection of HSV-1 in specimens/samples collected in the oral region was 97.5% in Aptima Multitest swab specimens and 81.5% in VTM samples. Of the 22 VTM samples with false negative results for HSV-1, 19 samples had negative culture results (Table 13). Specificity for detection of HSV-1 was 88.7% in Aptima Multitest swab specimens and 99.2% in VTM samples. Nine (9) of the 14 Aptima Multitest swab specimens with false positive results were from 2 of the 17 collection sites that collected specimens from the oral region (sites 1 and 18, Table 17).

Table 11 shows the sensitivity, specificity, PPV, and NPV of the Aptima HSV 1 & 2 assay for detection of HSV-1 and the prevalence of HSV-1 (based on the composite reference method) in oral lesions for each specimen type.

Table 11. Clinical Performance of the Aptima HSV 1 & 2 Assay for Detection of HSV-1 in Oral Lesions by Specimen Type

Specimen Type	N	TP	FP	TN	FN	Prev (%)	Sensitivity % (95% CI)[3]	Specificity % (95% CI)[3]	PPV % (95% CI)[4]	NPV % (95% CI)[4]
VTM	241	97	1	121	22[1]	49.4	81.5 (73.6-87.5)	99.2 (95.5-99.9)	99.0 (95.0-100)	84.6 (79.3-89.3)
Aptima swab STM	243	116	14	110	3[2]	49.0	97.5 (92.8-99.1)	88.7 (81.9-93.2)	89.2 (83.9-93.5)	97.3 (93.1-99.4)

Aptima swab STM = Aptima Multitest swab specimen, Prev = prevalence, VTM = VTM sample

[1]Nineteen samples had negative culture results and one had a non-typable HSV positive culture result.

[2]All three specimens had negative culture results.

[3]Score CI

[4]PPV 95% CI computed from the exact 95% CI for the positive likelihood ratio, NPV 95% CI computed from the exact 95% CI for the negative likelihood ratio

Because most oral HSV infections are caused by HSV-1, the prevalence of HSV-2 infections observed in the oral region was very low (0.9% to 1.3%) (Table 12). Of 235 VTM samples and 237 Aptima Multitest swab specimens, only 2 VTM samples and 3 Aptima Multitest swab specimens had positive results based on reference testing. Sensitivity for detection of HSV-2 in specimens/samples collected in the oral region was 66.7% in Aptima Multitest swab specimens and 100% in VTM samples. The one Aptima Multitest swab specimen collected from an oral lesion with a false negative result had a negative culture result. As described above, analytical sensitivity for detection of HSV-2 using contrived oral specimens was 100%. Specificity for detection of HSV-2 was 100% in Aptima Multitest swab specimens and 100% in VTM samples.

Table 12 shows the sensitivity, specificity, PPV, and NPV of the Aptima HSV 1 & 2 assay for detection of HSV-2 and the prevalence of HSV-2 (based on the composite reference method) in oral lesions for each specimen type.

Table 12. Clinical Performance of the Aptima HSV 1 & 2 Assay for Detection of HSV-2 in Oral Lesions by Specimen Type

Specimen Type	N	TP	FP	TN	FN	Prev (%)	Sensitivity % (95% CI)[2]	Specificity % (95% CI)[2]	PPV % (95% CI)[3]	NPV % (95% CI)[3]
VTM	235	2	0	233	0	0.9	100 (34.2-100)	100 (98.4-100)	100 (30.1-100)	100 (99.3-100)
Aptima swab STM	237	2	0	234	1[1]	1.3	66.7 (20.8-93.9)	100 (98.4-100)	100 (29.1-100)	99.6 (98.9-100)

Aptima swab STM = Aptima Multitest swab specimen, Prev = prevalence, VTM = VTM sample

[1]This sample had a negative culture result.

[2]Score CI

[3]PPV 95% CI computed from the exact 95% CI for the positive likelihood ratio, NPV 95% CI computed from the exact 95% CI for the negative likelihood ratio

Table 13 summarizes Aptima HSV 1 & 2 assay results that were discrepant with the composite reference method interpretation for HSV-1.

Table 13. Discrepant Results Between the Composite Reference Method Interpretation for HSV-1 and Aptima HSV 1 & 2 Assay by Lesion Location and Specimen Type

		Composite Reference Method				
Lesion Location	**Specimen Type**	**Culture Result**	**PCR/sequencing Result**	**Aptima HSV 1 & 2 Assay Result**	**Interpretation**	**Count**
Anogenital	VTM	Negative	Negative	Positive	False positive	1
	Aptima swab STM	Negative	Negative	Positive	False positive	2
Oral	VTM	Negative	Negative	Positive	False positive	1
	Aptima swab STM	Negative	Negative	Positive	False positive	14
Anogenital	VTM	Negative	Positive	Negative	False negative	2
	Aptima swab STM	Negative	Positive	Negative	False negative	1
Oral	VTM	Negative	Positive	Negative	False negative	19
	Aptima swab STM	Negative	Positive	Negative	False negative	3
Anogenital	VTM	Not typable[1]	Positive	Negative	False negative	1
	Aptima swab STM	Not typable[1]	Positive	Negative	False negative	1
Oral	VTM	Not typable[1]	Positive	Negative	False negative	1
Anogenital	VTM	Positive	Negative	Negative	False negative	2
	Aptima swab STM	Positive	Negative	Negative	False negative	2
Oral	VTM	Positive	Positive	Negative	False negative	2

Aptima swab STM = Aptima Multitest swab specimen, VTM = VTM sample

[1]Positive for HSV, type not determined.

Table 14 summarizes Aptima HSV 1 & 2 assay results that were discrepant with the composite reference method interpretation for HSV-2.

Table 14. Discrepant Results Between the Composite Reference Method Interpretation for HSV-2 and Aptima HSV 1 & 2 Assay by Lesion Location and Specimen Type

		Composite Reference Method				
Lesion Location	**Specimen Type**	**Culture Result**	**PCR/sequencing Result**	**Aptima HSV 1 & 2 Assay Result**	**Interpretation**	**Count**
Anogenital	VTM	Negative	Negative	Positive	False positive	6
		HSV-1 positive	Negative	Positive	False Positive	1
	Aptima swab STM	Negative	Negative	Positive	False positive	18
		HSV-1 positive	Negative	Positive	False positive	2
Anogenital	VTM	Negative	Positive	Negative	False negative	8
	Aptima swab STM	Negative	Positive	Negative	False negative	4
Oral	Aptima swab STM	Negative	Positive	Negative	False negative	1

Aptima swab STM = Aptima Multitest swab specimen, VTM = VTM sample

Table 15 shows the sensitivity, specificity, PPV, and NPV of the Aptima HSV 1 & 2 assay for detection of HSV-1 and the prevalence of HSV-1 (based on the composite reference method) in anogenital lesions for each specimen type and collection site.

Table 15. Clinical Performance of the Aptima HSV 1 & 2 Assay for Detection of HSV-1 in Anogenital Lesions by Specimen Type and Collection Site

Specimen Type	Site	N	TP	FP	TN	FN	Prev (%)	Sensitivity % (95% CI)[1]	Specificity % (95% CI)[1]	PPV % (95% CI)[2]	NPV % (95% CI)[2]
VTM	2	2	1	0	1	0	50.0	100 (20.7-100)	100 (20.7-100)	100 (9.1-100)	100 (9.1-100)
	3	14	1	0	13	0	7.1	100 (20.7-100)	100 (77.2-100)	100 (6.6-100)	100 (92.8-100)
	4	6	0	0	6	0	0.0	NC	100 (61.0-100)	NC	100 (NC)
	5	4	0	0	4	0	0.0	NC	100 (51.0-100)	NC	100 (NC)
	6	32	4	0	27	1	15.6	80.0 (37.6-96.4)	100 (87.5-100)	100 (54.6-100)	96.4 (88.3-99.9)
	7	7	0	0	7	0	0.0	NC	100 (64.6-100)	NC	100 (NC)
	8	67	6	0	60	1	10.4	85.7 (48.7-97.4)	100 (94.0-100)	100 (64.6-100)	98.4 (93.7-100)
	9	25	0	0	25	0	0.0	NC	100 (86.7-100)	NC	100 (NC)
	10	8	0	0	8	0	0.0	NC	100 (67.6-100)	NC	100 (NC)
	11	193	33	0	159	1	17.6	97.1 (85.1-99.5)	100 (97.6-100)	100 (90.3-100)	99.4 (96.8-100)
	12	27	12	0	15	0	44.4	100 (75.8-100)	100 (79.6-100)	100 (78.6-100)	100 (82.5-100)
	13	38	7	0	30	1	21.1	87.5 (52.9-97.8)	100 (88.6-100)	100 (68.6-100)	96.8 (87.7-99.9)
	14	4	0	0	4	0	0.0	NC	100 (51.0-100)	NC	100 (NC)
	15	4	0	0	4	0	0.0	NC	100 (51.0-100)	NC	100 (NC)
	17	46	3	1	41	1	8.7	75.0 (30.1-95.4)	97.6 (87.7-99.6)	75.0 (26.3-98.8)	97.6 (92.8-99.9)
	18	50	4	0	46	0	8.0	100 (51.0-100)	100 (92.3-100)	100 (53.0-100)	100 (95.0-100)
	19	1	0	0	1	0	0.0	NC	100 (20.7-100)	NC	100 (NC)
Aptima swab STM	2	2	1	0	1	0	50.0	100 (20.7-100)	100 (20.7-100)	100 (9.1-100)	100 (9.1-100)
	3	14	1	0	13	0	7.1	100 (20.7-100)	100 (77.2-100)	100 (6.6-100)	100 (92.8-100)
	4	5	0	0	5	0	0.0	NC	100 (56.6-100)	NC	100 (NC)
	5	3	0	0	3	0	0.0	NC	100 (43.9-100)	NC	100 (NC)
	6	32	4	0	27	1	15.6	80.0 (37.6-96.4)	100 (87.5-100)	100 (54.6-100)	96.4(88.3-99.9)
	7	7	0	0	7	0	0.0	NC	100 (64.6-100)	NC	100 (NC)
	8	70	7	0	62	1	11.4	87.5 (52.9-97.8)	100 (94.2-100)	100 (68.0-100)	98.4 (93.6-100)
	9	26	0	0	26	0	0.0	NC	100 (87.1-100)	NC	100 (NC)
	10	8	0	0	8	0	0.0	NC	100 (67.6-100)	NC	100 (NC)
	11	193	32	0	160	1	17.1	97.0 (84.7-99.5)	100 (97.7-100)	100 (90.0-100)	99.4 (96.9-100)
	12	27	12	0	15	0	44.4	100 (75.8-100)	100 (79.6-100)	100 (78.6-100)	100 (82.5-100)
	13	38	7	0	30	1	21.1	87.5 (52.9-97.8)	100 (88.6-100)	100 (68.6-100)	96.8 (87.7-99.9)
	14	4	0	0	4	0	0.0	NC	100 (51.0-100)	NC	100 (NC)
	15	4	0	0	4	0	0.0	NC	100 (51.0-100)	NC	100 (NC)
	17	47	4	2	41	0	8.5	100 (51.0-100)	95.3 (84.5-98.7)	66.7 (35.1-94.2)	100 (94.6-100)
	18	50	3	0	47	0	6.0	100 (43.9-100)	100 (92.4-100)	100 (45.1-100)	100 (95.7-100)
	19	1	0	0	1	0	0.0	NC	100 (20.7-100)	NC	100 (NC)

Aptima swab STM = Aptima Multitest swab specimen, NC = not calculable, Prev = prevalence, VTM = VTM sample

[1]Score CI

[2]PPV 95% CI computed from the exact 95% CI for the positive likelihood ratio, NPV 95% CI computed from the exact 95% CI for the negative likelihood ratio

Note: Sites 1 and 16 did not enroll any subjects with lesions that were categorized as anogenital.

Table 16 shows the sensitivity, specificity, PPV, and NPV of the Aptima HSV 1 & 2 assay for detection of HSV-2 and the prevalence of HSV-2 (based on the composite reference method) in anogenital lesions for each specimen type and collection site.

Table 16. Clinical Performance of the Aptima HSV 1 & 2 Assay for Detection of HSV-2 in Anogenital Lesions by Specimen Type and Collection Site

Specimen Type	Site	N	TP	FP	TN	FN	Prev (%)	Sensitivity % (95% CI)[1]	Specificity % (95% CI)[1]	PPV % (95% CI)[2]	NPV % (95% CI)[2]
VTM	2	2	1	0	1	0	50.0	100 (20.7-100)	100 (20.7-100)	100 (9.1-100)	100 (9.1-100)
	3	14	8	1	5	0	57.1	100 (67.6-100)	83.3 (43.6-97.0)	88.9 (67.5-99.7)	100 (63.8-100)
	4	7	4	0	3	0	57.1	100 (51.0-100)	100 (43.9-100)	100 (63.7-100)	100 (51.9-100)
	5	4	2	0	2	0	50.0	100 (34.2-100)	100 (34.2-100)	100 (38.7-100)	100 (38.7-100)
	6	32	15	1	16	0	46.9	100 (79.6-100)	94.1 (73.0-99.0)	93.8 (75.5-99.8)	100 (83.7-100)
	7	7	5	0	2	0	71.4	100 (56.6-100)	100 (34.2-100)	100 (73.4-100)	100 (33.9-100)
	8	66	24	1	40	1	37.9	96.0 (80.5-99.3)	97.6 (87.4-99.6)	96.0 (82.5-99.9)	97.6 (88.9-99.9)
	9	26	15	0	10	1	61.5	93.8 (71.7-98.9)	100 (72.2-100)	100 (83.7-100)	90.9 (67.4-99.7)
	10	8	3	0	5	0	37.5	100 (43.9-100)	100 (56.6-100)	100 (50.6-100)	100 (69.7-100)
	11	194	94	2	94	4	50.5	95.9 (90.0-98.4)	97.9 (92.7-99.4)	97.9 (93.2-99.7)	95.9 (90.6-98.8)
	12	29	7	0	22	0	24.1	100 (64.6-100)	100 (85.1-100)	100 (67.3-100)	100 (88.5-100)
	13	38	13	0	25	0	34.2	100 (77.2-100)	100 (86.7-100)	100 (79.1-100)	100 (88.6-100)
	14	4	1	0	3	0	25.0	100 (20.7-100)	100 (43.9-100)	100 (7.3-100)	100 (65.0-100)
	15	4	2	0	2	0	50.0	100 (34.2-100)	100 (34.2-100)	100 (38.7-100)	100 (38.7-100)
	17	46	22	1	22	1	50.0	95.7 (79.0-99.2)	95.7 (79.0-99.2)	95.7 (81.9-99.9)	95.7 (81.9-99.9)
	18	51	31	1	18	1	62.7	96.9 (84.3-99.4)	94.7 (75.4-99.1)	96.9 (86.6-99.9)	94.7 (78.4-99.8)
	19	1	1	0	0	0	100.0	100 (20.7-100)	NC	100 (NC)	NC
Aptima swab STM	2	2	1	0	1	0	50.0	100 (20.7-100)	100 (20.7-100)	100 (9.1-100)	100 (9.1-100)
	3	14	8	1	5	0	57.1	100 (67.6-100)	83.3 (43.6-97.0)	88.9 (67.5-99.7)	100 (63.8-100)
	4	5	2	0	3	0	40.0	100 (34.2-100)	100 (43.9-100)	100 (36.2-100)	100 (57.4-100)
	5	3	1	0	2	0	33.3	100 (20.7-100)	100 (34.2-100)	100 (7.8-100)	100 (45.1-100)
	6	32	15	2	15	0	46.9	100 (79.6-100)	88.2 (65.7-96.7)	88.2 (70.8-98.4)	100 (83.3-100)
	7	7	5	0	2	0	71.4	100 (56.6-100)	100 (34.2-100)	100 (73.4-100)	100 (33.9-100)
	8	69	27	3	39	0	39.1	100 (87.5-100)	92.9 (81.0-97.5)	90.0 (76.7-97.7)	100 (92.3-100)
	9	27	16	1	9	1	63.0	94.1 (73.0-99.0)	90.0 (59.6-98.2)	94.1 (78.9-99.8)	90.0 (65.8-99.6)
	10	8	3	1	4	0	37.5	100 (43.9-100)	80.0 (37.6-96.4)	75.0 (37.9-99.2)	100 (62.9-100)
	11	194	97	5	91	1	50.5	99.0 (94.4-99.8)	94.8 (88.4-97.8)	95.1 (89.7-98.3)	98.9 (94.5-100)
	12	29	7	1	21	0	24.1	100 (64.6-100)	95.5 (78.2-99.2)	87.5 (58.2-99.6)	100 (88.4-100)
	13	38	13	2	23	0	34.2	100 (77.2-100)	92.0 (75.0-97.8)	86.7 (66.6-98.2)	100 (88.4-100)
	14	4	1	1	2	0	25.0	100 (20.7-100)	66.7 (20.8-93.9)	50.0 (3.1-97.5)	100 (41.4-100)
	15	4	1	0	2	1	50.0	50.0 (9.5-90.5)	100 (34.2-100)	100 (7.8-100)	66.7 (24.0-98.8)
	17	47	23	2	21	1	51.1	95.8 (79.8-99.3)	91.3 (73.2-97.6)	92.0 (78.7-98.8)	95.5 (81.4-99.9)
	18	51	32	1	18	0	62.7	100 (89.3-100)	94.7 (75.4-99.1)	97.0 (86.6-99.9)	100 (84.4-100)
	19	1	1	0	0	0	100.0	100 (20.7-100)	NC	100 (NC)	NC

Aptima swab STM = Aptima Multitest swab specimen, NC = not calculable, Prev = prevalence, VTM = VTM sample

[1]Score CI

[2]PPV 95% CI computed from the exact 95% CI for the positive likelihood ratio, NPV 95% CI computed from the exact 95% CI for the negative likelihood ratio

Note: Sites 1 and 16 did not enroll any subjects with lesions that were categorized as anogenital.

Table 17 shows the sensitivity, specificity, PPV, and NPV of the Aptima HSV 1 & 2 assay for detection of HSV-1 and the prevalence of HSV-1 (based on the composite reference method) in oral lesions for each specimen type and collection site.

Table 17. Clinical Performance of the Aptima HSV 1 & 2 Assay for Detection of HSV-1 in Oral Lesions by Specimen Type and Collection Site

Specimen Type	Site	N	TP	FP	TN	FN	Prev (%)	Sensitivity % (95% CI)[1]	Specificity % (95% CI)[1]	PPV % (95% CI)[2]	NPV % (95% CI)[2]
VTM	1	11	7	0	4	0	63.6	100 (64.6-100)	100 (51.0-100)	100 (74.4-100)	100 (58.0-100)
	3	14	3	0	10	1	28.6	75.0 (30.1-95.4)	100 (72.2-100)	100 (47.0-100)	90.9 (75.5-99.7)
	4	15	10	0	5	0	66.7	100 (72.2-100)	100 (56.6-100)	100 (79.3-100)	100 (61.8-100)
	5	4	2	0	2	0	50.0	100 (34.2-100)	100 (34.2-100)	100 (38.7-100)	100 (38.7-100)
	7	1	0	0	0	1	100.0	0.0 (0.0-79.3)	NC	NC	0.0 (NC)
	8	7	3	0	3	1	57.1	75.0 (30.1-95.4)	100 (43.9-100)	100 (49.2-100)	75.0 (39.0-99.2)
	9	1	1	0	0	0	100.0	100 (20.7-100)	NC	100 (NC)	NC
	10	7	3	0	4	0	42.9	100 (43.9-100)	100 (51.0-100)	100 (51.9-100)	100 (63.7-100)
	11	38	9	0	29	0	23.7	100 (70.1-100)	100 (88.3-100)	100 (72.2-100)	100 (90.6-100)
	12	2	0	0	2	0	0.0	NC	100 (34.2-100)	NC	100 (NC)
	13	2	0	0	2	0	0.0	NC	100 (34.2-100)	NC	100 (NC)
	14	12	3	0	8	1	33.3	75.0 (30.1-95.4)	100 (67.6-100)	100 (47.7-100)	88.9 (70.7-99.7)
	15	66	39	1	17	9	72.7	81.3 (68.1-89.8)	94.4 (74.2-99.0)	97.5 (89.5-99.9)	65.4 (51.7-79.4)
	16	24	9	0	13	2	45.8	81.8 (52.3-94.9)	100 (77.2-100)	100 (75.5-100)	86.7 (69.5-98.1)
	17	4	0	0	4	0	0.0	NC	100 (51.0-100)	NC	100 (NC)
	18	31	7	0	17	7	45.2	50.0 (26.8-73.2)	100 (81.6-100)	100 (69.7-100)	70.8 (61.2-84.1)
	19	2	1	0	1	0	50.0	100 (20.7-100)	100 (20.7-100)	100 (9.1-100)	100 (9.1-100)
Aptima swab STM	1	12	7	4	1	0	58.3	100 (64.6-100)	20.0 (3.6-62.4)	63.6 (50.6-83.2)	100 (6.8-100)
	3	14	4	1	9	0	28.6	100 (51.0-100)	90.0 (59.6-98.2)	80.0 (43.1-99.4)	100 (79.8-100)
	4	15	10	0	5	0	66.7	100 (72.2-100)	100 (56.6-100)	100 (79.3-100)	100 (61.8-100)
	5	4	2	0	2	0	50.0	100 (34.2-100)	100 (34.2-100)	100 (38.7-100)	100 (38.7-100)
	7	1	1	0	0	0	100.0	100 (20.7-100)	NC	100 (NC)	NC
	8	7	4	0	3	0	57.1	100 (51.0-100)	100 (43.9-100)	100 (63.7-100)	100 (51.9-100)
	9	1	1	0	0	0	100.0	100 (20.7-100)	NC	100 (NC)	NC
	10	7	3	0	4	0	42.9	100 (43.9-100)	100 (51.0-100)	100 (51.9-100)	100 (63.7-100)
	11	39	9	0	30	0	23.1	100 (70.1-100)	100 (88.6-100)	100 (72.2-100)	100 (90.8-100)
	12	1	0	0	1	0	0.0	NC	100 (20.7-100)	NC	100 (NC)
	13	2	0	0	2	0	0.0	NC	100 (34.2-100)	NC	100 (NC)
	14	11	2	1	7	1	27.3	66.7(20.8-93.9)	87.5 (52.9-97.8)	66.7 (18.4-98.3)	87.5 (69.0-99.5)
	15	66	46	2	16	2	72.7	95.8 (86.0-98.8)	88.9 (67.2-96.9)	95.8 (88.4-99.4)	88.9 (71.0-98.3)
	16	25	11	1	13	0	44.0	100 (74.1-100)	92.9 (68.5-98.7)	91.7 (69.9-99.8)	100 (81.5-100)
	17	4	0	0	4	0	0.0	NC	100 (51.0-100)	NC	100 (NC)
	18	32	15	5	12	0	46.9	100 (79.6-100)	70.6 (46.9-86.7)	75.0 (61.2-89.5)	100 (80.6-100)
	19	2	1	0	1	0	50.0	100 (20.7-100)	100 (20.7-100)	100 (9.1-100)	100 (9.1-100)

Aptima swab STM = Aptima Multitest swab specimen, NC = not calculable, Prev = prevalence, VTM = VTM sample

Note: Sites 2 and 6 did not enroll any subjects with lesions that were categorized as oral.

[1]Score CI

[2]PPV 95% CI computed from the exact 95% CI for the positive likelihood ratio, NPV 95% CI computed from the exact 95% CI for the negative likelihood ratio

Table 18 shows the sensitivity, specificity, PPV, and NPV of the Aptima HSV 1 & 2 assay for detection of HSV-2 and the prevalence of HSV-2 (based on the composite reference method) in oral lesions for each specimen type and collection site.

Table 18. Clinical Performance of the Aptima HSV 1 & 2 Assay for Detection of HSV-2 in Oral Lesions by Specimen Type and Collection Site

Specimen Type	Site	N	TP	FP	TN	FN	Prev (%)	Sensitivity % (95% CI)[1]	Specificity % (95% CI)[1]	PPV % (95% CI)[2]	NPV % (95% CI)[2]
VTM	1	11	0	0	11	0	0.0	NC	100 (74.1-100)	NC	100 (NC)
	3	14	1	0	13	0	7.1	100 (20.7-100)	100 (77.2-100)	100 (6.6-100)	100 (92.8-100)
	4	13	0	0	13	0	0.0	NC	100 (77.2-100)	NC	100 (NC)
	5	4	0	0	4	0	0.0	NC	100 (51.0-100)	NC	100 (NC)
	7	1	0	0	1	0	0.0	NC	100 (20.7-100)	NC	100 (NC)
	8	7	0	0	7	0	0.0	NC	100 (64.6-100)	NC	100 (NC)
	9	1	0	0	1	0	0.0	NC	100 (20.7-100)	NC	100 (NC)
	10	7	0	0	7	0	0.0	NC	100 (64.6-100)	NC	100 (NC)
	11	38	0	0	38	0	0.0	NC	100 (90.8-100)	NC	100 (NC)
	12	2	0	0	2	0	0.0	NC	100 (34.2-100)	NC	100 (NC)
	13	2	0	0	2	0	0.0	NC	100 (34.2-100)	NC	100 (NC)
	14	12	1	0	11	0	8.3	100 (20.7-100)	100 (74.1-100)	100 (6.6-100)	100 (91.5-100)
	15	63	0	0	63	0	0.0	NC	100 (94.3-100)	NC	100 (NC)
	16	24	0	0	24	0	0.0	NC	100 (86.2-100)	NC	100 (NC)
	17	4	0	0	4	0	0.0	NC	100 (51.0-100)	NC	100 (NC)
	18	30	0	0	30	0	0.0	NC	100 (88.6-100)	NC	100 (NC)
	19	2	0	0	2	0	0.0	NC	100 (34.2-100)	NC	100 (NC)
Aptima swab STM	1	12	0	0	12	0	0.0	NC	100 (75.8-100)	NC	100 (NC)
	3	14	1	0	13	0	7.1	100 (20.7-100)	100 (77.2-100)	100 (6.6-100)	100 (92.8-100)
	4	13	0	0	13	0	0.0	NC	100 (77.2-100)	NC	100 (NC)
	5	4	0	0	4	0	0.0	NC	100 (51.0-100)	NC	100 (NC)
	7	1	0	0	1	0	0.0	NC	100 (20.7-100)	NC	100 (NC)
	8	7	0	0	7	0	0.0	NC	100 (64.6-100)	NC	100 (NC)
	9	1	0	0	1	0	0.0	NC	100 (20.7-100)	NC	100 (NC)
	10	7	0	0	7	0	0.0	NC	100 (64.6-100)	NC	100 (NC)
	11	39	0	0	38	1	2.6	0.0 (0.0-79.3)	100 (90.8-100)	NC	97.4 (96.8-99.9)
	12	1	0	0	1	0	0.0	NC	100 (20.7-100)	NC	100 (NC)
	13	2	0	0	2	0	0.0	NC	100 (34.2-100)	NC	100 (NC)
	14	11	1	0	10	0	9.1	100 (20.7-100)	100 (72.2-100)	100 (6.7-100)	100 (90.6-100)
	15	63	0	0	63	0	0.0	NC	100 (94.3-100)	NC	100 (NC)
	16	25	0	0	25	0	0.0	NC	100 (86.7-100)	NC	100 (NC)
	17	4	0	0	4	0	0.0	NC	100 (51.0-100)	NC	100 (NC)
	18	31	0	0	31	0	0.0	NC	100 (89.0-100)	NC	100 (NC)
	19	2	0	0	2	0	0.0	NC	100 (34.2-100)	NC	100 (NC)

Aptima swab STM = Aptima Multitest swab specimen, NC = not calculable, Prev = prevalence, VTM = VTM sample

[1]Score CI

[2]PPV 95% CI computed from the exact 95% CI for the positive likelihood ratio, NPV 95% CI computed from the exact 95% CI for the negative likelihood ratio

Note: Sites 2 and 6 did not enroll any subjects with lesions that were categorized as oral.

Reference Range and Expected Values

Prevalence

The prevalence of HSV-1 and HSV-2 in different populations depends on patient risk factors such as age, lifestyle, and the sensitivity of the test in detecting the infection. A summary of the prevalence of HSV-1 and HSV-2, by specimen type and age group, as determined by the Aptima HSV 1 & 2 assay in the clinical performance study is shown in Table 19.

Table 19. Aptima HSV 1 & 2 Assay Positivity by Lesion Location Category and Age Group[1]

	%Prevalence (# positive/# tested)			
	VTM Sample		Aptima Multitest Swab Specimen	
Lesion Location Age Group	**HSV-1 Positive**	**HSV-2 Positive**	**HSV-1 Positive**	**HSV-2 Positive**
All lesion locations				
All ages	21.9 (170/778)	33.0 (257/778)	26.0 (203/782)	35.3 (276/782)
<2 years	40.0 (2/5)	0.0 (0/5)	40.0 (2/5)	0.0 (0/5)
2 to 11 years	30.8 (4/13)	0.0 (0/13)	50.0 (7/14)	0.0 (0/14)
12 to 21 years	21.5 (23/107)	40.2 (43/107)	24.8 (27/109)	42.2 (46/109)
22 to 30 years	18.9 (63/334)	36.8 (123/334)	21.4 (72/337)	39.5 (133/337)
31 to 40 years	20.7 (30/145)	33.8 (49/145)	27.3 (39/143)	35.7 (51/143)
41 to 50 years	22.7 (17/75)	26.7 (20/75)	25.7 (19/74)	28.4 (21/74)
51 to 60 years	30.9 (21/68)	22.1 (15/68)	37.7 (26/69)	24.6 (17/69)
> 60 years	32.3 (10/31)	22.6 (7/31)	35.5 (11/31)	25.8 (8/31)
Anogenital lesions				
All ages	13.4 (72/537)	47.5 (255/537)	13.5 (73/539)	50.8 (274/539)
<2 years	0.0 (0/1)	0.0 (0/1)	0.0 (0/1)	0.0 (0/1)
2 to 11 years	0.0 (0/1)	0.0 (0/1)	0.0 (0/1)	0.0 (0/1)
12 to 21 years	20.7 (17/82)	52.4 (43/82)	20.2 (17/84)	54.8 (46/84)
22 to 30 years	14.2 (38/268)	45.5 (122/268)	14.4 (39/270)	48.9 (132/270)
31 to 40 years	11.5 (12/104)	47.1 (49/104)	12.6 (13/103)	49.5 (51/103)
41 to 50 years	9.1 (4/ 44)	45.5 (20/44)	4.8 (2/42)	50.0 (21/42)
51 to 60 years	3.7 (1/ 27)	51.9 (14/27)	7.1 (2/28)	57.1 (16/28)
> 60 years	0.0 (0/10)	70.0 (7/10)	0.0 (0/10)	80.0 (8/10)
Oral lesions				
All ages	40.7 (98/241)	0.8 (2/241)	53.5 (130/243)	0.8 (2/243)
<2 years	50.0 (2/4)	0.0 (0/4)	50.0 (2/4)	0.0 (0/4)
2 to 11 years	33.3 (4/12)	0.0 (0/12)	53.8 (7/13)	0.0 (0/13)
12 to 21 years	24.0 (6/25)	0.0 (0/25)	40.0 (10/25)	0.0 (0/25)
22 to 30 years	37.9 (25/66)	1.5 (1/66)	49.3 (33/67)	1.5 (1/67)
31 to 40 years	43.9 (18/41)	0.0 (0/41)	65.0 (26/40)	0.0 (0/40)
41 to 50 years	41.9 (13/31)	0.0 (0/31)	53.1 (17/32)	0.0 (0/32)
51 to 60 years	48.8 (20/41)	2.4 (1/41)	58.5 (24/41)	2.4 (1/41)
> 60 years	47.6 (10/21)	0.0 (0/21)	52.4 (11/21)	0.0 (0/21)

[1]No subjects had positive Aptima HSV 1 & 2 assay results for both HSV-1 and HSV-2.

Positive and Negative Predictive Values for Hypothetical Prevalence Rates

The estimated positive and negative predictive values (PPV and NPV) of the Aptima HSV 1 & 2 assay for detection of HSV-1 and HSV-2 across different hypothetical prevalence rates are shown for each specimen type in Table 20. These calculations are based on the overall estimated sensitivity and specificity for each specimen type as determined in the clinical performance study.

Table 20. Hypothetical PPV and NPV for Detection of HSV-1 and HSV-2 by Specimen Type and Lesion Location Category

			HSV-1		HSV-2	
Specimen Type	**Lesion Location**	**Prevalence (%)**	**PPV (%)**	**NPV (%)**	**PPV (%)**	**NPV (%)**
VTM sample	Anogenital	1	81.0	99.9	27.9	100
		2	89.6	99.9	43.9	99.9
		5	95.7	99.7	66.9	99.8
		10	97.9	99.3	81.0	99.6
		20	99.1	98.4	90.6	99.2
		30	99.5	97.3	94.3	98.6
		40	99.6	95.8	96.2	97.9
		50	99.8	93.8	97.5	96.9
	Oral	1	50.1	99.8	100	100
		2	67.0	99.6	100	100
		5	84.0	99.0	100	100
		10	91.7	98.0	100	100
		20	96.1	95.5	100	100
		30	97.7	92.6	100	100
		40	98.5	88.9	100	100
		50	99.0	84.3	100	100
Aptima swab STM	Anogenital	1	68.6	99.9	12.1	100
		2	81.5	99.9	21.8	100
		5	91.9	99.7	41.9	99.9
		10	96.0	99.4	60.3	99.8
		20	98.2	98.7	77.4	99.6
		30	98.9	97.8	85.4	99.3
		40	99.3	96.6	90.1	98.9
		50	99.5	94.9	93.2	98.4
	Oral	1	8.0	100	100	99.7
		2	15.0	99.9	100	99.3
		5	31.2	99.9	100	98.3
		10	49.0	99.7	100	96.4
		20	68.3	99.3	100	92.3
		30	78.7	98.8	100	87.5
		40	85.2	98.1	100	81.8
		50	89.6	97.2	100	75.0

Aptima swab STM = Aptima Multitest swab specimen, VTM = VTM sample

TTime Distribution for Aptima HSV 1 & 2 Assay Positive Controls

The distribution of the TTime values for the Aptima HSV 1 & 2 assay positive control from all valid Aptima HSV 1 & 2 assay runs performed during the clinical performance study is presented in Table 21.

Table 21. Distribution of TTimes for Aptima HSV 1 & 2 Assay Positive Controls

	TTime	
Statistics	**HSV-1**	**HSV-2**
N	107	107
Mean	20.03	22.01
Median	19.8	21.7
SD	1.198	1.612
CV (%)	6.0	7.3
Minimum	18.1	19.5
Maximum	22.9	26.2

CV = coefficient of variation, SD = standard deviation

Bibliography

1. **Gupta R., T. Warren, A. Wald**. 2007. Genital Herpes. *The Lancet* 370: 2127-2137.
2. **Bradley H., L. Markowitz, T. Gibson, G. McQuillan**. 2014. Seroprevalence of Herpes Simplex Virus Types 1 and 2 United States, 1999-2010. *Journal of Infectious Diseases* (JID) 209: 325-333.
3. **Whitley R., B. Roizman**. 2001. Herpes Simplex Virus Infections. *The Lancet* 357: 1513-1518.
4. **LeGoff J., H. Péré, L. Bélec**. 2014. Diagnosis of Genital Herpes Simplex Virus Infection in the Clinical Laboratory. *Virology Journal* 11: 83-99.
5. **Wald A., K. Link.** 2002. Risk of Human Immunodeficiency Virus Infection in Herpes Simplex Virus Type 2- Seropositive Persons: A Meta-Analysis. *Journal of Infectious Diseases* (JID) 185: 45-52.
6. **Brown A., A. Wald, R. Morrow, S. Selke, J. Zeh, L. Corey.** 2003. Effect of Serologic Status and Cesarean Delivery on Transmission Rates of Herpes Simplex Virus from Mother to Infant. *The Journal of the American Medical Association* (JAMA) 289(2): 203-209.
7. **Ashley RL., A. Wald.** 1999. Genital Herpes: Review of the Epidemic and Potential Use of Type-Specific Serology. *Clinical Microbiology Reviews* 12: 1-8.
8. **Swenson, et al.** 2016. Evaluation of a transcription mediated amplification assay for detection of herpes simplex virus types 1 and 2 mRNA in clinical specimens. *J Clin Virol* 80, 62-67.
9. **Sciortino MT., M. Suzuki, B. Taddeo, B. Roizman**. 2001. RNAs Extracted from Herpes Simplex Virus 1 Virions: Apparent Selectivity of Viral but Not Cellular RNAs Packaged in Virions. *Journal of Virology* 75(17):8105-8116.
10. **Clinical and Laboratory Standards Institute.** 2005. Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods; Approved Guideline. CLSI Document MM13-A. Wayne, PA**.**
11. 29 CFR Part 1910.1030. Occupational Exposure to Bloodborne Pathogens; current version.
12. **Centers for Disease Control and Prevention/National Institutes of Health.** Biosafety in Microbiological and Biomedical Laboratories (BMBL); current version.
13. **Clinical and Laboratory Standards Institute.** 2002. Clinical Laboratory Waste Management. CLSI Document GP5-A2. Villanova, PA.

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