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 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 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 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 supernatant 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/Panther Fusion™ System Operator's Manual* prior to performing this assay.

Laboratory Related

B. Use only supplied or specified disposable laboratory ware.

C. 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.

D. 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.

E. 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.

F. Use good standard practices for molecular laboratories including environmental monitoring.

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, 2 swabs or a swab not supplied by Hologic, the specimen must be rejected. Prior to rejecting a swab transport tube with no swab, verify that it is not an Aptima Specimen Transfer Tube as this specimen transport tube will not contain a swab.

Assay Related

M. Do not interchange, mix, or combine assay reagents from kits with different master lot numbers. Aptima controls and assay fluids (Panther system) can be from different lot numbers..

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.

Q. Some reagents of this kit are labeled with risk and safety symbols.

Note. *For information on any hazard and precautionary statements that may be associated with reagents refer to the Safety Data Sheet Library at www.hologicsds.com. For more information on the symbols, refer to the symbol legend on www.hologic.com/package-inserts.*

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. Controls are stable until the date indicated on the vials.

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

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

G. 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 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 (Aptima Multitest Swab Specimen Collection Kit, for specimens collected in STM, or Aptima™ Specimen Transfer Kit, for specimens collected in VTM).

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 1 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, foil barrier, or cap.
Note. *Specimens must be shipped in accordance with applicable national, international, and regional transportation regulations*.
Note. *Any condition resulting in loss or evaporation of media during transport, handling, or storage may impact the ability to pipette multiple aliquots.*

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	303095
Panther Fusion System	PRD-04172
Panther System Continuous Fluid and Waste (Panther Plus)	PRD-06067
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, filtered, conductive, liquid sensing, disposable *Not all products are available in all regions. Contact your representative for region-specific information.*	901121 (10612513 Tecan) 903031 (10612513 Tecan) MME-04134 (30180117 Tecan) MME-04128

Material	Cat. No.
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
Aptima Multitest Swab Specimen Collection Kit	PRD-03546
Bleach, 5.0% to 8.25% (0.7 M to 1.16 M)	—
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	—
Centrifuge	—

Optional Materials

	Cat. No.
Hologic Bleach Enhancer for Cleaning *for routine cleaning of surfaces and equipment*.	302101
Tube rocker	—

Panther System Test Procedure

Note. *See the Panther/Panther Fusion System Operator's Manual for additional Panther system 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 deionized water (DI) water rinse. Do not allow the sodium hypochlorite solution to dry. Cover the bench surface with clean, plastic-backed absorbent laboratory bench covers.
2. Clean a separate work surface where samples will be prepared. Use the procedure described above (step A.1).
3. Clean any pipettors. Use the cleaning procedure described above (Step A.1)..

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. Label caps of reconstitution solution bottles.
   d. Open the lyophilized reagent glass vial and firmly insert the notched end of the reconstitution collar into the glass 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 reconstitution solution bottle opening (Figure 1, Step 2).
   g. Slowly invert the assembled bottles. Allow the solution to drain from the reconstitution solution 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 reconstitution solution bottle.
   j. Remove the reconstitution collar and glass vial (Figure 1, Step 6).
   k. Recap the plastic bottle with either the saved label cap that corresponds to the reagent or a new cap. Do not mismatch caps. Record the operator initials and reconstitution date on the label (Figure 1, Step 7).
   l. Discard the reconstitution collar and glass vial (Figure 1, Step 8).

   Option: *Additional mixing of the Amplification, Enzyme and Promoter Reagents using a tube rocker is allowed. The reagents may be mixed by placing the recapped plastic bottle on a tube rocker set to 20 RPM (or equivalent) for a minimum of 5 minutes.*

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

***Warning**. **A**dequate mixing of the reagents is necessary to achieve expected assay results..*

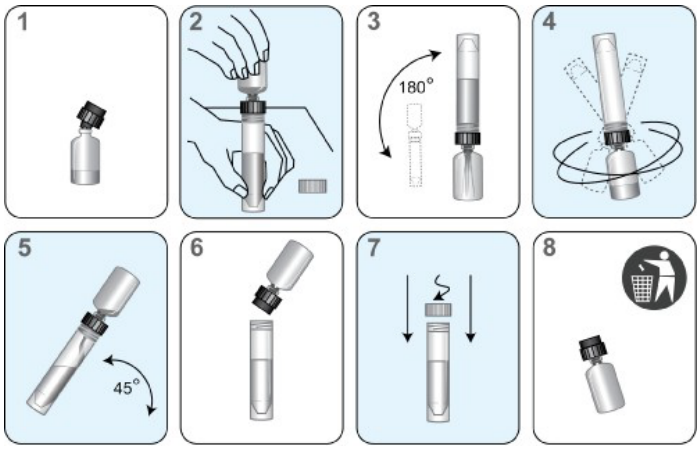

Figure 1. Reagent Reconstitution Process

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.

    ***Option:** Additional mixing of the Amplification, Enzyme and Promoter Reagents using a tube rocker is allowed. The reagents may be mixed by placing the recapped plastic bottle on a tube rocker set to 20 RPM (or equivalent) for a minimum of 25 minutes.*

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. Visually confirm that each specimen tube meets 1 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.
2. Allow the controls and specimens to reach room temperature prior to processing.

   Note. *Prior to testing and/or to resolve suspected specimen related invalid results, specimens may be vortexed at high speed for a minimum of 3 minutes, followed by low speed vortexing for 1 minute (to draw the fluid down in the tube).*
3. 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 3a-3b may result in liquid discharge from the specimen tube cap.*

Note. *Up to 5 separate aliquots can be tested from each specimen tube. Attempts to pipette more than 5 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/Panther Fusion System Operator's Manual* and "*Procedural Notes*".

   Note. *Make sure the appropriately sized reagent racks and TCR adapters are used.*
2. Load samples.

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 1 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. The first valid result is the result that should be reported. Samples with invalid test results should be retested. If the result is invalid upon retest, a new specimen should be collected.

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 40 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 performed 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 3 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 2 clinical isolates of HSV-1 and 2 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 4 clinical isolates and was conducted using 3 lots of reagents. All replicates for all clinical isolates at all 3 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 blood (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
protein	Casein	4% W/V
antiviral drug	acyclovir	5% W/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

Panther System Clinical Assay Performance

Reproducibility

Aptima HSV 1 & 2 assay reproducibility was evaluated at 3 external US sites. Testing was performed using 3 lots of assay reagents and 6 operators (2 at each site). At each site, testing was performed for at least 6 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.

HSV-negative panel members and panel members containing low (<1 X LoD and 1-2 X LoD) and moderate levels (2-3 X LoD) of HSV-1 and HSV-2 were tested with the Aptima HSV 1 & 2 assay. 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 8). The signal variability of the Aptima HSV 1 & 2 assay in the low and moderate positive panel members and in the positive control is summarized in Tables 9 and 10.

Table 8. Reproducibility - Agreement of Aptima HSV 1 & 2 Assay 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 = confidence interval, Conc = concentration, HNeg = high negative (<1 X LoD), LPos = low positive (1-2 X LoD), MPos = moderate positive (2-3 X LoD), Neg = negative, Pos = positive

Table 9. Reproducibility - Signal Variability of the Aptima HSV 1 & 2 Assay

Virus Conc	N	Mean TTime	Between Sites SD (%CV)	Between Lots SD (%CV)	Between Operators/ Days[1] SD (%CV)	Between Runs SD (%CV)	Within Runs SD (%CV)	Total SD (%CV)
HSV-1								
LPos	103	24.68	0	1.63	0.75	0.54	0.88	2.07
			(0)	(6.62)	(3.04)	(2.18)	(3.55)	(8.40)
LPos	97	23.91	0	2.18	0.86	0	1.60	2.84
			(0)	(9.11)	(3.58)	(0)	(6.71)	(11.87)
MPos	108	22.96	0	1.54	0.38	0.68	0.94	1.96
			(0)	(6.69)	(1.65)	(2.96)	(4.11)	(8.55)
HSV-2								
LPos	105	25.49	0	0.84	0.70	0	2.52	2.74
			(0)	(3.30)	(2.74)	(0)	(9.87)	(10.76)
LPos	108	25.34	0	1.54	0.86	0.59	2.67	3.26
			(0)	(6.08)	(3.41)	(2.34)	(10.53)	(12.85)
MPos	108	22.91	0	1.09	0.35	0.42	1.06	1.62
			(0)	(4.76)	(1.53)	(1.83)	(4.64)	(7.07)

Conc = concentration, CV = coefficient of variation, LPos = low positive (1-2 X LoD), MPos = moderate positive (2-3 X LoD), 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.

[1]Between Operators may be confounded with Between Days; therefore, Between Operators and Between Days estimates are combined in Between Operators/Days.

Table 10. Reproducibility - Signal Variability of the Aptima HSV 1 & 2 Assay in the Positive Control

Virus	N	Mean TTime	Between Sites SD (%CV)	Between Lots SD (%CV)	Between Operators/Days[1] SD (%CV)	Within Days SD (%CV)	Total SD (%CV)
HSV-1	36	20.11	0	1.30	0.40	1.09	1.75
			(0)	(6.48)	(1.99)	(5.42)	(8.68)
HSV-2	36	22.16	0	1.61	0.71	1.38	2.24
			(0)	(7.27)	(3.21)	(6.22)	(10.09)

CV = coefficient of variation, 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.

[1]Between Operators may be confounded with Between Days; therefore, Between Operators and Between Days estimates are combined in Between Operators/Days.

Clinical Performance

A prospective, multicenter clinical study was conducted to establish the performance characteristics of the Aptima HSV 1 & 2 assay. A total of 544 evaluable subjects (195 males and 349 females) with active anogenital lesions were enrolled from 17 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: 1 was collected with a swab from a commercially available VTM collection kit and 1 was collected with a swab from the Aptima Multitest Swab Specimen Collection Kit (STM). Specimens were processed in accordance with the appropriate package insert instructions. Aptima HSV 1 & 2 assay testing was performed at 3 external sites. The performance of the Aptima HSV-1 & 2 assay was evaluated relative to a composite reference. The composite reference method used the results from ELVIS HSV ID and D^3 Typing Test system viral culture and a validated bidirectional PCR/sequencing procedure. A third FDA-cleared assay for HSV-1 and HSV-2, was used to determine the final composite reference interpretation when the ELVIS D^3 culture and PCR/sequencing results did not agree on the type of HSV detected or when PCR/sequencing detected both HSV-1 and HSV-2. The performance of the Aptima HSV 1 & 2 assay was estimated for detection of HSV-1 and HSV-2 separately using each specimen type.

For HSV-1, of the 544 evaluable subjects, 528 VTM and 531 STM specimen types resulted in evaluable results for HSV-1 analysis (16 VTM and 13 STM were reported as unknown composite reference interpretation or withdrawn).

For HSV-2, of the 544 evaluable subjects, 533 VTM and 535 STM specimen types produced evaluable results for HSV-2 analysis (11 VTM and 9 STM were reported as unknown composite reference interpretation or withdrawn).

The performance of Aptima HSV 1 & 2 assay compared to the composite reference method is summarized for anogenital skin lesions in STM and VTM in Tables 11 and 12. Overall, for HSV-1, sensitivity was 93.4% for VTM and 94.7% for STM specimen types and specificity was 99.8% for VTM and 99.6% for STM specimen types (Table 11). For HSV-2, sensitivity was 96.9% for VTM and 98.4% for STM specimen types and specificity was 97.5% for VTM and 92.8% for STM specimen types (Table 12).

Table 11. Summary of HSV-1 Results by VTM and STM Specimen Type

Specimen Type	Gender	N	TP	FP	TN	FN	Prev (%)	Sensitivity % (95% CI)[1]	Specificity % (95% CI)[1]	PPV % (95% CI)[2]	NPV % (95% CI)[2]
VTM	Combined	528	71	1[3]	451	5[4]	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	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	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)
STM	Combined	531	71	2[5]	454	4[6]	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	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	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)

STM = Aptima Multitest Swab specimen, 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

[3]The sample had a negative culture result.

[4]Two (2) samples had negative culture results, 1 had a non-typable HSV positive culture result, and 2 were HSV-1 positive by culture.

[5]Both specimens had negative culture results.

[6]One (1) specimen had a negative culture result, 1 had a non-typable HSV positive culture result, and 2 were HSV-1 positive by culture.

Table 12. Summary of HSV-2 Results by VTM and STM Specimen Type

Specimen Type	Gender	N	TP	FP	TN	FN	Prev (%)	Sensitivity % (95% CI)[1]	Specificity % (95% CI)[1]	PPV % (95% CI)[2]	NPV % (95% CI)[2]
VTM	Combined	533	248	7[3]	270	8[4]	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	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	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)
STM	Combined	535	253	20[5]	258	4[6]	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	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	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)

STM = Aptima Multitest swab specimen, 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

[3]Six (6) samples had negative culture results and 1 was HSV-1 positive by culture.

[4]All 8 samples had negative culture results.

[5]Eighteen specimens had negative culture results and 2 were HSV-1 positive by culture.

[6]All 4 specimens had negative culture results.

Expected Values

Prevalence

The prevalence of HSV-1 and HSV-2 observed during a multi-center clinical study was calculated for the Aptima HSV 1 & 2 assay. The prevalence of HSV-1 and HSV-2 with the Aptima HSV 1 & 2 assay is summarized by age group, gender group, and specimen type in Table 13.

Table 13. Gender and Age Distribution by VTM and STM Specimen Type[1]

Gender / Age Group	%Prevalence (# positive results/# tested)			
	VTM		STM	
	HSV-1	HSV-2	HSV-1	HSV-2
Male				
All ages	10.4 (20/192)	41.8 (81/194)	11.5 (22/192)	43.8 (85/194)
<2 years	0.0 (0/0)	0.0 (0/0)	0.0 (0/0)	0.0 (0/0)
2 to 11 years	0.0 (0/1)	0.0 (0/1)	0.0 (0/1)	0.0 (0/1)
12 to 21 years	11.1 (2/18)	38.9 (7/18)	11.1 (2/18)	38.9 (7/18)
22 to 30 years	14.0 (13/93)	34.0 (32/94)	14.9 (14/94)	35.8 (34/95)
31 to 40 years	12.5 (5/40)	50.0 (20/40)	12.8 (5/39)	53.8 (21/39)
41 to 50 years	0.0 (0/20)	52.4 (11/21)	0.0 (0/20)	52.4 (11/21)
51 to 60 years	0.0 (0/14)	57.1 (8/14)	7.1 (1/14)	57.1 (8/14)
> 60 years	0.0 (0/6)	50.0 (3/6)	0.0 (0/6)	66.7 (4/6)
Female				
All ages	15.5 (52/336)	51.3 (174/339)	15.0 (51/339)	55.1 (188/341)
<2 years	0.0 (0/1)	0.0 (0/1)	0.0 (0/1)	0.0 (0/1)
2 to 11 years	0.0 (0/0)	0.0 (0/0)	0.0 (0/0)	0.0 (0/0)
12 to 21 years	23.8 (15/63)	56.3 (36/64)	23.1 (15/65)	59.1 (39/66)
22 to 30 years	14.6 (25/171)	52.6 (90/171)	14.5 (25/172)	56.4 (97/172)
31 to 40 years	11.1 (7/63)	46.0 (29/63)	12.5 (8/64)	47.6 (30/63)
41 to 50 years	18.2 (4/22)	39.1 (9/23)	10.0 (2/20)	47.6 (10/21)
51 to 60 years	7.7 (1/13)	46.2 (6/13)	7.1 (1/14)	57.1 (8/14)
> 60 years	0.0 (0/3)	100 (4/4)	0.0 (0/3)	100 (4/4)
Combined				
All ages	13.6 (72/528)	47.8 (255/533)	13.7 (73/531)	51.0 (273/535)
<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	21.0 (17/81)	52.4 (43/82)	20.5 (17/83)	54.8 (46/84)
22 to 30 years	14.4 (38/264)	46.0 (122/265)	14.7 (39/266)	49.1 (131/267)
31 to 40 years	11.7 (12/103)	47.6 (49/103)	12.6 (13/103)	50.0 (51/102)
41 to 50 years	9.5 (4/42)	45.5 (20/44)	5.0 (2/40)	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 (0/9)	70.0 (7/10)	0.0 (0/9)	80.0 (8/10)

[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 14. These calculations are based on the overall estimated sensitivity and specificity for each specimen type as determined in the clinical performance study.

Table 14. Prevalence vs Hypothetical PPV[1] and NPV[2] for Detection of HSV-1 and HSV-2 by Specimen Type

		HSV-1		HSV-2	
Specimen Type	Prevalence (%)	PPV (%)	NPV (%)	PPV (%)	NPV (%)
VTM	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
STM	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

STM = Aptima Multitest swab specimen, VTM = VTM sample

[1]PPV was calculated using:
(Sensitivity x Prevalence) / (Sensitivity x Prevalence + [1 - Specificity] x [1 - Prevalence]).

[2]NPV was calculated using:
(Specificity x [1 - Prevalence]) / ([1 - Sensitivity] x Prevalence + Specificity x [1 - Prevalence]).

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 15.

Table 15. 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

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AW-15735-001 Rev. 005
2026-03