



IsoAmp[®] HSV Assay

PART NO. DR-HS01

Contents

INTENDED USE	2
SUMMARY AND EXPLANATION OF THE TEST	2
ASSAY PRINCIPLE	3
MATERIALS PROVIDED	3
MATERIALS REQUIRED BUT NOT PROVIDED	4
WARNINGS AND PRECAUTIONS	4
STORAGE, HANDLING AND STABILITY	5
ASSAY PROCEDURE	6
INTERPRETATION OF RESULTS	9
QUALITY CONTROL	10
LIMITATIONS	10
EXPECTED RESULTS	11
PERFORMANCE CHARACTERISTICS	13
DISPOSAL	21
REFERENCES	21

INTENDED USE

The IsoAmp® HSV Assay is an in vitro diagnostic test for the direct, qualitative detection of herpes simplex virus (HSV-1 & HSV-2) DNA in male and female genital and oral lesions. The test is intended for use as an aid in diagnosis of HSV infection in symptomatic patients.

Warning: The IsoAmp® HSV Assay is not FDA cleared for use with cerebrospinal fluid (CSF). The assay does not provide specific typing information to differentiate HSV-1 and HSV-2. The assay is not intended to be used for prenatal screening.

SUMMARY AND EXPLANATION OF THE TEST

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

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

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

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

Recently, nucleic acid amplification tests for the detection of HSV DNA have been reported to be more sensitive and rapid than virus culture and antigen detection

methods [21, 22]. However, they usually require DNA extraction prior to amplification and specialized instrumentation for the process.

The IsoAmp® HSV Assay detects HSV-1 and HSV-2 DNA by an isothermal DNA amplification technology named Helicase-Dependent Amplification (HDA) which does not require DNA extraction. A clinical specimen is diluted in dilution buffer and added to the amplification reagent. After amplification for one hour in the presence of target-specific detection probes at a constant temperature, the reaction tube is inserted into the disposable detection cassette, where HSV-1 and HSV-2 DNA is captured, resulting in a colored line that is visually read. The results are available within 1.5 hours of obtaining the specimens and do not require complex instrumentation.

ASSAY PRINCIPLE

The IsoAmp® HSV Assay consists of three major steps: 1) specimen preparation: 2) isothermal Helicase-Dependent Amplification (HDA) of the HSV glycoprotein B (gB) gene using biotinylated primers [23-27]; and 3) detection of the amplified DNA by a target-specific hybridization probe via a colorimetric reaction on a lateral-flow strip which is embedded in a self-contained disposable cassette to prevent amplicon contamination [25, 28-31].

Specimen preparation includes a simple dilution step in which specimens in viral transport medium are diluted 40-fold in dilution buffer. The diluted samples are mixed with HDA reagents. Incubation at 64°C results in the release of the HSV DNA and subsequent isothermal amplification of the target sequence. A competitive internal control (IC) is included in the Amplification Reagents to monitor inhibitory substances in negative samples, reagent failure or device failure.

After incubation for one hour, the amplified DNA is detected by two detection probes, one labeled with fluorescein isothiocyanate (FITC) for hybridizing to the HSV target and the other labeled with digoxigenin (DIG) for binding to the IC target. The hybrid of FITC-labeled probe and HSV amplicon is captured at the Test Line (T-Line) on the lateral-flow strip by anti-FITC antibodies, while the DIG-labeled IC amplicon is captured at the Control Line (C-Line) on the strip by anti-DIG antibodies. The biotin label in each amplicon captures the streptavidin-conjugated color particles for visualization and the test result is shown as colored lines that are visually read.

The self-contained Type II BEST™ cassettes contain lateral-flow DNA detection strips coated with anti-FITC antibodies and anti-DIG antibodies that serve as T line and C line respectively in the assay. A positive result (detection of HSV DNA) is reported when the T line is visible through the detection window of the cassette. A negative result (no detection of HSV DNA) is reported when only the C line is displayed. The assay result is regarded as invalid when both the T line and C line are not present and the assay should be repeated.

MATERIALS PROVIDED

BioHelix IsoAmp® HSV Assay (50 tests), Part No. DR-HS01

1. IsoAmp® HSV Reagent Kit, Part No. CC-H006

- | | | |
|--------------------------|---|------------------|
| <input type="checkbox"/> | Amplification Reagents | 14 x 100 µL |
| <input type="checkbox"/> | Enzyme Reagents | 1 x 390 µL |
| 2. | Type II BESt™ Cassette, Part No. CD-C007 | 2 x 25 cassettes |
| 3. | IsoAmp® HSV Assay Device, Part No. CD-C006 | |
| <input type="checkbox"/> | Dilution Tubes | 50 tubes |
| <input type="checkbox"/> | Mineral Oil | 1 x 30 mL |
| <input type="checkbox"/> | Disposable transfer pipettes | 15 pipettes |
| <input type="checkbox"/> | Reaction Tubes | 52 tubes |
| <input type="checkbox"/> | Cassette Disposal Bags | 15 bags package |
| 4. | BioHelix IsoAmp® HSV External Control Kit, Part No. CC-H005 | |
| <input type="checkbox"/> | HSV-1 Assay Positive Control | 6 x 50 µL |
| <input type="checkbox"/> | HSV-2 Assay Positive Control | 6 x 50 µL |
| <input type="checkbox"/> | Assay Negative Control | 6 x 50 µL |

MATERIALS REQUIRED BUT NOT PROVIDED

1. Viral transport medium:

The following transport media have been tested and had assay performance verified: **Remel M4, Remel M4RT, BD Universal Viral Transport Media and Bartels VTM**

2. Appropriately sized pipettors
3. Sterile DNase-free filter-blocked or positive displacement pipette tips
4. Disposable gloves
5. Timer
6. Dry heating block specific for 0.2 mL tubes
7. Mini microcentrifuge
8. Cooling block for 1.5 mL and 0.2 mL tubes

WARNINGS AND PRECAUTIONS

Only medical technologists with appropriate training should perform the BioHelix IsoAmp® HSV Assay.

1. For In Vitro Diagnostic Use
2. Reagents are not interchangeable between different lots.
3. Do not use the reagents after their expiration date.
4. Do not interchange caps among reagents as contamination may occur and compromise test results.
5. Open the tubes only when adding aliquots into tubes or removing aliquots from tubes. Keep the tubes closed at any other time to avoid contamination.
6. To avoid contamination of the environment with HSV amplicons, do not open the

reaction tubes post-amplification.

7. Avoid microbial and deoxyribonuclease (DNase) contamination of reagents when removing aliquots from tubes. The use of sterile DNase-free disposable filter-blocked or positive displacement pipettor tips is recommended.
8. Use a new pipettor tip for each specimen or reagent.
9. Performing the assay outside of the recommended time ranges can produce invalid results. Assays not completed within specified time ranges should be repeated.
10. Additional controls may be tested according to guidelines or requirements of local, state, provincial and/or federal regulations or accrediting organizations.
11. Separated or segregated working areas should be used for pre-amplification activities (reagent and specimen preparation), amplification, and post-amplification activities (HDA /detection). Workflow in the laboratory must proceed in a unidirectional manner, beginning in the pre-amplification area and moving to the post-amplification area. Supplies and equipment should be dedicated to each area and should not be moved from one area to another. Gloves must always be worn and must be changed before going from one area to another. Gloves must be changed before manipulating the reagents.
12. Always handle specimens as if they are infectious and in accordance with safe laboratory procedures such as those described in CDC Biosafety in Microbiological and Biomedical Laboratories [32] and in CLSI Document M29 Protection of Laboratory Workers from Occupationally Acquired Infections [33].
13. Wear protective clothing and disposable gloves while handling kit reagents. Wash hands thoroughly after performing the test.
14. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
15. Dispose of unused reagents and waste in accordance with county, federal, provincial, state and local regulations.

STORAGE, HANDLING AND STABILITY

HSV Specimens

Swabs collected from lesions can be stored in the viral transport medium (Remel M4, Remel M4RT, BD Universal Viral Transport Media and Bartels VTM) at 2-8°C for up to 5 days before being tested. Protect specimens against freezing or exposure to excessive heat.

Reagents

Reagents and kit components should be stored in accordance with individual reagent labels.

Amplification Reagents, Enzyme Reagents, HSV-1 and HSV-2 Assay Positive Controls, and Assay Negative Control should be stored at -15°C or colder until the expiration date. Amplification Reagents, HSV-1 and HSV-2 Assay Positive Controls, and Assay Negative Control are provided as single-use tubes, and repeated freeze and thaw of

these reagents should be avoided.

All other reagents and BEST™ Cassettes should be stored at room temperature (15-30°C) until the expiration date.

ASSAY PROCEDURE

Reagent preparation

1. **Set a heat block to 64°C.**
2. **Thaw the required number of Amplification Reagent Tubes (blue cap), an HSV-1 Assay Positive Control Tube (yellow cap), an HSV-2 Assay Positive Control (green cap) and an Assay Negative Control Tube (white cap) at room temperature for approximately 5 minutes.**
Once thawed, briefly spin down the liquid in the tubes (5 seconds) in a mini microcentrifuge. Place tubes in a cooling block until ready for use. (Cooling block should be stored in the freezer at <15°C for a minimum of 2 hours prior to use, per manufacturer's instructions).
3. **One Amplification Reagent Tube contains enough reagents for 4 HSV tests. Use the table below as guidance for the required number of tubes. Do not set up more than 12 assays at a time.**

Number of HSV assays including controls	Required number of Amplification Reagent Tubes
1 – 4	1
5 – 8	2
9 – 12	3

4. **Remove the Enzyme Reagent Tube (red cap) from the freezer and place it in a cooling block.**

Note: The Assay Positive Controls are intended to monitor reagent and device failure. The Assay Negative Control is used to detect reagent or environmental contamination by HSV-1 or HSV-2 DNA or amplicons. As necessary, additional external control tests can be performed by using HSV-1 and/or HSV-2 virus as specimen processing controls in accordance with the guidelines or requirements of local, state and/or federal regulations or accreditation organizations. Both Assay Positive Controls and Assay Negative Control are designed for single use only.

Specimen Preparation

1. **Place the required number of 2.0 ml Dilution Tubes prefilled with dilution buffer in a rack.**
Mark the Dilution Tubes on the cap and/or side of the tube. If assay controls are used, the ones next to the last are for HSV-1 and HSV-2 Positive Controls (P1 and P2), the last one is for Negative Control (N).
2. **Transfer 25 µL of viral transport medium from each collection vial or from**

a control tube to a corresponding Dilution Tube. Close the cap and mix the solution well by inverting the tube 5 times.

Use a new pipette tip for each specimen.

- 3. Centrifuge the Dilution Tubes in a mini microcentrifuge for approximately 5 seconds.**
- 4. Place the same number of 0.2 mL Reaction Tubes in a cooling block.**
Mark the Reaction Tubes on the cap and/or side of the tube.
- 5. Transfer 25 µl of each of the diluted samples to the corresponding 0.2 mL Reaction Tubes.**
Keep the Tubes in a cooling block.

Amplification

The following steps (1-4) MUST be performed without stopping.

- 1. Prepare the Master Mix by transferring 25 µL of Enzyme Reagent (red cap) to each Amplification Reagent Tube (blue cap). Mix the solution THOROUGHLY by pipetting the solution up and down for a minimum of 5 times.**
Place the tubes in a cooling block.
- 2. Add 25 µL of the Master Mix to each 25 µl diluted sample in each of the 0.2 ml Reaction Tube.**
Use a new pipette tip for each diluted sample and place the tube back in a cooling block. The Master Mix can be frozen and used within 7 days: do not freeze and thaw more than once.
- 3. Drop two to three droplets of mineral oil to each Reaction Tube using a disposable transfer pipette.**
If the pipette tip touched the Reaction Tube, discard the pipette and use a new one for the next tube. Close the tubes and place them in a cooling block.
- 4. Place the Reaction Tubes in a 64°C heat block for 60 minutes.**
Note: Ensure that all tubes are in tight contact with the block.
- 5. Place the Enzyme Reagent Tube back in the -15°C freezer.**

Detection

- 1. Remove the Reaction Tubes from 64°C after 60 minutes.**
Place the tubes in a rack at room temperature.
- 2. Take the number of cassette packages (one for each reaction) out of the kit.**
- 3. Tear open a new cassette package.**
Make sure the buffer bulb is still attached in the correct position in the Amplicon Cartridge (Fig. 1: the bulb should be in the cavity adjacent to where the Reaction Tube is placed. If the bulb has been dislodged from the cavity, place it back into this space).
- 4. Place the Reaction Tube into the Amplicon Cartridge (Fig. 1, step 1).**
It is recommended to place the Amplicon Cartridge face up on a flat surface to

insert the Reaction Tube. **Be sure to place the HINGE of the tube cap into the largest slot adjacent to the buffer bulb.**

5. **Close the Amplicon Cartridge (Fig. 1, step 2) and MAKE SURE THAT IT SNAPS SHUT.**
6. **Insert the closed Amplicon Cartridge into the Detection Chamber (Fig. 1, step 3).**
Make sure the arrow, located on the top of the Amplicon Cartridge, faces the detection strip (Reaction Tube should be on top of the razor blade and the plastic bulb containing the running buffer should face the pin). Identify the cassette on the top and/or side of the outer casing.
7. **Keep the device upright and squeeze the handle of the outer casing to close the device (Fig. 1, step 4).**
The handle will lock into place when closed completely.
8. **Read the result after 15 minutes (but no longer than 60 minutes) through the front window and record the results (Fig. 1, step 5; see interpretation section that follows).**

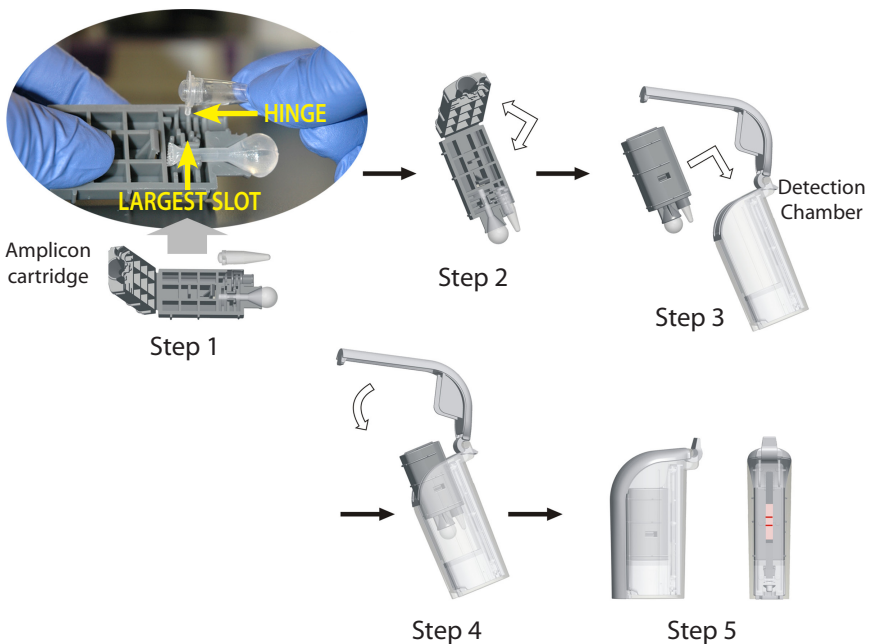


Figure 1. Cassette Detection

INTERPRETATION OF RESULTS

- ❑ **Positive:** Always read the Test (T) line first. When T line is visible (T+), report the assay result as “HSV DNA detected”.
- ❑ **Negative:** When no visible T line is present (T-), a visible C line indicates that the Internal Control DNA has been amplified and detected, eliminating the possibility of a false negative due to failure of amplification or device, and thus the assay result should be reported as negative - “no HSV DNA detected”.
- ❑ **Invalid:** If both T and C lines are not present (T-/C-), then the assay is invalid and the test needs to be repeated.
- ❑ Any visible T line and C line, regardless of intensity of that line, is recorded as a reactive test (“+”) for that line, while the complete absence of any visible line is recorded as a nonreactive test (“-”) for that particular line.

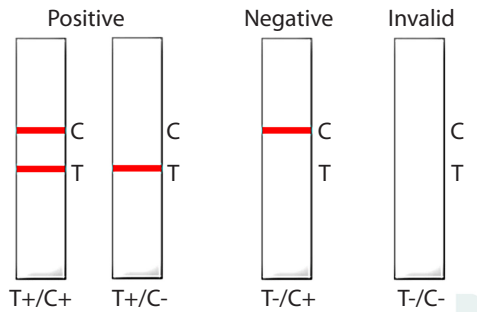


Figure 2. Interpretation of the assay results

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

T line and C line Result	Interpretation of Result
T+/C+ or T+/C-	Positive (T-line Reactive): HSV DNA detected
T-/C+	Negative (T-line Non-Reactive; C-line Reactive): HSV DNA not detected
T-/C-	Invalid: process or test failure – repeat assay

Note: Read the test and control lines after 15 minutes (but no longer than 60 minutes). Discard the cassettes after interpretation by placing them in the Cassette Disposal Bags provided. Seal the bag and dispose of it in accordance with the appropriate laboratory waste guidelines. DO NOT RECYCLE used cassettes.

QUALITY CONTROL

Internal control

Quality control procedures are designed to monitor assay performance. An internal control (IC) has been included in the IsoAmp® HSV assay. The internal control is used to detect HDA inhibitory specimens and to confirm the integrity of assay reagents and cassette detection.

The competitive internal control (IC) consists of plasmid DNA. The IC target sequence is amplified using the same primer set that amplifies the HSV target sequence. The internal sequence of the IC target is different from the HSV target sequence and is detected by an IC-specific probe. After amplification, the IC amplicon-probe complexes are detected as a visible Control line on the Type II BEST™ Cassette. The IC DNA and probe are pre-mixed in the Amplification Reagent.

External Assay (positive and negative) controls

The Assay Positive Controls are plasmid DNA containing HSV-1 or HSV-2 DNA and are intended to monitor reagent and cassette failure. The Assay Negative Control consists of blank viral transport medium and is used to detect reagent or environmental contamination (or carry-over) by either HSV DNA or amplicons. Unexpected control results should be repeated; patient samples should not be tested before valid and expected test results are achieved with the assay controls.

The user should determine quality control testing in accordance with guidelines or requirements of local, state and/or federal regulations or accreditation organizations.

Specimen processing controls

Additional controls may be tested in accordance with the guidelines or requirements of local, state and/or federal regulations or accreditation organizations. HSV-1 (Catalog Number: 10-110-000) and HSV-2 (Catalog Number: 10-111-000) can be purchased from Advanced Biotechnologies Inc (Columbia, MD) and they can be used as specimen processing controls with appropriate amount of viral titer.

For general QC guidance, refer to CLSI C24 [34].

LIMITATIONS

1. The IsoAmp® HSV Assay has been tested with male and female genital and oral lesion specimens only. Performance with other specimen types has not been assessed. The device is not FDA cleared for use with cerebrospinal fluid (CSF).
2. The assay is not intended to be used for prenatal screening.
3. The assay does not provide specific typing information to differentiate HSV-1 and HSV-2. It does not detect or differentiate any other Herpes virus types.
4. As with many diagnostic tests, results from the IsoAmp® HSV Assay should be interpreted in conjunction with other laboratory and clinical data available to the physician.
5. HSV viability and/or infectivity cannot be inferred from a positive test result since

target DNA may persist in the absence of infectious virus

6. A negative test does not exclude the possibility of infection because test results may be affected by improper specimen collection/transport/handling (inadequate specimen collection), presence of inhibitor(s), technical error, specimen mix-up, concurrent antiviral therapy, or the presence of insufficient DNA for detection.
7. A trained health care professional should interpret assay results in conjunction with the patient’s medical history, clinical signs and symptoms and the results of other diagnostic tests.

EXPECTED RESULTS

The prevalence of HSV-1 and HSV-2 in genital and oral swab specimens during the multi-site clinical study (n=962*) was estimated using the IsoAmp® HSV Assay.

* Note: Retrospective samples were not included in this tabulation.

**Table 1. Distribution of Prospective Population by Age Group:
Genital Lesion Swab Specimens**

Age Range	IsoAmp® HSV Assay Positive	Total Number of Specimens
<1 year to 17 years	10	58
18 to 25 years	113	268
26 to 30 years	44	110
31 to 35 years	32	83
36 to 40 years	22	68
41 to 45 years	19	56
46 to 50 years	14	45
51 to 55 years	15	42
56 to 60 years	9	21
61 to 65 years	7	23
66 to 70 years	8	16
71 to 75 years	1	3
76 to 80 years	3	3
81 to 85 years	2	6
86 to 90 years	0	0
90 to 95 years	0	1
Total	299	803
Prevalence	37.2%	N/A

**Table 2. IsoAmp® Distribution of Prospective Population by Age Group:
Oral Lesion Swab Specimens**

Age Range	IsoAmp® HSV Assay Positive	Total Number of Specimens
<1 year to 17 years	10	28
18 to 25 years	19	31
26 to 30 years	3	10
31 to 35 years	3	12
36 to 40 years	2	9
41 to 45 years	1	7
46 to 50 years	2	18
51 to 55 years	9	15
56 to 60 years	3	6
61 to 65 years	3	10
66 to 70 years	2	4
71 to 75 years	0	1
76 to 80 years	2	5
81 to 85 years	0	1
86 to 90 years	0	1
90 to 95 years	0	1
Total	59	159
Prevalence	37.1%	N/A

The combined prevalence was used to calculate the hypothetical positive predictive values (PPV) and hypothetical negative predictive values (NPV) of the IsoAmp® HSV Assay. The calculations are based on the positive agreement and negative agreement obtained from the clinical studies: positive agreement of 97.1% and negative agreement of 93.4% for genital lesion samples; positive agreement of 93.8% and negative agreement of 87.4% for oral lesion samples.

The prevalence observed by a laboratory may vary and the distribution in the table below may be used to establish the frequency distributions based on a specific laboratory's patient population.

**Table 3. Prevalence vs. Hypothetical Predictive Values:
Genital Lesion Swab Specimens**

Prevalence	Positive Predictive Value (PPV)	Negative Predictive Value (NPV)
50%	93.6%	97.0%
40%	90.7%	98.0%
30%	86.3%	98.7%
20%	78.6%	99.2%
10%	62.0%	99.7%
5%	43.6%	99.8%

**Table 4. Prevalence vs. Hypothetical Predictive Values:
Oral Lesion Swab Specimens**

Prevalence	Positive Predictive Value (PPV)	Negative Predictive Value (NPV)
50%	88.2%	93.4%
40%	83.2%	95.5%
30%	76.1%	97.0%
20%	65.0%	98.3%
10%	45.3%	99.2%
5%	28.2%	99.6%

PERFORMANCE CHARACTERISTICS

1. Clinical Performance

The performance of the IsoAmp® HSV Assay was evaluated at five geographically diverse locations within the United States from 2010 - 2011. A total of nine hundred and ninety-four (994) swab samples obtained from male and female genital and oral lesions were collected in Viral Transport Media (Remel M4, Remel M4RT, BD Universal Viral Transport and Bartels VTM) from the patient population ranging from <1 year to 92 years, and evaluated. Of the 994 specimens, 962 prospective samples and 32 retrospective

samples were tested. Of the 962 prospective samples, 803 genital samples and 159 oral samples were tested. Of the 32 retrospective samples, 15 genital and 17 oral samples were tested at a single study site.

Genital swab specimens were collected from vaginal, labial, penile and rectal lesions. Oral swab specimens were collected from lips, gums, and mouth.

The performance of the IsoAmp®HSV Assay was compared with a gold standard/reference method i.e., Cell Culture-based ELVIS® HSV ID/Typing Test System using an enzyme linked virus inducible system.

Table 5. PROSPECTIVE SAMPLE DATA: Genital Samples Only

IsoAmp® HSV Assay	Reference Method		
	Positive	Negative	Total
Positive	264	35*	299
Negative	8**	496	504
Total	272	531	803
		95% Confidence Interval	
Sensitivity	97.1% (264/272)	94.3 – 98.5%	
Specificity	93.4% (496/531)	91.0 – 95.2%	

* 35 samples were tested using bidirectional sequencing analysis. Sequence analysis detected HSV target in 29 [6 HSV-1, 23 HSV-2] of the 35 discordant samples identified as HSV Positive by the IsoAmp® HSV Assay. Sequence analysis did not detect HSV in six (6) of the discordant samples.

** Eight (8) samples were tested using bidirectional sequencing analysis. Sequence analysis did not detect HSV target in four (4) of the 8 samples identified as HSV Negative by the IsoAmp® HSV Assay. Sequence analysis did detect HSV in four (4) samples [2 HSV-1, 2 HSV-2]

Table 6. PROSPECTIVE SAMPLE DATA: Oral Samples Only

IsoAmp® HSV Assay	Reference Method		
	Positive	Negative	Total
Positive	45	14*	59
Negative	3**	97	100
Total	48	111	159
		95% Confidence Interval	
Sensitivity	93.8% (45/48)	83.2 – 97.9%	
Specificity	87.4% (97/111)	79.9 – 92.3%	

* 14 samples were tested using bidirectional sequencing analysis. Sequence analysis detected HSV target in 13 [12 HSV-1, 1 HSV-2] of the 14 discordant samples identified as HSV Positive by the IsoAmp® HSV Assay. Sequence analysis did not detect HSV in one (1) of the discordant samples.

** Three (3) samples were tested using bidirectional sequencing analysis. Sequence analysis did not detect HSV target in two (2) of the 3 samples identified as HSV Negative by the IsoAmp® HSV Assay. Sequence analysis did detect HSV in one (1) samples [1 HSV-1]

Retrospective Sample Data

All of the 32 retrospective samples, 15 genital and 17 oral samples were shown positive by both the IsoAmp®HSV Assay and the reference assay.

2. Precision/Reproducibility

The Precision/Reproducibility of the IsoAmp® HSV Assay was evaluated at three (3) test sites. A panel of seven (7) members was prepared containing one negative control sample (HSV negative pooled swab specimens) and six simulated HSV-1 and HSV-2 samples that included High Negative (below the assay limit of detection), Low Positive (near the assay limit of detection) and Moderate Positive (three times the assay limit of detection) samples. The panel (which included one replicate of each panel member), along with external HSV-1 and HSV-2 positive and negative controls, was tested at each site for five (5) days by two operators with each operator running the panel two times a day using a single lot of the IsoAmp® HSV Assay. One (1) site tested the panel using three (3) lots. Results of the Precision/Reproducibility study for the IsoAmp® HSV at three sites are presented in the table below.

Table 7. Overall Reproducibility Study

Category	Percent Agreement						Overall Percent Agreement		95% CI
	Site #1*		Site #2		Site #3				
HSV-1 High Negative	13/60	22%	13/20	65%	6/20	30%	32/100	32%	24% - 42%
HSV-1 Low Positive	60/60	100%	19/20	95%	20/20	100%	99/100	99%	94% - 100%
HSV-1 Moderate Positive	60/60	100%	20/20	100%	20/20	100%	100/100	100%	96% - 100%
HSV-2 High Negative	19/60	32%	7/20	35%	6/20	30%	32/100	32%	24% - 42%
HSV-2 Low Positive	60/60	100%	18/20	90%	18/20	89%	96/100	96%	90% - 98%
HSV-2 Moderate Positive	60/60	100%	20/20	100%	20/20	100%	100/100	100%	96% - 100%
Negative**	60/60	100%	20/20	100%	19/20	95%	99/100	99%	96% - 100%
HSV-1 Positive Control	60/60	100%	20/20	100%	20/20	100%	100/100	100%	96% - 100%
HSV-2 Positive Control	60/60	100%	20/20	100%	20/20	100%	100/100	100%	96% - 100%
Assay Negative Control***	60/60	100%	20/20	100%	20/20	100%	100/100	100%	96% - 100%

*Site#1 tested two additional lots.

** Negative pooled serum control

***Remel M4 transport media

3. Cross Reactivity

A cross-reactivity study was performed to determine if any organisms which may present with the same clinical symptoms as HSV, which are associated with bacterial vaginosis, or which are commonly found in the genital track and oral area could give positive results with the IsoAmp® HSV Assay. Forty-eight (48) specificity panel members including purified DNA and cultured organisms were tested with the IsoAmp® HSV assay in triplicate following instructions in the Package Insert. No cross-reactivity was observed with any panel member tested at clinically significant concentrations.

Table 8. Cross Reactivity Panel

Organisms	Member Type (GD , QC , IHC)*	Test Concentration
Acinetobacter calcoaceticus var. anitratus (ATCC 51432)	IHC	1.0E+06 CFU/mL
Acinetobacter lwoffii (ATCC 17925)	IHC	1.0E+07 CFU/mL
Adenovirus 2	QC	1.0E+06 TCID50/mL
Bacteroides fragilis	QC	1.0E+07 CFU/mL
Candida albicans (ATCC 14053)	IHC	1.0E+07 CFU/mL
Candida glabrata	QC	1.0E+07 CFU/mL
Candida guilliermondii	QC	1.0E+07 CFU/mL
Candida krusei	QC	1.0E+06 CFU/mL
Candida lusitanae	QC	1.0E+07 CFU/mL
Candida parapsilosis	QC	1.0E+07 CFU/mL
Candida tropicalis	QC	1.0E+07 CFU/mL
Chlamydia trachomatis LGV-II434	GD	1.0E+07 cp/mL
Cytomegalovirus	QC	1.0E+06 TCID50/mL
Enterobacter cloacae (ATCC 13047)	IHC	1.0E+07 CFU/mL
Enterovirus (Type 71)	QC	1.0E+05 TCID50/mL
Epstein-Barr Virus	GD	1.0E+06 cp/mL
Escherichia coli (ATCC 25922)	IHC	1.0E+07 CFU/mL
Fusobacterium nucleatum (ATCC 25586)	IHC	1.0E+07 CFU/mL
Gardnerella vaginalis (ATCC 14018)	IHC	1.0E+07 CFU/mL
Haemophilus ducreyi	QC	8.5E+05 CFU/mL
Human Herpes 6 virus (Z29 strain)	QC	1.0E+06 TCID50/mL
Human Herpes 7 virus (SB strain)	QC	1.0E+06 TCID50/mL

* GD: Genomic DNA; QC: Quantified Cultures; IHC: In-House Culture

Table 8. Cross Reactivity Panel - continued

Organisms	Member Type (GD , QC , IHC)*	Test Concentration
Human papilloma virus 16 (HPV16)	GD	1.0E+06 cp/mL
Human papilloma virus 18 (HPV18)	GD	1.0E+05 cp/mL
Klebsiella pneumonia	QC	1.0E+07 CFU/mL
Lactobacillus acidophilus Z048	QC	1.0E+07 CFU/mL
Mobiluncus curtisii V125 [DSM 2711]	QC	1.0E+07 CFU/mL
Mobiluncus mulieris BV 64-5	QC	1.0E+06 CFU/mL
Moraxella catarrhalis	QC	1.0E+07 CFU/mL
Mycoplasma hominis (ATCC 23114)	IHC	1.0E+07 CFU/mL
Neisseria gonorrhoeae (ATCC 21823)	IHC	1.0E+07 CFU/mL
Neisseria meningitides	QC	1.0E+07 CFU/mL
Prevotella melaninogenica	QC	1.0E+07 CFU/mL
Rubella virus	QC	4.17E+05 TCID50/mL
Simian Virus type 40 (SV40)	QC	1.0E+06 TCID50/mL
Staphylococcus aureus MRSA (ATCC 33591)	IHC	1.0E+07 CFU/mL
Staphylococcus aureus MSSA (ATCC 25923)	IHC	1.0E+07 CFU/mL
Staphylococcus epidermidis MRSE (ATCC700566)	IHC	1.0E+07 CFU/mL
Staphylococcus saprophyticus MRSE (ATCC 15305)	IHC	1.0E+07 CFU/mL
Streptococcus mitis clinical isolate	QC	1.0E+07 CFU/mL
Streptococcus mutans Z072	QC	1.0E+06 CFU/mL
Streptococcus pneumonia	QC	1.0E+07 CFU/mL
Streptococcus pyogenes: (ATCC19615)	IHC	1.0E+07 CFU/mL
Streptococcus salivarius (ATCC BAA-1024)	IHC	1.0E+07 CFU/mL
Toxoplasma gondii	QC	6.6E+06 CFU/mL
Treponema pallidum	QC	1.0E+07 TP/mL
Trichomonas vaginalis	QC	1.0E+06 CFU/mL
Varicella-Zoster Virus (VZV)	GD	1.0E+06 cp/mL

* GD: Genomic DNA; QC: Quantified Cultures; IHC: In-House Culture

4. Interfering Substances

Potentially interfering substances i.e. viral transport media, substances that might be present in clinical samples, and organisms/cross reactive panel members listed under cross reactivity were tested to confirm that they did not interfere with the performance of the IsoAmp® HSV Assay.

All interference testing was carried out in the presence of HSV-1 and HSV-2 at three times the observed LOD (3 x LOD). HSV-1 HF and HSV-2 MS strains were used. All test runs were conducted in triplicate. Controls were tested with each run.

a. Interfering Substances

Performance of the IsoAmp® HSV Assay was characterized in the presence of twenty-four (24) potentially interfering substances which could reasonably be expected to be present in genital and oral swab specimens.

Interfering substances were tested at the highest (“worst case”) concentration expected in clinical samples. By “worst case,” each interfering substance was introduced into the assay by directly wetting a clean, dry Remel M4 kit swab with the substance and placing the swab directly in transport media. Calculated concentrations are based on an estimated volume of 200µL of substance introduced by the swab. Each panel member was tested in triplicate spiked with HSV-1 HF and HSV-2 MS strains separately at 3 x LoD. The panel was also tested in triplicate in the absence of HSV transport media to see if the potentially interfering substances interfere with the detection of the internal control. No interference was observed in the presence of the potential interfering substances tested.

b. Viral Transport Media

The performance of the IsoAmp® HSV Assay was assessed with Remel M4, Remel M5, Remel M4RT, Bartels VTM, and BD Universal Viral Transport (UVT). Each medium was tested after spiking with HSV-1 HF and HSV-2 MS strain to a final concentration of approximately 3 x LOD to determine if the viral transport media interferes with the detection of HSV targets in positive samples. The media were tested in the absence of HSV-1 and HSV-2 (medium only) to see if the viral transport media interfere with the detection of the internal control in negative samples. There was no interference observed with the Remel M4, Remel M4RT, Remel M5, Bartels VTM, and BD UVT media for the detection of HSV-1 and HSV- 2 target or the internal control. M4, M4RT, M5, Bartels VTM, and BD UVT did not interfere with the detection of HSV-1 and HSV-target or the internal control.

c. Cross-Reactivity Panel Members

The performance of the IsoAmp® HSV Assay was characterized by testing the organisms that were evaluated for analytical specificity and cross reactivity in the presence of HSV-1 HF and HSV-2 MS at 3xLoD separately to see if the presence of these organisms interferes with the detection of HSV target. Each panel member was tested in triplicate. None of the cross reactivity panel members interfered with the detection of HSV-1 and HSV-2 target.

Table 9. Interfering Substance Panel

Substances	Calculated Concentration
Whole blood with EDTA	7% (v/v)
Female Urine	7% (v/v)
Male Urine	7% (v/v)
Acyclovir 10%	7 mg/mL
Albumin	3.3 mg/mL
Casein	7 mg/mL
K-Y Brand Jelly	7% (w/v)
Douche	7% (v/v)
Contraceptive Jelly	7% (w/v)
YeastGard	7% (w/v)
Monistat 1	7% (w/v)
Vagisil Creme	7% (w/v)
Monistat 3	7% (w/v)
Triconazole 1	7% (w/v)
Balneol Hygienic Cleansing Lotion	7% (w/v)
Clotrimazole 3 Vaginal Cream	7% (w/v)
CVS Anti-Itch Cream	7% (w/v)
Listerine Antiseptic Mouth Wash	7% (v/v)
Abreva	7% (w/v)
Carmex Cold Sore Lip Balm	7% (w/v)
Releev cold sore treatment	7% (w/v)
Lip clear Lysine+	7% (w/v)
Toothpaste	7% (w/v)
Buffy coat	7% (v/v)

5. Limit of Detection

A Limit of Detection (LoD) study was performed to determine the analytical sensitivity of the IsoAmp HSV Assay using two representative strains of HSV-1 (McIntyre & HF) and two representative strains of HSV-2 (G & MS). Quantified (TCID50/mL) cultures of the HSV-1 and HSV-2 strains were serially diluted to five (5) concentrations in HSV-negative matrix pools and tested in replicates of ten (10) on three (3) reagent lots. The observed LoD of a HSV strain was determined as the lowest concentration level that had a positivity rate of >95%. Since two (2) representative strains of HSV-1 and HSV-2 were used in the study, the higher LoD value was defined as the observed LoD for HSV-1 and HSV-2 respectively. Since the IsoAmp® HSV Assay does not differentiate viral types, the final assay LoD is defined as the higher of the HSV-1 and HSV-2 concentrations where 95% positivity was observed.

In addition, LoD confirmation studies were conducted to confirm the observed LoD for HSV-1 and HSV-2. The first confirmatory study included testing the four (4) representative HSV-1 and HSV-2 strains 20 times each at the corresponding observed LoD. Each strain was tested by three (3) reagent lots, and all four strains showed a positivity rate of 100%. In addition, twenty (20) HSV-1 and 20 HSV-2 clinical isolates were cultured and quantified in TCID50/mL. Each isolate was diluted to the corresponding LoD in HSV-negative matrix and tested in triplicate. IsoAmp® HSV Assay was able to detect all 20 HSV-1 and 20 HSV-2 clinical isolates.

a. HSV-1

The LoD for HSV-1 McIntyre was determined to be 3.7E+04 TCID50/mL. At this concentration, 97% of samples were detected with a 95% Confidence Interval of 83.33% – 99.41%. The LoD for HSV-1 HF was determined to be 1.1E+05 TCID50/mL. At this concentration, 100% of samples were detected with a 95% Confidence Interval of 88.65% – 100%. Therefore, the LoD for HSV-1 is 1.1E+05 TCID50/mL.

McIntyre (TCID50/mL)	Positive/Total	Positivity Rate	95% CI
3.3E+05	30/30	100%	88.65% - 100.00%
1.1E+05	30/30	100%	88.65% - 100.00%
3.7E+04	29/30	97%	83.33% - 99.41%
1.2E+04	18/30	60%	42.32% - 75.41%
4.1E+03	10/30	33%	19.23% - 51.22%
HF (TCID50/mL)	Positive/Total	Positivity Rate	95% CI
3.3E+05	30/30	100%	88.65% - 100.00%
1.1E+05	30/30	100%	88.65% - 100.00%
3.7E+04	28/30	93%	78.68% - 98.15%
1.2E+04	19/30	63%	45.51% - 78.13%
4.1E+03	9/30	30%	16.66% - 47.88%

b. HSV-2

The LoD for HSV-2 G was determined to be 1.1E+04 TCID50/mL. At this

concentration, 100% of samples were detected with a 95% Confidence Interval of 88.65% – 100%. The LoD for HSV-2 MS was determined to be 3.7E+03 TCID50/ mL. At this concentration, 100% of samples were detected with a 95% Confidence Interval of 88.30% – 100%. Therefore, the LoD for HSV-2 is 1.1E+04 TCID50/mL.

G (TCID50/mL)	Positive/Total	Positivity Rate	95% CI
3.3E+04	30/30	100%	88.65% - 100.00%
1.1E+04	30/30	100%	88.65% - 100.00%
3.7E+03	26/30	87%	70.32% - 94.69%
1.2E+03	14/30	47%	30.23% - 63.86%
4.1E+02	8/30	27%	14.18% - 44.45%
MS (TCID50/mL)	Positive/Total	Positivity Rate	95% CI
3.3E+04	30/30	100%	88.65% - 100.00%
1.1E+04	30/30	100%	88.65% - 100.00%
3.7E+03	29/29	100%	88.30% - 100.00%
1.2E+03	25/30	83%	66.44% - 92.66%
4.1E+02	8/30	27%	14.18% - 44.45%

c. Assay LoD

Since the IsoAmp® HSV Assay does not differentiate viral types, the final assay LoD is defined as the higher of the HSV-1 and HSV-2 concentrations where 95% positivity was observed. The final assay LoD claim is 1.1E+05 TCID50/mL.

6. Carry over/Cross contamination

Carry-over/Cross Contamination Study was done only with HSV-1 target since both HSV-1 and HSV-2 share a single set of primers and probes for target amplification and detection. The HSV-1 McIntyre (6.65E+08 TCID50/mL) was used directly without dilution. Remel M4 viral transport media was used as the negative sample. Ten (10) replicates of negative sample together with assay controls were run by two (2) operators to confirm that negative samples (Remel M4 viral transport media) generate a negative result 100% of the time. Five (5) replicates of high-concentration positive and negative samples were tested in a series, alternating sample types. All results were as expected. Negative samples tested were negative (10/10) and positive samples were positive (10/10).

DISPOSAL

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

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