Simplexa™ HSV 1 & 2 Direct

REF MOL2150
Rev. E

A real-time PCR assay intended for the *in vitro* qualitative detection and differentiation of HSV-1 and/or HSV-2 viral DNA.

For *in vitro* Diagnostic Use
CLIA – Moderate Complexity
Rx Use Only

INTENDED USE

The Focus Diagnostics Simplexa™ HSV 1 & 2 Direct assay is intended for use on the 3M Integrated Cycler instrument for the qualitative detection and differentiation of herpes simples virus (HSV-1 and HSV-2) DNA present in genital lesion swabs samples from patients with signs and symptoms of HSV-1 or HSV-2 infection of the genitalia. This test is an aid in the differential diagnosis of HSV-1 and HSV-2 genital infections.

The assay is not intended for use as a screening test for the presence of HSV-1 and HSV-2 in blood or blood products. The assay is for professional use only.

SUMMARY AND EXPLANATION

HSV-1 and HSV-2 are members of the alpha-herpesviridae subfamily. HSV is an enveloped virus with a capsid containing viral DNA. Although HSV-1 and HSV-2 are closely related, the two viruses are serologically and genetically distinct.[1,2]

Genital herpes caused by HSV is one of the most common sexually transmitted diseases. HSV primary infection or reactivation of latent disease can cause cutaneous or mucosal lesions.[1,3] These lesions are usually self-limited but severe disease can occur in immunocompromised patients, pregnant woman and infants.

Genital herpes is usually caused by HSV-2, with the minority of first genital episodes (5-30%) caused by HSV-1.[4] The Centers for Disease Control and Prevention (CDC) states that counseling is an important aspect of managing patients who have genital herpes.[2]

After primary HSV infection, the virus colonizes the sensory neurons, and the latent infection may reactivate causing recurrent infection.

PRINCIPLES OF THE PROCEDURE

The Simplexa™ HSV 1 & 2 Direct assay system is a real-time PCR that enables the direct amplification, detection and differentiation of HSV-1 and/or HSV-2 DNA from unprocessed genital swab specimens without nucleic acid extraction. The system consists of the Simplexa™ HSV 1 & 2 Direct assay, the 3M Integrated Cycler (with 3M Integrated Cycler Studio Software), the Direct Amplification Disc and associated accessories.

In the Simplexa™ HSV 1 & 2 Direct assay, bi-functional fluorescent probe-primers are used together with corresponding reverse primers to amplify HSV-1, HSV-2 and internal control targets. Well conserved regions of the HSV-1 and HSV-2 DNA polymerase genes are targeted to identify HSV-1 and HSV-2 DNA respectively in the specimen. An internal control is used to detect PCR failure and/or inhibition.

MATERIALS PROVIDED

The Focus Diagnostics Simplexa™ HSV 1 & 2 Direct assay contains sufficient reagents for 24 reactions. Upon receipt, store at -10 to -30 ºC (do not use a frost-free freezer). Each vial contains sufficient material for a single reaction. Use within 30 minutes of thawing.

Kit Description

Component Name	REF	EC SYMBOL ON LABEL		Abbreviated Name	Cap Color	Number of Vials	Reactions per Vial/Kit	Volume per Vial
Simplexa™ HSV 1 & 2 Direct Reaction Mix	MOL2151	REAG	A	RM	Brown	24	1/24	50 µL

Component Description

Kit Component	Contents
Simplexa™ HSV 1 & 2 Direct Reaction Mix (RM)	DNA polymerase, buffer, dNTPs, template DNA (Internal Control) dye-labeled fluorescent probe-primers specific for detection of HSV-1 and/or HSV-2 and for the DNA Internal Control.

Target	Probe Fluorophore (Dye)	Excitation (nm)	Emission (nm)	Targeted Gene
HSV-1	CFR610	590	610	HSV-1 DNA polymerase
HSV-2	FAM	495	520	HSV-2 DNA polymerase
DNA Internal Control	Q670	644	670	NA

Simplexa™ HSV 1 & 2 Kit Barcode Card	Assay specific parameters.

MATERIALS SUPPLIED SEPARATELY

1. Direct Amplification Disc Kit (REF MOL1455).
    a) Direct Amplification Discs for use on the 3M Integrated Cycler.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. 3M Integrated Cycler with 3M Integrated Cycler Studio Software version 6.0 or higher.
2. Simplexa™ HSV 1 & 2 Positive Control Pack (REF MOL2160).
3. 50 µL fixed volume pipette (VWR Signature™ Fixed Volume Ergonomic High-Performance Pipette Model VWR FE50 or equivalent).
4. Sterile, nuclease-free disposable pipette tips with filters (Extra Long tips ≥ 91 mm are recommended for pipetting directly from primary collection tubes).
5. Freezer (manual defrost) at -10 to -30 °C (for kit component and specimen frozen storage).
6. Refrigerator at 2 to 8 °C (for specimens).
7. Disposable, powder-free gloves.

RECOMMENDED MATERIALS

1. Universal Transport Media (UTM) to use as a No Template Control (NTC) when testing genital swab samples.

REAGENT HANDLING AND STORAGE

1. Store reagents at -10 to -30 °C (do not use a frost-free freezer).
2. Allow reagents to thaw at room temperature (approximate range 18 to 25 °C) before use.
3. Do not use kits or reagents beyond their expiration dates.
4. After removing Reaction Mix from freezer storage, initiate the test within 30 minutes.
5. Do not vortex the Reaction Mix.
6. Do not refreeze the Reaction Mix.

WARNINGS AND PRECAUTIONS

1. Wear personal protective equipment, such as (but not limited to) gloves and lab coats when handling kit reagents. Wash hands thoroughly when finished performing the test.
2. Do not smoke, drink, eat, handle contact lenses or apply make-up in areas where kit reagents and/or human specimens are being used.
3. Dispose of unused kit reagents and human specimens according to local, state and federal regulations.
4. Treat all specimens and discs as capable of transmitting infectious agents. Contamination of patient specimens or reagents can produce erroneous results. Use good laboratory practices and control workflow.[5,6]
5. Only use the protocol described in this insert. Deviations from the protocol or the use of times or temperatures other than those specified may give erroneous results.
6. Assay setup should be performed at room temperature (approximate range 18 to 25 °C).
7. Use fixed volume pipettes or equivalent for sample and Reaction Mix.
8. Avoid touching the underside of the foil that will be in contact with the wells and disc surface.
9. To prevent potentially erroneous results, make sure that the sample is added to the Sample input well.
10. Finish loading and applying adhesive foil cover to one set of Sample and Reaction wells before opening the foil of adjacent set(s)s of Sample and Reaction wells.
11. Initiate the run within 30 minutes of removing the Reaction Mix vial from the freezer.
12. Do not attempt to remove adhesive foil cover from wedges that have been used or attempt to re-use Sample and Reaction ports that have been used in previous runs.
13. Discs may be reused until all 8 wedges have been used. Dispose of used discs without detaching foil cover in a biohazardous waste container.
14. After each use store DAD discs flat with the numbered foil side up.
15. Reaction Mix contains > 1% glycerol, which may cause irritation upon inhalation or skin contact. Upon inhalation or skin contact, first aid measures should be taken.
16. If kit packaging or contents appear to be broken or damaged do not use and contact Focus Diagnostics. Contact information is on the last page of this document.
17. The spectral matrix must be installed in each 3M Integrated Cycler and should not be changed unless an updated QR code for the instrument is provided by Focus Diagnostics. The spectral matrix is unique to each 3M Integrated Cycler. The spectral matrix was provided with the 3M Integrated Cycler instrument on the cover of the 3M Integrated Cycler Hardware Manual. If the matrix label will not scan or cannot be found contact Focus Diagnostics. The contact information is on the last page of this document.
18. Not installing or changing the spectral matrix can result in false results.

INSTRUCTIONS FOR USE

A. SPECIMEN COLLECTION

Acceptable specimen type is swab samples collected from a herpetic genital lesion and stored in BD UVT, Remel M4, Remel M4RT, Remel M5, Remel M6 or UTM transport media. Follow the manufacturer's package inserts for collection media and acceptable swab types. Do not use calcium alginate swabs, as they may contain substances that inhibit PCR testing. Specimens should be transported on ice and stored at 2 to 8°C for up to 7 days post collection. If there is a greater than 7 days delay before processing of the specimen, store specimen at -70° C.

B. REAL-TIME PCR INSTRUMENT SETUP

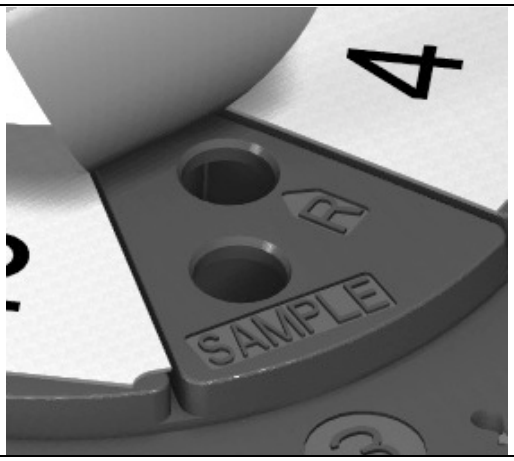
1. Refer to the 3M Integrated Cycler Operator Manual for details on how to configure the 3M Integrated Cycler Studio Software to add an assay definition set up and analyze runs on the 3M Integrated Cycler.

C. DIRECT AMPLIFICATION DISC LOADING AND REAL-TIME PCR AMPLIFICATION

NOTE: No sample extraction is needed prior to PCR amplification step.

1. Select samples that need to be tested.
2. Thaw Reaction Mix vials at room temperature (approximate range 18 to 25 °C). Thaw one Reaction Mix vial for each sample or control to be tested.
3. Scan the barcode on the Simplexa™ HSV 1 & 2 Direct Reaction Mix vial or barcode card.
4. Scan the disc barcode on the Direct Amplification Disc (DAD).
5. Scan or type in each sample identifier.
6. For one wedge at a time, peel the adhesive foil back to expose the Sample (SAMPLE) and Reaction (R) wells without completely removing the adhesive foil cover (Figure 1 & 2). Avoid touching the underside of the foil that will be in contact with the wells and disc surface.
7. Ensure that the Reaction Mix is completely thawed. Briefly spin down the tubes as needed. (Do not vortex the Reaction Mix).
8. Use the fixed volume pipette to transfer 50 µL of the Reaction Mix into Reaction (R) well.

9. Use the fixed volume pipette to transfer 50 µL of sample or control; pipette sample or control into Sample well (SAMPLE).
10. Cover the wedge sealing the wells with the peeled adhesive foil, pressing down firmly near the edge of the wedge. If the original foil is torn do not load the wells in the wedge. Instead load another wedge.
11. Tear off the tab portion of the foil cover along the perforation.
12. Repeat steps 6 to 11 for the next sample(s).
13. Load the sealed DAD into the 3M Integrated Cycler and start the run.

Figure 1 - Disc with pre-use foil lifted from Sample and Reaction Wells for wedge #3	Figure 2 –Sample [Sample] and Reaction [R] Wells
	

NOTES *(for informational purposes - no user action/interpretation required)*:

1. Focus Diagnostics kits may contain version numbers for Assay Definitions. If the version number exists, it will be appended to the Assay Definition i.e. 'Sample IVD Assay.2'. When multiple versions exist, the software automatically uses the assay definition associated with the scanned lot number.

QUALITY CONTROL

Simplexa™ HSV 1 & 2 Positive Control Pack (MOL2160) may be used as an external control for Quality Control (QC) testing, training or proficiency testing. UTM is recommended as a NTC when testing genital swab samples. Quality control ranges have been established as indicated in the table below. If the controls are not within these parameters, patient results should be considered invalid and the assay repeated. Focus Diagnostics recommends testing controls once per day. Each laboratory should establish its own QC ranges and frequency of QC testing based on applicable local laws, regulations and standard good laboratory practice. Refer to the Simplexa™ HSV 1 & 2 Positive Control package insert (PI.MOL2160) for instructions on testing the positive control.

Expected Control Results

Control Type	HSV-1	HSV-2	DNA Internal Control (DNA IC)
Simplexa™ HSV 1 & 2 Positive Control[1]	Detected	Detected	Not applicable[2]
No Template Control (NTC)	Not Detected	Not Detected	Valid

[1] Typical Ct values for the Positive Control range between 25 to ≤40.
[2] Detection of the Simplexa™ DNA Internal Control (DNA IC) is not required for a valid result when HSV is detected.

RESULTS

Upon completion of the run, the software automatically calculates and displays results.

1. For each accession ID (Sample ID) entered, the software displays a result ("Detected", "Not Detected", "Invalid" or "EC500") for HSV-1 and HSV-2.
    a. **"Detected"** result points to the presence of HSV-1 and/or HSV-2 DNA in the patient sample.
    b. **"Not Detected"** result points to the absence of HSV-1 and/or HSV-2 DNA in the patient sample.
    c. **"Invalid"** result points to the inability to determine presence or absence of HSV-1 and/or HSV-2 DNA in the patient sample. This result may be due to 1) DNA Internal Control (DNA IC) failure, or 2) failure to detect sufficient specimen. The sample needs to be re-tested. See "Invalid Results" section below.
    d. **"EC500"** result points to a data quality error for the particular viral analyte(s). The software was unable to determine a valid amplification for that analyte(s). The sample should be re-tested.

e. **"EC505"** result indicates a data quality error for the viral analyte. The software was unable to determine a valid amplification for the analyte. Contact Technical Assistance at Focus Diagnostics which can be found on the last page of this document.

2. Print the report as needed.
    a. Export the results as needed.

INVALID RESULTS

In case of an "Invalid" result, re-test the sample with a new Reaction Mix vial from the same kit or a new kit. If the problem is unresolved, contact Focus Diagnostics Technical Services department.

LIMITATIONS

1. For *in vitro* diagnostic use.
2. In the United States, this product is intended for use in healthcare facilities with a minimum CLIA certification of moderate complexity.
3. Results from this test must be considered in conjunction with the clinical history, epidemiological data and other laboratory information available to the clinician evaluating the patient.
4. The detection of viral nucleic acid is dependent upon proper sample collection, transport, handling and storage. Failure to observe proper procedures in any one of these steps can lead to incorrect results.
5. The prevalence of viral infections may affect the test's predictive value.
6. Negative results do not rule out HSV infection of the genitals and should not be used as the sole basis for treatment or other patient management decisions.
7. False-negative results may occur if the viruses are present at a level that is below the analytical sensitivity of the assay or if the virus has genomic mutations, insertions, deletions, or rearrangements or if performed very early in the course of illness.
8. As with other tests, false-positive results may occur. Repeat testing or testing with a different device may be indicated in some settings.
9. A positive result by this test cannot rule out infections caused by other viral or bacterial pathogens. Viral nucleic acids may persist *in vivo* independent of virus viability. Detection of target analyte(s) does not imply that the corresponding viruses are infectious or are the causative agent for clinical symptoms.
10. When very high levels of HSV-1 are present with very low levels of HSV-2, the signal from the HSV-2 reaction may not be adequate to be detected, due to competitive interference.
11. This test is a qualitative test and does not provide the quantitative value of detected virus present.
12. The performance of this test has not been established for screening of blood or blood products for the presence of HSV or for use with samples other than genital swabs.
13. The performance of this test has not been established for immunocompromised individuals.
14. The performance of this test has not been established for monitoring treatment of HSV infection of the genitals.
15. Information on the Simplexa™ HSV 1 & 2 Direct Reaction Mix vial can only be transferred into the 3M Integrated Cycler Studio through a bar-code scanner. If the scanner is not working, or if you are unable to transfer the information for any reason, contact Focus Diagnostics Technical Services.

PERFORMANCE CHARACTERISTICS

EXPECTED VALUES – Genital Swab Sample Type
The observed expected values using the Simplexa™ HSV 1 & 2 Direct assay are presented below for patients 17 years of age or older from the prospective study population. The data is stratified by age and by gender.

Gender	Age Group	Sample Demographics by Simplexa™ HSV 1 & 2 Direct Assay					
		All	Simplexa™ HSV 1 & 2 Direct Result				
			HSV-1 & HSV-2 Not Detected	HSV-1 Detected & HSV-2 Not Detected	HSV-1 Not Detected & HSV-2 Detected	HSV-1 & HSV-2 Dual Positive	Not-Evaluable
Female	17 Years of age to 21 Years of age	98	41.8% (41/98)	25.5% (25/98)	30.6% (30/98)	0.0% (0/98)	2.0% (2/98)
	More than 21 Years of age	503	54.3% (273/503)	16.9% (85/503)	25.8% (130/503)	0.8% (4/503)	2.2% (11/503)
	All	601	52.2% (314/601)	18.3% (110/601)	26.6% (160/601)	0.7% (4/601)	2.2% (13/601)
Male	17 Years of age to 21 Years of age	14	42.9% (6/14)	21.4% (3/14)	35.7% (5/14)	0.0% (0/14)	0.0% (0/14)
	More than 21 Years of age	66	68.2% (45/66)	10.6% (7/66)	18.2% (12/66)	1.5% (1/66)	1.5% (1/66)
	All	80	63.8% (51/80)	12.5% (10/80)	21.3% (17/80)	1.3% (1/80)	1.3% (1/80)
All		**681**	**53.6% (365/681)**	**17.6% (120/681)**	**26.0% (177/681)**	**0.7% (5/681)**	**0.7% (5/681)****

*Samples never tested due to temperature excursion or tested but "invalid" results obtained due to internal control failure, insufficient specimen volume, daily PC/NTC failure.
**Excluded from this table are 35 samples from patients less than 17 years of age and 2 samples where the age of the patient was not t available.

CLINICAL AGREEMENT – Genital Swab Sample Type

Prospective Study
A total of 718 genital swab samples were prospectively collected from patients with signs and symptoms of genital herpes simplex virus (HSV) infection from 6 geographically diverse locations. Of the 718 samples collected, 9 samples were removed from the analysis because they were either not tested or had invalid results on the 3 assays (Simplexa™ HSV 1 & 2 Direct, Culture or bi-directional sequencing). Of the 709 remaining samples, 13 samples were removed from the analysis because they were not tested on the tests included in the composite comparator method sufficient to generate a final comparator result. A total of 696 samples were used for the analysis. The clinical performance of the Simplexa HSV 1 & HSV 2 assay was evaluated by comparing the positive and negative percent agreement to a composite comparator algorithm consisting of; culture, bi-directional sequencing and an FDA cleared NAAT. A positive result for HSV-1 and/or HSV-2 was determined by a positive test result in either the culture or the bi-directional sequencing. If both the culture and the bi-directional sequencing yielded positive results but disagreed in the differentiation of HSV-1 versus HSV-2, the results of the FDA cleared NAAT were used and a 2 out of 3 rule was followed to determine the type of the virus (e.g. if two of the methods were positive for HSV-1, the final comparator result was HSV-1 positive). All sites collected and tested the genital swab samples on the Simplexa™ HSV-1 and HSV-2 Direct and sent samples to a central lab for culture testing. For culture, each sample was tested for HSV- 2 first and if positive for HSV-2 no further testing was performed. Samples that were HSV-2 culture negative were further tested for HSV-1 culture positivity. Dual positives could not be identified in the culture assay.

The available retained samples were sent to Focus Diagnostics and tested in a validated bi-directional sequencing assay. Of the 24 discordant samples that were positive for HSV-2 by the culture method but positive for HSV-1 by the bi-directional sequencing assay, 18 samples had valid results and 1 sample had an invalid result when tested on an FDA cleared NAAT. There were 5 samples that were not tested on the FDA cleared NAAT due to insufficient volume and therefore 6 out of 24 samples were excluded from the analysis. There were 2 samples that were positive for HSV-1 by the culture method but positive for HSV-2 by the bi-directional sequencing assay that were not tested on the FDA cleared NAAT for insufficient volume and therefore were

excluded from analysis. Results for Simplexa™ HSV 1 & 2 Direct compared to the composite comparator algorithm are presented in the following tables.

Simplexa™ HSV 1 & 2 Direct Compared to Composite Comparator Result (HSV-1)

Simplexa™ HSV 1 & 2 Direct Results HSV-1	Composite Comparator Result (HSV-1)		
	Detected	Not Detected	Total
Detected	111	10	121
Not Detected	3	560	563
Total	114	570	684
Sensitivity	97.4%(111/114) 95% CI: 92.5% to 99.1%	Specificity	98.2%(560/570) 95% CI: 96.8% to 99.0%
PPV	91.7%(111/121) 95% CI: 85.5% to 95.4%	NPV	99.5%(560/563) 95% CI: 98.4% to 99.8%

Simplexa™ HSV 1 & 2 Direct Compared to Composite Comparator Result (HSV-2)

Simplexa™ HSV 1 & 2 Direct Results HSV-2	Composite Comparator Result (HSV-2)		
	Detected	Not Detected	Total
Detected	175	11	186
Not Detected	5	497	502
Total	180	508	688
Sensitivity	97.2%(175/180) 95% CI: 93.7% to 98.8%	Specificity	97.8%(497/508) 95% CI: 96.2% to 98.8%
PPV	94.1%(175/186) 95% CI: 89.7% to 96.7%	NPV	99.0%(497/502) 95% CI: 97.7% to 99.6%

Retrospective Study
A total of 28 genital swab samples (14 positive HSV-1 and 14 positive HSV-2) were retrospectively collected from male patients with signs and symptoms of genital herpes simplex virus (HSV) infection and contained preselected positive and negative samples. The samples were tested at Focus Diagnostics using the Simplexa™ HSV 1 & 2 Direct and a validated bi-directional sequencing assay.

Simplexa™ HSV 1 & 2 Direct Compared to Composite Comparator Result (HSV-1)

Simplexa™ HSV 1 & 2 Direct Results	Composite Comparator Result (HSV-1)		
	Detected	Not Detected	Total
Detected	14	1	15
Not Detected	0	13	13
Total	14	14	28
PPA	100.0%(14/14) 95% CI: 78.5% to 100.0%	NPA	92.9%(13/14) 95% CI: 68.5% to 98.7%
PPV	93.3%(14/15) 95% CI: 70.2% to 98.8%	NPV	100.0%(13/13) 95% CI: 77.2% to 100.0%

Simplexa™ HSV 1 & 2 Direct Compared to Composite Comparator Result (HSV-2)

Simplexa™ HSV 1 & 2 Direct Results	Composite Comparator Result (HSV-2)		
	Detected	Not Detected	Total
Detected	14	0	14
Not Detected	0	14	14
Total	14	14	28
PPA	100.0%(14/14) 95% CI: 78.5% to 100.0%	NPA	100.0%(14/14) 95% CI: 78.5% to 100.0%
PPV	100.0%(14/14) 95% CI: 78.5% to 100.0%	NPV	100.0%(14/14) 95% CI: 78.5% to 100.0%

REPRODUCIBILITY – Genital Swab Sample Type

Reproducibility for the Simplexa™ HSV 1 & 2 Direct assay was evaluated. Three investigative sites assessed the device's inter-site, inter-day and inter/intra-assay reproducibility. Each of the laboratories tested the positive control and a panel of five contrived sample pools including a low (approximately 1-2 times LoD) and medium positive (approximately 2-4 times LoD) for each analyte and a high negative. The high negative sample contained a small amount of HSV-1 and HSV-2, and it was designed to be negative approximately 95% of the time. The assays were performed in triplicate on five different days. Each site had two operators; each operator assayed the entire sample panel and positive control once per day, for a total of two sets of data per day. Combined results for all sites are presented in the tables below.

	Sample	Site – 1			Site – 2			Site – 3			Total % Agreement With Expected Results	95% CI
		% Agreement With Expected Results	Avg. Ct	Total %CV	% Agreement With Expected Results	Avg. Ct	Total %CV	% Agreement With Expected Results	Avg. Ct	Total %CV		
HSV-1 Result	HSV-1 Low Positive	100.0% (30/30)	36.0	2.2	100.0% (30/30)	36.1	2.6	100.0% (30/30)	36.3	2.7	100.0% (90/90)	95.9 to 100.0%
	HSV-1 Medium Positive	100.0% (30/30)	34.4	1.7	100.0% (30/30)	34.8	1.2	100.0% (30/30)	34.6	1.9	100.0% (90/90)	95.9 to 100.0%
	HSV-2 Low Positive	100.0% (30/30) [a]	NA	NA	100.0% (30/30) [a]	NA	NA	96.7% (29/30) [a]	NA	NA	98.9% (89/90) [a]	94.0 to 99.8%
	HSV-2 Medium Positive	100.0% (30/30) [a]	NA	NA	96.7% (29/30) [a]	NA	NA	100.0% (30/30) [a]	NA	NA	98.9% (89/90) [a]	94.0 to 99.8%
	High Negative	96.7% (29/30) [a]	38.8	0.0	93.3% (28/30) [a]	38.7	0.5	90.0% (27/30) [a]	38.0	4.1	93.3% (84/90) [a]	86.2 to 96.9%
	Positive Control	100.0% (30/30)	29.9	0.8	100.0% (30/30)	30.4	1.3	100.0% (29/29)	29.9	2.8	100.0% (89/89)	95.9 to 100.0%
	Total Agreement	**99.4% (179/180)**			**98.3% (177/180)**			**97.8% (175/179)**			**98.5% (531/539)**	**97.1 to 99.2%**

a) Expected Results of HSV-2 Low Positive, HSV-2 Medium Positive and High Negative samples are “Negative” for HSV-1.

	Sample	Site – 1			Site – 2			Site – 3			Total % Agreement With Expected Results	95% CI
		% Agreement With Expected Results	Avg. Ct	Total %CV	% Agreement With Expected Results	Avg. Ct	Total %CV	% Agreement With Expected Results	Avg. Ct	Total %CV		
HSV-2 Result	HSV-1 Low Positive	100.0% (30/30)[b]	NA	NA	100.0% (30/30) [b]	NA	NA	96.7% (29/30) [b]	41.1	0.0	98.9% (89/90) [b]	94.0 to 99.8%
	HSV-1 Medium Positive	100.0% (30/30) [b]	NA	NA	100.0% (30/30) [b]	NA	NA	100.0% (30/30) [b]	NA	NA	100.0% (90/90) [b]	95.9 to 100.0%
	HSV-2 Low Positive	100.0% (30/30)	37.4	2.9	90.0% (27/30)	37.5	3.5	93.3% (28/30)	37.1	2.8	94.4% (85/90)	87.6 to 97.6%
	HSV-2 Medium Positive	100.0% (30/30)	35.5	1.9	100.0% (30/30)	35.6	2.0	100.0% (30/30)	35.3	1.6	100.0% (90/90)	95.9 to 100.0%
	High Negative	96.7% (29/30) [b]	39.5	0.0	86.7% (26/30) [b]	38.6	2.9	100.0% (30/30) [b]	NA	NA	94.4% (85/90) [b]	87.6 to 97.6%
	Positive Control	100.0% (30/30)	30.2	1.3	100.0% (30/30)	30.1	0.6	100.0% (29/29)	29.9	1.2	100.0% (89/89)	95.9 to 100.0%
	Total Agreement	**99.4% (179/180)**			**96.1% (173/180)**			**98.9% (176/179)**			**98.0% (528/539)**	**96.4 to 98.9%**

b) Expected Results of HSV-1 Low Positive, HSV-1 Medium Positive and High Negative samples are “Negative” for HSV-2.

	Sample	Site – 1			Site – 2			Site – 3			Total % Agreement With Expected Results	95% CI
		% Agreement With Expected Results	Avg. Ct	Total %CV	% Agreement With Expected Results	Avg. Ct	Total %CV	% Agreement With Expected Results	Avg. Ct	Total %CV		
DNA IC Result	HSV-1 Low Positive	100.0% (30/30)	29.6	0.7	100.0% (30/30)	29.8	1.2	100.0% (30/30)	29.7	1.0	100.0% (90/90)	95.9 to 100.0%
	HSV-1 Medium Positive	100.0% (30/30)	29.6	0.8	100.0% (30/30)	29.8	1.4	100.0% (30/30)	29.7	0.9	100.0% (90/90)	95.9 to 100.0%
	HSV-2 Low Positive	100.0% (30/30)	29.6	0.8	100.0% (30/30)	29.8	1.2	100.0% (30/30)	29.7	1.0	100.0% (90/90)	95.9 to 100.0%
	HSV-2 Medium Positive	100.0% (30/30)	29.5	0.6	100.0% (30/30)	29.7	1.4	100.0% (30/30)	29.8	1.4	100.0% (90/90)	95.9 to 100.0%
	High Negative	100.0% (30/30)	29.6	0.6	100.0% (30/30)	29.8	1.2	100.0% (30/30)	29.7	1.0	100.0% (90/90)	95.9 to 100.0%
	Positive Control	100.0% (30/30)	29.5	0.5	100.0% (30/30)	29.7	1.4	100.0% (29/29)	29.7	0.9	100.0% (89/89)	95.9 to 100.0%
	Total Agreement	**100.0% (180/180)**			**100.0% (180/180)**			**100.0% (179/179)**			**100.0% (539/539)**	**96.4 to 98.9%**

ANALYTICAL SENSITIVITY/LIMIT OF DETECTION – Genital Swab Sample Type

The Limit of Detection (LoD) was determined for the Simplexa™ HSV 1 & 2 Direct assay using quantified stocks of HSV-1 and HSV-2 serially diluted into negative genital swab matrix containing male and female genital swabs. LoD was determined to be the lowest concentration that could be detected positive $\geq$ 95% of the time.

Virus Strain	LoD Concentration ($TCID_{50}$/mL)	Qualitative Results (#Detected/#Total)	Mean Ct ± SD (from Detected Replicates only)
HSV-1 McIntyre	4	32/32	36.4 ± 1.16
HSV-1 HF	160	32/32	35.2 ± 1.03
HSV-2 G	2	32/32	37.5 ± 1.08
HSV-2 MS	10	31/32	37.9 ± 1.15

Analytical Reactivity –Genital Swab Sample Type

The analytical reactivity of the Simplexa™ HSV 1 & 2 Direct assay was evaluated using different strains of HSV-1 and HSV-2 that were not used in the determination of the limit of detection (LoD) for the assay. Quantified viral material was spiked into negative genital swab matrix containing male and female genital swabs using a single dilution and assayed in triplicate. The Simplexa™ HSV 1 & 2 Direct assay was able to detect other strains of HSV-1 and HSV-2 viruses.

HSV Strain/Isolate	Spiked Concentration [$TCID_{50}$/mL]	Qualitative Result (#Detected/#Total)	
		HSV-1	HSV-2
HSV-1 KOS	16	3/3	0/3
HSV-1 F	32	3/3	0/3
HSV-2 Isolate 1	8	0/3	3/3
HSV-2 Isolate 2	8	0/3	3/3
HSV-2 Isolate 3	8	0/3	3/3

Cross-Reactivity (Analytical Specificity) – Genital Swab Sample Type

The Simplexa™ HSV 1 & 2 Direct assay's analytical specificity was evaluated by testing the ability to exclusively identify HSV-1 and HSV-2 viruses with no cross-reactivity to organisms that are closely related, or cause similar clinical symptoms or may be present on swabs of the genital region. Thirty six (36) potential cross-reactants were spiked into negative genital swab matrix containing male and female genital swabs and assayed in triplicate. No cross-reactivity was observed.

No.	Potential Cross-Reactants	Tested Concentration	Qualitative Result (#Detected/#Total)	
			HSV-1	HSV-2
1	None (Baseline)	Not Applicable	0/15	0/15
2	*Bacteroides fragilis*	1.00 X 10^6 cfu/mL	0/3	0/3
3	*Bacteroides ureolyticus**	Not Applicable	Not Applicable	Not Applicable
4	*Candida albicans*	1.00 X 10^6 cfu/mL	0/3	0/3
5	*Chlamydia trachomatis*	1.00 X 10^6 IFU/mL	0/3	0/3
6	*Clostridium sordellii*	1.00 X 10^6 cfu/mL	0/3	0/3
7	*Corynebacterium genitalium*	1.00 X 10^6 cfu/mL	0/3	0/3
8	Cytomegalovirus AD169 strain	1.00 X 10^5 $TCID_{50}$/mL	0/3	0/3
9	*Enterococcus faecalis* vanB	1.00 X 10^6 cfu/mL	0/3	0/3
10	Enterovirus 71	1.00 X 10^5 $TCID_{50}$/mL	0/8	1/8
11	Epstein Barr Virus (B95-8)	1.00 X 10^5 copies/mL	0/3	0/3
12	*Escherichia coli* O157H7	1.00 X 10^6 cfu/mL	0/3	0/3
13	*Gardnerella vaginalis*	1.00 X 10^6 cfu/mL	0/3	0/3
14	Hepatitis B	1.00 X 10^5 IU/mL	0/3	0/3
15	Hepatitis C	1.00 X 10^5 IU/mL	0/3	0/3
16	HHV-6 (Z29 Strain)	1.00 X 10^5 $TCID_{50}$/mL	0/3	0/3
17	HHV-7 SB	1.00 X 10^5 $TCID_{50}$/mL	0/3	0/3
18	HIV-1 IIIB	1.00 X 10^5 copies/mL	0/3	0/3
19	HIV-2 NIHZ**	Not Available	0/3	0/3
20	HPV18 Recombinant	1.00 X 10^5 pfu/mL	0/3	0/3
21	*Lactobacillus acidophilus*	1.00 X 10^6 cfu/mL	0/3	0/3
22	*Mobiluncus mulieris*	1.00 X 10^6 cfu/mL	0/3	0/3
23	*Mycoplasma genitalium**	Not Applicable	Not Applicable	Not Applicable
24	*Mycoplasma hominis*	1.00 X 10^6 CCU/mL	0/3	0/3

No.	Potential Cross-Reactants	Tested Concentration	Qualitative Result (#Detected/#Total)	
			HSV-1	HSV-2
25	*Neisseria gonorrhoeae*	1.00 X 10^6 cfu/mL	0/3	0/3
26	*Proteus vulgaris*	1.00 X 10^6 cfu/mL	0/3	0/3
27	Rubella	1.00 X 10^5 $TCID_{50}$/mL	0/3	0/3
28	*Staphylococcus aureus* (MRSA), ATCC 700699	1.00 X 10^6 cfu/mL	0/3	0/3
29	*Staphylococcus epidermidis* (MRSE), ATCC 29887	1.00 X 10^6 cfu/mL	0/3	0/3
30	*Staphylococcus saprophyticus*	1.00 X 10^6 cfu/mL	0/3	0/3
31	*Streptococcus mitis*	1.00 X 10^6 cfu/mL	0/3	0/3
32	*Streptococcus pyogenes*, M1	1.00 X 10^6 cfu/mL	0/3	0/3
33	*Toxoplasma gondii*	1.00 X 10^6 tachyzooites/mL	0/3	0/3
34	*Treponema pallidum**	Not Applicable	Not Applicable	Not Applicable
35	*Trichomonas vaginalis*	1.00 X 10^6 trophozoites/ml	0/3	0/3
36	*Ureaplasma urealyticum*	1.00 X 10^6 CCU/mL	0/3	0/3
37	VZV	1.00 X 10^5 copies/mL	0/3	0/3

* Microorganism was not available for testing therefore in-silico NCBI BLAST analysis was performed and found no predicted cross reactivity.
** Quantified material was not available to test; instead the vendor provided a culture fluid with a known Ct value. The site was directed to dilute the stock to a relevant Ct value; 1:50 dilution factor.

INTERFERENCE – Genital Swab Sample Type

The performance of the Simplexa™ HSV 1 & 2 Direct assay was evaluated with potentially interfering substances that may be present on swabs of the genital region at the concentrations indicated in the table below. A total of five (26) potentially interfering substances were tested in a low positive HSV-1 and HSV-2 sample (4 times LoD) in negative genital swab matrix containing male and female genital swabs and assayed in triplicate. No interference was observed.

Potential Interferent	Interferent Concentration	#Detected/#Total		
		HSV-1	HSV-2	IC
Abreva cold sore treatment	7% w/v	3/3	3/3	3/3
Acyclovir	2.5 mg/mL	3/3	3/3	3/3
Acyclovir Cream*	7% w/v	8/8	5/8	8/8
Albumin	10 mg/mL	3/3	3/3	3/3
Balneol Hygienic Cleansing lotion	7% w/v	3/3	3/3	3/3
Casein	10 mg/mL	3/3	3/3	3/3
Cidofovir	2.5 mg/mL	3/3	3/3	3/3
Clotrimazole vaginal cream	7% w/v	3/3	3/3	3/3
Denavir	2.5 mg/mL	3/3	3/3	3/3
Douche	7% w/v	3/3	3/3	3/3
Famciclovir	2.5 mg/mL	3/3	3/3	3/3
Feces	2.5 mg/mL	3/3	3/3	3/3
Gynol II (Contraceptive jelly)	7% w/v	3/3	3/3	3/3
KY Jelly	5% v/v	3/3	3/3	3/3
Monistat 1	7% w/v	3/3	3/3	3/3
Monistat 3	7% w/v	3/3	3/3	3/3
Mucin	7% w/v	3/3	3/3	3/3

Potential Interferent	Interferent Concentration	#Detected/#Total		
		HSV-1	HSV-2	IC
Preparation H Hemorrhoid cream	7% w/v	3/3	3/3	3/3
Releev cold sore treatment	7% w/v	3/3	3/3	3/3
Urine	10% v/v	3/3	3/3	3/3
Vagicaine Anti-Itch Cream	7% w/v	3/3	3/3	3/3
Vagisil Creme	7% w/v	3/3	3/3	3/3
VagiStat 1	7% w/v	3/3	3/3	3/3
Valacyclovir	2.5 mg/mL	3/3	3/3	3/3
Whole Blood	10% v/v	3/3	3/3	3/3
YeastGard Suppositories	7% w/v	3/3	3/3	3/3
* 1/3 initial replicates and 2/5 confirmation replicates were negative for HSV-2.				

COMPETITIVE INTERFERENCE – Genital Swab Sample Type

Competitive interference was studied to evaluate the effects of clinically relevant co-infections with each of the analytes detected by the Simplexa™ HSV 1 & 2 Direct assay. The study assessed whether a high concentration of one virus in the sample could potentially affect the Simplexa™ HSV 1 & 2 Direct assay performance for another target present at low levels. A low sample was contrived at approximately 4 times LoD for each target (HSV-1 McIntryre strain and HSV-2 G strain), and a baseline Ct was determined for each sample. Each potential concomitant infecting virus was spiked into the low level sample and assayed in triplicate. Baseline sample results are also shown below. No competitive interference was observed.

Baseline (Low Level)		Competitive Interferent (High Concentration)		Qualitative Results (#Detected/#Total)	
Strain	Concentration ($TCID_{50}$/mL)	Strain	Concentration ($TCID_{50}$/mL)	HSV-1	HSV-2
HSV1 McIntyre	16	HSV2 G	0	5/5	0/5
HSV1 McIntyre	16	HSV2 G	1.00×10^6	3/3	3/3
HSV2 G	8	HSV1 McIntyre	0	0/5	5/5
HSV2 G	8	HSV1 McIntyre	1.71×10^3	3/3	3/3

INHIBITION BY OTHER MICROORGANISMS – Genital Swab Sample Type

The Simplexa™ HSV 1 & 2 Direct assay was evaluated by testing the ability to identify HSV-1 and HSV-2 viruses when other potentially inhibitory organisms are present. The panel of thirty six (36) potentially inhibitory organisms was individually spiked into a pool with a low concentration (approximately 4 times LoD) of HSV-1 and HSV-2 in genital swab matrix. Each microorganism sample was initially tested in triplicate and if any one of the replicates was "Not Detected" for either the HSV-1 or the HSV-2 targets then five additional replicates would be tested to confirm if any inhibition was caused by the microorganism. If the majority (>4/8) replicates were "Not Detected" then an inhibitory effect would be determined. None of the microorganisms caused >4/8 of the replicates to be "Not Detected".

No.	Microorganism	Tested Concentration	Qualitative Result (#Detected/#Total)	
			HSV-1	HSV-2
1	Baseline	Not Applicable	15/15	15/15
2	*Bacteroides fragilis*	1.00×10^6 cfu/mL	3/3	3/3
3	*Bacteroides ureolyticus***	Not Applicable	Not Applicable	Not Applicable
4	*Candida albicans*	1.00×10^6 cfu/mL	3/3	3/3
5	*Chlamydia trachomatis*	1.00×10^6 IFU/mL	3/3	3/3
6	*Clostridium sordellii*	1.00×10^6 cfu/mL	3/3	3/3
7	*Corynebacterium genitalium*	1.00×10^6 cfu/mL	3/3	3/3
8	Cytomegalovirus	1.00×10^5 $TCID_{50}$/mL	3/3	3/3

No.	Microorganism	Tested Concentration	Qualitative Result (#Detected/#Total)	
			HSV-1	HSV-2
9	*Enterococcus faecalis* vanB	1.00 X 10^6 cfu/mL	3/3	3/3
10	Enterovirus 71	1.00 X 10^5 $TCID_{50}$/mL	3/3	3/3
11	Epstein Barr Virus (B95-8)	1.00 X 10^5 copies/mL	3/3	3/3
12	*Escherichia coli* O157H7	1.00 X 10^6 cfu/mL	3/3	3/3
13	*Gardnerella vaginalis*	1.00 X 10^6 cfu/mL	3/3	3/3
14	Hepatitis B	1.00 X 10^5 IU/mL	3/3	3/3
15	Hepatitis C	1.00 X 10^5 IU/mL	3/3	3/3
16	HHV-6 (Z29 Strain)	1.00 X 10^5 $TCID_{50}$/mL	3/3	3/3
17	HHV-7 SB	1.00 X 10^5 $TCID_{50}$/mL	3/3	3/3
18	HIV-1 IIIB	1.00 X 10^5 copies/mL	3/3	3/3
19	HIV-2 NIHZ*	Not Available	3/3	3/3
20	HPV18 Recombinant	1.00 X 10^5 pfu/mL	3/3	3/3
21	*Lactobacillus acidophilus*	1.00 X 10^6 cfu/mL	3/3	3/3
22	*Mobiluncus mulieris*	1.00 X 10^6 cfu/mL	3/3	3/3
23	*Mycoplasma genitalium***	Not Applicable	Not Applicable	Not Applicable
24	*Mycoplasma hominis*	1.00 X 10^6 CCU/mL	3/3	3/3
25	*Neisseria gonorrhoeae*	1.00 X 10^6 cfu/mL	3/3	3/3
26	*Proteus vulgaris*	1.00 X 10^6 cfu/mL	3/3	3/3
27	Rubella	1.00 X 10^5 $TCID_{50}$/mL	3/3	3/3
28	*Staphylococcus aureus* (MRSA), ATCC 700699	1.00 X 10^6 cfu/mL	3/3	3/3
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30	*Staphylococcus saprophyticus*	1.00 X 10^6 cfu/mL	3/3	3/3
31	*Streptococcus mitis*	1.00 X 10^6 cfu/mL	3/3	3/3
32	*Streptococcus pyogenes*, M1	1.00 X 10^6 cfu/mL	3/3	3/3
33	*Toxoplasma gondii*	1.00 X 10^6 tachyzooites/mL	3/3	3/3
34	*Treponema pallidum***	Not Applicable	Not Applicable	Not Applicable
35	*Trichomonas vaginalis*	1.00 X 10^6 trophozoites/ml	3/3	3/3
36	*Ureaplasma urealyticum*	1.00 X 10^6 CCU/mL	3/3	3/3
37	VZV	1.00 X 10^5 copies/mL	3/3	3/3

*Quantified material was not available to test; instead the vendor provided a culture fluid with a known Ct value. The site was directed to dilute the stock to a relevant Ct value; 1:50 dilution factor.
**Microorganism was not available for testing therefore in-silico NCBI BLAST analysis was performed and found no cross reactivity.

CARRY-OVER CONTAMINATION

The amplification carry-over for the Simplexa™ assays including the Simplexa™ HSV 1 & 2 Direct assay was assessed from the Simplexa™ Flu A/B & RSV Direct REF MOL2650 (K120413) viral assay, and can be found on the FDA website. The study can be applied to the Simplexa™ HSV 1 & 2 Direct assays as the study is not analyte specific. In the Simplexa™ Flu A/B & RSV Direct REF MOL2650 (K120413), the amplification carry-over study searched for the presence of contamination in negative samples adjacent to strong positive samples. The study was designed by alternately placing high positive and negative samples on each disc. No evidence of carry-over contamination was observed.

FRESH VS FROZEN STUDY

Storage conditions were validated using the following transport media types BD VTM, M4, M4RT, M5, M6, and UTM by spiking media with organism at concentrations ranging from 3 times LoD to 50 times LoD and at different storage temperatures and durations.

Samples should be transported on ice and stored at 2 to 8°C for up to 7 days post collection. If there is a greater than 7 day delay before processing of the sample, store the sample at -70° C.

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