EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR
Lyra™ Direct HSV 1 + 2/VZV Assay

DECISION SUMMARY

A. 510(k) Number:

K133448

B. Purpose for Submission:

De novo request for evaluation of automatic class III designation for the Lyra™ Direct HSV 1 + 2/VZV Assay

C. Measurand:

Target DNA sequences from conserved regions of the herpes simplex virus type 1 (HSV-1), herpes simplex virus type 2 (HSV-2) and varicella-zoster virus (VZV) genes.

D. Type of Test:

A real-time Polymerase Chain Reaction (PCR) test for qualitative detection and differentiation of HSV-1, HSV- 2, and VZV DNA isolated and purified from cutaneous or mucocutaneous lesion samples.

E. Applicant:

Quidel Corporation.

F. Proprietary and Established Names:

Lyra™ Direct HSV 1 + 2/VZV Assay

G. Regulatory Information:

1. Regulation: 21 CFR 866.3309
2. Classification: Class II (special controls)
3. Product code: PGI
4. Panel: Microbiology (83)

H. Intended Use:

1. Intended use(s):

   The Lyra™ Direct HSV 1 + 2/VZV Assay is an in vitro multiplex Real-Time PCR test for qualitative detection and differentiation of herpes simplex virus type 1, herpes simplex virus type 2, and varicella-zoster virus DNA isolated and purified from cutaneous or mucocutaneous lesion samples obtained from symptomatic patients suspected of active herpes simplex virus 1, herpes simplex virus 2 and/or varicella-zoster infection. The Lyra™ Direct HSV 1 + 2/VZV Assay is intended to aid in the diagnosis of herpes simplex virus 1, herpes simplex virus 2 and varicella-zoster virus active cutaneous or mucocutaneous infections. Negative results do not preclude herpes simplex virus 1, herpes simplex virus 2 and varicella-zoster virus infections and should not be used as the sole basis for diagnosis, treatment or other management decisions. The Lyra™ Direct HSV 1 + 2/VZV Assay is not intended for use with cerebrospinal fluid or to aid in the diagnosis of HSV or VZV infections of the central nervous system (CNS). The Lyra™ Direct HSV 1 + 2/VZV Assay is not intended for use in prenatal screening. The device is not intended for point-of-care use.

2. Indication(s) for use:

   Same as intended use

3. Special conditions for use statement(s):

   For prescription use only in accordance with 21 CFR 801.109.

4. Special instrument requirements:

   To be used with:
   Life Technologies QuantStudio™ Dx (software version 1.0)
   Applied Biosystems® 7500 Fast Dx (software version 1.4)
   Cepheid SmartCycler® II System (software version 3.0b)

I. Device Description:

The Lyra™ Direct HSV 1 + 2/VZV Assay detects viral nucleic acids from a patient sample. A multiplex Real-Time PCR reaction is carried out under optimized conditions in a single tube or well generating amplicons for HSV-1, HSV-2, VZV, and the Process Control (PRC). Identification of amplicons for HSV-1, HSV-2, VZV, and the PRC occurs by the use of target-specific primers and fluorescent-labeled probes that hybridize to conserved regions in the genomes of HSV-1, HSV-2, and VZV and to the PRC, respectively.

Lyra™ Direct Probe Labels

Target	**Dye**
HSV-1	FAM
HSV-2	CAL Fluor® Orange 560
VZV	CAL Fluor® Red 610
PRC	Quasar® 670

The following table and list describe: the reagents provided in the kit, the materials which are required but not provided with the kit and the optional reagents and materials.

Materials Provided With The Kit:

The Lyra™ Direct HSV 1 + 2/VZV Assay kit consists of the following:

- Rehydration Solution
- Process Buffer Part M5050 (contains the PRC)
- Lyra™ Direct HSV 1 + 2/VZV Master Mix Part M5012
- Lyophilized Contents:
    - DNA polymerase enzyme
    - Primers and probes
    - dNTPs
    - Stabilizers

Materials Required But Not Provided:

- Micropipettors (range between 2 to 20 µL and 20 to 200 µL)
- Non-aerosol pipette tips
- Life Technologies QuantStudio™ Dx or the Applied Biosystems® 7500 Fast Dx
- Applied Biosystems Fast Dx 96 well PCR plate
- Optical plate films
- Plate centrifuge for Applied Biosystems 96 well plate
- Dry heating block, capable of heating 1.5 mL tubes at 60°C for 5 minutes
- Empty microcentrifuge tubes

Or

- Micropipettors (range between 2 to 20 µL and 20 to 200 µL)
- Non-aerosol pipette tips
- Cepheid SmartCycler® II
- SmartCycler disposables
- SmartCycler centrifuge
- Smartcycler reaction tube racks. Dry heating block, capable of heating 1.5 mL tubes at 60°C for 5 minutes.
- Empty microcentrifuge tubes

Optional Materials:

- Positive controls for HSV-1, HSV-2 and VZV (i.e., Lyra™ Direct HSV 1 + 2/VZV Control Set which serves as an external assay control)

J. Standard/Guidance Documents Referenced:

1. Guidance for Industry and FDA Staff - Establishing the Performance Characteristics of In Vitro Diagnostic Devices for the Detection or Detection and Differentiation of Influenza Viruses (July 15, 2011) http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm079171.htm
2. Guidance for Industry and FDA Staff - Class II Special Controls Guidance Document: Respiratory Viral Panel Multiplex Nucleic Acid Assay (October 9, 2009) http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm180307.htm
3. Guidance on Informed Consent for In Vitro Diagnostic Device Studies Leftover Human Specimens that are Not Individually Identifiable (April 2006) http://www.fda.gov/cdrh/oivd/guidance/1588.pdf.
4. Guidance for Industry and Food and Drug Administration Staff - eCopy Program for Medical Device (December 2012) http://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/UCM313794.pdf
5. Guidance for Industry, FDA Reviewers and Compliance on Off-The-Shelf Software Use in Medical Devices (September 9, 1999) http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm073778.htm

The following documents were referenced in one or more of the Guidance Documents above:

1. CLSI EP17-A: Guidance for Protocols for Determination of Limits of Detection and Limits of Quantitation (Vol. 2, No. 34) (Oct 2004).
2. CLSI MM13-A: Guidance for the Collection, Transport, Preparation and Storage of Specimens for Molecular Methods (Vol. 25, No. 31) (Dec 2005).
3. CLSI EP7-A2: Guidance for Interference Testing in Clinical Chemistry (Vol. 25, No.27 Second Ed) (Nov 2005).
4. CLSI EP12-A: Guidance for User Protocol for Evaluation of Qualitative Test Performance (Vol. 22, No. 14) (Sept 2002).
5. CLSI MM6-A: Guidance for the Quantitative Molecular Methods for Infectious Diseases (Vol. 23, No.28) (Oct 2003).
6. CLSI EP5-A2: Guidance for Evaluation of Precision Performance of Quantitative Measurement Methods (Vol. 24, No. 25 Second Ed.) (Aug 2004).

K. Test Principle:

The Lyra™ Direct HSV 1 + 2/VZV assay is based on TaqMan® chemistry and uses an enzyme with DNA polymerase and 5'-3' exonuclease activities. During DNA amplification, this enzyme cleaves the probe bound to the complementary DNA sequence, separating the quencher dye from the reporter dye. This step generates an increase in fluorescent signal upon excitation by a light source of the appropriate wavelength. With each cycle, additional dye molecules are separated from their quenchers resulting in additional signal. If sufficient

fluorescence is achieved on the Life Technologies QuantStudio™ Dx, the Applied Biosystems® 7500 Fast Dx, or the Cepheid SmartCycler® II System, the sample is reported as positive for the detected nucleic acid.

The following is a summary of the procedure:

Sample Collection: Obtain lesion swabs using standard techniques from symptomatic patients. Transport, store, and process these specimens according to established laboratory procedures.

Sample Preparation: Remove 100 µL of each clinical specimen from the original collection tube and place into a clean microcentrifuge tube. Heat at 60°C for 5 minutes, then remove from heat and add 25 µL of Process Buffer within 60 minutes according to the detailed instructions for use. The Process Buffer contains a buffer and diluents as well as the PRC. The PRC serves to monitor inhibitors in the prepared specimen, assures that adequate amplification has taken place, and confirms that the overall process was performed correctly.

Rehydration of Master Mix: Rehydrate the lyophilized Master Mix using 135 µL of Rehydration Solution. The Master Mix contains oligonucleotide primers, fluorophore and quencher-labeled probes targeting conserved regions of HSV-1, HSV-2, and VZV, as well as the PRC sequence.

Nucleic Acid Amplification and Detection: Add 15 µL of the rehydrated Master Mix to each reaction tube or plate well. Then add 5 µL of nucleic acids (specimen with PRC) to the plate well or appropriately labeled reaction tube. Place the plate or tube into either the QuantStudio™ Dx, Applied Biosystems® 7500 Fast Dx or SmartCycler® II instruments, respectively. Once the reaction tube or plate is added to the instrument, initiate the assay protocol. This protocol initiates amplification of the DNA targets.

L. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Reproducibility/ Precision

Reproducibility: The reproducibility of the Lyra™ Direct HSV 1 + 2/VZV Assay was evaluated at 3 laboratory sites (two external, one in-house). Reproducibility was assessed using a panel of 6 simulated samples that include medium positive, low positive, high negative and negative HSV-1, HSV-2, and VZV samples.

The panels and controls were processed and tested on the Life Technologies QuantStudio™ Dx, the Applied Biosystems® 7500 Fast Dx, and the Cepheid SmartCycler® II System. Panels and controls were tested at each site by 2 operators for 5 days (triplicate testing x 2 operators x 5 days x 3 sites = 90 results per level for each virus).

Reproducibility Results - Life Technologies QuantStudio™ Dx												
Panel Member ID	Site 1			Site 2			Site 3			Combined Site Data		
	Rate of Detection	AVE Ct	% CV	Rate of Detection	AVE Ct	% CV	Rate of Detection	AVE Ct	% CV	Rate of Detection	AVE Ct	%CV
HSV-1 High Negative	10/30	37.0	4.9	16/30	34.5	8.9	17/30	34.0	7.6	43/90	34.8	8.2
HSV-1 Low Positive	30/30	29.8	1.6	30/30	29.0	2.6	30/30	29.5	3.6	90/90	29.4	2.9
HSV-1 Moderate Positive	30/30	28.3	1.9	30/30	27.3	2.4	30/30	27.9	3.7	90/90	27.8	3.1
HSV-1 Negative	0/30	N/A	N/A	0/29*	N/A	N/A	0/30	N/A	N/A	0/89*	N/A	N/A
HSV-2 High Negative	30/30	34.8	3.0	30/30	31.9	4.7	28/30	34.1	6.2	88/90	33.6	6.0
HSV-2 Low Positive	30/30	32.8	1.4	30/30	32.0	2.3	30/30	31.8	3.6	90/90	32.2	2.9
HSV-2 Moderate Positive	30/30	30.8	3.8	30/30	29.8	2.8	30/30	30.8	4.2	90/90	30.5	3.9
HSV-2 Negative	0/30	N/A	N/A	0/29*	N/A	N/A	0/30	N/A	N/A	0/89*	N/A	N/A
VZV High Negative	9/30	37.4	4.2	10/30	35.2	6.4	5/30	34.4	6.6	24/90	35.9	6.5
VZV Low Positive	30/30	29.2	1.7	30/30	28.7	1.2	29/30	29.9	5.5	89/90	29.3	3.7
VZV Moderate Positive	30/30	27.6	1.3	30/30	27.2	1.0	30/30	27.7	2.6	90/90	27.5	2.9
VZV Negative	0/30	N/A	N/A	0/29*	N/A	N/A	0/30	N/A	N/A	0/89*	N/A	N/A
HSV-1 Positive Control	30/30	27.4	2.6	30/30	25.1	0.9	30/30	26.8	2.7	90/90	26.4	7.0
HSV-2 Positive Control	30/30	26.7	6.6	30/30	29.8	1.5	30/30	25.4	3.7	90/90	22.8	7.9
VZV Positive Control	30/30	22.1	13.4	30/30	20.0	0.8	30/30	21.1	4.5	90/90	21.1	9.2
Negative Control	0/30	N/A	N/A	0/30	N/A	N/A	0/30	N/A	N/A	0/90	N/A	N/A

* One replicate's PRC was not detected. The replicate was reported as invalid.

Reproducibility Results - Applied Biosystems® 7500 Fast Dx												
Panel Member ID	Site 1			Site 2			Site 3			Combined Site Data		
	Rate of Detection	AVE Ct	% CV	Rate of Detection	AVE Ct	% CV	Rate of Detection	AVE Ct	% CV	Rate of Detection	AVE Ct	%CV
HSV-1 High Negative	9/30	36.2	3.6	9/30	35.0	10.1	12/29	36.5	6.0	30/89	36.1	7.6
HSV-1 Low Positive	30/30	30.3	1.8	30/30	30.3	3.4	30/30	30.2	2.6	90/90	30.3	2.7
HSV-1 Moderate Positive	30/30	28.8	2.4	30/30	28.5	2.6	30/30	29.5	8.1	90/90	28.9	5.4
HSV-1 Negative	0/30	N/A	N/A	0/30	N/A	N/A	0/30	N/A	N/A	0/90	N/A	N/A
HSV-2 High Negative	30/30	35.4	3.7	30/30	34.2	5.8	24/30	36.0	6.0	84/90	35.2	5.5
HSV-2 Low Positive	30/30	32.8	4.2	30/30	33.6	3.8	30/30	32.7	5.9	90/90	33.0	4.8
HSV-2 Moderate Positive	30/30	31.2	2.1	30/30	31.3	2.9	30/30	31.9	4.3	90/90	31.5	3.4
HSV-2 Negative	0/30	N/A	N/A	0/30	N/A	N/A	0/30	N/A	N/A	0/90	N/A	N/A
VZV High Negative	0/30	N/A	N/A	3/30	33.8	6.2	0/30	N/A	N/A	3/90	33.8	6.2
VZV Low Positive	29/30	31.5	4.4	29/30	31.5	4.6	30/30	32.1	6.2	88/90	31.7	5.2
VZV Moderate Positive	30/30	29.4	2.5	30/30	29.2	2.1	30/30	30.9	9.1	90/90	29.8	6.2
VZV Negative	0/30	N/A	N/A	0/30	N/A	N/A	0/30	N/A	N/A	0/90	N/A	N/A
HSV-1 Positive Control	30/30	27.1	11.6	30/30	25.9	1.3	30/30	26.2	2.2	90/90	26.4	7.2
HSV-2 Positive Control	30/30	26.1	8.1	30/30	24.9	1.2	30/30	25.7	2.0	90/90	25.6	5.2
VZV Positive Control	30/30	23.6	13.2	30/30	21.8	1.1	30/30	22.1	1.7	90/90	22.5	8.6
Negative Control	0/30	N/A	N/A	0/30	N/A	N/A	0/30	N/A	N/A	0/90	N/A	N/A

Reproducibility Results - Cepheid SmartCycler® II												
Panel Member ID	Site 1			Site 2			Site 3			Combined Site Data		
	Rate of Detection	AVE Ct	% CV	Rate of Detection	AVE Ct	% CV	Rate of Detection	AVE Ct	% CV	Rate of Detection	AVE Ct	%CV
HSV-1 High Negative	5/30	37.8	1.9	5/30	33.4	7.5	13/30	35.3	2.4	23/30	35.4	6.0
HSV-1 Low Positive	30/30	31.3	1.3	30/30	31.0	4.5	30/30	31.5	2.4	90/90	31.3	3.1
HSV-1 Moderate Positive	29/29*	29.4	2.9	30/30	28.9	4.2	30/30	30.0	2.4	89/89*	29.5	3.6
HSV-1 Negative	0/30	N/A	N/A	0/30	N/A	N/A	0/30	N/A	N/A	0/90	N/A	N/A
HSV-2 High Negative	24/30	38.5	5.1	27/30	36.6	6.5	17/30	37.3	4.4	68/90	37.4	5.8
HSV-2 Low Positive	30/30	36.2	3.3	29/30	35.8	2.5	30/30	35.8	4.9	89/90	36.0	3.7
HSV-2 Moderate Positive	29/29*	33.6	1.6	30/30	33.3	4.6	30/30	33.8	3.4	89/89*	33.6	2.5
HSV-2 Negative	0/30	N/A	N/A	0/30	N/A	N/A	0/30	N/A	N/A	0/90	N/A	N/A
VZV High Negative	0/30	N/A	N/A	1/30	N/A	N/A	0/30	N/A	N/A	1/90	N/A	N/A
VZV Low Positive	29/30	33.4	7.0	29/30	32.7	2.8	30/30	36.2	10.7	88/90	34.1	8.9
VZV Moderate Positive	29/29*	30.9	2.3	30/30	30.6	1.1	30/30	33.4	8.5	89/89*	31.6	6.7
VZV Negative	0/30	N/A	N/A	0/30	N/A	N/A	0/30	N/A	N/A	0/90	N/A	N/A
HSV-1 Positive Control	30/30	27.6	8.6	30/30	26.4	1.8	30/30	27.4	2.2	90/90	27.2	5.6
HSV-2 Positive Control	30/30	28.0	7.0	30/30	26.5	1.8	30/30	28.1	9.3	90/90	27.5	7.3
VZV Positive Control	30/30	24.7	12.1	30/30	22.9	1.1	30/30	23.7	1.9	90/90	23.8	7.9
Negative Control	0/30	N/A	N/A	0/30	N/A	N/A	0/30	N/A	N/A	0/90	N/A	N/A

* One replicate's PRC was not detected. The replicate was reported as invalid.

Precision: For the Precision/Within Laboratory Repeatability study, a blinded four-member panel consisting of HSV-1, HSV-2, and VZV positive and negative samples was tested by two (2) operators (Op 1 and Op 2), twice a day (2X) for twelve (12) days on all three instruments.

Life Technologies QuantStudio™ Dx Results Summary						
Target		Positive Control	5X LoD	2X LoD	≤0.25X LoD	Negative Matrix
HSV-1	Op 1[a] Avg Ct	25.0	27.7	29.4	36.1	Neg
	Op 2[b] Avg Ct	25.4	27.6	28.9	36.2	Neg
	Positivity (%)	100	100	100	51	0
HSV-2	Op 1 Avg Ct	26.3	28.3	29.0	37.2	Neg
	Op 2 Avg Ct	26.3	28.2	29.0	37.6	Neg
	Positivity (%)	100	100	100	63	0
VZV	Op 1 Avg Ct	18.8	27.8	29.3	34.3	Neg
	Op 2 Avg Ct	18.7	27.9	29.2	34.7	Neg
	Positivity (%)	100	100	100	51	0

[a] Operator 1; [b] Operator 2

Applied Biosystems® 7500 Fast Dx Results Summary						
Target		Positive Control	5X LoD	2X LoD	≤0.25X LoD	Negative Matrix
HSV-1	Op 1 Avg Ct	26.2	28.3	29.9	38.6	Neg
	Op 2 Avg Ct	25.9	27.5	29.0	37.4	Neg
	Positivity (%)	100	100	100	32	0
HSV-2	Op 1 Avg Ct	25.4	29.1	30.1	37.2	Neg
	Op 2 Avg Ct	25.8	28.4	29.6	36.8	Neg
	Positivity (%)	100	100	100	75	0
VZV	Op 1 Avg Ct	19.6	29.0	33.1	36.9	Neg
	Op 2 Avg Ct	19.5	29.1	30.7	36.7	Neg
	Positivity (%)	100	100	99	25	0

Cepheid SmartCycler® II Results Summary						
Target		Positive Control	5X LoD	2X LoD	≤0.25X LoD	Negative Matrix
HSV-1	Op 1 Avg Ct	25.6	30.8	32.8	37.7	Neg
	Op 2 Avg Ct	25.5	30.9	32.6	36.4	Neg
	Positivity (%)	100	100	100	38	0
HSV-2	Op 1 Avg Ct	27.2	32.4	33.5	37.9	Neg
	Op 2 Avg Ct	27.6	32.2	33.4	37.7	Neg
	Positivity (%)	100	100	100	65	0
VZV	Op 1 Avg Ct	21.0	30.5	31.9	39.5	Neg
	Op 2 Avg Ct	21.0	30.7	32.5	40.2	Neg
	Positivity (%)	100	100	100	54	0

b. Linearity/assay reportable range:

Not applicable.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Kit Stability:
The stability of the Lyra Direct HSV 1 + 2/VZV Assay is currently being assessed in a real-time study using 3 production lots of the kits. All kits used in the study are being stored at 2° to 8°C. The test protocol utilizes 2x LoD virus dilutions of HSV-1, HSV-2, and VZV. These dilutions are being tested in triplicate according to the product insert on the Applied Biosystems® 7500 Fast Dx. The Ct values for each analyte (HSV-1, HSV-2, and VZV) generated at each time point is being compared to the Ct values generated at the time of production of the kits. The Ct values at a stability time point must be within 3 Cts of the result at the initial time point for a kit to pass. The real-time testing data collected thus far demonstrates stability up to 243 days at 2° to 8°C. All time points have passed for each analyte.

Rehydrated Master Mix Stability:
A study was performed on the Applied Biosystems® 7500 Fast Dx to determine the effect of time and temperature on rehydrated Master Mix stability. The Lyra™ DirectHSV 1 + 2/VZV Master Mix was rehydrated, stored at -20°C, 2° to 8°C and room temperature (RT) (20° to 25°C) for various times and then tested with samples at 2x LoD HSV-1, HSV-2 and VZV. Based on the data generated in this study, the final stability claims for rehydrated Lyra™ Direct HSV 1 + 2/VZV Master Mix are summarized in the tables below:

HSV/VZV Rehydrated Master Mix Stability Summary			
Target	Storage Temperature		
	RT	2-8°C	-20°C
HSV-1	4hrs	24hrs	76 hrs
HSV-2	4hrs	24hrs	76 hrs
VZV	1.5hrs	24hrs	76 hrs

Stability for RehydratedMaster Mix		
Storage Temperature and Stability Claim		
RT	2-8°C	-20°C
1 hr	9 hrs	72 hrs

Processed Sample Stability:
The stability of processed samples was evaluated when stored at room temperature, 2 to 8°C, -20°C, and -70°C for various periods of time. This study was conducted on the Applied Biosystems® 7500 Fast Dx. HSV-1, HSV-2, and VZV stocks were diluted to 2x LoD, processed, and then stored at the various temperatures listed below.

Number of Processed Samples Required for Each Storage Condition				
	Control and Room Temp	2° to 8°C	-20°C	-70°C
HSV-1	3	21	12	12
HSV-2	3	21	12	12
VZV	3	21	12	12
Negative	1	7	4	4

HSV-1, HSV-2, and VZV processed samples tested positive with little or no Ct shift (see table below) at all tested time points and for all storage conditions. Based on results at the tested time points, processed samples are stable for 48 hours when stored at room temperature (20-25ºC), 48 hours when stored at 2° to 8°C, 31 days when stored at -20°C, and 31 days when stored at -70°C.

	Control Ct Value (Ave.)	Ct Average 20° to 25°C (50 hours)	Ct Shift	Ct Average 2° to 8°C (50 hours)	Ct Shift	Ct Average -20°C (32 days)	Ct Shift	Ct Average -20°C (32 days)	Ct Shift
HSV-1	30.5	31.7	+ 1.2	30.5	0	28.9	- 1.6	33.7	+3.2
HSV-2	33.9	30.3	- 3.6	35.7	+1.8	34.6	+0.7	34.8	+0.9
VZV	30.5	30.7	+0.2	30.6	+0.1	30.3	-0.2	30.8	+0.3

d. Assay cut-off:

See limit of detection.

e. Detection limit (LoD)

The analytical sensitivity (Limit of Detection or LoD) of the Lyra™ Direct HSV 1 + 2/VZV Assay was determined on each instrument in three separate studies using quantified ($TCID_{50}$/mL) stocks of two strains of HSV-1, two strains of HSV-2 and two strains of VZV serially diluted in a negative matrix. Testing was performed concurrently using the same serial dilutions on each instrument. Analytical sensitivity (LoD) is defined as the lowest concentration at which 95% of all replicates tested positive.

Limit of Detection						
Instrument	Virus $TCID_{50}$/mL					
	HSV-1 Macintyre	HSV-1 QC 316	HSV-2 Strain G	HSV-2 QC Comp	VZV Ellen	VZV 130
Life Technology QuantStudio Dx	2.18E+03	5.69E+03	2.44E+03	2.53E+02	1.68E+00	2.33E+01
Applied Biosystems® 7500 Fast Dx	4.35E+03	1.14E+04	2.44E+03	5.05E+02	3.35E+00	2.33E+01
Cepheid SmartCycler II	5.44E+02	1.14E+04	2.44E+03	1.01E+03	8.38E-01	2.33E+01

The data from this LoD study support the claim that the performance of the assay is substantially equivalent on the three platforms (the LoD for each virus tested is within three doubling dilutions on the platforms).

f. Analytical Reactivity

A study was performed on the Applied Biosystems® 7500 Fast Dx to verify that the Lyra™ Direct HSV 1 + 2/VZV Assay detects multiple strains of HSV-1, HSV-2 and VZV at concentrations near the limit of detection.

Sixteen (16) viruses (four HSV-1, five HSV-2, and seven VZV) were tested and detected by the Lyra™ Direct HSV 1 + 2/VZV Assay.

HSV-1 Results								
Replicate #	HSV-1 Strain and Test Concentration ($TCID_{50}$/mL)							
	MacIntyre (Control)		QC 316		SFG-029		Isolate 7	
	8.70E+03		8.70E+03		4.84E+03		8.70E+03	
	HSV-1	PRC	HSV-1	PRC	HSV-1	PRC	HSV-1	PRC
1	30.0	30.3	32.4	29.0	38.4	29.4	32.3	29.6
2	30.0	29.8	33.8	30.0	38.7	30.3	32.0	29.8
3	30.0	30.2	33.8	30.0	38.6	30.2	31.7	29.7
Avg. Ct	30.0	30.1	33.3	29.7	38.6	29.9	32.0	29.7
SD	0.0	0.3	0.8	0.6	0.2	0.5	0.3	0.1
CV %	0.0%	0.9%	2.4%	1.9%	0.4%	1.7%	1.0%	0.4%

HSV-2 Results										
Replicate #	HSV-2 Strain and Test Concentration ($TCID_{50}$/mL)									
	Strain G (control)		Isolate 6		Isolate 9		Isolate 10		MS	
	4.88E+03		4.88E+03		4.88E+03		4.88E+03		4.88E+03	
	HSV-2	PRC	HSV-2	PRC	HSV-2	PRC	HSV-2	PRC	HSV-2	PRC
1	29.2	30.6	31.8	26.2	31.0	26.1	30.8	26.6	26.4	26.1
2	29.4	30.3	32.2	26.3	31.3	26.3	31.0	26.6	28.2	26.2

HSV-2 Results										
Replicate #	HSV-2 Strain and Test Concentration ($TCID_{50}$/mL)									
	Strain G (control)		Isolate 6		Isolate 9		Isolate 10		MS	
	4.88E+03		4.88E+03		4.88E+03		4.88E+03		4.88E+03	
	HSV-2	PRC	HSV-2	PRC	HSV-2	PRC	HSV-2	PRC	HSV-2	PRC
3	29.3	30.2	32.3	26.4	30.8	26.2	30.3	25.8	28.2	26.1
Avg. Ct	29.3	30.4	32.1	26.3	31.1	26.2	30.7	26.3	27.6	26.1
SD	0.1	0.2	0.3	0.1	0.2	0.1	0.3	0.4	1.0	0.1
CV %	0.4%	0.7%	0.8%	0.4%	0.7%	0.3%	1.1%	1.6%	3.6%	0.2%

VZV Results														
Replicate #	VZV Strain and Test Concentration ($TCID_{50}$/mL)													
	130 (Control)		AV-92-3		Ellen		Isolate B		Isolate D		Strain 82		Strain 275	
	4.67E+01		4.67E+01		4.67E+01		4.67E+01		4.67E+01		4.67E+01		4.67E+01	
	VZV	PRC	VZV	PRC	VZV	PRC	VZV	PRC	VZV	PRC	VZV	PRC	VZV	PRC
1	30.2	32.1	29.0	26.5	27.0	26.1	29.8	26.5	26.5	26.4	28.4	26.9	27.6	26.3
2	30.8	30.7	29.8	26.2	26.9	26.3	27.4	26.7	27.7	26.5	27.9	26.4	28.0	26.5
3	30.8	30.5	29.5	26.1	26.9	26.3	29.2	26.3	27.4	26.8	28.5	26.7	27.8	26.4
Avg. Ct	30.6	31.1	29.4	26.3	26.9	26.2	28.8	26.5	27.2	26.6	28.3	26.7	27.8	26.4
SD	0.3	0.9	0.4	0.2	0.1	0.1	1.2	0.2	0.6	0.2	0.3	0.3	0.2	0.1
CV %	1.1%	2.8%	1.4%	0.9%	0.2%	0.3%	4.3%	0.7%	2.2%	0.8%	1.2%	1.0%	0.8%	0.4%

g. Analytical specificity:

Cross-Reactivity and Inhibition by Other Microorganisms:
A study was performed on the Applied Biosystems® 7500 Fast Dx to evaluate the performance of the Lyra™ Direct HSV 1 +2 /VZV Assay in the presence of seventy (70) other microorganisms that might be found in lesion specimens. The study evaluated the potential cross reactivity with the assay (using negative samples) and the potential inhibition by other microorganisms as follows. Each potentially interfering or cross-reactive microorganism was tested in negative matrix (for cross-reactivity) or in the presence of 2x LoD HSV-1, HSV-2 and VZV viruses (for inhibition). Clinically relevant levels of viruses and bacteria are typically 10^6cfu/ml or higher for bacteria and 10^5pfu/ml or higher for viruses.

Analytical Specificity – Interfering or Cross-Reactive Microorganism					
Organism	Test Concentration	Lyra™ Direct HSV 1 + 2/VZV Assay Result			
		Negative Matrix	HSV-1	HSV-2	VZV
Adenovirus 7	9.38E+05 $TCID_{50}$/mL	Neg	Pos	Pos	Pos
Coronavirus OC43	1.79E+05 $TCID_{50}$/mL	Neg	Pos	Pos	Pos

Analytical Specificity – Interfering or Cross-Reactive Microorganism					
Organism	Test Concentration	Lyra™ Direct HSV 1 + 2/VZV Assay Result			
		Negative Matrix	HSV-1	HSV-2	VZV
Coxsackievirus B4	2.60E+06 $TCID_{50}$/mL	Neg	Pos	Pos	Pos
Cytomegalovirus Towne VR-977	1.55E+05 $TCID_{50}$/mL	Neg	Pos	Pos	Pos
Echovirus 11	1.61E+05 $TCID_{50}$/mL	Neg	Pos	Pos	Pos
HHV-6	1.46E+06 $TCID_{50}$/mL	Neg	Pos	Pos	Pos
HHV-7	8.63E+05 $TCID_{50}$/mL	Neg	Pos	Pos	Pos
HIV Virus	1.05E+05 $TCID_{50}$/mL	Neg	Pos	Pos	Pos
hMPV A1	1.24E+05 $TCID_{50}$/mL	Neg	Pos	Pos	Pos
HSV-1 SFG029	3.27E+05 $TCID_{50}$/mL	Neg	Pos	Pos	Pos
HSV-1 Macintyre	2.14E+05 $TCID_{50}$/mL	Neg	Pos	Pos	Pos
HSV-2 MS	6.44E+06 $TCID_{50}$/mL	Neg	Pos	Pos	Pos
HSV-2 Strain G	1.38E+05 $TCID_{50}$/mL	Neg	Pos	Pos	Pos
Influenza A/Mexico/4108/2009	2.17E+06 $TCID_{50}$/mL	Neg	Pos	Pos	Pos
Influenza B Hong Kong VR-791	7.15E+05 $TCID_{50}$/mL	Neg	Pos	Pos	Pos
Measles virus	1.46E+05 $TCID_{50}$/mL	Neg	Pos	Pos	Pos
Mumps virus	2.07E+07 $TCID_{50}$/mL	Neg	Pos	Pos	Pos
Parainfluenza Type 1	4.02E+05 $TCID_{50}$/mL	Neg	Pos	Pos	Pos
Parainfluenza Type 3	3.25E+05 $TCID_{50}$/mL	Neg	Pos	Pos	Pos
Parainfluenza Type 4	1.28E+05 $TCID_{50}$/mL	Neg	Pos	Pos	Pos
RSV A Long	7.13E+05 $TCID_{50}$/mL	Neg	Pos	Pos	Pos
RSV B Washington	1.01E+06 $TCID_{50}$/mL	Neg	Pos	Pos	Pos
Rubella Virus	9.45E+05 $TCID_{50}$/mL	Neg	Pos	Pos	Pos
Acholeplasma laidlawi	5.33E+06 cfu/mL	Neg	Pos	Pos	Pos
Bordetella bronchiseptica	8.78E+07 cfu/mL	Neg	Pos	Pos	Pos
Bordetella pertussis	1.41E+07 cfu/mL	Neg	Pos	Pos	Pos
Clostridium perfringens	1.18E+06 cfu/mL	Neg	Pos	Pos	Pos
Mycoplasma hominis	9.75E+06 cfu/mL	Neg	Pos	Pos	Pos
Mycoplasma hyorhinis	4.95E+06 cfu/mL	Neg	Pos	Pos	Pos
Mycoplasma orale	1.16E+07 cfu/mL	Neg	Pos	Pos	Pos
Mycoplasma pneumoniae	7.50E+06 ccu/mL	Neg	Pos	Pos	Pos
Mycoplasma salivarium	1.25E+07 cfu/mL	Neg	Pos	Pos	Pos
Enterovirus 70	3.83E+04 $TCID_{50}$/mL	Neg	Pos	Pos	Pos
Epstein Barr Virus	1.67E+04 $TCID_{50}$/mL	Neg	Pos	Pos	Pos
HBV	1.67E+03 $TCID_{50}$/mL	Neg	Pos	Pos	Pos
HCV	1.67E+04 $TCID_{50}$/mL	Neg	Pos	Pos	Pos
HHV-8	6.95E+04 $TCID_{50}$/mL	Neg	Pos	Pos	Pos
HPV	Not Available	Neg	Pos	Pos	Pos
Parainfluenza Type 2	5.23E+04 $TCID_{50}$/mL	Neg	Pos	Pos	Pos

Analytical Specificity – Interfering or Cross-Reactive Microorganism					
Organism	Test Concentration	Lyra™ Direct HSV 1 + 2/VZV Assay Result			
		Negative Matrix	HSV-1	HSV-2	VZV
VZV Ellen	8.75E+02 $TCID_{50}$/mL	Neg	Pos	Pos	Pos
VZV Webster	1.85E+03 $TCID_{50}$/mL	Neg	Pos	Pos	Pos
Chlamydia trachomatis	5.00E+05 cfu/mL	Neg	Pos	Pos	Pos
Chlamydophila pneumoniae	2.67E+03 cp/mL	Neg	Pos	Pos	Pos
Toxoplasma gondii	1.10E+06 tachyzoites/mL	Neg	Pos	Pos	Pos
Trichomonas vaginalis	2.75E+05 trophozoites/mL	Neg	Pos	Pos	Pos
Acinetobacter calcoaceticus	7.35E+08 cfu/mL	Neg	Pos	Pos	Pos
Bacteroides fragilis	5.67E+05 cfu/mL	Neg	Pos	Pos	Pos
Candida albicans	1.50E+06 cfu/mL	Neg	Pos	Pos	Pos
Candida glabrata	1.50E+06 cfu/mL	Neg	Pos	Pos	Pos
Corynebacterium diphtheriae	2.40E+08 cfu/mL	Neg	Pos	Pos	Pos
Enterococcus faecalis	1.65E+08 cfu/mL	Neg	Pos	Pos	Pos
Escherichia coli	4.35E+07 cfu/mL	Neg	Pos	Pos	Pos
Gardnerella vaginalis	9.00E+06 cfu/mL	Neg	Pos	Pos	Pos
Haemophilis influenzae type A	6.30E+08 cfu/mL	Neg	Pos	Pos	Pos
Klebsiella pneumoniae	3.90E+07 cfu/mL	Neg	Pos	Pos	Pos
Lactobacillus acidophilus	1.35E+06 cfu/mL	Neg	Pos	Pos	Pos
Legionella pneumophila	2.85E+07 cfu/mL	Neg	Pos	Pos	Pos
Mobiluncus mulieris	1.91E+08 cfu/mL	Neg	Pos	Pos	Pos
Moraxella cartarrhalis	2.25E+07 cfu/mL	Neg	Pos	Pos	Pos
Neisseria gonorrhoeae	2.40E+07 cfu/mL	Neg	Pos	Pos	Pos
Proteus mirabilis	9.60E+07 cfu/mL	Neg	Pos	Pos	Pos
Pseudomonas aeruginosa	5.25E+07 cfu/mL	Neg	Pos	Pos	Pos
Salmonella enteriditis	4.05E+07 cfu/mL	Neg	Pos	Pos	Pos
Salmonella typhimurium	3.45E+07 cfu/mL	Neg	Pos	Pos	Pos
Staphylococcus aureus	1.38E+09 cfu/mL	Neg	Pos	Pos	Pos
Staphylococcus saprophyticus	2.25E+08 cfu/mL	Neg	Pos	Pos	Pos
Streptococcus agalactiae	1.65E+08 cfu/mL	Neg	Pos	Pos	Pos
Streptococcus pneumoniae	2.10E+06 cfu/mL	Neg	Pos	Pos	Pos
Streptococcus pyogenes	2.85E+08 cfu/mL	Neg	Pos	Pos	Pos
Ureaplasma uralyticum	Unable to titer	Neg	Pos	Pos	Pos

Treponema pallidum could not be sourced for the Lyra™ Direct HSV 1 + 2/VZV Assay study and therefore an *in silico* analysis was required to confirm no cross-reactivity of this organism with the HSV-1, HSV-2, or VZV primers. The *in silico* analysis determined that the three primer pairs will not cross-react with *T. pallidum* under the Lyra™ Directassay conditions. Therefore, the presence of the

organism should not interfere with the assay.

None of the seventy (70) microorganisms listed above cross-reacted with the the assay or interfered with the ability of the assay to detect 2x LoD HSV1, HSV2 or VZV.

Interfering Substances:
A study was performed on the Applied Biosystems® 7500 Fast Dx to evaluate the performance of the Lyra™ Direct HSV 1 + 2/VZV Assay in the presence of twenty-six (26) clinically relevant levels of potentially interfering substances that might be present in lesion specimens.

Analytical Specificity – Interfering or Cross-Reactive Substances					
Substance Name	Substance Final Concentration	Lyra™ Direct HSV 1 + 2/VZV Assay Result			
		Negative Matrix	HSV-1	HSV-2	VZV
Seminal Fluid	7%	Negative	Positive	Positive	Positive
Cornstarch	2.5 mg/mL	Negative	Positive	Positive	Positive
Acetamidophenol	10 mg/mL	Negative	Positive	Positive	Positive
Feces	0.219%	Negative	Positive	Positive	Positive
Chlorpheniramine	5 mg/mL	Negative	Positive	Positive	Positive
Dextromethorphan	5 mg/mL	Negative	Positive	Positive	Positive
Blood/EDTA	0.625%	Negative	Positive	Positive	Positive
Female Urine	7%	Negative	Positive	Positive	Positive
Male Urine	7%	Negative	Positive	Positive	Positive
Acyclovir	7 mg/mL	Negative	Positive	Positive	Positive
Albumin	3.3 mg/mL	Negative	Positive	Positive	Positive
Casein	7 mg/mL	Negative	Positive	Positive	Positive
KY Jelly	7%	Negative	Positive	Positive	Positive
Douche	7%	Negative	Positive	Positive	Positive
Miconazole 1	7%	Negative	Positive	Positive	Positive
Miconazole 3	7%	Negative	Positive	Positive	Positive
Tioconazole 1	Approx. 7%	Negative	Positive	Positive	Positive
Preparation H	7%	Negative	Positive	Positive	Positive
Lanacane	3.5%	Negative	Positive	Positive	Positive
Listerine	7%	Negative	Positive	Positive	Positive
Abreva	7%	Negative	Positive	Positive	Positive
Carmex	7%	Negative	Positive	Positive	Positive
Releev	7%	Negative	Positive	Positive	Positive
Colgate	7%	Negative	Positive	Positive	Positive
Mucin	60 µg/mL	Negative	Positive	Positive	Positive
Leukocytes	2.5e5 cells/mL	Negative	Positive	Positive	Positive

None of the twenty-six (26) potentially interfering substances interfered with the

detection of 2x LoD HSV-1, HSV-2, or VZV, or were cross-reactive with the Lyra™ Direct HSV 1 + 2/VZV Assay.

Competitive Interference:

To evaluate whether competitive interference exists in the Lyra™ Direct HSV 1 + 2/VZV Assay when HSV-1, HSV-2 and VZV are present in the same reaction a study was performed on all three instruments. HSV-1, HSV-2 and VZV stocks at 2x LoD concentrations were tested in the presence of varying amounts of another analyte in order to determine if competitive interference exists.

No competitive interference was observed on any instrument with 2x LoD concentrations of HSV-1, HSV-2 and VZV when multiple two-analyte combinations of HSV-1, HSV-2 and VZV were tested in the same reaction with 2x, 10x, 100x, 1000x, and 10,000x LoD concentrations.

HSV-1: 2x Results Summary: Competitive Interference							
Sample Number	Combined Analytes		HSV1	HSV2	VZV	PRC	Interference
Control 2x HSV-1	N/A		29.8	NEG	NEG	25.8	N/A
1	2x HSV-1	2x HSV-2	29.2	30.8	NEG	23.5	NO
2	2x HSV-1	10x HSV-2	29.7	28.8	NEG	22.6	NO
3	2x HSV-1	100x HSV-2	29.3	26.2	NEG	22.8	NO
4	2x HSV-1	2x VZV	29.2	NEG	29.0	23.1	NO
5	2x HSV-1	10x VZV	29.9	NEG	26.7	24.2	NO
6	2x HSV-1	100x VZV	29.6	NEG	23.4	23.6	NO
16	2x HSV-1	1000x VZV	29.8	NEG	20.3	22.7	NO
17	2x HSV-1	10,000x VZV	30.6	NEG	18.3	21.6	NO

HSV-2: 2x Results Summary: Competitive Interference							
Sample Number	Combined Analytes		HSV1	HSV2	VZV	PRC	Interference
Control 2x HSV-2	N/A		NEG	32.1	NEG	25.6	N/A
1	2x HSV-2	2x HSV-1	29.2	30.8	NEG	23.5	NO
7	2x HSV-2	10x HSV-1	27.6	32.1	NEG	23.8	NO
8	2x HSV-2	100x HSV-1	23.4	32.4	NEG	23.4	NO
9	2x HSV-2	2x VZV	NEG	31.6	29.5	24.3	NO
10	2x HSV-2	10x VZV	NEG	33.0	26.9	23.7	NO
11	2x HSV-2	100x VZV	NEG	33.6	23.7	25.1	NO
18	2x HSV-2	1000x VZV	NEG	29.8	20.5	22.3	NO
19	2x HSV-2	10,000x VZV	NEG	29.1	18.4	22.7	NO

VZV: 2x Results Summary: Competitive Interference						
Sample Number	Combined Analytes	HSV1	HSV2	VZV	PRC	Interference

Control 2x VZV	N/A		NEG	NEG	29.0	26.0	N/A
4	2x VZV	2x HSV-1	29.2	NEG	29.0	23.1	NO
9	2x VZV	2x HSV-2	NEG	31.6	29.5	24.3	NO
12	2x VZV	10x HSV-1	27.5	NEG	29.2	24.2	NO
13	2x VZV	100x HSV-1	24.1	NEG	29.4	22.3	NO
14	2x VZV	10x HSV-2	NEG	29.4	29.1	24.6	NO
15	2x VZV	100x HSV-2	NEG	25.9	29.0	22.9	NO

HSV-1: 2x Results Summary: Competitive Interference (Avg C_t)							
Sample Number	Combined Analytes		HSV1	HSV2	VZV	PRC	Interference
Control 2x HSV-1	N/A		31.0	NEG	NEG	26.2	N/A
1	2x HSV-1	2x HSV-2	29.9	31.4	NEG	28.8	NO
2	2x HSV-1	10x HSV-2	30.8	31.1	NEG	27.0	NO
3	2x HSV-1	100x HSV-2	30.7	28.1	NEG	30.0	NO
4	2x HSV-1	2x VZV	30.3	NEG	30.2	29.0	NO
5	2x HSV-1	10x VZV	29.6	NEG	28.1	26.9	NO
6	2x HSV-1	100x VZV	31.2	NEG	25.2	28.2	NO
16	2x HSV-1	1000x VZV	29.8	NEG	21.7	30.1	NO
17	2x HSV-1	10,000x VZV	31.0	NEG	19.4	30.4	NO

HSV-2: 2x Results Summary: Competitive Interference (Avg C_t)							
Sample Number	Combined Analytes		HSV1	HSV2	VZV	PRC	Interference
Control 2x HSV-2	N/A		NEG	32.4	NEG	25.6	N/A
1	2x HSV-2	2x HSV-1	29.9	31.4	NEG	28.8	NO
7	2x HSV-2	10x HSV-1	27.4	30.4	NEG	28.9	NO
8	2x HSV-2	100x HSV-1	24.0	33.5	NEG	27.4	NO
9	2x HSV-2	2x VZV	NEG	31.1	30.4	28.9	NO
10	2x HSV-2	10x VZV	NEG	31.5	28.0	29.3	NO
11	2x HSV-2	100x VZV	NEG	33.0	25.0	27.2	NO
18	2x HSV-2	1000x VZV	NEG	29.3	21.6	30.2	NO
19	2x HSV-2	10,000x VZV	NEG	28.8	19.3	30.3	NO

VZV: 2x Results Summary: Competitive Interference (Avg C_t)							
Sample Number	Combined Analytes		HSV1	HSV2	VZV	PRC	Interference
Control 2x VZV	N/A		NEG	NEG	31.1	26.3	N/A
4	2x VZV	2x HSV-1	30.3	NEG	30.2	29.0	NO
9	2x VZV	2x HSV-2	NEG	31.1	30.4	28.9	NO
12	2x VZV	10x HSV-1	26.3	NEG	31.6	28.7	NO
13	2x VZV	100x HSV-1	24.4	NEG	30.4	28.1	NO

14	2x VZV	10x HSV-2	NEG	28.7	30.2	27.1	NO
15	2x VZV	100x HSV-2	NEG	26.5	30.5	27.1	NO

HSV-1: 2x Results Summary: Competitive Interference							
Sample Number	Combined Analytes		HSV1	HSV2	VZV	PRC	Interference
Control 2x HSV-1	N/A		33.1	NEG	NEG	28.8	N/A
1	2x HSV-1	2x HSV-2	32.4	36.6	NEG	28.4	NO
2	2x HSV-1	10x HSV-2	33.0	32.8	NEG	28.8	NO
3	2x HSV-1	100x HSV-2	32.9	29.2	NEG	28.5	NO
4	2x HSV-1	2x VZV	33.1	NEG	31.7	28.8	NO
5	2x HSV-1	10x VZV	33.6	NEG	28.7	28.7	NO
6	2x HSV-1	100x VZV	32.6	NEG	25.4	28.4	NO
16	2x HSV-1	1000x VZV	32.6	NEG	23.0	31.5	NO
17	2x HSV-1	10,000x VZV	34.4	NEG	20.4	31.9	NO

HSV-2: 2x Results Summary: Competitive Interference							
Sample Number	Combined Analytes		HSV1	HSV2	VZV	PRC	Interference
Control 2x HSV-2	N/A		NEG	36.2	NEG	28.7	N/A
1	2x HSV-1	2x HSV-2	32.4	36.6	NEG	28.4	NO
7	2x HSV-2	10x HSV-1	28.8	35.3	NEG	28.5	NO
8	2x HSV-2	100x HSV-1	27.3	35.8	NEG	28.4	NO
9	2x HSV-2	2x VZV	NEG	34.9	31.0	28.9	NO
10	2x HSV-2	10x VZV	NEG	35.4	28.7	28.2	NO
11	2x HSV-2	100x VZV	NEG	35.6	25.9	28.4	NO
18	2x HSV-2	1000x VZV	NEG	32.8	23.0	31.2	NO
19	2x HSV-2	10,000x VZV	NEG	32.8	20.4	31.8	NO
20	2x HSV-2	1000x HSV-1	23.3	33.1	NEG	30.3	NO

VZV: 2x Results Summary: Competitive Interference							
Sample Number	Combined Analytes		HSV1	HSV2	VZV	PRC	Interference
Control 2x VZV	N/A		NEG	NEG	31.9	28.7	N/A
4	2x VZV	2x HSV-1	33.1	NEG	31.7	28.8	NO
9	2x VZV	2x HSV-2	NEG	34.9	31.0	28.9	NO
12	2x VZV	10x HSV-1	30.0	NEG	31.8	28.4	NO
13	2x VZV	100x HSV-1	27.2	NEG	31.5	28.0	NO
14	2x VZV	10x HSV-2	NEG	32.7	31.5	28.5	NO
15	2x VZV	100x HSV-2	NEG	28.7	32.4	28.4	NO
21	2x VZV	1000x HSV-1	23.3	NEG	31.3	29.7	NO

i. Sample stability studies

This study was performed to assess whether fresh and frozen samples provided comparable results when tested in the Lyra™ Direct HSV 1 + 2/VZV Assay. The study was performed on the Applied Biosystems® 7500 Fast Dx using a panel of contrived specimens at near LoD levels (2x and 5x) for each virus. Five (5) panels were created that contained randomized 2x LoD, 5x LoD, and negative samples. Each panel was prepared with 30 aliquots of 5x LoD and 30 aliquots of 2x LoD for each virus and 10 aliquots of negative matrix. Panel 1 was tested on the day of preparation. Panels 2 and 3 were stored at -20°C and then tested on day 7 and day 8, respectively. Panels 4 and 5 were stored at 2° to 8°C and then tested on day 7 and day 8, respectively. Each panel member was tested in singlet. Positive and Negative Controls were analyzed on each instrument run. The Positive Controls were 5x LoD HSV-1, HSV-2 and VZV. The Negative Control was negative matrix. For each panel, one set of Positive Controls and one Negative Control was prepared.

Summary of Results from the Applied Biosystems® 7500 Fast Dx								
Panel		2x HSV-1	5x HSV-1	2x HSV-2	5x HSV-2	2x VZV	5x VZV	Negative
-20°C DAY 8	Mean C_t of positives	29.6	27.9	30.9	29.5	30.8	28.9	26.7
	C_t Std. Dev	0.9	0.8	1.2	1.2	0.7	0.4	0.5
	CV %	3.1	2.8	4.0	4.2	2.3	1.5	1.7
2-8°C DAY 7	Number detected #/30	30/30	30/30	30/30	30/30	30/30	30/30	10/10
	% Detection	100%	100%	100%	100%	100%	100%	100%
	Mean C_t of positives	30.0	28.3	30.4	28.9	30.9	29.0	27.4
	C_t Std. Dev	0.8	0.6	1.6	1.0	1.0	0.2	0.7
	CV %	2.7	2.1	5.3	3.5	3.3	0.8	2.5
2-8°C DAY 8	Number detected #/30	30/30	30/30	30/30	30/30	30/30	30/30	10/10
	% Detection	100%	100%	100%	100%	100%	100%	100%
	Mean C_t of positives	29.6	28.3	30.4	28.6	31.3	29.1	25.9
	C_t Std. Dev	0.8	0.6	1.5	1.5	2.0	0.4	0.9
	CV %	2.6	2.0	4.9	5.4	6.5	1.3	3.3

There was ≥95% agreement between the results obtained with the contrived specimens on the day of preparation and after storage at 2° to 8°C for 7 and 8 days. There was ≥95% agreement between the results obtained with the contrived specimens on the day of preparation and after storage at -20°C for 7 and 8 days. Based on this study, specimens may be stored at either 2° to 8°C or -20°C for up to 7days with no effect on the performance of the Lyra™ Direct HSV 1 + 2/VZV Assay.

j. Comparison of Transport Media

The following study was performed to determine whether various transport media impact the performance in the Lyra™ Direct HSV 1 + 2/VZV Assay on the Applied Biosystems 7500® Fast Dx instrument. HSV-1, HSV-2, and VZV virus stocks were diluted to 2x LoD concentrations using negative matrix collected in five different media: UTM, M4, M4-RT, M5, and M6. The acceptance criteria for this study were met for all media. The Lyra™ Direct HSV 1 + 2/VZV Assay can be used with samples collected in UTM, M4, M4RT, M5, or M6 transport media.

k. Carry-over Contamination

An evaluation of potential carry–over contamination during the sample processing and amplification steps was performed on each instrument. The study was conducted using a 96-sample panel consisting of 48 high positives and 48 negative specimens. Each high positive specimen contained 1.00E+05 $TCID_{50}$/mL of each analyte (combined into one specimen). The negative specimen was negative matrix. The high positive samples were analyzed in series alternating with the negative samples. The testing was repeated over a 5-day period. Over the course of 5days, cross-contamination and amplicon carry-over did not occur with the Lyra™ Direct HSV 1+2/VZV Assay on any of the three instruments.

2. Comparison studies:

a. Method comparison with predicate device:

Not applicable. Refer to the Clinical Studies section of this document.

b. Matrix comparison:

Not applicable.

3. Clinical studies:

A multi-center study was performed between April, 2013 and October, 2013 to evaluate the Lyra™ Direct HSV 1 + 2/VZV Assay using lesion swab specimens obtained from cutaneous or mucocutaneous lesions and submitted for HSV and/or VZV culture. These specimens were processed with the Lyra™ Direct HSV 1 + 2/VZV Assay kit and tested on the Life Technologies QuantStudio™ Dx, the Applied Biosystems® 7500 Fast Dx, and the Cepheid SmartCycler® II at three locations. Each specimen was also processed and inoculated into two (2) different cell culture systems within 72 hours of collection: one system for the isolation and identification of HSV-1 and HSV-2 and the other system for the isolation and identification of VZV. Cells isolated from the specimens were also stained for the presence of VZV.

The specimens were categorized as cutaneous (skin lesion, penis), or mucocutaneous

(anorectal, vaginal/cervical, and oral lesion). The gender and age demographics for each category are listed below.

Combined Study – Age and Gender Distribution (Cutaneous)		
Gender	Female	Male
Age		
≤ 5 years	6	16
6 to 21 years	18	12
22 to 59 years	72	98
≥ 60 years	37	20
Total	133	146
Combined Study – Age and Gender Distribution (Mucocutaneous)		
Gender	Female	Male
Age		
≤ 5 years	10	8
6 to 21 years	106	24
22 to 59 years	403	49
≥ 60 years	47	6
Total	566	84

Performance on the Life Technologies QuantStudio™ Dx:

Cutaneous Lesions

Two-hundred and seventy-nine (279) active cutaneous lesion specimens were cultured for HSV-1 using the FDA-cleared ELVIS cell culture system and were also tested with the subject device for HSV-1 viral DNA. One (1) specimen was invalid in the Lyra™ Direct HSV 1 + 2/VZV Assay. This specimen has been excluded from further analysis. The table below details the HSV-1 results for the remaining two-hundred and seventy-eight (278) specimens.

HSV-1			
	Comparator: ELVIS® HSV ID and D³ Typing Test		
Lyra™ Direct HSV 1 + 2/VZV Assay	Positive	Negative	Total
Positive	24	4*	28
Negative	0	250	250
Total	24	254	278
			95% CI
Sensitivity	24/24	100%	86.2% to 100%
Specificity	250/254	98.4%	96.0% to 99.4%

* One (1) of the four (4) positives was positive by an additional RT-PCR assay.

Two-hundred and seventy-nine (279) active cutaneous lesion specimens were cultured for HSV-2 using the FDA-cleared ELVIS cell culture system and were also tested with the subject device for HSV-2 viral DNA. One (1) specimen was invalid in the Lyra™ Direct

HSV 1 + 2/VZV Assay. This specimen has been excluded from further analysis. The table below details the HSV-2 results for the remaining two-hundred and seventy-eight (278) specimens.

HSV-2			
	Comparator: ELVIS® HSV ID and D³ Typing Test		
Lyra™ Direct HSV 1 + 2/VZV Assay	Positive	Negative	Total
Positive	35	9*	44
Negative	0	234	250
Total	35	243	278
			95% CI
Sensitivity	35/35	100%	90.1% to 100%
Specificity	234/243	96.3%	93.1% to 98.0%

* Nine (9) of the nine (9) positives were positive by an additional RT-PCR assay.

Two-hundred and seventy-nine (279) active cutaneous lesion specimens were cultured for VZV and were also tested with the subject device for VZV viral DNA. The detection and isolation of VZV was performed by staining cells present in the samples with a FDA-cleared VZV detection reagent (DSFA) and by culturing the specimen for 96-hours using a mixed cell culture (H&V mixed cells) consisting of MRC-5 cells (human diploid fibroblast) and CV-1 cells (african green monkey kidney), and staining the cultures with the same FDA-cleared reagent used for DSFA. Due the presence of either HSV-1 or HSV-2, fifty-six (56) specimens have been excluded from analysis. One (1) specimen was invalid in the Lyra™ Direct HSV 1 + 2/VZV Assay. Thus, a total of fifty-seven (57) specimens have been excluded from analysis. The table below details the VZV results for the remaining two-hundred and twenty-two (222) specimens.

VZV			
	Comparator: DSFA and Culture with DFA		
Lyra™ Direct HSV 1 + 2/VZV Assay	Positive	Negative	Total
Positive	27	8*	35
Negative	0	187	187
Total	27	195	222
			95% CI
Sensitivity	27/27	100%	87.5% to 100%
Specificity	187/195	95.9%	92.1% to 97.9%

* Seven (7) of the eight (8) positives were positive by an additional RT-PCR assay.

Mucocutaneous Lesions

Six-hundred and fifty (650) active mucocutaneous lesion specimens were cultured for HSV-1 using the FDA-cleared ELVIS cell culture system and were also tested with the subject device for HSV-1 viral DNA. Three (3) specimens were contaminated in the ELVIS cell culture and one (1) specimen was invalid in the Lyra™ Direct HSV 1 + 2/VZV Assay. These four (4) specimens have been excluded from further analysis. The

table below summarizes the HSV-1 results for the remaining six-hundred forty-six (646) specimens.

HSV-1			
	Comparator: ELVIS® HSV ID and D³ Typing Test		
Lyra™ Direct HSV 1 + 2/VZV Assay	Positive	Negative	Total
Positive	100	16*	116
Negative	3**	527	530
Total	103	543	646
			95% CI
Sensitivity	100/103	97.1%	91.8% to 99.0%
Specificity	527/543	97.1%	95.3% to 98.2%

* Thirteen (13) of the sixteen (16) positives were positive by an additional RT-PCR assay.
** Three (3) of the three (3) positives were positive by an additional RT-PCR assay.

Six-hundred and fifty (650) active mucocutaneous lesion specimens were cultured for HSV-2 using the FDA-cleared ELVIS cell culture system and were also tested with the subject device for HSV-2 viral DNA. Three (3) specimens were contaminated in the ELVIS cell culture, and one (1) specimen was invalid in the Lyra™ Direct HSV 1 + 2/VZV Assay. These four (4) specimens have been excluded from further analysis. The table below details the HSV-2 results for the remaining six-hundred forty-six (646) specimens.

HSV-2			
	Comparator: ELVIS® HSV ID and D³ Typing Test		
Lyra™ Direct HSV 1 + 2/VZV Assay	Positive	Negative	Total
Positive	95	21*	116
Negative	0	530	530
Total	95	551	646
			95% CI
Sensitivity	95/95	100%	96.1% to 100%
Specificity	530/551	96.2%	94.2% to 97.5%

* Eighteen (18) of the twenty-one (21) positives were positive by an additional RT-PCR assay.

Six-hundred and fifty (650) active mucocutaneous lesion specimens were cultured for VZV and were also tested with the subject device for VZV viral DNA. The detection and isolation of VZV was performed by staining cells present in the samples with a FDA-cleared VZV detection reagent (DSFA) and by culturing the specimen for 96-hours using a mixed cell culture (H&V mixed cells) consisting of MRC-5 cells (human diploid fibroblast) and CV-1 cells (african green monkey kidney), and staining the cultures with the same FDA-cleared reagent used for DSFA. Due the presence of either HSV-1 or HSV-2, two hundred seventeen (217) specimens have been excluded from analysis. One

(1) specimen was invalid in the Lyra™ Direct HSV 1 + 2/VZV Assay. These two hundred eighteen (218) specimens have been excluded from analysis. The table below details the VZV results for the remaining four-hundred and thirty-two (432) specimens.

VZV			
	Comparator: DSFA and Culture with DFA		
Lyra™ Direct HSV 1 + 2/VZV Assay	Positive	Negative	Total
Positive	4	5*	9
Negative	0	423	423
Total	4	428	432
			95% CI
Sensitivity	4/4	100%	51.0% to 100%
Specificity	423/428	98.8%	97.3% to 99.5%

* Five (5) of the five (5) positives were positive by an additional RT-PCR assay.

Performance on the Applied Biosystems® 7500 Fast Dx

Cutaneous Lesions

Two-hundred and seventy-nine (279) active cutaneous lesion specimens were cultured for HSV-1 using the FDA-cleared ELVIS cell culture system and were also tested with the subject device for HSV-1 viral DNA. The table below details the HSV-1 results for the two-hundred and seventy-nine (279) specimens.

HSV-1			
	Comparator: ELVIS® HSV ID and D³ Typing Test		
Lyra™ Direct HSV 1 + 2/VZV Assay	Positive	Negative	Total
Positive	24	3*	27
Negative	0	252	252
Total	24	255	279
			95% CI
Sensitivity	24/24	100%	86.2% to 100%
Specificity	252/254	98.8%	96.6% to 99.6%

* One (1) of the three (3) positives was positive by an additional RT-PCR assay.

Two-hundred and seventy-nine (279) active cutaneous lesion specimens were cultured for HSV-2 using the FDA-cleared ELVIS cell culture system and were also tested with the subject device for HSV-2 viral DNA. The table below details the HSV-2 results for the two-hundred and seventy-nine (279) specimens.

HSV-2			
	Comparator: ELVIS® HSV ID and D³ Typing Test		
Lyra™ Direct HSV 1 + 2/VZV Assay	Positive	Negative	Total
Positive	34	8*	42
Negative	1**	236	251
Total	35	244	279
			95% CI
Sensitivity	34/35	97.1%	85.5% to 99.5%
Specificity	236/244	96.7%	93.7% to 98.3%

* Seven (7) of the eight (8) positives were positive by an additional RT-PCR assay.
** One (1) of the one (1) negative was positive by an additional RT-PCR assay.

Two-hundred and seventy-nine (279) active cutaneous lesion specimens were cultured for VZV and were also tested with the subject device for VZV viral DNA. The detection and isolation of VZV was performed by staining cells present in the samples with a FDA-cleared VZV detection reagent (DSFA) and by culturing the specimen for 96-hours using a mixed cell culture (H&V mixed cells) consisting of MRC-5 cells (human diploid fibroblast) and CV-1 cells (african green monkey kidney), and staining the cultures with the same FDA-cleared reagent used for DSFA. Due the presence of either HSV-1 or HSV-2, fifty-six (56) specimens have been excluded from analysis. The table below details the VZV results for the two-hundred and twenty-three (223) specimens.

VZV			
	Comparator: DSFA and Culture with DFA		
Lyra™ Direct HSV 1 + 2/VZV Assay	Positive	Negative	Total
Positive	26	7*	33
Negative	1**	189	190
Total	27	196	223
			95% CI
Sensitivity	26/27	100%	87.5% to 100%
Specificity	189/196	95.9%	92.1% to 97.9%

* Seven (7) of the seven (7) positives were positive by an additional RT-PCR assay.
** One (1) of the one (1) negative was positive by an additional RT-PCR assay.

Mucocutaneous Lesions

Six-hundred and fifty (650) active mucocutaneous lesion specimens were cultured for HSV-1 using the FDA-cleared ELVIS cell culture system and were also tested with the subject device for HSV-1 viral DNA. Three (3) specimens were contaminated in the ELVIS cell culture, and three (3) specimens were invalid in the Lyra™ Direct HSV 1 + 2/VZV Assay. These six (6) specimens have been excluded from further analysis. The table below details the HSV-1 results for the remaining six-hundred forty-four (644) specimens.

HSV-1			
	Comparator: ELVIS® HSV ID and D³ Typing Test		
Lyra™ Direct HSV 1 + 2/VZV Assay	Positive	Negative	Total
Positive	98	10*	108
Negative	5**	531	536
Total	103	541	644
			95% CI
Sensitivity	98/103	95.1%	89.1% to 97.9%
Specificity	531/541	98.2%	96.6% to 99.0%

* Ten (10) of the ten (10) positives were positive by an additional RT-PCR assay.
** Five (5) of the five (5) negatives were positive by an additional RT-PCR assay.

Six-hundred and fifty (650) active mucocutaneous lesion specimens were cultured for HSV-2 using the FDA-cleared ELVIS cell culture system and were also tested with the subject device for HSV-2 viral DNA. Three (3) specimens were contaminated in the ELVIS cell culture, and three (3) specimens were invalid in the Lyra™ Direct HSV 1 + 2/VZV Assay. These six (6) specimens have been excluded from further analysis. The table below details the HSV-2 results for the remaining six-hundred forty-four (644) specimens.

HSV-2			
	Comparator: ELVIS® HSV ID and D³ Typing Test		
Lyra™ Direct HSV 1 + 2/VZV Assay	Positive	Negative	Total
Positive	93	16*	109
Negative	2**	533	535
Total	95	549	644
			95% CI
Sensitivity	93/95	97.9%	92.6% to 99.4%
Specificity	533/549	97.1%	95.3% to 98.2%

* Sixteen (16) of the sixteen (16) positives were positive by an additional RT-PCR assay.
** Two (2) of the two (2) negatives were positive by an additional RT-PCR assay.

Six-hundred and fifty (650) active mucocutaneous lesion specimens were cultured for VZV and were also tested with the subject device for VZV viral DNA. The detection and isolation of VZV was performed by staining cells present in the samples with a FDA-cleared VZV detection reagent (DSFA) and by culturing the specimen for 96-hours using a mixed cell culture (H&V mixed cells) consisting of MRC-5 cells (human diploid fibroblast) and CV-1 cells (african green monkey kidney), and staining the cultures with the same FDA-cleared reagent used for DSFA. Due the presence of either HSV-1 or HSV-2, two hundred seventeen (217) specimens have been excluded from analysis. Three (3) specimens were invalid in the Lyra™ Direct HSV 1 + 2/VZV Assay. These two hundred twenty (220) specimens have been excluded from analysis. The table below details the VZV results for the remaining four-hundred and thirty (430) specimens.

VZV			
	Comparator: DSFA and Culture with DFA		
Lyra™ Direct HSV 1 + 2/VZV Assay	Positive	Negative	Total
Positive	4	3*	7
Negative	0	423	423
Total	4	426	430
			95% CI
Sensitivity	4/4	100%	51.0% to 100%
Specificity	423/426	99.3%	98.0% to 99.8%

* Three (3) of the three (3) positives were positive by an additional RT-PCR assay.

Performance on the Cepheid SmartCycler® II

Cutaneous Lesions

Two-hundred and seventy-nine (279) active cutaneous lesion specimens were cultured for HSV-1 using the FDA-cleared ELVIS cell culture system and were also tested with the subject device for HSV-1 viral DNA. One (1) specimen was invalid in the Lyra™ Direct HSV 1 + 2/VZV Assay. This specimen has been excluded from further analysis. The table below details the HSV-1 results for the two-hundred and seventy-eight (278) specimens.

HSV-1			
	Comparator: ELVIS® HSV ID and D³ Typing Test		
Lyra™ Direct HSV 1 + 2/VZV Assay	Positive	Negative	Total
Positive	24	3*	27
Negative	0	251	251
Total	24	254	278
			95% CI
Sensitivity	24/24	100%	86.2% to 100%
Specificity	251/254	98.8%	96.6% to 99.6%

* One (1) of the three (3) positives was positive by an additional RT-PCR assay.

Two-hundred and seventy-nine (279) active cutaneous lesion specimens were cultured for HSV-2 using the FDA-cleared ELVIS cell culture system and were also tested with the subject device for HSV-2 viral DNA. One (1) specimen was invalid in the Lyra™ Direct HSV 1 + 2/VZV Assay. This specimen has been excluded from further analysis. The table below details the HSV-1 results for the two-hundred and seventy-eight (278) specimens.

HSV-2			
	Comparator: ELVIS® HSV ID and D³ Typing Test		
Lyra™ Direct HSV 1 + 2/VZV Assay	Positive	Negative	Total
Positive	35	8*	43
Negative	0	235	235
Total	35	243	278
			95% CI
Sensitivity	35/35	100%	90.1% to 100%
Specificity	235/243	96.7%	93.6% to 98.3%

* Seven (7) of the eight (8) positives were positive by an additional RT-PCR assay.

Two-hundred and seventy-nine (279) active cutaneous lesion specimens were cultured for VZV and were also tested with the subject device for VZV viral DNA. The detection and isolation of VZV was performed by staining cells present in the samples with a FDA-cleared VZV detection reagent (DSFA) and by culturing the specimen for 96-hours using a mixed cell culture (H&V mixed cells) consisting of MRC-5 cells (human diploid fibroblast) and CV-1 cells (african green monkey kidney), and staining the cultures with the same FDA-cleared reagent used for DSFA. Due the presence of either HSV-1 or HSV-2, fifty-six (56) specimens have been excluded from analysis. One (1) specimen was invalid in the Lyra™ Direct HSV 1 + 2/VZV Assay. The table below details the VZV results for the two-hundred and twenty-two (222) specimens.

VZV			
	Comparator: DSFA and Culture with DFA		
Lyra™ Direct HSV 1 + 2/VZV Assay	Positive	Negative	Total
Positive	27	10*	37
Negative	0	185	185
Total	27	195	222
			95% CI
Sensitivity	27/27	100%	87.5% to 100%
Specificity	185/195	94.9%	90.8% to 97.2%

* Six (6) of the ten (10) positives were positive by an additional RT-PCR assay.

Mucocutaneous Lesions

Six-hundred and fifty (650) active mucocutaneous lesion specimens were cultured for HSV-1 using the FDA-cleared ELVIS cell culture system and were also tested with the subject device for HSV-1 viral DNA. Three (3) specimens were contaminated in the ELVIS cell culture, and four (4) specimens were invalid in the Lyra™ Direct HSV 1 + 2/VZV Assay. These seven (7) specimens have been excluded from further analysis. The table below details the HSV-1 results for the remaining six-hundred forty-three (643) specimens.

HSV-1			
	Comparator: ELVIS® HSV ID and D³ Typing Test		
Lyra™ Direct HSV 1 + 2/VZV Assay	Positive	Negative	Total
Positive	101	15*	116
Negative	2**	525	527
Total	103	540	643
			95% CI
Sensitivity	101/103	98.1%	93.2% to 99.5%
Specificity	525/540	97.2%	95.5% to 98.3%

* Fourteen (14) of the fifteen (15) positives were positive by an additional RT-PCR assay.
** Two (2) of the two (2) negatives were positive by an additional RT-PCR assay.

Six-hundred and fifty (650) active mucocutaneous lesion specimens were cultured for HSV-2 using the FDA-cleared ELVIS cell culture system and were also tested with the subject device for HSV-2 viral DNA. Three (3) specimens were contaminated in the ELVIS cell culture, and four (4) specimens were invalid in the Lyra™ Direct HSV 1 + 2/VZV Assay. These seven (7) specimens have been excluded from further analysis. The table below details the HSV-2 results for the remaining six-hundred forty-three (643) specimens.

HSV-2			
	Comparator: ELVIS® HSV ID and D³ Typing Test		
Lyra™ Direct HSV 1 + 2/VZV Assay	Positive	Negative	Total
Positive	94	16*	110
Negative	1**	532	533
Total	95	548	643
			95% CI
Sensitivity	94/95	98.9%	94.3% to 99.8%
Specificity	532/548	97.1%	95.3% to 98.2%

* Sixteen (16) of the sixteen (16) positives were positive by an additional RT-PCR assay.
** The one (1) negative was positive by an additional RT-PCR assay.

Six-hundred and fifty (650) active mucocutaneous lesion specimens were cultured for VZV and were also tested with the subject device for VZV viral DNA. The detection and isolation of VZV was performed by staining cells present in the samples with a FDA-cleared VZV detection reagent (DSFA) and by culturing the specimen for 96-hours using a mixed cell culture (H&V mixed cells) consisting of MRC-5 cells (human diploid fibroblast) and CV-1 cells (african green monkey kidney), and staining the cultures with the same FDA-cleared reagent used for DSFA. Due the presence of either HSV-1 or HSV-2, two hundred seventeen (217) specimens have been excluded from analysis. Four (4) specimens were invalid in the Lyra™ Direct HSV 1 + 2/VZV Assay. These two hundred twenty-one (221) specimens have been excluded from analysis. The table below

details the VZV results for the remaining four-hundred and twenty-nine (429) specimens.

VZV			
	Comparator: DSFA and Culture with DFA		
Lyra™ Direct HSV 1 + 2/VZV Assay	Positive	Negative	Total
Positive	4	5*	9
Negative	0	420	423
Total	4	425	429
			95% CI
Sensitivity	4/4	100%	51.0% to 100%
Specificity	420/425	98.8%	97.3% to 99.5%

* Five (5) of the five (5) positives were positive by an additional RT-PCR assay.

4. Expected Values

The observed expected values in the clinical study are presented below for the Life Technologies QuantStudio™ Dx, the Applied Biosystems® 7500 Fast Dx, and the Cepheid SmartCycler® II System. The tables below provide the expected value for each virus detected on the three instruments based on patient age and the specific lesion categories. The study design did not differentiate samples in which an HSV-1, HSV-2, VZV test was requested or if all of the tests were requested for each of the samples. The samples included in the study consisted of any sample which was sent for HSV or VZV testing which may have included samples in which both the HSV and VZV testing was ordered. The expected values shown below, therefore, reflect the expected values for this study design only (samples submitted for HSV-1, or HSV-2, or VZV testing) and are not reflective of the prevalence values of VZV DNA in patients suspected of VZV cutaneous and mucocutaneous infections, or the prevalence of HSV-1 and HSV-2 DNA in patients suspected of HSV cutaneous and mucocutaneous infections.

Life Technologies QuantStudio™ Dx

Expected Values (Cutaneous) (N=279)*									
Age	**HSV- 1**			**HSV-2**			**VZV**		
	Total #	Total Positive	Prevalence	Total #	Total Positive	Prevalence	Total #	Total Positive	Prevalence
< 5 years	22	1	4.5%	22	1	4.5%	22	2	9.1%
6 to 21 years	30	8	26.7%	30	1	3.3%	30	1	3.3%
22 to 59 years	170	14	8.2%	170	33	19.4%	170	21	12.4%
> 60 years*	56	5	8.9%	56	9	16.1%	56	12	21.4%

*1 Specimen was invalid when tested on the Life Technologies QuantStudio™ Dx. It has been removed from analysis.

Expected Values (Cutaneous) (N=279)*									
	HSV- 1			**HSV-2**			**VZV**		
	Total #	Total Positive	Prevalence	Total #	Total Positive	Prevalence	Total #	Total Positive	Prevalence
skin lesion	213*	24	11.3%	213*	27	12.7%	213*	35	16.4%

genital - penis	65	4	6.2%	65	17	26.2%	65	1	1.5%

*1 Specimen was invalid when tested on the Life Technologies QuantStudio™ Dx. It has been removed from analysis.

Expected Values (Mucocutaneous) (N=650)*									
Age	**HSV- 1**			**HSV-2**			**VZV**		
	Total #	Total Positive	Prevalence	Total #	Total Positive	Prevalence	Total #	Total Positive	Prevalence
≤ 5 years	18	5	27.8%	18	0	N/A	18	0	N/A
6 to 21 years	130	26	20.0%	130	25	19.2%	130	1	0.8%
22 to 59 years*	448	81	18.1%	448	81	18.1%	448	6	1.3%
≥ 60 years	53	4	7.5%	53	10	18.9%	53	2	3.8%

*1 Specimen was invalid when tested on the Life Technologies QuantStudio™ Dx. It has been removed from analysis.

Expected Values (Mucocutaneous) (N=650)*									
	HSV- 1			HSV-2			VZV		
	Total #	Total Positive	Prevalence	Total #	Total Positive	Prevalence	Total #	Total Positive	Prevalence
anorectal	26	4	15.4%	26	6	23.1%	26	1	3.8%
genital – vaginal/cervical	473*	75	15.9%	473*	107	22.6%	473*	4	0.8%
Nares	9	2	22.2%	9	0	N/A	9	0	N/A
Ocular	6	0	N/A	6	0	N/A	6	1	16.6%
oral lesion	135	35	25.9%	135	3	2.2%	135	3	2.2%

*1 Specimen was invalid when tested on the Life Technologies QuantStudio™ Dx. It has been removed from analysis.

Applied Biosystems® 7500 Fast Dx

Expected Values (Cutaneous) (N=279)									
Age	HSV- 1			HSV-2			VZV		
	Total #	Total Positive	Prevalence	Total #	Total Positive	Prevalence	Total #	Total Positive	Prevalence
≤ 5 years	22	1	4.5%	22	1	4.5%	22	2	9.1%
6 to 21 years	30	8	26.7%	30	2	6.7%	30	1	3.3%
22 to 59 years	170	14	8.2%	170	31	18.2%	170	20	11.8%
≥ 60 years	57	4	7.0%	57	8	14.0%	57	10	17.5%

Expected Values (Cutaneous) (N=279)									
	HSV- 1			**HSV-2**			**VZV**		
	Total #	Total Positive	Prevalence	Total #	Total Positive	Prevalence	Total #	Total Positive	Prevalence
skin lesion	214	24	11.2%	214	26	12.1%	214	33	15.4%
genital - penis	65	3	4.6%	65	16	24.6%	65	0	N/A

Expected Values (Mucocutaneous) (N=650)*			
Age	HSV- 1	HSV-2	VZV

	Tota l #	Total Positive	Prevalence	Total #	Total Positive	Prevalence	Total #	Total Positive	Prevalence
< 5 years	18	5	27.8%	18	0	N/A	18	0	N/A
6 to 21 years	130	25	19.2%	130	24	18.5%	130	1	0.8%
22 to 59 years*	446	75	16.8%	446	75	16.8%	446	5	1.1%
> 60 years	53	3	5.7%	53	10	18.9%	53	1	1.9%

* Three (3) specimens were invalid when tested on the Applied Biosystems® 7500 Fast Dx. They have been removed from analysis.

Expected Values (Mucocutaneous) (N=656)*									
	HSV- 1			HSV-2			VZV		
	Total #	Total Positive	Prevalence	Total #	Total Positive	Prevalence	Total #	Total Positive	Prevalence
anorectal	26	4	15.4%	26	6	23.1%	26	1	3.8%
genital – vaginal/cervical	471*	70	14.9%	471*	102	21.7%	471*	3	0.6%
Nares	9	1	11.1%	9	0	N/A	9	0	N/A
Ocular	6	0	N/A	6	0	N/A	6	1	16.6%
oral lesion	135	33	24.4%	135	1	0.7%	135	2	1.5%

* Three (3) specimens were invalid when tested on the Applied Biosystems® 7500 Fast Dx. They have been removed from analysis.

Cepheid SmartCycler® II System

Expected Values (Cutaneous) (N=279)*									
Age	HSV- 1			HSV-2			VZV		
	Total #	Total Positive	Prevalence	Total #	Total Positive	Prevalence	Total #	Total Positive	Prevalence
< 5 years	22	1	4.5%	22	1	4.5%	22	2	9.1%
6 to 21 years	30	8	26.7%	30	1	3.3%	30	1	3.3%
22 to 59 years*	169	15	8.9%	169	32	18.9%	169	23	13.6%
> 60 years	57	3	5.3%	57	9	15.8%	57	11	19.3%

*One (1) specimen was invalid when tested on the Cepheid SmartCycler® II. It has been removed from analysis.

Expected Values (Cutaneous) (N=279)*									
	HSV- 1			**HSV-2**			**VZV**		
	Total #	**Total Positive**	**Prevalence**	**Total #**	**Total Positive**	**Prevalence**	**Total #**	**Total Positive**	**Prevalence**
skin lesion	213*	22	10.3%	213*	26	12.2%	213*	37	17.4%
genital - penis	65	5	7.7%	65	17	26.2%	65	0	N/A

*One (1) specimen was invalid when tested on the Cepheid SmartCycler® II. It has been removed from analysis.

Expected Values (Mucocutaneous) (N=650)*									
Age	HSV- 1			HSV-2			VZV		
	Total #	Total Positive	Prevalence	Total #	Total Positive	Prevalence	Total #	Total Positive	Prevalence
≤ 5 years	18	5	27.8%	18	0	N/A	18	0	N/A
6 to 21 years	130	29	22.3%	130	24	18.5%	130	1	0.8%
22 to 59 years*	445	78	17.5%	445	76	17.1%	445	6	1.3%
≥ 60 years	53	4	7.5%	53	10	18.9%	53	2	3.8%

*Four (4) specimens were invalid when tested on the Cepheid SmartCycler® II. They have been removed from analysis.

Expected Values (Mucocutaneous) (N=650)*									
	HSV- 1			HSV-2			VZV		
	Total #	Total Positive	Prevalence	Total #	Total Positive	Prevalence	Total #	Total Positive	Prevalence
anorectal	26	4	15.4%	26	6	23.1%	26	1	3.8%
genital – vaginal/cervical	470*	75	16.0%	470*	103	21.9%	470*	4	0.9%
nares	9	2	22.2%	9	0	N/A	9	0	N/A
ocular	6	0	N/A	6	0	N/A	6	1	16.6%
oral lesion	135	35	25.9%	135	1	0.7%	135	3	2.2%

*Four (4) specimens were invalid when tested on the Cepheid SmartCycler® II. They have been removed from analysis.

M. Instrument Names:

The manufacturer intends to market this assay for use with three instrument platforms:
- Life Technologies QuantStudio™ Dx
- Applied Biosystems® 7500 Fast Dx
- Cepheid SmartCycler® II System

N. System Description:

1. Modes of Operation:

1. Modes of Operation
For this application, Quidel assessed the software for each of the three systems and provided documentation on these platforms per FDA *Guidance for Industry, FDA Reviewers and Compliance- Off-The-Shelf Software Use in Medical Devices.* The firm's assessment determined that the software packages, when used as intended, presents a Moderate Level of Concern. Quidel states they have full access to the instruments with the current software. Each instrument is under a quality service plan with the manufacturers that includes both maintenance and software upgrades and will be in effect for the life of the Lyra™ Direct HSV 1+2/VZV Assay.The Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument with the SDS Software version 1.4 is a real-time nucleic acid amplification and detection system that measures fluorescence signal output from dual-labeled hydrolysis probes. The Sequence Detection Software (SDS) version 1.4 for the 7500 Fast Dx Instrument is used for instrument control, data collection and data analysis. The software can analyze cycle-by-cycle real-time signals from the sample. Life Technologies QuantStudio™ Dx uses fluorescent-based polymerase chain reaction (PCR) reagents to provide quantitative detection of target nucleic acid sequences (targets) using real-time analysis. After a test run, the version 1.0 software uses calibration data to determine the location and intensity of the fluorescent signals in each read, the dye associated with each fluorescent signal, and the significance of the signal. The Cepheid SmartCycler II with the Software version 3.0b is a real-time nucleic acid amplification and detection system that measures fluorescence signal output from dual-labeled hydrolysis probes. Software version 3.0b is used for instrument control, data collection and data analysis. The software can measure cycle-by-cycle real-time signals from the sample.

2. Specimen Identification:
Not Applicable

3. Specimen Sampling and Handling:

Specimens used for the validation of the Lyra™ Direct HSV 1 + 2/VZV Assay were obtained using standard techniques from patients with lesion infection symptoms. These specimens were collected, transported, stored, and processed according to CLSI M41-A.2. Samples can be stored at 2° to 8°C or –20°C for up to 7 days prior to processing. The performance of the assay was evaluated using: M4, M4-RT, M5, M6, and UTM. No

significant difference in assay performance was seen between the five different types of viral transport media. Prepared samples can be stored for 48 hours when stored at 2° to 8°C, 31 days when stored at either -20°C or -70°C.

4. Calibration:
   Calibration is not required or recommended.

5. Quality Control:

   The Lyra™ Direct HSV 1 + 2/VZV Assay incorporates several controls to monitor assay performance.

   1. The Process Control should be used during sample preparation and amplification in the assay. This control should be added to each sample aliquot prior to PCR.
   2. Commercially available external positive HSV-1, HSV-2, and VZV controls may be treated as a patient specimen and should be used in accordance with your lab standards. Previously characterized positive HSV-1, HSV-2, and VZV specimens may be used in lieu of commercial HSV-1, HSV-2, and VZV controls.
   3. Viral transport media or previously characterized negative specimen may be used as an external negative control. This must be treated as a patient specimen and should be performed in accordance with current lab standards.

6. Software:
   FDA has reviewed applicant's Hazard Analysis and Software Development processes for this line of product types:
   Yes___X_____ or No_____

O. Other Supportive Instrument Performance Characteristics Data Not Covered In the "Performance Characteristics" Section above:

Not Applicable

P. Proposed Labeling:

The labeling is sufficient and satisfies the requirements of 21 CFR Part 809.10, 21 CFR 801.109, and the special controls.

Q. Identified Potential Risks and Required Mitigation Measures

Identified Potential Risks	Required Mitigation Measures

Risk of false results	Special controls (1), (2), and (3)
Failure to correctly interpret test results	Special controls (4) and (5)
Failure to correctly operate the instrument	Special controls (6) and (7)

R. Benefit/Risk Analysis

Summary	
Summary of the Benefit(s)	When used for the proposed intended use, the benefits to the clinician and the patient include: (1) establishment of the device performance in a manner that demonstrates consistent accurate test results; and 2) ability to use a well validated device to diagnose HSV 1, HSV 2 and VZV in cutaneous and mucocutaneous lesion swab specimens, which will allow prompt initiation of disease specific treatment when that is indicated.
Summary of the Risk(s)	The risks associated with the device, when used as intended, are those related to the risk of false test results, failure to correctly interpret the test results and failure to correctly operate the instrument. Inaccurate test results may lead to error or delay in the diagnosis of HSV 1, HSV 2 and VZV infections, error or delay in appropriate treatment of these infections, failure or delay in implementing infection control measures, unnecessary use of anti-viral therapy, and delay in establishing the patient's true diagnosis.
Summary of Other Factors	HSV 1, HSV 2 and VZV infections may present as acute clinical episodes that are likely to recur. In most patients these infections are mild, but in certain populations they can be severe and life threatening. Severe infections are treated with antiviral drugs such as acyclovir, but initiation of therapy in the first few days of the infection is critical for a successful outcome. The analytical studies conducted by the sponsor were robust while the clinical study was limited by the number of VZV positive mucocutaneous lesion specimens available to evaluate the device. The data provided is adequate to demonstrate the device's performance characteristics. No post-market information is available.

Conclusions Do the probable benefits outweigh the probable risks?	The probable benefits of this device outweigh the probable risks associated with its use. There are no substantial clinical concerns with the classification of this device in Class II given the combination of general and special controls.

S. Conclusion:

The information provided in this *de novo* submission is sufficient to classify this device into class II under regulation 21 CFR 866.3309 with special controls. FDA believes that special controls, along with the applicable general controls, provide reasonable assurance of the safety and effectiveness of the device type. The device is classified under the following:

Device Type:	Herpes virus nucleic acid-based cutaneous and mucocutaneous lesion panel
Class:	II (special controls)
Regulation:	21 CFR 866.3309

(a) *Identification*. A herpes virus nucleic acid- based cutaneous and mucocutaneous panel is a qualitative *in vitro* diagnostic device intended for the simultaneous detection and differentiation of different herpes viruses in cutaneous and mucocutaneous lesion samples from symptomatic patients suspected of Herpetic infections. Negative results do not preclude infection and should not be used as the sole basis for treatment or other patient management decisions. The assay is not intended for use in cerebrospinal fluid samples.
(b) *Classification*. Class II (special controls). The special controls for this device are:

1) Premarket notification submissions must include detailed documentation for the device description, including the device components, ancillary reagents required but not provided, and a detailed explanation of the methodology including primer design and selection.

2) Premarket notification submissions must include detailed documentation from the following analytical and clinical performance studies: Analytical sensitivity (Limit of Detection), reactivity, inclusivity, precision, reproducibility, interference, cross reactivity, carry-over, and cross contamination.

3) Premarket notification submissions must include detailed documentation of a clinical study using lesion samples in which Herpes Simplex Virus 1, Herpes Simplex Virus 2, or Varicella Zoster Virus DNA detection was requested. The study must compare the device performance to an appropriate well established reference method.

4) A detailed explanation of the interpretation of results and acceptance criteria must be included in the device's 21 CFR 809.10(b)(9) compliant labeling.

5) The device labeling must include a limitation statement that reads: “The device is not intended for use with cerebrospinal fluid or to aid in the diagnosis of HSV or VZV infections of the central nervous system (CNS).”

6) Premarket notification submissions must include quality assurance protocols and a detailed documentation for device software, including, but not limited to, standalone software applications and hardware-based devices that incorporate software.

7) The risk management activities performed as part of the manufacturer’s 21 CFR 820.30 design controls must document an appropriate end user device training program that will be offered as part of efforts to mitigate the risk of failure to correctly operate the instrument.