This Procedural Bulletin is intended to provide a ready outline reference for performance of the assay. These abbreviated directions for use are not intended to replace the complete Package Insert. It is the obligation of every manufacturer of medical devices labeled FOR *IN VITRO* DIAGNOSTIC USE to provide a complete Package Insert in accordance with FDA labeling regulation (21 CFR 809.10).

Quidel Corporation provides CLSI procedures for your use. The procedures are required to include the same information as listed in the Package Insert. Any modifications to this document are the sole responsibility of the Laboratory.

Solana HSV 1+2/VZV Assay

CLIA Complexity: Moderate

INTENDED USE

The Solana HSV 1+2/VZV Assay is an *in vitro* diagnostic test, using isothermal amplification technology (helicase-dependent amplification, HDA), for the 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 Solana 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 Solana HSV 1+2/VZV Assay is intended for use only with the Solana instrument.

> ***Warning:*** The Solana 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. The Solana HSV 1 + 2/VZV Assay is not intended for use in prenatal screening.

SUMMARY AND EXPLANATION

HSV-1 and HSV-2: *Herpes simplex* virus types 1 and 2 (HSV-1 and HSV-2), also known as Human herpes virus 1 and 2 (HHV-1 and HHV-2), are DNA viruses of the family Herpesviridae. HSV infections in humans can cause lesions at a variety of cutaneous and mucocutaneous sites. These lesions can be a result of the primary infection by the virus or they can result from a reactivation of the latent virus, causing recurrent episodes of the disease. HSV-1 and HSV-2 are genetically and antigenically distinct forms of HSV. HSV-2 is the most common cause of genital infections, due to venereal transmission; HSV-1 is commonly associated with other disease locations although both serotypes have been shown to cause disease in all locations of the body. Studies have shown an increasing prevalence of genital HSV infections with a concomitant increase of the disease in neonates.

VZV: *Varicella-zoster* virus (VZV), also known as Human herpes virus 3 (HHV-3), is a DNA virus of the family Herpesviridae. Primary VZV infection results in chickenpox (varicella), which may rarely result in complications including encephalitis or pneumonia. Even when clinical symptoms of chickenpox have resolved, VZV remains dormant in the nervous system of the infected person (virus latency). In approximately 10 to 20% of cases, VZV reactivates later in life producing shingles. Serious complications of shingles include postherpetic neuralgia, zoster multiplex, myelitis, herpes ophthalmicus, or zoster sine herpete.

PRINCIPLE OF THE TEST

The Solana HSV 1+2/VZV Assay amplifies and detects viral DNA isolated 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 assay consists of two (2) major steps: 1) specimen preparation, and 2) amplification and detection of target sequence specific to HSV-1, HSV-2 and/or VZV using isothermal Helicase-Dependent Amplification (HDA) in the presence of a target- specific fluorescence probe.

Patient specimen is transferred to a Process Tube, subjected to heat treatment at 95 ± 2°C for 5 minutes and mixed and vortexed. The processed sample is transferred to a Reaction Tube and mixed. The Reaction Tube contains lyophilized HDA reagents, dNTPs, primers and probes. Once rehydrated with the diluted sample, the Reaction Tube is placed in Solana for amplification and detection of specific target sequence. In Solana, the target sequences are amplified by HSV-1, HSV-2 and/or VZV specific primers and detected by HSV-1, HSV-2 and/or VZV specific fluorescence probes included in the Reaction Tube. A competitive process control (PRC) is included in the Process Tube to monitor sample processing, inhibitory substances in clinical samples, reagent failure or device failure. The PRC target is amplified by specific primers and detected by a PRC specific fluorescence probe.

The target and PRC probes are labeled with a quencher on one end and a fluorophore on the other end. In addition, the target and PRC probes carry a ribonucleic acid. Upon annealing to HSV-1, HSV-2, VZV or PRC amplicons, the fluorescence probes are cleaved by RNaseH2 and the fluorescence signal increases due to physical separation of fluorophore from quencher. Solana measures and interprets the fluorescent signal, using on-board method-specific algorithms. Solana will then report the test results to the user on its display screen, and the results can be printed via an attached printer.

MATERIALS PROVIDED

Cat. #M302
48 Tests per Kit

Component	Quantity	Storage
Process Buffer Tubes	48 tubes/kit 1.6 mL	2°C to 8°C
Reaction Tubes	48 tubes/kit	2°C to 8°C

MATERIALS REQUIRED BUT NOT PROVIDED

- External controls for HSV-1, HSV-2, or VZV (i.e. the laboratory's own internal control materials from isolated and characterized clinical specimen previously submitted for interpretation which serves as an external processing and extraction control or Solana HSV 1+2/VZV Control Set, Cat. #M118, which contains positive and negative controls, serves as an external processing control)
- Sterile DNAse-free filter-blocked or positive displacement micropipettor tips
- Micropipettor
- Stopwatch or timer
- Vortex Mixer
- Scissors or a blade
- Workflow tray
- Transfer Rack
- Heat block capable of 95 ± 2°C temperature
- Solana instrument
- Transport Media (eg. BDTM Universal Viral Transport Medium (UVT) / COPAN Universal Transport Medium (UTM™) 3.0 mL, Thermo Fisher Scientific™ Remel™ MicroTest™ M4® 3.0 mL, Remel™ MicroTest™ M4RT® 3.0 mL, Remel™ MicroTest™ M5® 3.0 mL, Remel™ MicroTest™ M6® 3.0 mL)

WARNINGS AND PRECAUTIONS

- All reagents are for *in vitro* diagnostic use only.
- Refer to the Solana Operator's Manual for further information regarding instrument installation and operation.
- Only use the protocol described in this package insert. Deviations from the protocol may give erroneous results.
- Treat all specimen/samples as potentially infectious. Follow universal precautions when handling samples, this kit and its contents.
- All tubes should be capped tightly prior to vortexing.
- Proper sample collection, storage and transport are essential for correct results.
- Store assay reagents as indicated on their individual labels.
- Reagents are not interchangeable between lots.
- Never pool reagents from different tubes even if they are from the same lot.
- Do not use the reagents after their expiration date.

- Do not interchange caps among reagents as contamination may occur and compromise test results.
- Only open the tubes when adding aliquots into tubes or removing aliquots from tubes. Keep the tubes closed at any other time to avoid contamination.
- To avoid contamination of the environment with amplicons, do not open the reaction tubes post-amplification.
- Avoid microbial and deoxyribonuclease (DNAse) contamination of reagents when removing aliquots from tubes. The use of sterile DNAse-free disposable filter-blocked or positive displacement pipettor tips is recommended.
- Use a new pipettor tip for each specimen or reagents.
- Additional controls may be tested according to guidelines or requirements of local, state, provincial and/or federal regulations or accrediting organizations.
- Do not pipette by mouth.
- Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
- For accurate results, pipette carefully using only calibrated equipment. Use of inaccurate volumes may give erroneous results.
- Maintenance and decontamination of workspace and equipment should follow and be performed according to established laboratory protocols and schedules.
- Use micropipettes with an aerosol barrier or positive displacement tips for all procedures.
- Testing should be performed in an area with adequate ventilation.
- Dispose of containers and unused contents in accordance with Federal, State and Local regulatory requirements.
- Wear suitable protective clothing, gloves, and eye/face protection when handling the contents of this kit.
- Wash hands thoroughly after handling.
- For additional information on hazard symbols, safety, handling and disposal of the components within this kit, please refer to the Safety Data Sheet (SDS) located at quidel.com.

STORAGE AND HANDLING OF KIT REAGENTS

Store the Assay Kit at 2°C to 8°C until the expiration date listed on the outer kit box.

SPECIMEN COLLECTION, STORAGE AND HANDLING

Specimens used for the validation of the Solana HSV 1+2/VZV Assay were obtained using standard techniques from patients with lesion infection symptoms.[1] These specimens were collected, transported, stored, and processed according to CLSI M41-A.[2]

Samples can be stored at room temperature (up to 30 °C) for up to 48 hours, 2° C to 8°C or –20°C for up to 7 days prior to processing.

A series of studies were performed evaluating a number of routinely used viral transport medium at a volume of 2 mL: M4, M4-RT, M5, M6, UVT and UTM. No significant difference in assay performance was seen between the five different types of viral transport media.

TEST PROCEDURE

1. Turn on Solana by pressing the power button and wait until it completes self-testing.
   Note: Do not open the lid during the self-testing.
2. Place the required number of Process Buffer Tubes in the Workflow tray. Mark the Process Buffer Tubes on the cap and/or side of the tube.
   Note: One (1) Process Buffer Tube is required for each specimen or control to be tested.
   Note: A maximum of 12 tests can be performed in a single Solana instrument.
3. Vortex the collection device for 5 seconds and transfer 20 µL of the transport medium to a patient-identified Process Buffer Tube.
   Note: The specimens in Process Buffer Tubes may be stored at 2°C to 8°C for up to 72-hours.
4. Close the cap and mix the solution well by vortexing the tubes for 5 seconds.
   Note: Use a new pipette tip for each specimen.
5. Heat the Process Buffer Tubes at 95 ± 2°C for 5 minutes and then vortex the Tubes for 5 seconds.
   Note: Begin 5-minute lysis procedure when the heat block measures 95° ± 2°C. The timer must be stopped if the temperature falls out of range at any time during the 5-minute period and cannot be restarted until the heat block returns to 95° ± 2°C.

Note: The lysed specimens in Process Buffer Tubes may be stored at 2°C to 8°C for up to 72-hours.

6. Remove the required number of Reaction Tubes from the protective pouch and place into Transfer Rack. Mark the Reaction Tubes on the cap.
   Note: Remove the excess air and reseal the bag.
7. Transfer 50 µL of the diluted specimen to the labeled Reaction Tube, mix the solution by pipetting vigorously up and down a minimum of 5 times and close the cap. The solution should be clear, free of solid material.
   Note: Use a new pipette tip for each diluted sample.
   Note: Proceed immediately to the next step. Do not allow reconstituted reaction mix to sit for longer than 15 minutes.
8. Using the Solana Transfer Rack to hold Reaction Tubes at eye-level, visually inspect each Reaction Tube to ensure pellet rehydration.
9. Open the lid and transfer the Reaction Tubes to Solana via Transfer Rack.
   Note: Be sure that all tubes are in tight contact with Solana.
10. Select "NEW TEST". If Solana displays a different screen, go to the home screen.
11. Select the tube positions to use.
12. Scan the assay to enter Lot ID/Exp Date, and press "▶."
13. Select sample type (patient or QC) from the drop-down menu and enter Sample IDs (optional; see 2nd Note in next step).
14. Close the lid and press "Start" to initiate the Solana HSV 1+2/VZV Assay. Solana will display the progress and the count-down to assay completion. Test results will be displayed on the screen in approximately 50 minutes.
    Note: To avoid laboratory contamination, once the tube has been closed and the amplification reaction started, **DO NOT** open the Reaction Tube.
    Note: While the test is running, sample ID can be entered or edited by pressing the pencil icon.
15. After the run is completed press the arrow to move to the Test Results screen. The results can be printed by selecting the print button.
    Note: Do not navigate away from this screen before printing results. Once the screen is gone it cannot be revisited. If this occurs the results can be viewed individually by going to Home then selecting Review Results.

INTERPRETATION OF RESULTS

Samples	Assay Result	Interpretation
Patient specimen	HSV-1 POSITIVE	HSV-1 DNA detected
	HSV-1 NEGATIVE	No HSV-1 DNA detected, and other virus or PRC detected
	HSV-2 POSITIVE	HSV-2 DNA detected
	HSV-2 NEGATIVE	No HSV-2 DNA detected, and other virus or PRC detected
	VZV POSITIVE	VZV DNA detected
	VZV NEGATIVE	No VZV DNA detected, and other virus or PRC detected
	INVALID	No HSV-1, HSV-2, VZV DNA and No PRC detected; for invalid test results, retest the same processed sample first. If the test is invalid upon retesting with the processed sample, re-process another aliquot of the same sample or obtain a new sample and re-test.

QUALITY CONTROL

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

- The process control is used to monitor sample processing, to detect HDA inhibitory specimens, to confirm the integrity of assay reagents and the operation of the Solana instrument. The process control is included in the Process Buffer tube.
- The external positive control may be treated as a patient specimen. The control should be sampled and tested as if it were a patient specimen and processed as described above in the Assay Procedure. The external positive control is intended to monitor substantial reagent and instrument failure.
- The external negative control may be treated as a patient specimen. The control should be sampled and tested as if it were a patient specimen and processed as described above in the Assay Procedure. The external negative control is used to detect reagent or environmental contamination (or carry-over) by HSV 1+2/VZV DNA or amplicon.

LIMITATIONS

- Specimens should be tested with Solana HSV 1 + 2/VZV for only those viruses requested by the physician. Testing and reporting the additional, unrequested virus(es) may lead to confusion and delayed diagnosis due to an unexpected positive result.
- The samples used for the device should be limited to samples from lesions, indicating an active infection.
- Negative results do not preclude infection with HSV-1, HSV-2, or VZV and should not be the sole basis of a treatment decision.
- As with other assays of this type, there is a risk of false negative results due to the presence of sequence variants in the viral target.
- Improper collection, storage, or transport may lead to false negative results.
- Inhibitors present in the sample and/or errors in following the assay procedure may lead to false negative results.
- The programming instructions provided for each instrument are for use with the Solana HSV 1 + 2/VZV Assay. Their use for other instruments or assays has not been established.

EXPECTED VALUES

The expected values of the Solana HSV 1+2/VZV Assay were established during a prospective study conducted between February and May 2016. One thousand sixty-two (1062) specimens have been included in this study at three (3) sites across the United States. A single specimen was collected per patient. The specimens were processed and tested with Solana HSV 1+2/VZV Assay on the Solana instrument at the sites.

The expected value of HSV-1, HSV-2, and VZV with the Solana HSV 1+2/VZV Assay has been calculated for the combined sites based on the category of specimen (cutaneous, mucocutaneous, or uncategorized lesion source) and the age of the patient.

Combined Study – Expected Values (Cutaneous) (N=275)									
	HSV-1			**HSV-2**			**VZV**		
Age	Total #	Total Positive	Prevalence	Total #	Total Positive	Prevalence	Total #	Total Positive	Prevalence
≤ 5 years	9	1	11.1%	9	0	N/A	9	4	44.4%
6 to 21 years	43	10	23.3%	43	4	9.3%	43	0	N/A
22 to 59 years	180	16	8.9%	180	26	14.4%	180	19	10.6%
≥ 60 years	43	0	N/A	43	8	18.6%	43	6	14.0%

Expected Values (Cutaneous) (N=275)									
	HSV-1			**HSV-2**			**VZV**		
Specimen Source	Total #	Total Positive	Prevalence	Total #	Total Positive	Prevalence	Total #	Total Positive	Prevalence
Genital – penis	105	10	9.5%	105	19	18.1%	105	1	1.0%
skin lesion	170	17	10.0%	170	19	11.2%	170	28	16.5%

Combined Study – Expected Values (Mucocutaneous) (N=617)*									
	HSV-1			**HSV-2**			**VZV**		
Age	Total #	Total Positive	Prevalence	Total #	Total Positive	Prevalence	Total #	Total Positive	Prevalence
≤ 5 years	21	4	19.0%	21	0	N/A	21	1	4.8%
6 to 21 years	158	41	25.9%	158	29	18.4%	158	0	N/A
22 to 59 years	385	74	19.2%	385	89	23.1%	385	8	2.1%
≥ 60 years	53	11	20.8%	53	5	9.4%	53	1	1.9%

* Four (4) specimens were invalid and removed from analysis

Expected Values (Mucocutaneous) (N=617)*									
	HSV-1			HSV-2			VZV		
Specimen Source	Total #	Total Positive	Prevalence	Total #	Total Positive	Prevalence	Total #	Total Positive	Prevalence
Anorectal	26	7	26.9%	26	7	26.9%	26	1	3.8%
genital – vaginal/cervical	449	80	17.8%	449	112	24.9%	449	5	1.1%
Nares	23	5	21.7%	23	1	4.3%	23	3	13.0%
Ocular	7	2	28.6%	7	0	N/A	7	1	14.3%
Oral lesion	112	36	32.1%	112	3	2.7%	112	0	N/A

* Four (4) specimens were invalid and removed from analysis

Combined Study – Expected Values (Uncategorized Lesion Source) (N=166)									
	HSV-1			HSV-2			VZV		
Age	Total #	Total Positive	Prevalence	Total #	Total Positive	Prevalence	Total #	Total Positive	Prevalence
≤ 5 years	11	1	9.1%	11	0	N/A	11	0	N/A
6 to 21 years	28	10	35.7%	28	0	N/A	28	2	7.1%
22 to 59 years	89	15	16.9%	89	18	20.2%	89	14	15.7%
≥ 60 years	38	2	5.3%	38	5	13.2%	38	8	21.1%

CLINICAL PERFORMANCE

Performance characteristics of the Solana HSV 1+2/VZV Assay were established during a prospective study between February and May 2016. One thousand sixty-two (1062) fresh lesion specimens, collected for *herpes simplex*/*varicella-zoster* identification, have been included in this study at three (3) sites across the United States. A single specimen was collected per patient. The specimens were processed and tested with Solana HSV 1+2/VZV Assay on the Solana instrument at the sites. Each specimen was also processed and inoculated into two (2) different cell culture systems within 72 hours of collection. The isolation and identification of HSV-1 and HSV-2 was performed using the FDA-cleared ELVIS® HSV ID and D³ Typing Test. This testing was performed according to the manufacturer's product insert. The detection and isolation of VZV was performed by staining cells present in the specimen with a FDA-cleared VZV detection reagent (DSFA) and by culturing the specimen for 96-hours using commercially available mixed cell culture (H&V mixed cells from Diagnostic Hybrids, a Quidel Company) 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. A specimen was considered positive for VZV if either the DSFA or the culture with DFA were positive. Testing of the comparator methods (culture with DFA) were performed at one central location.

The gender and age demographics of the patients enrolled in the study are shown below.

Combined Study – Age and Gender Distribution (Cutaneous)		
Gender	Female	Male
Total	111	164
Age		
≤ 5 years	4	5
6 to 21 years	10	33
22 to 59 years	68	112
≥ 60 years	29	14

Combined Study – Age and Gender Distribution (Mucocutaneous)		
Gender	Female	Male
Total	552	69
Age		
≤ 5 years	5	16
6 to 21 years	141	18
22 to 59 years	366	22
≥ 60 years	40	13

Combined Study – Age and Gender Distribution (Uncategorized Lesion Source)		
Gender	Female	Male
Total	129	37
Age		
≤ 5 years	5	6
6 to 21 years	20	8
22 to 59 years	75	14
≥ 60 years	29	9

Combined Data

Cutaneous Lesions

Two-hundred seventy-five (275) 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. Samples which gave HSV-2 positive results from the ELVIS method were excluded from the HSV-1 performance calculation because of the inability of the ELVIS method to distinguish an HSV-1 positive sample when HSV-2 was detected first (n=26). The table below details the HSV-1 results for the remaining two-hundred forty-nine (249).

HSV-1 Results			
	Comparator: ELVIS HSV ID and D³ Typing Test		
Solana HSV 1 + 2/VZV Assay	Positive	Negative	Total
Positive	22	5*	27
Negative	0	222	222
Total	22	227	249
			95% CI
Sensitivity	22/22	100%	85.1% to 100%
Specificity	222/227	97.8%	95.0% to 99.1%

* Three (3) of the five (5) positives was positive by an additional RT-PCR assay.

Two-hundred seventy-five (275) 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 seventy-five (275).

HSV-2 Results			
	Comparator: ELVIS HSV ID and D³ Typing Test		
Solana HSV 1 + 2/VZV Assay	Positive	Negative	Total
Positive	24	14*	38
Negative	2**	235	237
Total	26	249	275
			95% CI
Sensitivity	24/26	92.3%	75.9% to 97.9%
Specificity	235/249	94.4%	90.8% to 96.6%

* Thirteen (13) of the fourteen (14) positives were positive by an additional RT-PCR assay.

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

Two-hundred and seventy-five (275) active cutaneous lesion specimens were cultured for VZV using the H&V mixed cells with DFA cell culture systems and were also tested with the subject device for VZV viral DNA. Due the presence of either HSV-1 or HSV-2, fifty-one (51) specimens have been excluded from analysis. Two (2) specimens were contaminated or had toxic cultures. These fifty-three (53) specimens have been excluded from analysis. The table below details the VZV results for the remaining two-hundred and twenty-two (222) specimens.

VZV Results			
	Comparator: DSFA and Culture with DFA		
Solana HSV 1 + 2/VZV Assay	Positive	Negative	Total
Positive	22	7*	29
Negative	0	193	193
Total	22	200	222
			95% CI
Sensitivity	22/22	100%	85.1% to 100%
Specificity	193/200	96.5%	93.0% to 98.3%

*Six (6) of the seven (7) positives were positive by an additional RT-PCR assay.

Mucocutaneous Lesions

Six-hundred twenty-one (621) 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. Seven (7) specimens were contaminated in the ELVIS cell culture. Two (2) specimens were invalid in Solana HSV 1 + 2/VZV Assay. Two (2) specimens were contaminated and invalid. Samples which gave HSV-2 positive results from the ELVIS method were excluded from the HSV-1 performance calculation because of the inability of the ELVIS method to distinguish an HSV-1 positive sample when HSV-2 was detected first (n=109). Thus, one hundred twenty (120) specimens have been excluded from further analysis. The table below details the HSV-1 results for the remaining five-hundred one (501) specimens.

HSV-1 Results			
	Comparator: ELVIS HSV ID and D³ Typing Test		
Solana HSV 1 + 2/VZV Assay	Positive	Negative	Total
Positive	113	14*	127
Negative	0	374	374
Total	113	388	501
			95% CI
Sensitivity	113/113	100%	96.7% to 100%
Specificity	374/388	96.4%	94.0% to 97.8

*Six (6) of the fourteen (14) positives were positive by an additional RT-PCR assay.

Six-hundred twenty-one (621) 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. Seven (7) specimens were contaminated in the ELVIS cell culture. Two (2) specimens were invalid in Solana HSV 1 + 2/VZV Assay. Two (2) specimens were contaminated and invalid. These eleven (11) specimens have been excluded from further analysis. The table below details the HSV-2 results for the remaining six-hundred ten (610) specimens.

HSV-2 Results			
	Comparator: ELVIS HSV ID and D³ Typing Test		
Solana HSV 1 + 2/VZV Assay	Positive	Negative	Total
Positive	108	14*	122
Negative	1**	487	488
Total	109	501	610
			95% CI
Sensitivity	108/109	99.1%	95.0% to 99.8%
Specificity	487/501	97.2%	95.4% to 98.3%

*Eleven (11) of the fourteen (14) positives were positive by an additional RT-PCR assay.

** One (1) of one (1) negative was positive by an additional RT-PCR assay.

Six-hundred twenty-one (621) active mucocutaneous lesion specimens were cultured for VZV using the H&V mixed cells with DFA cell culture systems and were also tested with the subject device for VZV viral DNA. Due the presence of either HSV-1 or HSV-2, two hundred thirty-six (236) specimens have been excluded from analysis. Nine (9) specimens were contaminated in culture, and four (4) specimens were invalid in Solana HSV 1 + 2/VZV Assay. These two hundred forty-nine (249) specimens have been excluded from analysis. The table below details the VZV results for the remaining three hundred seventy-two (372) specimens.

VZV Results			
	Comparator: DSFA and Culture with DFA		
Solana HSV 1 + 2/VZV Assay	Positive	Negative	Total
Positive	4	5*	9
Negative	0	363	363
Total	4	368	372
			95% CI
Sensitivity	4/4	100%	51.0% to 100%
Specificity	363/368	98.6%	96.9% to 99.4%

*One (1) of the five (5) positives was positive by an additional RT-PCR assay.

Note: The data presented for the detection of VZV is consistent with limited presence of VZV in mucocutaneous lesions. The use of mucocutaneous lesions has no discernible impact on the performance characteristics of Solana HSV 1 + 2/VZV Assay.

Uncategorized Lesions

One hundred sixty-six (166) active lesion specimens (not categorized as cutaneous or mucocutaneous) 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. Samples which gave HSV-2 positive results from the ELVIS method were excluded from the HSV-1 performance calculation because of the inability of the ELVIS method to distinguish an HSV-1 positive sample when HSV-2 was detected first (n=18). The table below details the HSV-1 results for the remaining one hundred forty-eight (148).

HSV-1 Results			
	Comparator: ELVIS HSV ID and D³ Typing Test		
Solana HSV 1 + 2/VZV Assay	Positive	Negative	Total
Positive	25	3*	28
Negative	0	120	120
Total	25	123	148
			95% CI
Sensitivity	22/22	100%	86.7% to 100%
Specificity	120/123	97.6%	93.1% to 99.2%

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

One hundred sixty-six (166) active lesion specimens (not categorized as cutaneous or mucocutaneous) 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 one hundred sixty-six (166).

HSV-2 Results			
	Comparator: ELVIS HSV ID and D³ Typing Test		
Solana HSV 1 + 2/VZV Assay	Positive	Negative	Total
Positive	18	5*	23
Negative	0	143	143
Total	18	148	166
			95% CI
Sensitivity	18/18	100%	82.4% to 100%
Specificity	143/148	96.6%	92.3% to 98.5%

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

One hundred sixty-six (166) active lesion specimens (not categorized as cutaneous or mucocutaneous) were cultured for VZV using the H&V mixed cells with DFA cell culture systems and were also tested with the subject device for VZV viral DNA. Due the presence of either HSV-1 or HSV-2, forty-six (46) specimens have been excluded from analysis. One (1) specimen was contaminated in culture. These forty-seven (47) specimens have been excluded from analysis. The table below details the VZV results for the remaining one hundred nineteen (119) specimens.

VZV Results			
	Comparator: DSFA and Culture with DFA		
Solana HSV 1 + 2/VZV Assay	Positive	Negative	Total
Positive	17	6*	23
Negative	0	96	96
Total	17	102	119
			95% CI
Sensitivity	17/17	100%	81.6% to 100%
Specificity	96/102	94.1%	87.8% to 97.3%

*Five (5) of the six (6) positives were positive by an additional RT-PCR assay.

ANALYTICAL PERFORMANCE

Limit of Detection

The analytical sensitivity (limit of detection or LOD) of the Solana HSV 1+2/VZV Assay was determined using quantified ($TCID_{50}$/mL) cultures of two (2) HSV-1 strains, two (2) HSV-2 strains, and two (2) VZV strains, serially diluted in negative matrix. Each dilution was run as 20 replicates in the Solana HSV 1+2/VZV assay. Analytical sensitivity (LOD) is defined as the lowest concentration at which at least 95% of all replicates tested positive. The demonstrated LOD for each strain tested is shown below:

LOD Values	
Virus	$TCID_{50}$/mL
HSV-1 MacIntyre	2.10×10^{2}
HSV-1 316	1.82×10^{4}
HSV-2 G	6.67×10^{3}
HSV-2 COMP	1.62×10^{5}
VZV Ellen	1.49×10^{-1}
VZV 9939	1.65×10^{2}

Analytical Reactivity (Inclusivity)

The inclusivity of the Solana HSV 1+2/VZV Assay was further evaluated by functional testing of viral strains in addition to those strains used in the LOD study. The clinical panel consisted of two (2) strains of HSV-1, three (3) strains of HSV-2, and five (5) strains of VZV at concentrations near the level of detection (LOD) of the assay.

Inclusivity Strains		
Strain	$TCID_{50}$/mL	Inclusive (Yes or No)
HSV-1 Isolate #1	1.26×10^3	Yes
HSV-1 Isolate #3	1.26×10^3	Yes
HSV-2 Strain MS	1.33×10^4	Yes
HSV-2 Isolate #25	1.33×10^4	Yes
HSV-2 Isolate #32	1.33×10^4	Yes
VZV Strain 82	8.05×10^0	Yes
VZV Strain 130	2.41×10^1	Yes
VZV Strain 275	8.05×10^0	Yes
VZV Strain B	2.41×10^1	Yes
VZV Strain D	8.05×10^0	Yes

Repeatability Study

The repeatability of the Solana HSV 1+2/VZV Assay was evaluated at the internal testing site. A panel containing 30 contrived samples, manufactured as high negative samples [n=3; 1/18x or 1/27x LOD (C_{20} – C_{80} concentration)] for HSV-1 MacIntyre, HSV-2 G, and VZV Ellen strains, low positive samples (n=3; near the assay limit of detection) for HSV-1, HSV-2 and VZV, moderate positive samples (n=3; 3x LOD) for HSV-1, HSV-2 and VZV and negative samples (n=3) was used for the study. The samples were randomized and blind-coded within each panel, and the operator tested one (1) panel, together three (3) positive and three (3) negative external controls, in three (3) runs. The panels were run by two (2) operators for twelve (12) non-consecutive days.

Summary of Repeatability Result Percent Detection ($TCID_{50}$/mL)				
HSV-1 MacIntyre	1.89×10^3	6.30×10^2	3.50×10^1	Negative
	100% (72/72)	100% (72/72)	32% (23/72)	0% (0/72)
HSV-2 G Strain	2.00×10^4	6.67×10^3	3.71×10^2	Negative
	100% (72/72)	100% (72/72)	19.4% (14/72)	0% (0/72)
VZV Ellen	4.47×10^{-1}	1.49×10^{-1}	5.50×10^{-3}	Negative
	100% (72/72)	100% (72/72)	19.4% (14/72)	0% (0/72)

Reproducibility Study

The reproducibility of the Solana HSV 1+2/VZV Assay was evaluated at three laboratory sites. A reproducibility panel containing 30 contrived samples, manufactured as high negative samples (n=3; 1/18x or 1/27x LOD (C_{20} – C_{80} concentration)) for HSV-1 MacIntyre, HSV-2 G, and VZV Ellen strain, low positive samples (n=3; near the assay limit of detection) for HSV-1, HSV-2 and VZV, moderate positive samples (n=3; 3x LOD) for HSV-1, HSV-2 and VZV and negative samples (n=3) was used for the study. The samples were randomized and blind-coded within each panel, and the operator tested one (1) panel, together with three (3) positive and three (3) negative external controls, in three (3) runs. The panels were run by two (2) operators at each of three (3) testing sites for five (5) non-consecutive days using a different lot of reagent at each site.

Reproducibility Summary									
HSV-1 MacIntyre	**SITE**						**Overall Percent Agreement**		**95% Confidence Interval**
	Site #1		**Site #2**		**Site #3**				
	Rate of Detection	*% Agreement*	*Rate of Detection*	*% Agreement*	*Rate of Detection*	*% Agreement*	*Rate of Detection*	*% Agreement*	
High Negative (3.50×10^{1} $TCID_{50}$/mL)	19/30	63.3	25/30	83.3	16/30	53.3	60/90	66.7	54.6 to 75.5
Low Positive (6.30×10^{2} $TCID_{50}$/mL)	30/30	100	30/30	100	30/30	100	90/90	100	96.5 to 100
Moderate Positive (1.89×10^{3} $TCID_{50}$/mL)	30/30	100	30/30	100	30/30	100	90/90	100	96.5 to 100
Negative	30/30	100	30/30	100	30/30	100	90/90	100	96.5 to 100
Positive Control	30/30	100	30/30	100	30/30	100	90/90	100	96.5 to 100
Negative Control	30/30	100	30/30	100	30/30	100	90/90	100	96.5 to 100
HSV-2 G	**SITE**						**Overall Percent Agreement**		**95% Confidence Interval**
	Site #1		**Site #2**		**Site #3**				
	Rate of Detection	*% Agreement*	*Rate of Detection*	*% Agreement*	*Rate of Detection*	*% Agreement*	*Rate of Detection*	*% Agreement*	
High Negative (3.71×10^{2} $TCID_{50}$/mL)	18/30	60	22/30	73.2	23/30	76.7	63/90	70.0	59.9 to 78.5
Low Positive (6.67×10^{3} $TCID_{50}$/mL)	30/30	100	30/30	100	30/30	100	90/90	100	96.5 to 100
Moderate Positive (2.00×10^{4} $TCID_{50}$/mL)	30/30	100	30/30	100	30/30	100	90/90	100	96.5 to 100
Negative	30/30	100	30/30	100	30/30	100	90/90	100	96.5 to 100
Positive Control	30/30	100	30/30	100	30/30	100	90/90	100	96.5 to 100
Negative Control	30/30	100	30/30	100	30/30	100	90/90	100	96.5 to 100
VZV Ellen	**SITE**						**Overall Percent Agreement**		**95% Confidence Interval**
	Site #1		**Site #2**		**Site #3**				
	Rate of Detection	*% Agreement*	*Rate of Detection*	*% Agreement*	*Rate of Detection*	*% Agreement*	*Rate of Detection*	*% Agreement*	
High Negative (5.50×10^{-3} $TCID_{50}$/mL)	19/30	63.3	28/30	93.3	22/30	73.3	69/90	76.7	66.9 to 84.2
Low Positive (1.49×10^{-1} $TCID_{50}$/mL)	30/30	100	30/30	100	30/30	100	90/90	100	96.5 to 100
Moderate Positive (4.46×10^{-1} $TCID_{50}$/mL)	30/30	100	30/30	100	30/30	100	90/90	100	96.5 to 100
Negative	30/30	100	30/30	100	30/30	100	90/90	100	96.5 to 100
Positive Control	30/30	100	30/30	100	30/30	100	90/90	100	96.5 to 100
Negative Control	30/30	100	30/30	100	30/30	100	90/90	100	96.5 to 100

Analytical Specificity – Microbial Interference

A study was performed to evaluate the performance of the Solana HSV 1+2/VZV Assay in the presence of sixty-four (64) organisms that might be found in lesion specimens. Each potentially interfering microorganism was tested in the

presence of 2x LOD HSV-1, HSV-2 and VZV viruses, or negative matrix at clinically relevant levels of viruses and bacteria: ≥10^6 CFU/mL or IFU/mL; viruses: ≥ 10^5 copies (cp), viral particles (vp) or $TCID_{50}$/mL.

Potentially Interfering Organisms			
Organism	**Test Concentration**	**Organism**	**Test Concentration**
Acholeplasma laidlawi	7.10E+06 CFU/mL	*Klebsiella pneumoniae*	1.61E+06 CFU/mL
Acinetobacter calcoaceticus	9.27E+06 CFU/mL	*Lactobacillus acidophilus*	2.49E+06 CFU/mL
Adenovirus 7	1.58E+05 $TCID_{50}$/mL	*Legionella pneumophila*	1.76E+06 CFU/mL
Bacteroides fragilis	1.19E+06 CFU/mL	Measles virus	1.95E+05 $TCID_{50}$/mL
Bordetella bronchiseptica	1.97E+06 CFU/mL	*Mobiluncus mulieris*	2.54E+06 CFU/mL
Bordetella pertussis	7.21E+06 CFU/mL	*Moraxella cartarrhalis*	1.26E+06 CFU/mL
Candida albicans	2.00E+06 CFU/mL	Mumps virus	5.89E+05 $TCID_{50}$/mL
Candida glabrata	3.93E+06 CFU/mL	*Mycoplasma hominis*	1.30E+06 CFU/mL
Chlamydia trachomatis	3.00E+06 CFU/mL	*Mycoplasma hyorhinis*	6.60E+06 CFU/mL
Chlamydophila pneumoniae	1.25E+06 IFU/mL	*Mycoplasma orale*	3.08E+06 CFU/mL
Clostridium perfringens	1.06E+06 CFU/mL	*Mycoplasma pneumoniae*	3.16E+06 CFU/mL
Coronavirus OC43	8.51E+05 $TCID_{50}$/mL	*Mycoplasma salivarium*	1.67E+06 CFU/mL
Corynebacterium diphtheriae	1.51E+06 CFU/mL	*Neisseria gonorrhoeae*	1.23E+06 CFU/mL
Coxsackievirus B4	3.16E+05 $TCID_{50}$/mL	Parainfluenza Type 1	3.97E+05 $TCID_{50}$/mL
Cytomegalovirus Towne VR-977	2.14E+05 $TCID_{50}$/mL	Parainfluenza Type 2	3.15E+05 $TCID_{50}$ /mL
Echovirus 11	2.14E+05 $TCID_{50}$/mL	Parainfluenza Type 3	2.56E+05 $TCID_{50}$/mL
Enterococcus faecalis	3.45E+06 CFU/mL	Parainfluenza Type 4	1.37E+05 $TCID_{50}$/mL
Enterovirus 70	1.78E+05 $TCID_{50}$/mL	*Proteus mirabilis*	1.19E+06 CFU/mL
Epstein Barr Virus	1.34E+05 vp/mL	*Pseudomonas aeruginosa*	1.32E+06 CFU/mL
Escherichia coli	8.42E+06 CFU/mL	RSV A Long	1.95E+05 $TCID_{50}$/mL
Gardnerella vaginalis	1.20E+06 CFU/mL	RSV B Washington	3.43E+05 $TCID_{50}$/mL
Haemophilis influenza type A	5.33E+06 CFU/mL	Rubella Virus	2.09E+05 $TCID_{50}$/mL
HBV synthetic DNA	6.80E+05 cp/mL	*Salmonella enteriditis*	5.40E+06 CFU/mL
HCV synthetic RNA	1.96E+05 cp/mL	*Salmonella typhimurium*	1.01E+06 CFU/mL
HHV-6	3.30E+05 $TCID_{50}$/mL	*Staphylococcus aureus*	1.02E+06 CFU/mL
HHV-7	1.15E+05 $TCID_{50}$/mL	*Staphylococcus saprophyticus*	2.00E+06 CFU/mL
HHV-8	1.26E+05 $TCID_{50}$/mL	*Streptococcus agalactiae*	2.20E+06 CFU/mL
HIV purified RNA	1.60E+05 cp/mL	*Streptococcus pneumoniae*	2.18E+06 CFU/mL
hMPV A1	3.66E+05 $TCID_{50}$/mL	*Streptococcus pyogenes*	1.29E+06 CFU/mL
HPV	4.30E+05 cp/uL	*Toxoplasma gondii*	1.06E+06 tachyzoites/mL
Influenza A/Mexico/4108/2009	2.88E+05 vp/mL	*Trichomonas vaginalis*	1.00E+06 trophozoites/mL
Influenza B Hong Kong VR-791	1.91E+05 $TCID_{50}$/mL	*Ureaplasma uralyticum*	1.23E+06 CFU/mL

No interference was observed with the sixty-four (64) microorganisms tested with the Solana HSV 1+2/VZV Assay.

Analytical Specificity – Cross-Reactivity

A study was performed to evaluate the performance of the Solana HSV 1+2/VZV Assay in the presence of sixty-four (64) organisms that might be found in lesion specimens. Each potentially cross-reactive microorganism was tested at clinically relevant levels of viruses and bacteria: ≥10^6 CFU/mL or IFU/mL; viruses: ≥ 10^5 copies (cp), viral particles (vp) or $TCID_{50}$/mL (Table 2.).

Potentially Cross-reactive Organisms			
Organism	**Test Concentration**	**Organism**	**Test Concentration**
Acholeplasma laidlawi	7.10E+06 CFU/mL	*Klebsiella pneumoniae*	1.61E+06 CFU/mL
Acinetobacter calcoaceticus	9.27E+06 CFU/mL	*Lactobacillus acidophilus*	2.49E+06 CFU/mL
Adenovirus 7	1.58E+05 $TCID_{50}$/mL	*Legionella pneumophila*	1.76E+06 CFU/mL
Bacteroides fragilis	1.19E+06 CFU/mL	Measles virus	1.95E+05 $TCID_{50}$/mL
Bordetella bronchiseptica	1.97E+06 CFU/mL	*Mobiluncus mulieris*	2.54E+06 CFU/mL
Bordetella pertussis	7.21E+06 CFU/mL	*Moraxella cartarrhalis*	1.26E+06 CFU/mL
Candida albicans	2.00E+06 CFU/mL	Mumps virus	5.89E+05 $TCID_{50}$/mL
Candida glabrata	3.93E+06 CFU/mL	*Mycoplasma hominis*	1.30E+06 CFU/mL
Chlamydia trachomatis	3.00E+06 CFU/mL	*Mycoplasma hyorhinis*	6.60E+06 CFU/mL
Chlamydophila pneumoniae	1.25E+06 IFU/mL	*Mycoplasma orale*	3.08E+06 CFU/mL
Clostridium perfringens	1.06E+06 CFU/mL	*Mycoplasma pneumoniae*	3.16E+06 CFU/mL
Coronavirus OC43	8.51E+05 $TCID_{50}$/mL	*Mycoplasma salivarium*	1.67E+06 CFU/mL
Corynebacterium diphtheriae	1.51E+06 CFU/mL	*Neisseria gonorrhoeae*	1.23E+06 CFU/mL
Coxsackievirus B4	3.16E+05 $TCID_{50}$/mL	Parainfluenza Type 1	3.97E+05 $TCID_{50}$/mL
Cytomegalovirus Towne VR-977	2.14E+05 $TCID_{50}$/mL	Parainfluenza Type 2	3.15E+05 $TCID_{50}$ /mL
Echovirus 11	2.14E+05 $TCID_{50}$/mL	Parainfluenza Type 3	2.56E+05 $TCID_{50}$/mL
Enterococcus faecalis	3.45E+06 CFU/mL	Parainfluenza Type 4	1.37E+05 $TCID_{50}$/mL
Enterovirus 70	1.78E+05 $TCID_{50}$/mL	*Proteus mirabilis*	1.19E+06 CFU/mL
Epstein Barr Virus	1.34E+05 vp/mL	*Pseudomonas aeruginosa*	1.32E+06 CFU/mL
Escherichia coli	8.42E+06 CFU/mL	RSV A Long	1.95E+05 $TCID_{50}$/mL
Gardnerella vaginalis	1.20E+06 CFU/mL	RSV B Washington	3.43E+05 $TCID_{50}$/mL
Haemophilis influenza type A	5.33E+06 CFU/mL	Rubella Virus	2.09E+05 $TCID_{50}$/mL
HBV synthetic DNA	6.80E+05 cp/mL	*Salmonella enteriditis*	5.40E+06 CFU/mL
HCV synthetic RNA	1.96E+05 cp/mL	*Salmonella typhimurium*	1.01E+06 CFU/mL
HHV-6	3.30E+05 $TCID_{50}$/mL	*Staphylococcus aureus*	1.02E+06 CFU/mL
HHV-7	1.15E+05 $TCID_{50}$/mL	*Staphylococcus saprophyticus*	2.00E+06 CFU/mL
HHV-8	1.26E+05 $TCID_{50}$/mL	*Streptococcus agalactiae*	2.20E+06 CFU/mL
HIV purified RNA	1.60E+05 cp/mL	*Streptococcus pneumoniae*	2.18E+06 CFU/mL
hMPV A1	3.66E+05 $TCID_{50}$/mL	*Streptococcus pyogenes*	1.29E+06 CFU/mL
HPV	4.30E+05 cp/uL	*Toxoplasma gondii*	1.06E+06 tachyzoites/mL
Influenza A/Mexico/4108/2009	2.88E+05 vp/mL	*Trichomonas vaginalis*	1.00E+06 trophozoites/mL
Influenza B Hong Kong VR-791	1.91E+05 $TCID_{50}$/mL	*Ureaplasma uralyticum*	1.23E+06 CFU/mL

No cross-reactivity was observed with the sixty-four (64) microorganisms tested with the Solana HSV 1+2/VZV Assay.

Analytical Specificity – Interfering Substances

The performance of Solana HSV 1+2/VZV Assay was evaluated with potentially interfering substances that may be present in lesion specimens. A panel composed of twenty-six (26) substances was tested in the absence or presence of HSV-1, HSV-2, or VZV (MacIntyre, G, Ellen strains, respectively) at 2X LOD in the Solana HSV 1+2/VZV Assay. There was no evidence of interference caused by the substances tested at the concentrations shown below.

Potential Interfering Substances and Concentrations Tested			
Substance	**Test Concentration**	**Substance**	**Test Concentration**
Abreva	7%	Female Urine	7%
Acetamidophenol	10 mg/mL	KY Jelly	7%
Acyclovir	7 mg/mL	Lanacane	3.50%
Albumin	3.3 mg/mL	Leukocytes	$2.5x10^5$ cells/mL
Blood/EDTA	0.63%	Listerine	7%
Carmex	7%	Male Urine	7%
Casein	7 mg/mL	Miconazole 1	7%

Potential Interfering Substances and Concentrations Tested			
Substance	**Test Concentration**	**Substance**	**Test Concentration**
Chlorpheniramine	5 mg/mL	Miconazole 3	7%
Colgate	7%	Mucin	60 µg/mL
Cornstarch	2.5 mg/mL	Preparation H	7%
Dextromethorphan	5 mg/mL	Releev	7%
Douche	7%	Seminal Fluid	2%
Feces	0.22%	Tioconazole 1	7%

Carryover and Cross-Contamination Studies

Positive samples consisting of HSV-1, HSV-2, and VZV formulated in pooled negative matrix at concentrations greater or equal to $1x10^5$ $TCID_{50}$/mL each. The negative samples consisted of pooled negative matrix. In each round of testing, 6 positive samples and 6 negative samples were tested in alternating order to assess the risk of cross contamination.

Consecutive testing of alternating high positive samples and negative samples resulted in no carry over or cross contamination as 53/53 positive samples tested positive and 53/53 negative samples tested negative.

CUSTOMER AND TECHNICAL ASSISTANCE

If you have any questions regarding the use of this product, please contact Quidel Technical Support at 1.800.874.1517 (in the U.S.) or technicalsupport@quidel.com. If outside the U.S., further information can be obtained from your distributor, or directly from Quidel at one of the numbers listed below. Reference **quidel.com** to see more options for Support.

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