Evaluation of a VZV Molecular Assay and Its Comparison to a VZV Analyte Specific Reagent

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Revised Abstract

INTRODUCTION: Varicella zoster virus (VZV) is the infectious agent responsible for chicken pox, with primary infection usually occurring in childhood. Activation of latent VZV leads to herpes zoster (shingles). VZV is also associated with other harmful conditions including Mollaret's meningitis, inflammation of arteries in the brain leading to stroke, post-herpetic neuralgia, myelitis and death. Prompt detection of VZV infections can assist in patient management, and is especially important in cases of debilitating conditions. DiaSorin Molecular's Simplexa™ VZV Direct kit is a sample-to-answer detection assay performed on the LIAISON® MDX system. Cerebrospinal fluid (CSF) specimens are loaded directly onto a Direct Amplification Disc without nucleic acid extraction or other specimen preparation. The analytical performance of the Simplexa™ VZV Direct kit was demonstrated with the following studies: Analytical Sensitivity (Limit of Detection, LoD), Analytical Reactivity, Analytical Specificity, Microbial Inhibition, and Interference. A sensitivity comparison between DiaSorin Molecular's sample-to-answer Simplexa™ VZV Direct kit and DiaSorin Molecular's VZV analyte specific reagent (ASR) using extracted nucleic acid with an internal test protocol was also performed.

METHODS: VZV Ellen and VZV 9939 strains were diluted into negative human CSF matrix for determining the LoD. A 2-fold serial dilution to obtain eight (8) different VZV concentrations was prepared for the two (2) VZV strains. Each concentration was tested with thirty-two (32) replicates. Analytical reactivity was performed with five (5) additional VZV strains at ~2X LoD by spiking quantified viral material into negative human CSF matrix. Analytical specificity was tested using a panel of one hundred five (105) microorganisms spiked into negative human CSF matrix at 1e5 PFU/mL for virus and 1e6 CFU/mL for bacteria. Microbial inhibition was tested with both VZV Ellen and VZV 9939 strains by spiking each strain at ~2X LoD into negative human CSF matrix in the presence of one hundred six (106) potentially inhibitory organisms. Interference was tested by evaluating the detection of VZV Ellen and VZV 9939 (both at ~2X LoD) in the presence of sixteen (16) potentially interfering substances in negative human CSF matrix. For sensitivity comparison, a testing panel was created using VZV Ellen and VZV 9939 strains diluted into negative human CSF matrix. Two-fold serial dilutions were performed to obtain five (5) different VZV concentrations for each of the two (2) VZV strains. Twelve (12) replicates of each concentration were tested. For DiaSorin Molecular's VZV ASR, 200 µl of sample was extracted using the Roche MagNA Pure 2.0, and 5 µl extracted nucleic acid was used in a 10 µl amplification reaction on the Universal Disc. Results were compared to testing with 50 µl of unextracted sample using Simplexa™ VZV Direct.

RESULTS: The LoD for Simplexa™ VZV Direct was 1,505 copies/mL for the VZV Ellen strain and 1,614 copies/mL for the VZV 9939 strain. All five (5) VZV strains (each at ~2X LoD) tested for analytical reactivity were detected. No cross-reactivity was observed for the one hundred five (105) microorganisms tested. There was no inhibition of VZV Ellen or VZV 9939 detection (both at ~2X LoD) in the presence of the one hundred five (105) microorganisms tested or with the sixteen (16) potentially interfering substances. The Simplexa™ VZV Direct kit used with unextracted samples was more sensitive than the VZV ASR using extracted nucleic acid (~2-fold more sensitive for VZV Ellen and ~16-fold more sensitive for VZV 9939).

CONCLUSIONS: The Simplexa™ VZV Direct kit demonstrated excellent assay performance across numerous analytical studies as well as for sensitivity and specificity. In addition, when compared to the DiaSorin Molecular VZV ASR, the Simplexa™ VZV Direct kit demonstrated superior sensitivity without the need for nucleic acid extraction from CSF samples. Simplexa™ VZV Direct is CE Marked and was submitted to the FDA for 510(k) clearance.

Methods

REAL-TIME PCR AMPLIFICATION AND DETECTION:

Simplexa™ VZV Direct Assay (MOL3650) contains all-in-one reagent vials for real-time PCR. No sample extraction or preparation is required. For each reaction on the Direct Amplification Disc, 50 µL of VZV Direct reaction mix was loaded into the reaction mix port and 50 µL of sample was loaded into the sample port. All testing was performed using the LIAISON® MDX instrument. (DiaSorin Molecular, Cypress, CA)

DiaSorin Molecular's VZV ASR requires formulation of the reaction mix. 5 µl extracted nucleic acid is used in a 10 µl amplification reaction on the Universal Disc. Testing was performed with an internal test protocol on the LIAISON® MDX instrument. (DiaSorin Molecular, Cypress, CA)

LIMIT OF DETECTION:

Stocks of VZV Ellen and VZV 9939 were quantified using droplet digital PCR (ddPCR) and serially diluted in negative human CSF matrix. Thirty-two (32) replicates were tested at each dilution. The LoD for VZV Ellen and 9939 strains was determined as the lowest concentration with ≥95% detection.

Methods (continued)

ANALYTICAL REACTIVITY:

Five (5) VZV strains were individually spiked at ~2X LoD (average LoD of VZV Ellen and 9939 in $TCID_{50}$/mL) into negative human CSF matrix and tested in triplicate.

INTERFERENCE:

VZV Ellen and VZV 9939 were spiked at ~2X LoD into negative human CSF matrix in the presence of sixteen (16) potentially interfering substances and tested in triplicate.

ANALYTICAL SPECIFICITY:

A total of one hundred fifty-nine (159) organisms were evaluated. One hundred five (105) organisms that are closely related, may cause similar clinical symptoms or may be present in CSF, were tested (1e5 PFU/mL for virus and 1e6 CFU/mL for bacteria) in triplicate while the remaining fifty-four (54) organisms were evaluated for potential cross-reactivity via *in silico* analysis due to unavailability of the organism.

MICROBIAL INHIBITION:

VZV Ellen and VZV 9939 were spiked at ~2X LoD into negative human CSF matrix in the presence of one hundred five (105) potentially inhibitory organisms and tested in triplicate.

SENSITIVITY COMPARISON BETWEEN THE SIMPLEXA™ VZV DIRECT KIT AND THE VZV ASR:

A testing panel consisting of two-fold serial dilutions of VZV Ellen and VZV 9939 negative human CSF matrix was performed to obtain five (5) different concentrations. Each concentration was tested with twelve (12) replicates per each strain. Samples were directly added onto the Direct Amplification Disc for the Simplexa ™ VZV Direct kit while samples were extracted (200 µL) on the Roche MagNA Pure prior to testing with the VZV ASR.

Results

Limit of detection for Simplexa™ VZV Direct : See Table 1 below.

Table 1. Limit of Detection

Strain	Concentration		% Detection of Channel	Mean +/- SD (%CV)
	($TCID_{50}$/mL)	(Copies/mL)		
Ellen	0.000063	94	12.50% (4/32)	39.2 ± 0.59 (1.49%)
	0.000130	188	28.13% (9/32)	39.4 ± 0.87 (2.21%)
	0.000250	376	59.38% (19/32)	39.2 ± 1.02 (2.59%)
	0.000500	753	84.38% (27/32)	38.8 ± 0.96 (2.46%)
	0.001000	**1,505**	**96.88% (31/32)**	**37.8 ± 0.92 (2.44%)**
	0.002000	3,010	100% (32/32)	36.7 ± 0.87 (2.38%)
	0.004000	6,020	100% (32/32)	35.8 ± 0.48 (1.34%)
	0.008000	12,040	100% (32/32)	34.9 ± 0.39 (1.13%)
9939	0.254	202	34.38% (11/32)	39.1 ± 1.00 (2.57%)
	0.508	404	59.38% (19/32)	39.0 ± 2.10 (5.40%)
	1.015	807	87.5% (28/32)	38.6 ± 1.47 (3.81%)
	2.030	**1,614**	**100% (32/32)**	**37.0 ± 1.57 (4.26%)**
	4.060	3,228	100% (32/32)	36.5 ± 1.01 (2.77%)
	8.120	6,456	100% (32/32)	34.4 ± 1.44 (4.17%)
	16.240	12,912	100% (32/32)	34.0 ± 1.16 (3.42%)
	32.480	25,824	100% (32/32)	32.6 ± 1.12 (3.43%)

Analytical Reactivity: All five (5) VZV strains tested were detected at ~2X LoD (Table 2).

Table 2. Analytical Reactivity

Strain	Result (#Detected /#Tested)	Average Ct
Strain 82	3/3	32.7
Strain 275	3/3	35.1
Strain 1700	3/3	38.0
Isolate A	3/3	31.7
Isolate B	3/3	33.9

Results (continued)

Interference: No interference was observed with the potentially interfering substances at the concentrations indicated in Table 3.

Table 3. Interfering Substances

No.	Potential Interferent	Concentration
1	Acyclovir	9.4 mg/mL
2	Albumin	50 mg/mL
3	Bilirubin	0.0125 mg/mL
4	Casein	9.0 mg/mL
5	Foscarnet	0.6 mg/mL
6	Gamma globulin	10.4 mg/mL
7	Glucose	11 mg/mL
8	Hemoglobin	3.5 mg/mL
9	Human Genomic DNA	72 µg/mL
10	Immunoglobulin	10 mg/mL
11	Lactate	2.2 mg/mL
12	Topical Antiseptic	5% (v/v)
13	Trans-Isolate Medium	50% (v/v)
14	UTM	50% (v/v)
15	White blood cells	$2x10^7$ WBC/mL
16	Whole Blood in EDTA	10% (v/v)

Analytical Specificity: None of the one hundred five (105) organisms tested were detected (Table 4) and the remaining fifty-four (54) organisms analyzed *in silico* did not show cross-reactivity (Table 5).

Table 4. Analytical Specificity and Microbial Inhibition – Tested

Bacterium	Virus (continued)	Virus (continued)
Bacillus cereus	Adenovirus C1	HIV-2 NIHZ
Bacillus subtilis	Adenovirus C2	HPeV-3
Citrobacter freundii	Adenovirus D20	HSV-1 (MacIntyre)
Citrobacter koseri	BK virus	HSV-2 (G)
Cronobacter sakazakii	Coronavirus 229E*	Human Rhinovirus A16
Enterobacter aerogenes	Coronavirus NL63*	Influenza A/California/7/2009
Enterobacter cloacae	Coronavirus OC43	Influenza A/H1N1
Escherichia coli (O157:H7)	Coxsackievirus A16	Influenza B/Florida/02/2006
Haemophilus ducreyi	Coxsackievirus A21*	JC virus (MAD-4 strain)
Haemophilus influenza Type B	Coxsackievirus A9	La Crosse encephalitis virus
Haemophilus influenzae Type A	Coxsackievirus B1	Measles virus
Haemophilus parainfluenzae	Coxsackievirus B2	Mumps virus
Klebsiella pneumoniae	Coxsackievirus B3	Parainfluenza Type 1
Listeria monocytogenes	Coxsackievirus B4	Parainfluenza Type 2
Mycobacterium tuberculosis genomic DNA	Coxsackievirus B5*	Parainfluenza Type 3
Neisseria gonorrhoeae	Cytomegalovirus (AD169 Strain)	Parainfluenza Type 4
Neisseria meningitidis (serogroup A)	Dengue virus (Type 1)*	Parvovirus B19
Neisseria mucosa	Dengue virus (Type 2)	Poliovirus (Type 3)
Propionibacterium acnes	Echovirus 1	Rabies virus
Proteus mirabilis Z050	Echovirus 11	Respiratory syncytial virus
Pseudomonas aeruginosa	Echovirus 4	Rhinovirus 1A
Serratia marcescens	Echovirus 6	Rotavirus (Type Wa)*
Shigella flexneri	Echovirus 7	Rubella virus
Staphylococcus aureus (MRSA), COL	Echovirus 9	Simian Virus type 40
Staphylococcus epidermidis (MRSE)	Encephalomyocarditis virus	St. Louis encephalitis virus
Staphylococcus saprophyticus	Enterovirus 70	West Nile virus
Streptococcus agalactiae	Enterovirus 71	**Other**
Streptococcus dysgalactiae	Epstein-Barr virus (B95-8 Strain)	*Aspergillus fumigatus* (fungus)
Streptococcus intermedius	Hepatitis A virus	*Candida albicans* (fungus)
Streptococcus mutans	Hepatitis B virus	*Candida krusei* (fungus)
Streptococcus pneumoniae	Hepatitis C virus	*Candida parapsilosis* (fungus)
Streptococcus pyogenes Z018	HHV-6A	*Candida tropicalis* (fungus)
Streptococcus salivarius	HHV-6B	*Cryptococcus neoformans* (fungus)
Toxoplasma gondii	HHV-7 SB	*Naegleria fowleri** (amoeba)
Virus	HHV-8	White Blood Cells (Human Genomic DNA)
Adenovirus B7A	HIV-1 IIIB	

* In silico analysis was also performed due to low concentration of stock and results confirmed organism to not be cross reactive with VZV

Table 5. Analytical Specificity – Evaluated *In Silico*

Bacterium	Bacterium (continued)	Virus
Corynebacterium striatum	*Staphylococcus capitis*	Adenovirus A12
Corynebacterium urealyticum	*Staphylococcus haemolyticus*	Adenovirus B35
Escherichia coli K1	*Staphylococcus hominis*	Adenovirus E4
Escherichia fergusonii	*Staphylococcus lugdunensis*	Adenovirus F41
Escherichia hermanii	*Streptococcus anginosus*	Coxsackievirus A10
Escherichia vulneris	*Streptococcus bovis*	Coxsackievirus A17
Haemophilus haemolyticus	*Streptococcus oralis*	Coxsackievirus A24
Haemophilus parahaemolyticus	*Streptococcus pseudopneumoniae*	Coxsackievirus A6
Listeria innocua	*Streptococcus sanguinis*	Echovirus 18
Listeria ivanovii	*Treponema pallidum*	Enterovirus 68
Morganella morganii	*Tropheryma whipplei*	Hepatitis D virus
Neisseria lactamica	**Fungus**	HPeV-1
Neisseria meningitidis (unencapsulated)	*Cryptococcus albidus*	HPeV-2
Neisseria sicca	*Cryptococcus amylolentus*	HPeV-4
Pantoea agglomerans	*Cryptococcus gattii*	HPeV-5
Salmonella bongori	*Cryptococcus laurentii*	HPeV-6
Salmonella enterica	*Cryptococcus uniguttulatus*	Human Rhinovirus B3
Shigella boydii	*Filobasidium capsuligenum*	Human Rhinovirus B83
Shigella sonnei		

Results (continued)

Microbial Inhibition: VZV Ellen and VZV 9939 strains were detected at ~2X LoD without any inhibition in the presence of the one hundred five (105) organisms tested (Table 4).

Sensitivity Comparison Between DiaSorin Molecular's Simplexa™ VZV Direct Kit and DiaSorin Molecular's VZV ASR: The Simplexa™ VZV Direct kit demonstrated better sensitivity than the VZV ASR (Tables 6 and 7).

Table 6. Simplexa™ VZV Direct Kit vs. VZV ASR - #Detected/#Tested

Concentration (Copies/mL)	# Detected/# Tested			
	VZV Ellen		VZV 9939	
	Simplexa	ASR	Simplexa	ASR
2,250	12/12	12/12	12/12	12/12
1,125	12/12	7/12	12/12	12/12
565	11/12	1/12	12/12	11/12
280	11/12	1/12	12/12	6/12
140	7/12	0/12	12/12	3/12

Table 7. Simplexa™ VZV Direct Kit vs. VZV ASR – Average Ct

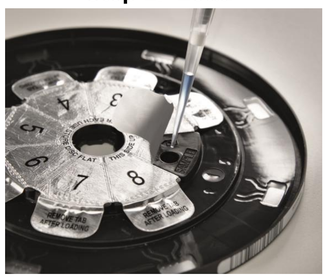
Concentration (Copies/mL)	Average Ct in VZV Channel*			
	VZV Ellen		VZV 9939	
	Simplexa	ASR	Simplexa	ASR
2,250	36.2	34.7	34.0	33.1
1,125	37.4	33.8	35.1	34.0
565	38.3	39.7	35.9	35.1
280	38.9	36.8	37.1	36.9
140	39.6	0.0	38.3	36.0

* Average Cts include only runs that were positive (cycle cut-off = 42)

Conclusions

- The Simplexa™ VZV Direct kit demonstrated excellent assay performance in all analytical studies performed based on ~2X LoD of the VZV Ellen (LoD = 1,505 copies/mL) and VZV 9939 (LoD = 1,614 copies/mL) strains.
- DiaSorin Molecular's sample-to-answer Simplexa™ VZV Direct kit without sample extraction was more sensitive than the DiaSorin Molecular VZV ASR with sample extraction (performed with an internal test protocol) in the side by side comparison study.

Step 1. Load



Step 2. Run

DiaSorin Molecular