Evaluation of the Luminex ARIES® System for the Detection of Varicella Zoster DNA directly From Clinical Samples

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Introduction

Primary Varicella zoster virus (VZV) infection results in chickenpox. Complications are rare, but can manifest as encephalitis, pneumonia or bronchitis. After infection VZV remains dormant in the nervous system of the infected person, and can reactivate later in life to cause shingles (herpes zoster). The most common complications of shingles include postherpetic neuralgia and meningitis. In the past laboratory diagnosis of VZV infections was limited to culture and/or direct immunologic staining methodologies. VZV culture was of limited value due to the fragility of the virus during transport. Over the past several years molecular methodologies such as the polymerase chain reaction (PCR) have become the gold-standard for VZV detection in clinical samples. Recently, Luminex has developed a new "sample to answer" automated instrument, the ARIES, which is designed for their proprietary MultiCode® PCR technology. The instrument utilizes test cassettes into which the sample is added. The appropriate MultiCode PCR primers are also manually added to a small tube that clips on to the end of the cassette. Once placed into the ARIES instrument, nucleic acid extraction and PCR analysis are fully automated. The purpose of this study was to determine the suitability of the ARIES platform for detection of VZV DNA directly from patient samples. The performance of the ARIES VZV method was compared to our current TaqMan PCR-based laboratory developed procedure (LDP) and a rapid cell culture assay.

Materials and Methods

MultiCode primers for VZV PCR and ARIES test cassettes were obtained from Luminex (Austin, TX). Testing was performed according to standard instrument settings supplied by Luminex using their proprietary SYNCT software. For our TaqMan PCR LDP method, the forward and reverse primers and the FAM-labeled probe, targeting a highly conserved region of the VZV DNA polymerase gene (Table 1), were designed using Primer Express software, (Life Technologies Inc., Grand Island, NY), and synthesized by Life Technologies Inc. Real-time PCR analysis was performed on the Roche Cobas z480 analyzer (Roche Molecular Diagnostics, Indianapolis, IN). Samples collected on swabs and submitted to the laboratory in M4 viral transport medium were prepared for testing as follows: a 500 µL sample of transport medium was placed into a 1.5 mL microcentrifuge tube with a locking cap and placed into a 100°C heat block for 10 minutes. After heating, the tubes were centrifuged at 14,000 x g at ambient temperature. A volume of 10 µL of the sample was added to 40 uL of PCR master mix containing 1X TaqMan® Universal PCR Master Mix (supplied at a 10X concentration, Life Technologies Inc., Grand Island, NY), 500 nM each of forward and reverse primers, and 200 nM of the probe. The reaction also contained an RNase P exogenous control primer and probe (VIC) mixture (Life Technologies Inc., Grand Island, NY). PCR analysis settings on the z480 were as follows: one cycle each of 50°C for 2 minutes and 95°C for 10 minutes followed by 45 cycles of 95°C for 15 seconds and 60°C for 1 minute. Rapid VZV culture was performed by inoculating the patient sample into H&V mix cell culture, and staining with VZV-specific fluorescent antibodies (Diagnostic Hybrids, Athens, OH) at 48 and 96 hours.

Table 1: VZV TaqMan PCR Primers and Probes

Primer/Probe	Sequence
VZV Forward primer	5' CCG GCA AGT CGC CAA TTA 3'
VZV Reverse primer	5' GTT TGG TAT AGC CTG CAG ATT ATC C3'
VZV Probe	5' 6FAM-ACA TGC AGT CAA TTT CAA CGT CGC TTA ACG-TAMRA 3'

Figure 1

TABLE 2

Sample Number	Virology Results	Roche z480			Luminex ARIES			Virology Results	Virology Results	Roche z480			Luminex ARIES		
		FAM	VIC (IC)	Result	FAM	VIC (IC)	Result			FAM	VIC (IC)	Result	FAM	VIC (IC)	Result
VZV NEG	VZV NEG			Pass	NEG		Pass	21	POS	24.05	Pass	POS	19.30	Pass	POS
1	POS	29.68	Pass	POS	23.20	Pass	POS	22	NEG		Pass	NEG		Pass	NEG
2	NEG		Pass	NEG		Pass	NEG	23	POS	27.03	Pass	POS	23.60	Pass	POS
3	POS	26.42	Pass	POS	18.10	Pass	POS	24	NEG		Pass	NEG		Pass	NEG
4	**NEG**	27.70	Pass	POS	25.30	Pass	POS	25	**POS**		Pass	NEG		Pass	NEG
5	POS	29.02	Pass	POS	32.70	Pass	POS	26	NEG		Pass	NEG		Pass	NEG
6	NEG		Pass	NEG		Pass	NEG	27	POS	28.56	Pass	POS	18.10	Pass	POS
7	POS	25.98	Pass	POS	19.10	Pass	POS	28	NEG		Pass	NEG		Pass	NEG
8	NEG		Pass	NEG		Pass	NEG	29	POS	21.62	Pass	POS	17.30	Pass	POS
9	POS	24.17	Pass	POS	19.10	Pass	POS	30	NEG		Pass	NEG		Pass	NEG
10	NEG		Pass	NEG		Pass	NEG	31	POS	26.95	Pass	POS	18.60	Pass	POS
11	POS	25.61	Pass	POS	20.10	Pass	POS	32	NEG		Pass	NEG		Pass	NEG
12	NEG		Pass	NEG		Pass	NEG	33	POS	26.80	Pass	POS	21.77	Pass	POS
13	POS	22.38	Pass	POS	16.30	Pass	POS	34	NEG		Pass	NEG		Pass	NEG
14	NEG		Pass	NEG		Pass	NEG	35	POS	28.62	Pass	POS	23.10	Pass	POS
15	POS	24.66	Pass	POS	18.30	Pass	POS	36	NEG		Pass	NEG		Pass	NEG
16	NEG		Pass	NEG		Pass	NEG	37	POS	26.12	Pass	POS	19.10	Pass	POS
17	POS	24.54	Pass	POS	16.70	Pass	POS	38	NEG		Pass	NEG		Pass	NEG
18	NEG		Pass	NEG		Pass	NEG	39	POS	38.20	Pass	POS	34.30	Pass	POS
19	POS	23.84	Pass	POS	18.00	Pass	POS	40	NEG		Pass	NEG		Pass	NEG
20	NEG		Pass	NEG		Pass	NEG								

Results

The limit of detection (LOD) for the Luminex and LDP methods was determined by testing 20 replicates of serial dilutions prepared from the NATtrol Varicella Zoster Virus (VZV) control (ZeptoMetrix, Buffalo, NY) starting at 50,000 copies/mL. For the VZV LDP, 500 µL of each dilution was heated for 10 minutes at 100°C followed by centrifugation to remove the precipitated proteins. For the ARIES, 200 µL of each dilution was added to the test cassette. The results determined the LOD of the VZV LDP and VZV ARIES methods to be equivalent at 500 copies of VZV DNA/mL. Next, a total of 40 patient samples were collected using Dacron swabs and transported in M4 viral transport medium (Remel Products, Lenexa, KS). The samples were tested for viable VZV by culture and for VZV DNA by both our VZV LDP PCR method and the VZV ARIES method. Agreement between the two PCR methods was 100%. One negative sample by culture was positive by both PCR methods. Conversely, one positive sample by culture was repeatedly negative by both the ARIES and LDP PCR methods.

Discussion

Results of the LOD experiments demonstrated equal analytical sensitivity for the z480 LDP method compared to the ARIES with an LOD of 1000 DNA copies/mL. The Luminex SYNCT software was used to develop the VZV User Defined Protocol (UDP) on the ARIES. The four-step work flow (Figure 1) reduces the amount of hands-on time required to perform molecular testing. An additional step is required if performing a UDP on the ARIES, which is to pipette PCR primers into the Ready Mix PCR tube and snap the PCR tube onto the extraction cassette. Once the sample is added into the cassette, the cassette is placed into the magazine, which is then placed into the instrument. Once the magazine is placed into the instrument, the ARIES run will start automatically. For a run of 6 samples (the number of tests slots per bay), total time to results is 2 hours 20 minutes (20 minutes of hands-on time and 2 hours for the instrument runtime). The total time to result for the TaqMan LDP is approximately 3 hours, including sample processing, 96 well plate setup, and instrument runtime. The level of automation gained by using the ARIES is very useful for performing low volume assays since samples can be tested as they come in, rather than waiting to batch test them in order to make testing more cost effective. The possibility of amplicon contamination is also significantly reduced with the ARIES by using a fully self-contained test cassette that is not open to the environment.

Conclusions

The ARIES instrument is a robust, simple to use, sample to result platform. The performance of the ARIES VZV ASR compared very well to our VZV LDP assay. The major advantages of the VZV ARIES method are the elimination of any up front sample processing and increased cost effectiveness by reducing the need for batch testing.