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

Tong Her*, Sama Najjar and Ted E. Schutzbank
St. John Hospital and Medical Center, Detroit, Michigan

Introduction

Primary Varicella zoster virus (VZV) infection results in chickenpox. Complications are rare, but can manifest as encephalitis, pneumonia or bronchiolitis. 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 detection in clinical samples. The instrument utilizes test cassettes into which the sample is added. The appropriate MultiCode PCR primers for VZV PCR and ARIES test cassettes were obtained from Luminex by Life Technologies Inc.

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 from 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 µL 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 99°C for 15 seconds and 60°C for 1 minute. Rapid VZV culture was performed by inoculating the patient sample into HIK/V mix cell culture, and staining with VZV-specific fluorescent antibodies (Diagnostic Hybrids, Athens, OH) at 48 and 96 hours.

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.

Table 2

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Figure 1

- Start
- Step 1: Load sample into magazine
- Step 2: Load cassette into magazine
- Step 3: Run test on instrument
- Step 4: Result displayed