Evaluation of the Luminex ARIES® System for the Detection and Quantification of BK virus DNA in Plasma Samples from Kidney Transplant Recipients

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Background

- BK virus (BKV) is found throughout the world. Most infections occur in early childhood with no known associated disease. BKV establishes a latent infection in the kidneys. Seroprevalence in adults is ~90%. Roughly 40% of renal allograft recipients shed BKV in the urine, either transiently or continuously over weeks to months. BKV Nephropathy is asymptomatic, and is usually discovered due to increase in serum creatinine levels. Nearly 50% of renal transplant patients with BKV nephropathy experience a significant loss of function of the transplanted kidney. It is routine practice to screen renal transplant recipients regularly for BK viremia.
- Luminex has developed a “sample to answer” automated instrument, the ARIES®, which is designed for their proprietary MultiCode® PCR technology. It can perform up to 12 of the same or different tests simultaneously. The instrument utilizes test cassettes into which the patient sample is added. The appropriate MultiCode® PCR primers are 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 (Figure 1).
- **Study Purpose:** To determine the suitability of the ARIES® platform for detection and quantification of BKV DNA directly from patient plasma samples. The performance of the ARIES® BKV laboratory developed procedure (LDP) was compared to our current TaqMan PCR-based LDP using the Roche Cobas z480 platform.

Materials and Methods

- The BKV DNA TaqMan PCR-based LDP was performed using ELITech 20X MGB Alert® BK Virus Primers and 20X MGB Alert® BK Virus Probe analyte specific reagents (ELITech, Logan, UT).
- The 25 µL PCR reaction consisted of 1X each of the primers, probe and MGB Alert® Hot Start Master (supplied as 2X). The MGB Alert® BK Virus Internal Control (ELITech) was added to each reaction. DNA was extracted from 1.0 mL of plasma with the bioMerieux easyMag extractor and eluted into 50 uL of elution buffer. A volume of 5 µL was added to the PCR reaction mix. Analysis was performed in the Roche Cobas z480 real-time PCR platform.
- MultiCode® primers (analyte specific reagents) for BKV PCR and ARIES® test cassettes were obtained from Luminex Corporation (Austin, TX). Testing was performed according to standard instrument settings supplied by Luminex using their proprietary SYNCT software. Sample volume for the ARIES® platform was 200 µL; the final elution volume post-extraction was 150 µL, 50 µL of which was added to the reaction tube containing the BKV MultiCode® primers and the reaction master mix (Ready Mix).

Figure 1. The ARIES® “sample to answer” automated instrument workflow

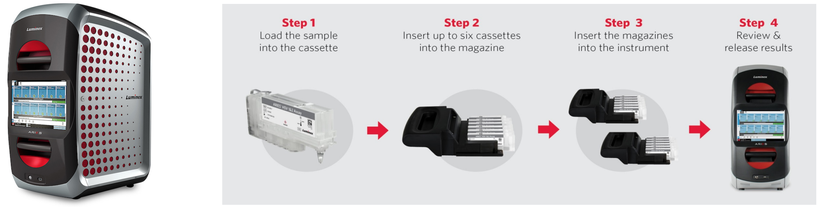
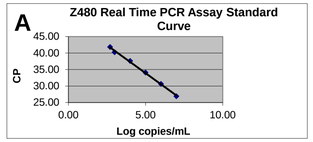


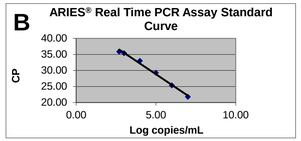
Table 1. ARIES®/z480 LDP Comparison

Sample			Roche z480 LDP		LOG Difference
	cp/mL	Log cp/mL	cp/mL	Log cp/mL	
313611	39	1.6	787	2.9	-1.3
318856	52	1.72	436	2.64	-0.92
147329	79	1.9	ND	ND	2.35
416826	111	2.05	473	2.67	-0.63
147329	254	2.4	ND	ND	-
497868	714	2.85	4,410	3.64	-0.79
211229	940	2.97	2,427	3.39	-0.41
211229	1,079	3.03	2,427	3.39	-0.35
299629	1,872	3.27	1,309	3.12	0.15
299521	2,149	3.33	9,756	3.99	-0.66
162904	2,831	3.45	8,346	3.92	-0.47
417959	3,729	3.57	5,406	3.73	-0.15
162904	7,428	3.87	8,346	3.92	-0.05
596591	9,785	3.99	44,607	4.65	-0.66
308868	14,795	4.17	25,571	4.41	-0.24
724744	23,966	4.38	27,553	4.44	-0.06
531684	23,966	4.38	52,497	4.72	-0.34
299531	23,966	4.38	54,309	4.73	-0.36
234871	25,675	4.41	52,497	4.72	-0.31
234871	27,507	4.44	52,497	4.72	-0.28
123044	41,590	4.62	71,244	4.85	-0.23
596643	44,557	4.65	94,739	4.98	-0.33
433255	47,735	4.68	93,462	4.97	-0.29
40181	62,884	4.8	33,773	4.53	0.27
240660	125,252	5.1	221,263	5.34	-0.25
240660	134,187	5.13	221,263	5.34	-0.22
355063	286,340	5.46	427,340	5.63	-0.17
597242	654,604	5.82	1671,599	6.22	-0.41
473995	804,920	5.91	2,237,977	6.35	-0.44
551855	862,338	5.94	1,150,917	6.06	-0.13
40052	923,852	5.97	853,834	5.93	0.03
228172	2,112,027	6.32	787,062	5.9	0.43
149337	40,875,563	7.61	51,103,299	7.71	-0.1
149337	43,791,366	7.64	51,103,299	7.71	-0.07
433174	ND	ND	368	2.57	
131845	ND	ND	ND	ND	
131845	ND	ND	ND	ND	
293718	ND	ND	ND	ND	
				Average Δ log cp/mL	-0.22

Figure 2. BKV PCR standard curves for z480 LDP and ARIES®

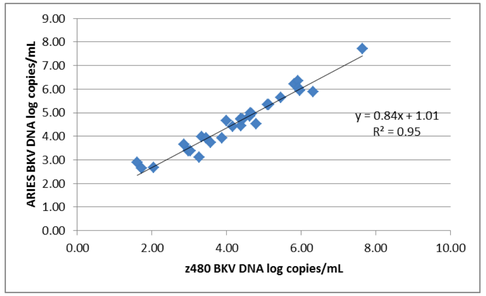


Slope: -3.3932
R squared: 0.9975



Equation y = -3.3417x + 45.535
Slope: -3.3417
R squared: 0..9905

Figure 3. ARIES®/ z480 LDP Correlation



Disclosure: Dr. Schutzbank is a member of the Luminex Laboratory Advisory Board

Results and Discussion

- A standard curve was established for both the Roche z480 and ARIES® Instruments using serial dilutions of BKV DNA (ATCC VR-3249SD) ranging from 1 X 10^7 to 500 copies/mL. Each concentration was tested in triplicate for the z480, and in duplicate for the ARIES® (Figure 2). Generation of the standard curve was performed automatically with the z480, which permits multiple data points for each DNA copy number panel member. The standard curve can be stored and imported for subsequent assay runs. The standard curve for the ARIES® was generated using duplicate samples of each panel member. The data, when imported into Excel, generated the standard curve shown in Figure 2.
- The ARIES® copy number data shown in Table 1 were calculated externally in Excel using the formula for the standard curve shown in Figure 1. The lower limit of quantification (LOQ) (results not shown) was performed by testing 20 replicates of BK DNA at 1000, 500 and 250 BKV DNA copies/mL. The LOD for both methods was determined to be 500 DNA copies per mL of original sample. A panel of 37 patient samples previously tested using the Roche z480 LDP was tested on the ARIES® platform. Results shown in Table 1.
- The correlation of results between both methods was excellent (r^2= 0.95) (Figure 3), especially considering that both methods used very different chemistries, sample processing, and amplification conditions. Typically, results between 2 different methods are considered to be equivalent if the difference between them is 0.3 log copies/mL or less. This is the case for the majority of the results obtained by the z480 LDP and the ARIES® BKV LDP. The average log difference between both methods was -0.22 log copies/mL.
- Total time to result for the z480 LDP, including DNA extraction, reaction plate assembly, and instrument run time ranges from 3.5 – 4 hours. With the ARIES®, total run time is shortened to 2 hours 20 minutes.
- Our laboratory performs BKV qPCR testing every other day to maximize the number of samples being tested in order to maximize cost effectiveness. A major advantage of ARIES® BK virus LDP is that samples can be tested as they arrive in the laboratory, providing a very quick turn-around-time for reporting results to the ordering physicians.

Conclusions

- Reproducible quantification is achievable using the Luminex ARIES® platform.
  - BKV quantification results were closely matched between the Luminex ARIES® and Roche z480 platforms using totally different reagents, sample extraction, and PCR technologies.
- More efficient workflow with the ARIES® System.
  - No up-front sample processing.
  - Simple assembly of the testing cassette after addition of primers to reaction tube.
  - Faster time to result.
- Increased cost effectiveness by elimination of up front sample processing and the need for batch testing.