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Evaluation of the Luminex ARIES® system for the detection and quantification of BK virus (BKV) DNA in plasma samples from kidney transplant recipients

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ABSTRACT

BK virus (BKV) nephropathy is a serious complication in renal transplant recipients due to the need for immunosuppression. 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. In this study, we compared the performance of BKV quantitative polymerase chain reaction analyte specific reagents by ELITech and Luminex for measuring BKV viral load in plasma using the Roche Cobas® z480 instrument and the Luminex ARIES® platform, respectively. A total of 34 patients previously tested on the z480, with results spanning the test's linear range, were analyzed on the ARIES®. The BKV DNA copy number correlation between the 2 methods was very good, with an R^2 value of 0.96. The average difference in log copy number between the 2 methods was −0.3, indicating that the ARIES® method may have slightly greater analytical sensitivity. BKV quantification results were closely matched between the 2 different methods. The workflow with the ARIES® System is greatly simplified by elimination of DNA extraction and most hands-on steps. The high degree of automation allows samples to be tested as they arrive into the laboratory, resulting in enhanced patient care due to more rapid turnaround time for results back to the ordering physician.

1. Introduction

BK virus (BKV), a member of the human polyomaviruses family, was first discovered in the urine of renal transplant patients with postoperative ureteral stenosis (Coleman and Hulme, 1971). BKV is ubiquitous in adults in the United States and Europe; seroprevalence ranges from 60% to 80%. Epidemiological studies indicate that BKV is contracted during childhood, between 3 and 4 years of age (Knowles et al., 2003). Very little is known about the transmission of this virus or about events during primary infection. The majority of primary infections are asymptomatic; however, BKV may cause mild upper respiratory disease in young children (Mäntyjärvi et al., 1973). A latent infection is established in the kidneys of the infected host by BKV.

BKV is recognized as a significant cause of nephropathy in renal transplant patients and is a significant cause of graft failure in 1% to 10% of patients (Hirsch et al., 2005; Nickeleit et al., 2000a). Approximately 40% of renal allograft recipients shed BKV in the urine, either transiently or continuously over weeks to months (Arthur et al., 1986; Arthur and Shah, 1989). Nephropathy caused by BKV is asymptomatic and associated with increased serum creatinine levels. Nearly 50% of renal transplant patients diagnosed with BKV nephropathy experience a significant loss of function of the transplanted kidney.

BKV viruria has also been demonstrated in approximately 10–25% of bone marrow transplant (BMT) patients, typically 2 months posttransplantation (Arthur et al., 1986). The frequency of cystitis is higher in adult allogeneic compared to autologous BMT recipients. BKV can be demonstrated in the peripheral blood of both groups (Drachenberg et al., 2007). Because detection of BKV in the urine of BMT patients in the absence of disease is common, it is difficult to correlate BKV as the cause of cystitis in these individuals (Bogdanovic et al., 1998).

Surveillance testing for BKV in the blood and urine of renal transplant recipients has become routine. In 2013, an expert panel recommended the use of either urine cytology or nucleic acid–based testing to screen renal transplant recipients monthly during the first 6 months posttransplantation and then every 3 months until 2 years after transplantation (Nickeleit et al., 2000a). These guidelines included quantitative cutoffs for BKV loads that indicate additional testing. Urine DNA loads of $>10^7$ copies/mL or plasma DNA loads of $>10^4$ copies/mL that persist for more than 3 weeks constitute a diagnosis of "presumptive polyomavirus-associated nephropathy" and should be followed up with a renal biopsy (Nickeleit et al., 2000a).

There are no antiviral agents for the treatment of BKV. Treatment of kidney transplant recipients with BKV infection consists of lowering the

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dosage of immunosuppressive drugs used to prevent rejection of the transplanted organ to allow the patient's immune response to clear the infection (Binggeli et al., 2007; Hirsch et al., 2013; Krymskaya et al., 2005; Schachtner et al., 2011). For this reason, timely turnaround time for quantitative BKV determination is important to initiate such treatment and then to resume proper immunosuppressive therapy once the infection is cleared.

There are no FDA-approved assays commercially available for BKV, so molecular testing is performed using laboratory developed tests; reagents for BKV nucleic acid amplification tests are available as either analyte specific reagents (ASR) or research use only from several commercial sources. In this paper, we describe a rapid, sample-to-answer quantitative BKV quantitative polymerase chain reaction (qPCR) assay based on Luminex MultiCode® PCR technology on the ARIES® platform (Luminex Corp., Austin, TX), which allows for samples to be analyzed as they are received in the laboratory as opposed to batch testing.

2. Materials and methods

2.1. Clinical Samples

All clinical samples used in this study were obtained from kidney transplant recipients as part of routine screening for BKV viremia, submitted to the St. John Hospital and Medical Center Specialized Testing Laboratory for testing. All samples were deidentified prior to testing in this study, eliminating the requirement for patient informed consent.

2.2. Specimen processing

Plasma samples from kidney transplant recipients were submitted to the St. John Hospital and Medical Center Specialized Testing Laboratory for BK qPCR virus testing. DNA was extracted from 1 mL of each sample using a bioMerieux NucliSENS® easyMag® nucleic acid extractor (bioMerieux, Durham, NC). The extracted nucleic acids from each sample were eluted into 50 μL of extraction buffer.

2.3. BKV TaqMan qPCR

BKV qPCR testing based on TaqMan technology was performed using ELITech (ELITech Group, Logan, UT) 20X MGB Alert® BK Virus Primers and 20X MGB Alert® Probe ASR. The 25-μL reaction consisted of 1× of the primers, probe, MGB Alert® Hot Start Master, and MGB Alert® BK Virus Internal Control primers and probes and 5 μL of the extracted nucleic acid solution. Analysis was performed using the Roche Cobas z480 platform with their proprietary user-defined software package (Roche Diagnostics, Indianapolis, IN). Thermocycling conditions were 1 cycle each of 50 °C for 2 min and 93 °C for 2 min, followed by 45 cycles of 93 °C for 15 s, 56 °C for 30 s, and 72 °C for 30 s. The standard curve was established using 5 μL of dilutions of a quantified synthetic BK virus DNA (ATCC, Manassas, VA) tested in triplicate.

2.4. MultiCode® PCR analysis on the ARIES® Platform

The MultiCode® primer mix (Luminex Corp., Austin, TX) was prepared by adding 2 μL of MultiCode® BK virus ASR primer and 2 μL of Control Primer (CP3) per sample to a 1.5-mL microcentrifuge tube. A volume of 4 μL of this primer mix was added to a MultiCode® Ready Mix tube (Luminex, Austin, TX). The MultiCode® Ready Mix tube contains all of reagents required for the MultiCode® PCR reaction to occur. The prepared MultiCode® Ready Mix tube is then attached to the appropriate position on the ARIES® Extraction Cassette. Two hundred microliters of plasma is added to the sample chamber of the cassette which is placed into the ARIES® cassette holder and loaded into the ARIES® instrument. Testing was performed according to standard instrument settings supplied by Luminex using their proprietary SYNCT™ software and the User-Defined Protocol application. To generate the standard curve, a 200-μL sample of each of the above BKV DNA dilutions was added to the sample chamber of the ARIES® cassette. Each dilution was tested in duplicate.

3. Results

3.1. Generation of the BKV qPCR standard curve

The BKV qPCR standard curve on the Cobas z480 and ARIES® platforms was generated using dilutions made from quantified synthetic BKV DNA. The 6-member panel ranged from 1×10^7 to 5×10^2 DNA copies/mL (10-fold dilutions down to 1×10^3 copies/mL, followed by one 2-fold dilution to yield 500 copies/mL). Each dilution was tested in triplicate on the Cobas z480 and in duplicate on the ARIES®. The z480 software automatically generates a standard curve that can be saved and imported for subsequent analyses, meaning that a standard curve need not be generated for each run. The same dilution panel was used to generate the BKV DNA standard curve for the ARIES® platform. In this case, 200 μL of each panel member was added to ARIES® Extraction Cassettes containing the MultiCode® BK virus primers. The results of the standard curve are exported to an Excel file. Since the ARIES® software was not designed for quantitative PCR analyses, it is necessary to import the standard curve C_ts and patient data from the ARIES® printout into Excel for analysis. There is no need to perform a standard curve with each test run. The standard curve generated as just described can be applied to subsequent test runs. High- and low-QC samples are run daily. The standard curve is regenerated if 1 or both QC samples fail or with new lots of any of the reagents used in the testing. The results, shown in Fig. 2A and B for the Cobas z480 and ARIES® platforms, respectively, are very similar. Based on the slopes of both standard curves, the efficiency of amplification for the ELITech reagents on the z480 and the MultiCode® BKV primers on the ARIES® platform was determined to be very close at 97.12% and 99.18%, respectively (where a slope of 3.322 = 100% efficiency) (https://www.thermofisher.com/us/en/home/brands/thermo-scientific/molecular-biology/molecular-biology-learning-center/molecular-biology-resource-library/thermo-scientific-web-tools/qpcr-efficiency-calculator.html).

3.2. Determination of linearity of ARIES® BKV viral load test and determination of lower limits of quantification (LoQ) and detection (LoD)

The linearity of the ARIES® BKV viral load test was determined by testing triplicate samples of serial dilutions prepared from the ATCC quantified BKV DNA. The concentrations tested ranged from 1×10^7 to 250 copies per mL (10-fold dilutions down to 1×10^3 copies/mL followed by two 2-fold dilutions to yield 500 and 250 copies/mL). Results from this analysis were entered into EP Evaluator (Data Innovations, Burlington, VT). The EP evaluator results (not shown) demonstrated linearity of the assay over the tested range. From this analysis, we set the LoQ at 250 BKV copies/mL. The LoDs for both methods were determined by testing dilutions made from the quantified BKV DNA used to generate the standard curve. Each dilution was tested 20 times. The lowest dilution resulting in a positivity of ≥95% was set as the LoD. The LoD for the ELITech/z480 method was determined to be 500 copies/mL of original sample and 250 copies/mL for the MultiCode®/ARIES® procedure (data not shown).

3.3. Reproducibility of BKV viral load testing on the ARIES® platform

Five previously tested patient samples were used to determine reproducibility of the BKV qPCR test on the ARIES® platform. Three of these were positive and 2 were undetectable for BKV DNA. The results shown in Table 1 demonstrate a high degree of reproducibility as demonstrated by the very low percent coefficient of variation between each of the replicate analyses.

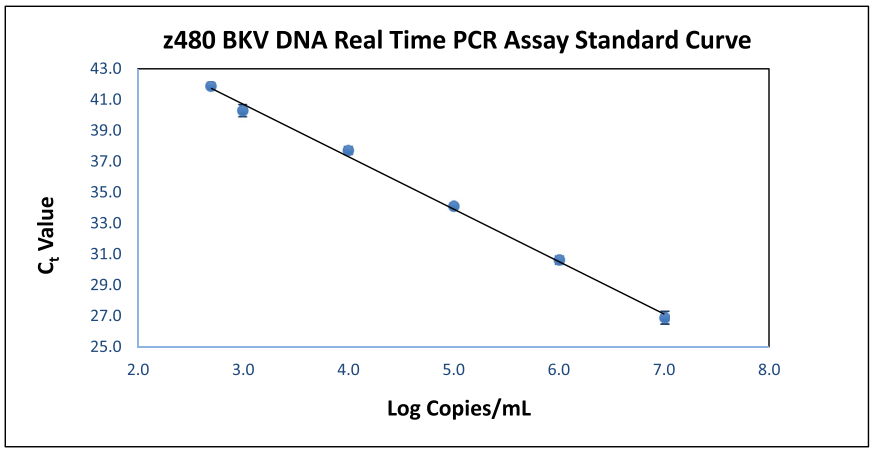
Table 1
Reproducibility of BKV qPCR on the ARIES® Platform.

Sample	Replicate 1		Replicate 2		Replicate 3				
	C_t	log cp/mL	C_t	log cp/mL	C_t	log cp/mL	Avg. log cp/mL	SD	%CV
413531	32.2	3.99	31.6	4.17	31.0	4.35	4.17	0.18	4.32
412173	ND	ND	ND	ND	ND	ND	-	-	-
410089	31.3	4.26	32.0	4.05	31.5	4.20	4.17	0.11	2.59
428857	26.2	5.79	25.8	5.91	25.9	5.88	5.86	0.06	1.07
341582	ND	ND	ND	ND	ND	ND	-	-	-

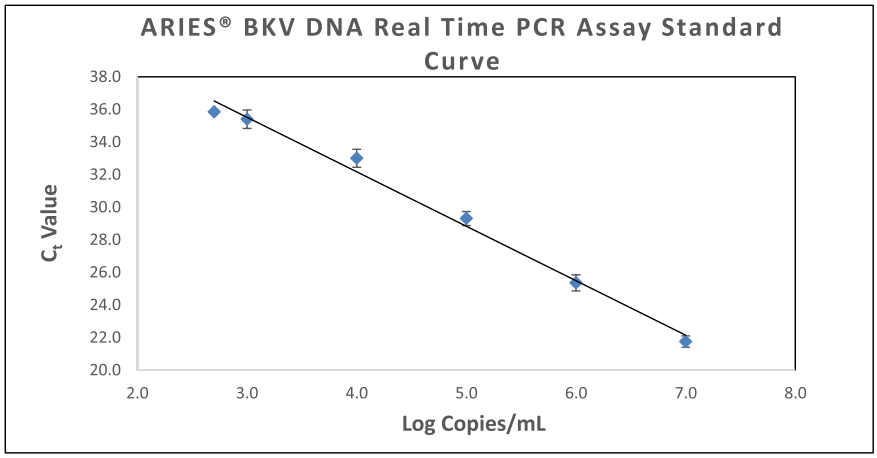
ND = not detected; Log cp/mL = log copies/mL.

3.4. Comparison of testing with clinical samples between the ELITech and ARIES® qPCR assays

To compare the performance of both assays with actual clinical samples, a panel of 34 plasma samples submitted previously for BKV DNA qPCR testing were analyzed using the ELITech reagents on the z480 and the MultiCode® BKV primers on the ARIES® Platform. The results are shown in Table 1 and Fig. 2. Test results for 2 different methods are considered equivalent if the log difference is ≤0.3 log10 copies/mL since the reproducibility of viral load assays (95% confidence limits) is 0.3 log10/mL, indicating that 10,000 copies/mL has a 2–standard deviation range of approximately 5000 to 20,000 copies/mL (Clinical and Laboratory Standards Institute (CLSI), 2011). The overall average Δ log difference between the ARIES® and z480 BKV quantification



Equation: y = -3.3417x + 5.535
Slope: 3.39
R^2 0.99



Equation: 3.3417x + 45.535
Slope: 3.34
R^2 0.9905

Fig. 1. Generation of BKV qPCR standard curves. Upper panel: standard curve generated for BKV DNA qPCR assay on the Roche Cobas z480 platform. Each dilution of BKV purified DNA was tested in triplicate. See text for details. Lower panel: standard curve generated for BKV DNA qPCR assay on the Luminex ARIES® platform. Each dilution of BKV purified DNA was tested in duplicate. See text for details.

tests was determined to be −0.3 logs. This value decreases to −0.24 if the samples with viral loads <500 copies per mL are removed from the analysis since 500 BKV DNA copies/mL is the lower limit of detection for the ELITech/z480 methodology. Linear regression analysis of the data in Table 1 was performed using Excel and is shown in Fig. 2. Comparison of the ELITech and ARIES® methods yielded a correlation coefficient (R^2) value of 0.96, indicating very close agreement of the results obtained by both methods. The residual plot in panel B clearly demonstrates the validity of the linear regression plot in panel A and also demonstrates lack bias of the ARIES® BKV qPCR method compared to the comparator ELITech assay as evidenced by the tight and even scatter of the data points across the *x*-axis.

4. Discussion

Due to their highly immunocompromised state, kidney transplant recipients are at high risk of loss of their transplanted organ due to reactivated BKV infection (Arthur et al., 1986; Arthur and Shah, 1989; Hirsch et al., 2005; Nickeleit et al., 2000a). In the absence of antiviral agents that are active against BKV, "treatment" of BKV infection in this population is limited to reduction of the use of immunosuppressive drugs to allow the patient's immune system to clear the infection (Hirsch et al., 2013). As a result, the patient is placed at greatly increased risk of immunological rejection of the transplanted kidney (Clinical and Laboratory Standards Institute (CLSI), 2011; Hirsch et al., 2002; Nickeleit et al., 2000b; Viscount et al., 2007). Therefore, it is important that BKV viral load testing be performed as expeditiously as possible to determine the need for reduction of immunosuppression, with follow-up monitoring of BKV viral load to determine when appropriate levels of immunosuppressive therapy can be resumed (Binggeli et al., 2007; Ginevri et al., 2007; Krymskaya et al., 2005; Schachtner et al., 2011). Batch testing of plasma samples for BKV qPCR can result in a delay of testing, especially if the volume of testing is not sufficiently high to permit testing daily. Similarly, sending samples to reference laboratories can result in longer-than-desirable turnaround times to receiving results. In this study, we evaluated the use of the Luminex MultiCode® PCR primers on the ARIES® real-time PCR platform to determine its applicability for performing quantitative PCR analysis, specifically for quantification of BKV DNA in plasma samples. The ARIES® BKV test, utilizing primers based on MultiCode® technology, was compared to our standard method with BKV PCR primers and an MGB probe from ELITech. For the latter, analysis was performed on the Roche Cobas z480 real-time PCR platform. Unlike the z480 platform, the ARIES® instrument is not designed for qPCR analysis, so it is necessary to import the standard curve C_ts and patient data from the ARIES® printout into Excel for analysis. Since it would be neither convenient nor economically prudent to perform a standard curve each for each BK qPCR test run on the ARIES®, a standard curve was established using serial dilutions made from commercially sourced quantified BKV DNA, the results of which were stored and applied to successive test runs. The standard curves generated using both the ELITech and ARIES® methods were nearly identical in terms of both slope and R^2 values (Fig. 1). In addition, the slope of the standard curve generated on the ARIES® platform of −3.34 demonstrates an amplification efficiency of >99% indicating a high degree of robustness of this assay design (Svec et al., 2015). A new ARIES® standard curve was generated when there was a lot change in any of the test reagents (ARIES® test cartridges or MultiCode® primers).

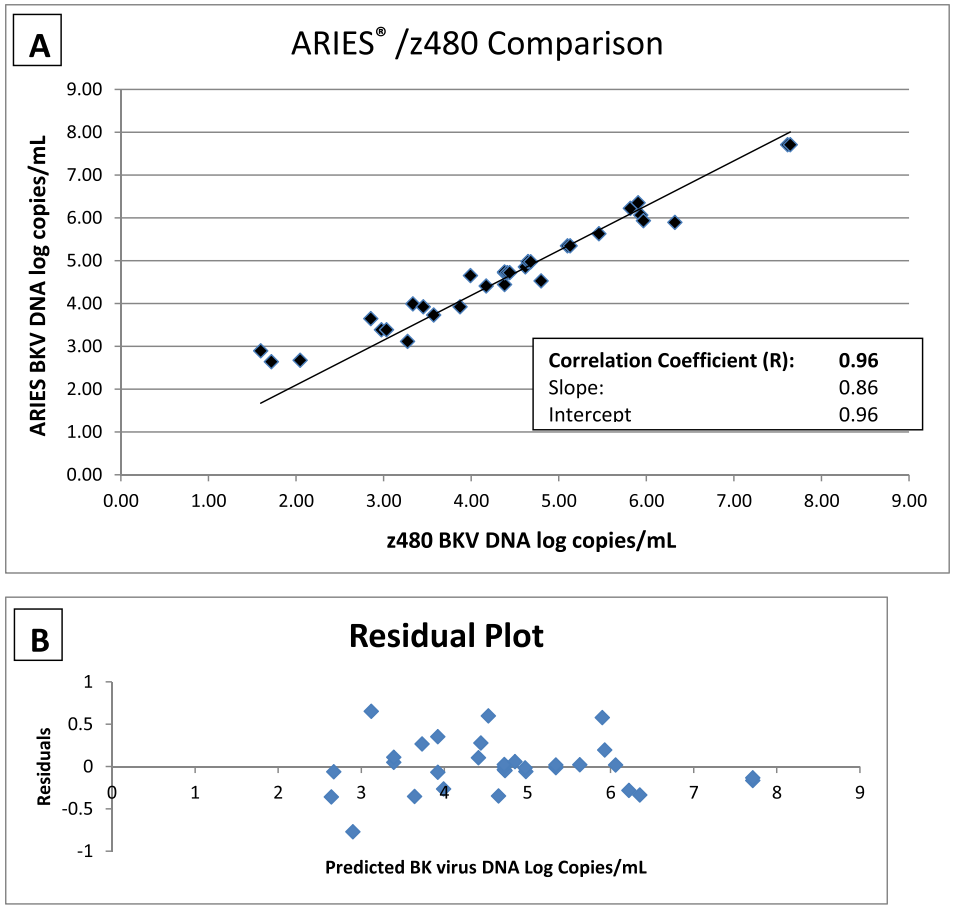


Fig. 2. ARIES® /z480 comparison: quantification of BKV DNA in clinical samples. (A) Excel generated linear regression analysis of data from Table 2. See text for experimental details (B) Residual plot generated from regression analysis in panel A to determine validity of the linear regression plot in panel A as well as assay bias.

Table 2
Comparison of testing with clinical samples between the ELITech and ARIES® qPCR assays.

Sample	MultiCode®/ARIES®		ELITech/z480		Δ log LOG difference ARIES®/z480
	Copies/mL	Log copies/mL	Copies/mL	Log copies/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	-
416826	111	2.05	473	2.67	−0.62
147329	254	2.4	ND	ND	-
497868	714	2.85	4410	3.64	−0.79
211229	1079	3.03	2427	3.39	−0.36
299629	1872	3.27	1309	3.12	0.15
299521	2149	3.33	9756	3.99	−0.66
162904	2831	3.45	8346	3.92	−0.47
417959	3729	3.57	5406	3.73	−0.16
162904	7428	3.87	8346	3.92	−0.05
596591	9785	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	−0.35
234871	25,675	4.41	52,497	4.72	−0.31
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	−0.24
355063	286,340	5.46	427,340	5.63	−0.17
597242	654,604	5.82	1,671,599	6.22	−0.4
473995	804,920	5.91	2,237,977	6.35	−0.44
551855	862,338	5.94	1,150,917	6.06	−0.12
40052	923,852	5.97	853,834	5.93	0.04
228172	2,112,027	6.32	787,062	5.9	0.42
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	-
293718	ND	ND	ND	ND	-
			Average Δ log copies/mL		**−0.3**

The robustness of this test design was clearly demonstrated by the results of the reproducibility experiment shown in Table 1. The log copy numbers of each of the samples in 3 different test runs resulted in low percent coefficients of variation demonstrating very low variability from run to run.

Comparison of testing with clinical samples between the ELITech and ARIES® qPCR assays demonstrated essentially equivalent results between the 2 methods. The average difference between log copies per mL generated by both methods for each sample was −0.3. Linear regression analysis of the 2 sets of results (Fig. 2) demonstrated 98% correlation between both methods. The high degree of correlation between results was somewhat surprising considering the large number of differences involved in the mechanics of performing both methodologies. The ELITech method on the z480 platform is entirely manual, including DNA extraction and assembly of the qPCR reactions. The ARIES® method is completely automated, including the DNA extraction and reaction setup processes. Another major difference is the qPCR chemistries used for both methods. The ELITech method utilizes TaqMan PCR technology and measures an increase in fluorescence with each PCR cycle. Due to its unique chemistry, MultiCode® technology relies on loss of fluorescent signal per amplification cycle (Sherrill et al., 2004).

The high degree of correlation between both methods —and lack of bias—is important since it negates the need to rebaseline our kidney transplant population that has been previously tested with the ELITech methodology when switching to the ARIES® BKV qPCR test.

In summary, our results clearly demonstrate that BKV quantification results were closely matched between the 2 different methods described above. The workflow with the ARIES® System is greatly simplified due to elimination of DNA extraction and most hands-on steps. The high degree of automation allows samples to be tested as they arrive in the laboratory, resulting in enhanced patient care due to more timely turnaround time for results back to the ordering physician.

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