

## PHYSICIAN UPDATE

# BK Virus Detection and Viral Load Testing by Real-Time PCR Now Available

### BENEFITS

- Useful to exclude a diagnosis of BKVAN (BKV associated renal allograft nephropathy) and to monitor the course of BKV infection.<sup>1</sup>
- Prospective monitoring of patients at risk for BKVAN can identify those with active BKV infections before the development of nephropathy and deterioration of renal function.
- Shown to have both very high precision and reproducibility.

### SUMMARY

Effective February 1, 2012, PeaceHealth Laboratories will offer quantitative BK virus (BKV) testing of urine, plasma and whole blood at the laboratory in Springfield, Oregon.

BKV is a common infection in early childhood with seroprevalence ranging from 60–100% in adults.<sup>2</sup> Following primary infection, BKV establishes latency in the urogenital tract, but clinical disease is rare in the immunocompetent adult.

BKV may reactivate spontaneously or in an immunocompromised patient where infections are most commonly described causing disease in

patients with hematologic malignancies and in renal transplant recipients.

BKV reactivation with urinary shedding occurs in 10-60% of renal transplant patients. BKVAN is a major cause of renal allograft dysfunction in 1–10% of recipients.<sup>3</sup> Hemorrhagic cystitis is more commonly described in hematopoietic stem cell transplant (HSCT) patients infected with BKV.

It has been demonstrated that measurement of BKV DNA in urine and blood by quantitative real-time PCR is useful to exclude a diagnosis of BKVAN and to monitor the course of BKV infection.<sup>1</sup>

Prospective monitoring of patients at risk for BKVAN can identify patients with active BKV infections before the development of nephropathy and deterioration of renal function. The indications to perform this assay include the detection and monitoring of patients post-transplantation for changes in the level of BKV DNA in blood and urine during disease progression or treatment.

### DETECTION AND VIRAL LOAD TESTING

#### Methodology

PeaceHealth Laboratories performed an extensive analytical verification and clinical validation process for the introduction of this

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laboratory-developed test. The real-time PCR assay targets conserved regions of the genes for the major capsid protein and the large T-antigen genes of BKV to provide optimal amplification of the currently known BKV subtypes.<sup>4</sup>

The real-time quantitative BKV PCR assay has been shown to have both very high precision and reproducibility. This assay has a dynamic range that permits the quantitation of BKV, with acceptable precision and linearity, between 2,000 and 1,250,000 copies/mL. The limit of quantification for this DNA assay is 3.3 log copies/mL (2,000 copies/mL).

### Urine or Blood to Monitor for BKV Associated Renal Allograft Nephropathy

Quantitative assays for BKV in urine and blood are used as both screening and diagnostic tests for BKVAN in renal transplant recipients. Prospective studies of renal allograft patients have demonstrated that high levels of BKV viruria (virus in the urine) precede sustained viremia (virus in the blood), which in turn precedes evidence of allograft dysfunction.<sup>5</sup>

The detection of clinically silent viruria allows for the early identification of patients that have been infected with BKV. The presence of virus circulating in the blood is also associated with active nephropathy because BKV enters the circulation through peritubular capillaries following tubular damage.

Unlike the role of urinary BKV viral load in the early stages of BKVAN management, detection and quantification of BKV DNA in blood is very

specific for BKVAN and is commonly used to monitor progression of active disease.<sup>7</sup>

### Thresholds for Intervention

There are no clear threshold levels for urinary viral load that can predict viremia and no universal viruria and viremia cutoff values for BKVAN.<sup>3</sup>

Urine specimens may have very high copy viral loads of BKV DNA and may overlap between symptomatic and asymptomatic patients. Consequently, urinary viral load of more than  $10^7$  copies/mL has now been proposed to be a significant risk factor for BKVAN.<sup>6</sup> A rising titer of several log orders can also be of clinical significance.

In 2005, an international consensus panel proposed a plasma or serum titer of  $>10,000$  copies/mL to be a significant marker of BKVAN with a specificity of 93%.<sup>3,4</sup> It has been suggested that when assessing the risk for BKVAN that some factors in particular should be taken into consideration:<sup>1</sup>

- plasma viral loads of more than 5,000 copies/mL indicate a greater risk for disease
- serial monitoring of viral load is preferable and can provide early detection of active BKV replication
- a viral load increase of less than 5- to 10-fold may not be clinically significant

## Renal Allograft Biopsy for BK Inclusions Required – Definitive Diagnosis BKVAN

A definite diagnosis of BKVAN requires the demonstration of BKV inclusions in tubular and glomerular epithelial cells in renal allograft biopsy specimens.

Because of the focal nature of BKV replication in the kidney, negative biopsy results cannot rule out BKVAN. Molecular techniques such as PCR can help avoid false-negatives caused by biopsy sampling errors.<sup>1</sup>

The diagnosis of BKV infection should not rely solely upon the result of PCR testing. A positive PCR result should be considered in conjunction with clinical presentation and additional established clinical testing. A negative PCR result indicates an absence of BKV DNA in the specimen that was tested and does not exclude the diagnosis of a BKV infection.

## QUESTIONS?

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### ORDERING INFORMATION

<b>58140</b>	BKV Quantitative PCR, Plasma
<b>58142</b>	BKV Quantitative PCR, Urine
<b>58144</b>	BKV Quantitative PCR, Whole Blood
<b>58146</b>	BKV Qualitative Detection by PCR
<b>Methodology:</b>	Real-Time PCR
<b>Performed:</b>	Monday, Wednesday, Saturday
<b>Reported:</b>	Same day as tested
<b>CPT Code:</b>	87799

### SPECIMEN REQUIREMENTS

<b>Collect:</b>	One 4 mL lavender (EDTA) tube or urine in a sterile container.
<b>Handling:</b>	Whole Blood: Do not freeze. Ship refrigerated at 4-8°C. Plasma: Centrifuge and separate plasma from cells within 6 hours and pour into a plastic vial. Refrigerate. Freeze at -20°C if specimen cannot be assayed within 3 days. Urine: Do not ship ambient or refrigerated. Freeze urine immediately at -20°C.
<b>Stability:</b>	Ambient: 24 hours for whole blood and plasma Refrigerated: 3 days for whole blood and plasma, 24 hours for urine. Frozen: 2 weeks plasma and urine
<b>Standard Volume:</b>	1 mL plasma, whole blood or urine
<b>Transport:</b>	Whole Blood: Refrigerated Plasma: Refrigerated or Frozen Urine: Frozen
<b>Rejection Criteria:</b>	Non-sterile or leaking container, amniotic fluid, ocular fluid, bone marrow, BAL or biopsy tissue, heparinized, frozen, hemolyzed or clotted whole blood.
<b>Reference Ranges:</b>	Not detected