BKV, Quantitative- PCR / real-time PCR

Use: For detection of BKV DNA; early detection will help in pre-emptive de-escalation of immunosuppressive therapy and prevent the disease progression. Quantitative BKV DNA PCR testing provides a "viral load" value useful to identify the BKV as the aetiological agent and for follow up.

Clinical Significance: BK virus is a non-enveloped Polyomavirus virus which commonly causes infection during childhood. The primary infection is usually asymptomatic or mild and the virus will be latent in urothelium. Reactivation can occur in immunocompromised patients especially in renal transplant population. BKV associated nephropathy can occur and progressive illness may lead to ultimate graft failure and graft loss.

Assay Availability: Batch testing on every **Wednesday. Samples should reach the laboratory by 09.00am of the test date.**

Results Reported (Turnaround time): 3-7 days

Specimen: Whole blood: (EDTA K3) commercial tube (in-house tubes not accepted)
Urine – in a screw cap plastic bottle (standard plastic urine culture bottle)

Volume: 2 ml (up to marked level of the EDTA tube)
5 ml urine in a sterile bottle

Storage: Transport in ICE on the same day. If cannot transport immediately, store the sample refrigerated for 72 hours. Avoid freezing

Detail clinical history and BKV specific form is MANDATORY for interpretation and selecting for the test. Specific BKV form can be downloaded at MRI website.

Incomplete forms or forms that does not accompany will be rejected.

Specimens should be labeled with patient name, BHT number, date of collection, and then sent at 4^0C temperature to the laboratory.

Specimens used for testing in other departments/laboratories are unacceptable due to possible contamination.

Causes for Rejection:

- Heparinized blood specimen
- Specimens in 'glass **penicillin** bottle'
- Quantity not sufficient for analysis
- Specimen grossly contaminated; specimen too old
- Frozen whole blood specimen
- Specimen leaky or tube broken
- Incompletely filled request forms

Methodology:

Extraction of BKV DNA from serum is followed by amplification and detection using polymerase chain reaction (PCR). The assay detects the presence of BKV DNA by amplifying viral genomic DNA. An internal control is added to each sample to assure that the extraction was performed correctly and to ensure that the PCR reaction was not inhibited. Four standards (Quantification Standards- QS1-1.00E+04, QS2-1.00E+03, QS3-1.00E+02, QS4-1.00E+01) are run in each test.

Absolute Quantification: is determined by plotting a standard curve using above standards.

Verification: is performed using below mentioned Control Parameters of the standard curve

Results are expressed in BKV DNA Iu/mL.

Calibrated against 1st WHO International Standard for BK Virus for Nucleic Acid Amplification Techniques (NAT) (NIBSC code 14/212)

Platform: Realtime ABI PCR system

Assay: Current assay is an in-vitro diagnostic (IVD) and CE certificated commercial assay manufactured in Germany (GmBh)

Normal Range: BKV is detectable in blood during reactivation/ recent infection. Spontaneous reactivation of in immunocompetent person can occur and usually gives rise to shedding of virus in urine (viruria). Immunosuppressed patients may have stable low viral loads in the absence of disease. Increasing viral load over time suggests progression of active disease.

Reportable Range:

1.00E+09 to 1.00E+00 IU/µl

Interpretative Data: As with any diagnostic test, this result should be interpreted in consideration of all clinical findings and laboratory investigations.

Significant Viruria > 10^7 IU / mL in urine

Significant viraemia > 10^4 IU /mL in blood[1]

Not significant –low copy numbers of BKV

Virus not detected - negative for BKV DNA (however does not rule out disease)

Laboratory Contact: For further information, please call the Department of Virology BKV Laboratory at 011-2693532-4 Ext 452 (virology reception) 463 (Medical Officer)

Reference:

1. Viscount, H.B., Eid, A.J., Espy, M.J., Griffin, M.D., Thomsen, K.M., Harmsen, W.S.,Razonable &R.R., Smith, T.F. (2007) Polyomavirus polymerase chain reaction as a surrogate marker of polyomavirus-associated nephropathy. Transplantation **84**:3; 340–345.