BK Virus Viral Load by Real Time PCR

BACKGROUND:

The Molecular Diagnostics Laboratory at The University of Toledo Medical Center performs a real time PCR assay for detection and quantification of BK viral load in plasma and urine specimens. The assay has a lower limit of detection and quantitation of 500 copies/mL, and an upper limit of quantitation of 1 X 10^{10} copies/mL.

This assay is useful serial monitoring in patients that are at risk for development of BK virus associated nephropathy (BKVAN) and to follow response to therapy. Patients who have >10^4 copies/mL BKV in plasma or >10^7 copies/mL BKV in urine have been shown to be at risk for development of BKVAN, although serial monitoring may be more useful than detection of a single time point. It may also be useful for monitoring bone marrow transplant patients who are at risk for hemorrhagic cystitis. As with other BK virus assays, because of the sequence similarity of JC virus with BK virus, high concentrations of JC virus (10^{11}copies/mL) could produce a low, false-positive result with this assay, but these levels are unlikely to occur in any clinical situation. Rarely, false negative results might possibly occur with inhibitors in patient samples, but this is monitored in each sample by utilization of an internal control and a DNA extraction method that removes inhibitors. It is suggested that patients monitored by another assay be retested by this new assay to establish an accurate baseline viral load for subsequent monitoring.

METHOD:

BK viral load in patient samples is determined by rapid, real-time, quantitative polymerase chain reaction (PCR). This assay has been developed and validated by our laboratory for use on the Abbott m2000 System. Oligonucleotide primers targeting a conserved sequence within the VP1 locus of the BK viral genome are utilized for PCR amplification and the target is then detected through the use of a fluorescent-labeled oligonucleotide probe that is specific for the amplified region. Serial dilutions of a known quantity of intact BK virus are used to calculate the copies per mL of BK virus in the patient's sample by interpolation from a standard curve.

SAMPLE REQUIREMENTS: 1) peripheral blood collected in a lavender top tube; 2) separated plasma; 3) separated serum; 4) urine ***Note: ACD tubes or samples anticoagulated with heparin are not acceptable**

RESULTS REPORTING:

ASSAY QUANTITATIVE RESULT	REPORTED RESULT
0 copies/mL	**Not Detected**
less than 500 copies/mL	**detected but <500 copies/mL** **detected but <log 2.70 copies/mL**
500 copies/mL to 1 X 10^{10} copies/mL	**calculated number in copies/mL** **calculated number in log copies/mL**
greater than 1 X 10^{10} copies/mL	**> 10,000,000,000 copies/mL** **> log 10.00 copies/mL**

MAXIMUM TURN AROUND TIME: 7 days

REFERENCES: 1) Luo C, et. al. Journal of Medical Virology 80:1850–1857 (2008), 2) Boothpur R, et. al. Journal of Clinical Virology 47:306–312 (2010)

For any questions regarding BK viral load testing, please contact the Molecular Diagnostics laboratory at 5636 or the director at 6444. Further information can be found on the Molecular Diagnostics web site at: http://www.utoledo.edu/med/depts/path/moldx/index.html