LAB NEWS

From the Department of Laboratory Medicine - Yale-New Haven Hospital Medical Center

Clinical Virology Laboratory Newsletter

Vol. 14 (4) **June 2005**

Quantitative BK Virus DNA PCR for Diagnosis in Compromised Hosts

Human polyomaviruses BK and JC infect 80-100% of individuals in childhood, establishing subclinical and persistent infection predominantly in the kidney. Although asymptomatic reactivation with virus shedding in urine is common in pregnancy, older age groups, and immunosuppressed states, overt disease is rare and appears to be related to the degree of immunosuppression.

In compromised hosts, BK virus (BKV) primarily causes disease in the urinary tract. BKV was first isolated from a renal transplant recipient (initials B.K.) with ureteral stenosis, but has since been associated with hemorrhagic cystitis in bone marrow and stem cell transplant recipients, and with interstitial nephritis predominantly in renal transplant recipients. Risk factors for BKV virus-associated nephropathy are not known, but newer, more potent immunosuppressive agents may play a role. Effective treatment requires reduction in immunosuppression and/or a change in immunosuppressive drugs. Although cidofovir has antiviral activity, it is nephrotoxic. Disseminated disease due to BKV has been reported in patients with AIDS, CLL, and congenital immunodeficiency, in whom immunosuppression could not be reduced.

BKV-associated nephropathy results in a decline in renal function. Definitive diagnosis is made by documenting characteristic pathologic changes on renal biopsy, followed by EM or immunohistochemistry. Recently, PCR to detect and quantitate BKV DNA in plasma has been promoted as a non-invasive way to identify patients at risk for BK nephropathy and to monitor response to therapy (1-4). Detection of BKV in plasma has a stronger correlation with disease than BKV in urine. A similar rationale has been recently applied to hematopoietic transplant patients with hemorrhagic cystitis, but less supporting data is available (5,6).

Laboratory diagnosis at YNHH:

1) **Quantitative BKV PCR on plasma**: Plasma can be tested in renal transplant patients to assess risk for BKV nephropathy. Please note the following;
    a. Viral loads of $> 5 \times 10^3$ copies/ml of plasma indicate greater risk for disease.
    b. Some patients with high viral loads in plasma do not develop renal disease.
    c. Serial monitoring of viral load is preferred rather than a single sample.
    d. Variations of less than 5-10 fold may not be clinically significant.
    e. A decline in renal function should prompt renal biopsy to rule out rejection, as well as to confirm pathologic changes of BKV.
2) **Quantitative BKV PCR on urine**: Urine can be tested in parallel with plasma for BKV nephropathy, and is still the standard sample to test in hemorrhagic cystitis. Viral loads of $>10^7$ copies/ml or urine are more likely associated with disease.

Note discontinued test: Rapid BKV culture provides qualitative results in 2-3 days. Rapid culture detects only high titers of infectious virus in urine ($>10^7$ copies/ml), however such high titers correlate fairly well with clinical disease. This test will now be replaced at YNHH by PCR.

Benefits of quantitative PCR: The main benefits of PCR are faster time to result, ability to monitor viral load and thus response to therapy, and ability to test plasma. The negative predictive value of a negative BKV PCR in urine is very high.

Pitfalls of quantitative PCR: 1) Although higher viral loads in plasma and urine have a greater positive predictive value for disease, there is no precise viral load cut-off. 2) Variations in sample types, quantitative standards, gene targets and test protocols make comparisons of viral loads among different laboratories impossible without direct comparative studies. 3) Mutations in primer or probe binding sites can lead to under quantification or even false-negative results. Therefore if suspicion remains high, the Virology Laboratory should be notified and an alternative test method (e.g. rapid culture or a different PCR) for the suspected virus should be employed.

Sample requirements: 1) 3-6 ml EDTA blood (lavender tube), received in Virology within 2-3 hrs of collection; 2) 2-5 ml urine

Test method: Real-time TaqMan PCR targeting BKV large T antigen gene. Test protocol developed at NIH (7) and validated at YNHH.
Test Availability: Once a day, Monday-Friday, if sample received by 8 AM.
Time to result: Generally within one working day for preliminary qualitative results, excluding weekends and holidays when staffing is limited. Quantitation of positive samples will be done once a week.

References

1. Hirsch H et al. Testing for polyomavirus type BK DNA in plasma to identify renal-allograft recipients with viral nephropathy. N Engl J Med 342:1309-15, 2000.
2. Limaye A, et al. Quantitation of BK virus load in serum for the diagnosis of BK virus-associated nephropathy in renal transplant recipients. J Infect Dis 183:1669-72, 2001.
3. Hirsch H et al. Prospective study of polyomavirus type BK replication and nephropathy in renal transplant recipients. N Engl J Med 347:488-96, 2002.
4. Randhawa P et al. Correlates of quantitative measurement of BK polyomavirus (BKV) DNA with clinical course of BKV infection in renal transplant recipients. J Clin Microbiol 42:1176-80, 2004.
5. Erard V, et al. BK virus infection in hematopoietic stem cell transplant recipients: frequency, risk factors, and association with postengraftment hemorrhagic cystitis. Clin Infect Dis 39:1861-5, 2004.
6. Bogdanovic G et al. Association between a high BK virus load in urine samples of patients with graft-versus-host disease and development of hemorrhagic cystitis after hematopoietic stem cell transplantation. J Clin Microbiol 42:5394-6, 2004.
7. Ryschkewitsch C, et al. Comparison of PCR-Southern hybridization and quantitative real time PCR for the detection of JC and BK viral nucleotide sequences in urine and cerebrospinal fluid. J Virol Methods 121:217-21, 2004.

UPDATE in Respiratory Viruses Detected: October 2004 to April 2005

Virus	Oct	Nov	Dec	Jan	Feb	Mar	April	Total Positive
RSV[a]	4	43	180	203	139	80	22	671
Influenza A[a]	1	4	99	374	214	50	4	746
Influenza B[a]	0	2	0	16	67	140	42	267
Parainfluenza[a]	8	13	19	21	20	22	20	123
Adenovirus[a]	1	11	10	5	5	11	7	50
Metapneumovirus[b]	n.d.	n.d.	2	0	2	10	20	34
Total Positive	14	73	310	619	447	313	115	1891

a, Direct fluorescence antibody (DFA); 7670 samples tested by respiratory DFA from October to April
b, RT-PCR; 163 samples were tested by RT-PCR for human metapneumovirus (HMPV)
n.d., not done

Questions or comments: Call Marie L. Landry, M.D., Laboratory Director, at 688-3475, or David Ferguson, Laboratory Manager, Clinical Virology Laboratory at 688-3524.