



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

Clinical Virology Laboratory Newsletter

Vol. 21 (1)

June 2012

Epstein Barr Virus (EBV) Viral Load: Use and Interpretation

Transfer to the Virology Laboratory: On July 2, 2012, Epstein-Barr virus (EBV) viral load testing will be transferred to the Virology Laboratory from the Molecular Diagnostic Laboratory at YNHH. For over 10 years, the test has been performed on isolated and enumerated peripheral blood leukocytes, which is very labor intensive, and results have been reported as genome equivalents per 100,000 leukocytes.

In the Virology Laboratory, a different EBV real-time PCR assay is used (1, 2) and the plasma compartment is tested similar to viral load assays performed for CMV, BKV, adenovirus and HHV-6. Since EBV persists in B cells for the lifetime of the host, latent virus can be detected in leukocytes. Testing plasma detects only actively replicating virus released from cells, correlates better with clinical disease, allows testing on leukopenic patients, and may provide more accurate results in patients treated with rituximab (3).

EBV viral load measurement: EBV viral load determination has been used for the prevention, early detection and monitoring of EBV-associated posttransplant lymphoproliferative disorder (PTLD). A variety of methods, gene targets, and blood compartments (leukocytes, whole blood, plasma, serum) have been used. Inter-laboratory variability in results is considerable (100-10,000 fold differences), making comparisons across institutions difficult (4). Prospective studies correlating specific viral loads to risk of PTLD have been inconsistent, especially in adult seropositive recipients, and there are no clear guidelines (5). Unfortunately, while EBV viral load has become easy to measure, results are often difficult to interpret.

In general, only high-risk transplant recipients (EBV+ donor / EBV- recipient) are prospectively monitored by EBV PCR during the first year after transplant and during periods of acute rejection (6). Since infection is almost universal by age 30, high-risk patients tend to be children, adolescents or young adults.

Indications for plasma viral load at YNHH: 1) Monitor high-risk patients during first year posttransplant and at times of antirejection therapy. 2) Monitor response of EBV associated-tumors to therapy and as indicator of relapse. 3) Diagnose atypical or early primary EBV, or when serology is non-diagnostic.

EBV PCR in Virology: Since 2005, the Virology Laboratory has performed real-time EBV PCR for the qualitative detection of EBV in CSF and is now offering the same assay as a quantitative test (see **Appendix** for additional info). This EBV PCR test was originally developed as a quantitative plasma PCR test for transplant patients (1,2).

- Testing requires 3 ml of EDTA blood (lavender top tube) and is performed once a day, Monday through Friday.
- Results are reported as both copies and log₁₀/mL plasma, with a linear range of 10³ to 10⁸ copies/mL.

Result interpretation: Detection of EBV DNA in plasma indicates active replication of virus, rather than latent infection. The significance of absolute levels will vary with the patient, risk factors and degree of immune suppression. **Rapidly rising titers are of greatest concern for impending disease.** Normal adults will have no detectable EBV DNA in plasma. In infectious mono, EBV is only detectable in plasma for about 1-2 weeks after onset of symptoms, but persists in leukocytes for life.

Diagnosis of EBV CNS lymphoma. EBV-associated CNS lymphoma patients may have negative or low EBV viral load in blood, but high titers in CSF. Qualitative EBV PCR is used to screen CSF and if positive, a viral load can be obtained. Positive predictive value for lymphoma correlates with compatible MRI findings, low CSF lymphocytosis, and high EBV viral load in CSF. In contrast, a high CSF lymphocytosis with a low EBV viral load usually does not correlate with EBV disease.

For questions or concerns, call Marie L. Landry, MD, the Virology Laboratory Director at 688-3475 (marie.landry@yale.edu), or David Ferguson, the Laboratory Manager at 688-3524.

References

1. Niesters HGM, et al. Development of a real-time quantitative assay for detection of Epstein-Barr virus. J Clin Microbiol 38:712-715, 2000

- van Esser JW, Niesters HG, et al. Molecular quantification of viral load in plasma allows for fast and accurate prediction of response to therapy of Epstein-Barr virus-associated lymphoproliferative disease after allogeneic stem cell transplantation. *Brit J Haematol.* 2001, 113:814-21.
- Gartner B and Preiksaitis JK. EBV viral load detection in clinical virology. *J. Clin Virol* 48:82-90, 2010.
- Preiksaitis JK, et al; Interlaboratory comparison of Epstein-Barr virus viral load assays. *Am J Transplant.* 2009, 9:269-79.
- Tsai DE, et al. EBV PCR in the diagnosis and monitoring of posttransplant lymphoproliferative disorder: results of a two-arm prospective trial. *Am J Transplant.* 2008, 8:1016-24.
- Kasiske BL, et al; Kidney Disease: Improving Global Outcomes. KDIGO clinical practice guideline for the care of kidney transplant recipients: a summary. *Kidney Int.* 2010, 77:299-311.

APPENDIX:

I. Clinical Disease: Primary EBV: EBV infection is universal. In lower socioeconomic groups, infection is acquired in early childhood and is often subclinical. In higher socioeconomic groups, infection is often delayed until adolescence or young adulthood and is commonly manifested by infectious mononucleosis (IM), characterized by fever, pharyngitis, lymphadenopathy and “atypical” peripheral blood lymphocytes. EBV infects and immortalizes B lymphocytes and I.M. symptoms are due to the vigorous T cell response (the “atypical” lymphocytes) called forth to control the proliferating EBV-transformed B cells. Primary EBV manifestations can also include hepatosplenomegaly, hepatitis, hemolytic anemia, airway obstruction, splenic rupture, pneumonia, meningitis, encephalitis, and rarely hemophagocytic syndrome. **Serology remains the mainstay for diagnosis of acute primary EBV infection.** *PCR should be done only for very early or atypical cases or if serology is non-diagnostic.*

EBV and malignancy: EBV is associated with a variety of neoplasms in normal hosts, such as Burkitt lymphoma, Hodgkin lymphoma, T and/or NK cell lymphoma, nasopharyngeal carcinoma, gastric carcinoma, and leiomyosarcoma. In addition, EBV has been associated with lymphomas in immunosuppressed patients including AIDS patients, transplant recipients (PTLD), and congenital immunodeficiencies such as X-linked lymphoproliferative disease (XLP).

II. EBV viral load at YNHH: Past Results at YNHH using leukocyte-based assay: At YNHH, essentially all cases of PTLD have been diagnosed *after presentation with clinical disease* and *not* by prospective monitoring of EBV viral load. In 13 cases of PTLD at YNHH in the past 7 years for which records were available (9 kidney, 3 liver, 1 heart), 3 were EBV-negative lymphomas. For the 10 EBV-positive PTLD, EBV viral loads at the time of tumor presentation ranged from 16 to 8620 genome equivalents/100,000 leukocytes, with a median of 705 g.e. Only 2 of 10 patients had high values over 2500 g.e. at presentation whereas 3 of 10 had the lowest values (5-50 g.e.). EBV-PTLD occurred at 7, 7, 11, 14, and 20 months, and 3, 6, 8, 12 and 14 years post-transplant. Patients were 10, 20, 28, 37, 45, 46, 56, 56, 61 and 63 years of age at presentation. Patients developing PTLD within 7-14 months were EBV seronegative at transplant.

Clinical validation studies for plasma assay in Virology:

1. Virology participated in an **international study of 28 transplant centers** that received 12 blinded plasma samples for EBV viral load testing, with results reported in the *American Journal of Transplantation* (4). Quantifications among labs testing the same samples varied by 100-10,000 fold. Virology results fell on the geometric mean of expected results.

2. Over a 5 month period in 2012, **Virology tested in parallel 170 plasma samples from 126 patients** that were also tested by the leukocyte-based assay in the Molecular Diagnostics Laboratory. Of 90 positive by the leukocyte assay, only 25 were positive in plasma. None of the leukocyte-based assay positives that were negative by the plasma assay had EBV disease. The plasma assay detected the one patient with PTLD in this period (below). In one case of *acute primary* EBV, the leukocyte based test result was 445 g.e./100,000 cells, whereas the plasma assay detected 43,972 EBV DNA copies/mL plasma.

3. A subset of **samples with discrepant results** between the plasma and leukocyte-based tests were sent to the University of Washington (UWASH) Clinical Virology Laboratory, which also serves the Fred Hutchinson Cancer Research Center in Seattle. UWASH uses a different lab-developed PCR test, tests plasma not leukocytes, and publishes widely on EBV in both solid organ and hematopoietic transplant patients. UWASH results matched our plasma assay results.

4. The **only current PTLD patient** available for comparative study in 2012 using the two EBV assays is a young adult who was EBV seronegative prior to transplant and who presented 14 months after transplant with a mass in his kidney. In this one case, the plasma EBV viral load assay was superior to the leukocyte-based assay, showing rising titers prior to diagnosis, and correlating well with disease and response to therapy (see table below).

Sample date	Genome equivalents/ 100,000 leukocytes	Plasma copies/mL	Plasma log ₁₀ /mL*	Comment
12/15/11	16	1407	3.15	Prior to disease diagnosis (routine screen)
12/16/11	16	3079	3.49	
2/21/12		5465	3.74	
3/8/12	14	8011	3.90	Presented with mass in transplanted kidney; biopsy confirmed PTLD
3/16/12	14	15,129	4.18	
4/4/12	14	17,081	4.23	
4/10/12		28,256	4.45	Rituxan started 4/10/12
4/23/12		1311	3.12	CHOP started 4/27/12
5/11/12	<5	Not detected	Not detected	

*Note: In general, a change of ≥ 0.5 log₁₀ copies/mL is considered a significant change.