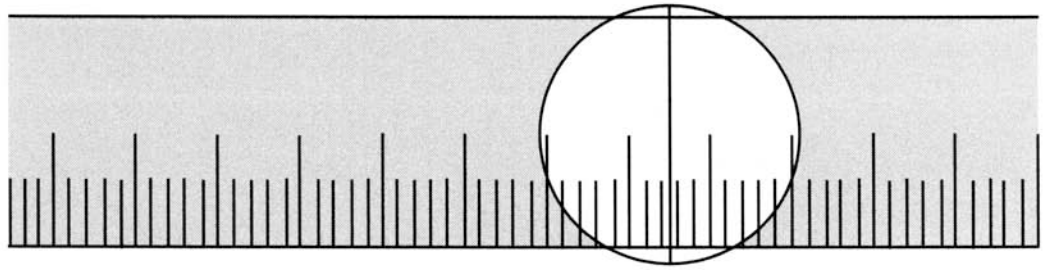


# LAB NEWS



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

## Clinical Virology Laboratory Newsletter

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### CMV Viral Load: PCR to Replace Antigenemia at YNH

CMV pp65 antigenemia, detected by immunostain of peripheral blood leukocytes, was the first clinically available test to measure viral load in blood and was implemented at YNH in 1992 (1). Antigenemia is more sensitive, flexible, cheaper and faster than the commercial Roche Amplicor CMV PCR or CMV DNA Hybrid Capture tests used in many centers (2). However, it is manual, labor-intensive test and cannot be readily “scaled up” beyond 15-20 per day.

In 2006, the Clinical Virology Laboratory introduced a “home-brew” real-time TaqMan CMV PCR viral load test developed at Vanderbilt University. CMV DNA PCR allows: testing of plasma in cases of leukopenia, delayed sample processing due to greater stability of DNA, and more automation to handle increased sample numbers. Until now, we have not converted from antigenemia to our in-house PCR due to 1) the risk of over-reaction to the higher copy numbers in CMV PCR, and 2) detection of many clinically irrelevant low level positives. Both can lead to unnecessary anti-CMV therapies, which can have deleterious side effects. In addition, time to result for quantitative PCR is longer than for antigenemia.

An additional concern is interpretation of CMV copy numbers due to the tremendous variation (2 log<sub>10</sub>) in quantitative results between laboratories (3). To address this, we have compared and validated our quantitative results via sample exchanges with Univ. of Washington, Seattle and in an international study of 37 transplant centers (3). In the report by Pang et al, our CMV results were virtually identical to the “expected results” (3).

Due to the success of our transplant programs and the increased demand for CMV viral load, the Virology Laboratory is compelled to convert to CMV PCR. Therefore, in **June-July 2009** (pending computer modifications) CMV antigenemia will no longer be orderable via the computer and CMV PCR will become our main CMV test. To aid in difficult cases\*, CMV antigenemia can still be obtained as a special request by calling the laboratory.

#### Availability of CMV PCR:

1. Submit blood, 2 lavender tubes. If received by **11 AM Mon-Fri** and **9 AM Sat**, results reported by 5 PM.
2. CMV DNA PCR will be done 6 days a week, Monday through Saturday.
3. Samples for CMV PCR must be processed within 24 hrs of collection (instead of 6 hrs for antigenemia).

#### Relationship between CMV Antigenemia and CMV DNA PCR

CMV pp65 protein in infected neutrophils and CMV virion DNA in plasma are different entities and thus their relationship is inconsistent. However, CMV pp65 antigenemia and CMV DNA PCR trend up and down together. *CMV genome copy numbers in plasma are much higher than the number of infected neutrophils.* Since our CMV PCR is more sensitive than antigenemia, PCR also will become positive earlier and stay positive longer.

#### SIMPLIFIED comparison of CMV antigenemia to PCR copy numbers at YNH (based on median values)

CMV pp65 Antigenemia	CMV PCR at YNH	Interpretation and relevance to pre-emptive treatment (details page 2)
0-1 antigen positive cell	<200 DNA copies/ml plasma	VERY LOW: Usually not significant <sup>a</sup>
1-10	200-1000 DNA copies/ml plasma	LOW: Usually not significant <sup>a</sup>
11-49 antigen positive cells	>1,000-5,000 DNA copies/ml plasma	INDETERMINATE: Can be significant in high risk patients. Clinical correlation and assessment of risk factors are needed.
≥50 antigen positive cells	>5,000-10,000 DNA copies/ml plasma	HIGH: Often significant

a, Except HSCT patients, until day 100 post-transplant (see page 2). Also CMV end organ disease (e.g. GI) can have negative/low viral load.

The following information is intended as a starting point to aid clinicians in interpretation of PCR results. The guidelines were compiled from multiple sources including YNHH, Vanderbilt University, Univ. of Washington-Seattle, Mt. Sinai NYC, and Univ. of Calgary, Alberta Canada (organizer of the international study in ref. 3), and adjusted for the different methods used. As we gain experience in the use of our PCR, these guidelines should be re-evaluated and modified.

#### A. Three Approaches to CMV Therapy

1. **Prophylactic treatment:** Give anti-CMV therapy to prevent CMV disease during periods of high risk.
2. **Pre-emptive treatment:** Monitor CMV viral load prospectively and treat when viral load reaches a level that has a reasonable predictive value for development of disease. This level will vary with the risk factors of the host (i.e. primary vs reactivation infection and degree of immunosuppression).
3. **Treatment of symptomatic CMV disease:** Test only when symptomatic and treat if active CMV is confirmed by diagnostic tests.

#### B. Overview of General Guidelines for Initiating Therapy in Different Hosts

Consultation with an infectious disease specialist is recommended when needed to correlate lab results with patient findings. Whether antigenemia or PCR, there are no absolute CMV viral load cut-offs for pre-emptive therapy because the viral load is only part of the story. Other factors in predicting CMV disease are the type of host, the host's immune competence, whether immune competence is improving or worsening, and whether the viral load is rising.

##### 1. Bone marrow/ stem cell transplant: Pre-emptive therapy (requires monitoring viral load)

- a. From conditioning until day 100, cut off levels for treatment vary from 100-500-1000 copies/ml depending on risk factors.
- b. Beyond day 100, cut off levels for treatment are 1000 copies/ml or rising levels (i.e. an increase to >5x baseline within 2 wk period). Protocol developed by Dr. M. Boeckh at Fred Hutchinson Cancer Research Center.

##### 2. Solid organ transplants: Prophylactic or pre-emptive therapy

a. **Prophylaxis** is given for first 90 days post-transplant for all adults, highest risk (D+/R-) children, and possibly other pediatric risk groups.

b. **Pre-emptive therapy** may be used at YNHH for some groups, such as D+/R+, D-/R+, or D-/R- children. If antiviral prophylaxis is not given, CMV viral load must be checked every 2 weeks for the first 90 days post-transplant. Current 2009 YNHH transplant protocol guidelines indicate that if the patient is viremic but **asymptomatic** and viral load is <2,500 copies/ml, liver transaminases and platelet count should be checked. If transaminases/platelet counts are normal and CMV load <2,500 copies/ml, testing may be repeated in 1 week. If tests are abnormal and/or CMV load >2,500 copies/ml, the patient should be treated. **The Infectious Disease Service should be consulted for guidance and management.**

c. **After the first 3 months**, CMV viral load is obtained if patient is symptomatic. Treatment is given if viral load or other test is CMV positive. If symptoms are uncertain (e.g. leukopenia only) and viral load is low, testing can be repeated in 1 week. See YNHH transplant program protocol for details.

##### 3. HIV patients: Treat symptomatic disease

- a. Perform CMV viral load if symptomatic disease
- b. Treat if i) CMV end organ disease confirmed by CMV diagnostic test; or ii) viral load rising rapidly or **>10,000 copies/ml** with compatible clinical symptoms. Clinical correlation is essential as low level viremia is common in AIDS patients.

#### Footnote:

*\*Examples of difficult cases for which antigenemia may be useful: clinical features strongly suggestive of CMV, but PCR negative or very low; aid decision to terminate antiviral therapy when PCR persists at very low level for prolonged periods.*

#### References

1. Landry, ML, Ferguson D. Comparison of quantitative cytomegalovirus antigenemia assay with culture methods and correlation with clinical disease. J Clin Microbiol 31:2851-2856, 1993.
2. Bankowski MJ, Belzer SW, Ferguson D, Bryndon D, Lembke BD, Landry ML. A comparison of cytomegalovirus (CMV) pp65 antigenemia levels with CMV viral load (Cobas Amplicor CMV Monitor) in plasma and cell pellets from transplant and other immunocompromised patients. Poster presentation, Clinical Virology Symposium, Clearwater Beach FL, April 2002.
3. Pang XL et al. Interlaboratory comparison of cytomegalovirus viral load assays. Am J Transplant. 9:258-68, 2009.

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