

UPMC Clinical Virology
Laboratory update August 2006
New PCR test for quantitation of cytomegalovirus

On 26 June 2006, the UPMC Clinical Virology Laboratory implemented a new quantitative polymerase chain reaction (PCR) test for monitoring cytomegalovirus (CMV) viral load in whole blood. This assay has been extensively field-tested by the laboratory and replaces the CMV pp65 antigenemia (Ag) test.

The new PCR test is superior to CMV antigenemia because (1) it requires less blood and can be reliably performed with blood that is up to three days old and/or with low leukocyte counts, (2) the PCR test offers increased sensitivity, resulting in detection of virus up to two weeks earlier than antigenemia, and (3) the new test gives faster turnaround times for reporting of results.

The new PCR test tracks extremely well with CMV antigenemia in longitudinal testing (see graphs below). Results from our field testing of over 3,000 bloods for both CMV antigenemia (Ag) and PCR show:

CMV pp65 Ag Results	No. of Samples	CMV US17 positive (%)	CMV US17 negative (%)
Negative	3163	195 (6.1)	2968 (93.9)
Positive	179	158 (88.3)	21 (11.7)
Total (%)	3342	353 (10.5)	2989

The following laboratory interpretation of CMV PCR results is recommended:

- Negative : < 15 CMV DNA copies/ml (Approx. CMV Ag results = 0)
- Low positive: 15-100 CMV DNA copies/ml (Approx. CMV Ag results ~ 0-10)
- High positive: >100 CMV DNA copies/ml (Approx. CMV Ag results ~ 10->200+)

The test requires 3 to 5 ml of whole blood collected in acid citrate dextrose (ACD) tubes (yellow top) and sent to the UPMC Clinical Virology Laboratory, Room A910, UPMC Presbyterian. The test can be ordered by using the code CNAT. Blood samples are accepted daily, with results reported Monday through Saturday.

For more information or a consultation, contact **Charles Rinaldo, PhD**, professor of pathology and assistant director, Clinical Microbiology Laboratory, at 412-647-3764, in house pager 6940, or rinaldocr@upmc.edu.

QUESTIONS AND ANSWERS

(1) The CMV Ag test was working well for many years. Why replace it?

The basic reasons why the PCR test is superior to the CMV Ag test are given above. With the new test: (a) You will be able to detect CMV in blood by PCR days to weeks before it can be detected the Ag test, thus allowing you to make important decisions on preemptive therapy; (b) You will be able to detect changes in levels of CMV essentially the same as you have been doing with the CMV Ag test, as these track extremely well; (c) You will be able to know with greater accuracy when to change or stop antiviral treatment.

(2) Why did you choose to use an in-house PCR test instead of a commercially available PCR test?

It is important to note that the UPMC Clinical Virology Laboratory has been a leader in the fields of CMV diagnosis and immunopathogenesis since the late 1970's. We developed and implemented improved diagnosis of CMV for the UPMC by long term cell cultures (1970s), CMV p72 Ag shell vial testing (1980s) and CMV pp65 Ag (1990s). In our quest to keep enhancing our diagnostic testing to improve patient care, we examined different commercial PCR assays for CMV DNA and RNA in multicenter studies, as well as assessed the newest, state-of-the-art, real time PCR testing for quantitation of CMV.

After 3 years of development and testing, we concluded that our real time PCR assay was the most sensitive and specific assay for CMV in blood. It also should be noted that there is no accepted "standard" for quantitative CMV DNA testing. However, our PCR format is the basic one used by the best clinical virology laboratories.

(3) I was not fully aware of this new test when it was placed on-line. How was this new test implemented?

We worked closely with clinicians in the UPMC Division of Infectious Diseases for 3 years in developing and implementing this test. As part of this process, we changed our anticoagulant from heparin (which inhibits the PCR reaction) to acid citrate dextrose 2 years ago. Because there is no accepted standard for PCR assay of CMV, we first established the best primers and probes for detection of a low number of copies of CMV DNA by the quantitative real-time (RT) PCR assay (i.e., ≥ 15 copies of CMV DNA), and that there was no cross-reaction with other herpesviruses. We then compared the PCR assay to the Ag assay on cryopreserved samples from longitudinal bloods that we had collected from UPMC organ transplant recipients. These results showed an extremely close tracking of the two assays (PCR and Ag), and that the PCR assay could detect CMV infection days to weeks earlier than Ag, and also for days after Ag turned negative (see data below).

We also field tested over 1,000 bloods in 2004-2005, and over 3,000 bloods in June of this year for both PCR and Ag, and found the PCR to be more sensitive (see Table above).

After careful analysis of these data with UPMC Infectious Disease clinicians, the consensus was that the PCR assay was superior to the Ag assay and was ready to be implemented. We alerted UPMC medical staff as follows:

- (a) In May of this year, we sent email letters with an alert about this new test to transplant surgeons at the UPMC Presbyterian Hospital, cancer specialists at Shadyside Hospital, and infectious disease clinicians at Children's Hospital and the VA Hospital.
- (b) We placed an alert about the new PCR test in the June UPMC Presbyterian Physicians Newsletter.
- (c) UPMC Infectious Disease clinicians gave personal explanations of the new test to UPMC transplant surgeons.

Unfortunately, some of you were not aware of this changeover, and we apologize for missing you in this outreach process.

(4) I have been confused by these new CMV PCR data. Is there a simple way to translate these results similar to our use of the Ag results?

We believe that these problems can be best addressed by close monitoring of the new results with the UPMC Infectious Diseases clinical staff and the UPMC Clinical Virology laboratory. A "cheat sheet" transformation for PCR and Ag is given above. Basically, with high risk transplant recipients (e.g., D+R-), the rule of thumb is to assume that PCR values over 15 are "positive", and that PCR values of 16-100 are roughly equivalent to Ag counts = 1-10.

Notably, it is expected that there will be some differences in these test results, in that these tests assess very different aspects of the virus replication cycle and pathogenesis. CMV Ag assesses the number of PMNs in the blood that stain positive for CMV pp65 matrix antigen. This includes some infectious virus but is mostly the detritus of CMV replication scavenged by the PMNs from other non-blood cells, as PMNs do not support CMV replication. The CMV PCR assay is detecting the number of copies of CMV DNA genome, both infectious and noninfectious, in whole blood.

(5) Why are you "normalizing" the results? Won't this make it difficult to compare our PCR results with PCR results from other, non-UPMC institutions?

We "spike" all of our blood specimens received for CMV testing with a known, equal amount of seal herpesvirus DNA. We process and quantitate this seal virus DNA in parallel with the human CMV DNA quantitation. This allows us to control for any variations in the assay, for example, inhibition of the PCR reaction in a particular blood sample. We use the seal virus data to transform the "raw" CMV DNA counts to give a more accurate result. We have found that this decreases the CMV DNA counts about

100-fold from the raw values. Since there is no industry-wide standard for CMV testing (either PCR or Ag), you should not assume exact comparability of CMV test results among different laboratories. Indeed, the Roche Amplicor PCR assay uses blood plasma for quantitation of CMV DNA, which contains very little infectious virus, while many clinical labs such as ours use whole blood in their PCR assays.

(6) What about the small percentage of blood samples that are PCR positive and pp65 Ag negative. Are these results specific?

Few diagnostic tests can completely rule out false positives. Our longitudinal analysis indicates that many of these results are due to higher sensitivity of PCR in detecting CMV DNA either before Ag is positive or after the Ag test becomes negative.

(6) What about the small percentage of blood samples that are PCR negative and pp65 Ag positive?

The PCR and Ag tests measure very different biological parameters of CMV infection and replication (see #4 above). Notably, the CMV Ag test relies on the judgment of the technician who is manually reading the immunofluorescence slide preparations, with little quality control. In contrast, the CMV RT PCR assay has several, highly stringent internal controls and standards, including our seal virus DNA control. Thus, we believe that PCR negative/Ag positive samples are a result of false positive Ag readings, or Ag positive cells that have minimal clinical significance. Thus, in the cross sectional results shown in the table above, of the 21 PCR negative / Ag positive blood specimens, two had 2 Ag positive cells, two had 4 Ag positive cells and the remaining 17 specimens had only 1 Ag positive cell.

(7) Are there any longitudinal results that show the comparative patterns of CMV PCR and Ag results in UPMC patients?

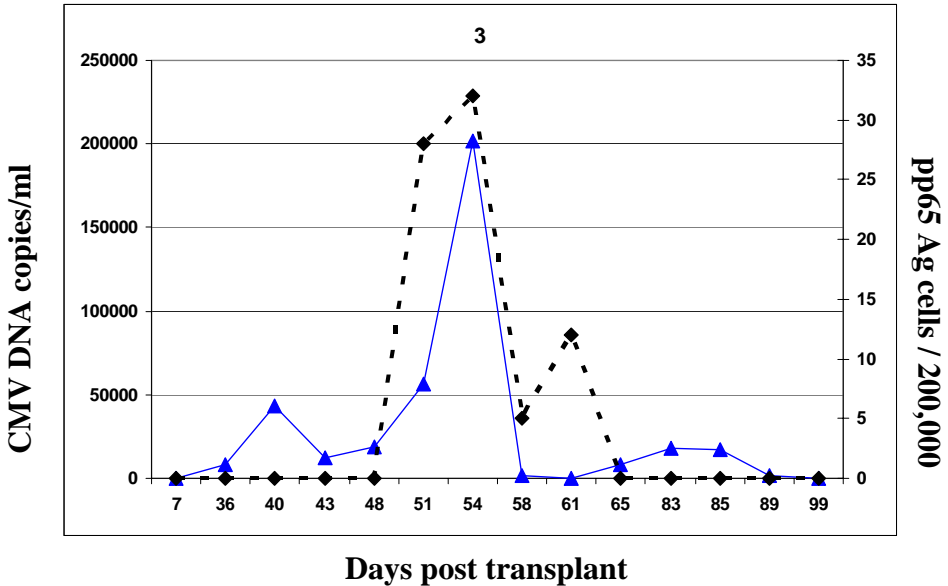
On the next series of pages, we show longitudinal results on CMV PCR and Ag from a group of UPMC transplant recipients. The data indicate a very close concordance between the two tests (US17 = PCR; pp65 Ag = Ag). Because of the greater sensitivity of the PCR assay, we see that the CMV PCR results appear positive prior to the CMV Ag results in some cases, and remain positive longer during antiviral drug therapy in some cases. Please note that based on our IRB exemption, we have not yet been able to add in the clinical outcomes. Also, because these tests were done on archived, cryopreserved blood samples, we could not “spike” them with our seal virus control (see #5 above). Thus, these are “raw” CMV DNA counts. Normalized results are approximately 100-fold less than these raw DNA counts. Also, note that the scales for the two tests are quite different.

3. Renal transplant D+/R-

Antiviral drug: Days post Transplant

GCV:	43 - 67	ACV:	0 - 42
	85 - 102		54 - 85

▲ — CMV US17
◆ - - CMV pp65 Ag

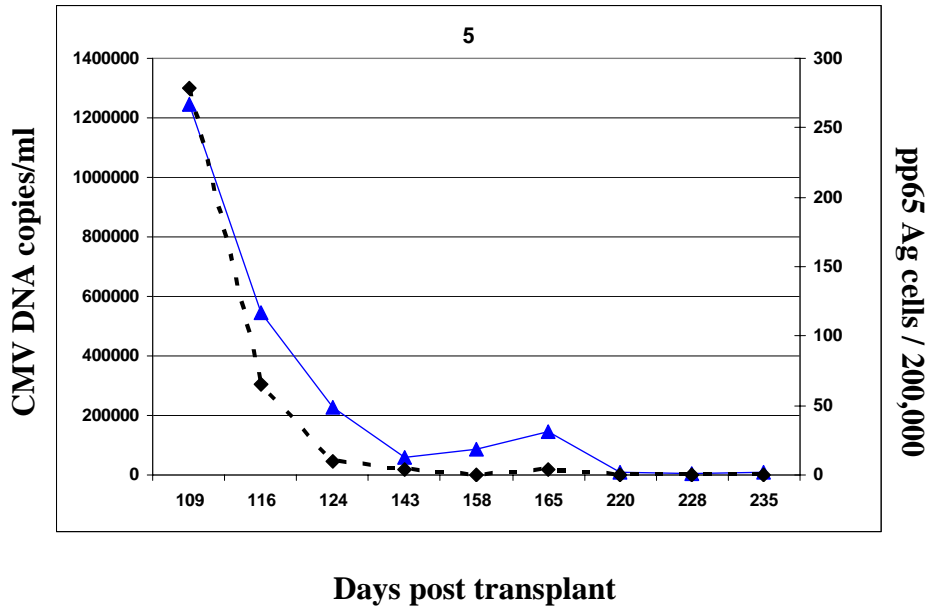


5. Renal transplant D+/R-

Antiviral drug: Days post Transplant

GCV: 40 - 88 ACV: 8 - 144
108 - 176

—▲— CMV US17
-◆- CMV pp65 Ag



6. Liver transplant D+/R-

Antiviral drug: Days post Transplant

GCV: 29 - 52
64 - 272 **ACV:** 25 - 47 **Cytogam:** 40 - 43
117 - 162 **Foscarnet:** 37 - 63

—▲— CMV US17
-◆- CMV pp65 Ag

