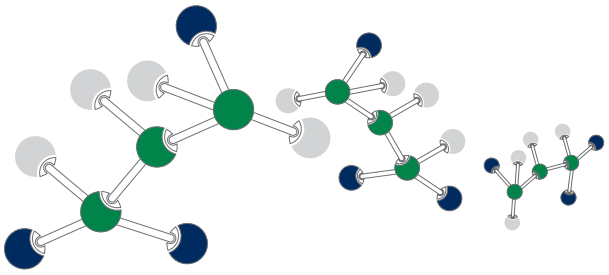

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CMV VIRAL LOAD TESTING

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What is Cytomegalovirus?

Cytomegalovirus (CMV) is a double-stranded DNA virus that belongs to the *Betaherpesvirinae* subfamily of the *Herpesviridae* family. CMV can infect and establish latency in a broad range of cell types, including epithelial cells, endothelial cells, polymorphonuclear cells, T lymphocytes, fibroblasts, monocytes and macrophages. The virus can be transmitted via saliva, urine, genital secretions, blood and blood products, and stem cell and solid organ transplantation. In pregnant women with primary CMV infection, the virus may be transmitted transplacentally to the foetus, with significant sequelae, including cognitive deficits, sensorineural hearing loss and vision impairment. The infant can also be infected via genital secretions during birth, or later in infancy via breastfeeding, but these seem to result in milder clinical illness. CMV disease ranges from asymptomatic infection in immunocompetent people to severe and life-threatening infections in neonates and immunocompromised patients. CMV disease can occur during primary infection, reactivation, or re-infection with a different strain.

What is CMV viral load testing?

The CMV viral load can help to diagnose CMV disease, assist with determining an asymptomatically-infected patient's risk of developing disease, and monitor response to therapy. CMV viral load testing measures the quantity of viral DNA circulating in the patient's blood. Real-time molecular assays are preferred due to their low limits of quantification and detection, their broad linear range, shorter turn-around-time, and diminished risk of sample contamination during processing. However, viral load testing is not without certain limitations that need to be kept in mind when interpreting these results.

Technical aspects of CMV viral load testing

All tests performed in the laboratory will be influenced by certain pre-analytical (patient characteristics, sample collection and transport), analytical (testing) and post-analytical (reporting and interpretation of results) considerations.

Pre-analytical considerations

The **sample type** analysed is an important pre-analytical variable. CMV viral load testing is most often performed on either whole blood or plasma. CMV DNA is detected more frequently and usually at higher viral load values in whole blood, as both intracellular and cell-free viruses are detected in this sample type. The clinical significance of low level CMV DNA detected in whole blood is not always clear, as CMV persists latently in the cellular compartment. Therefore the plasma viral load is thought to be more representative of active infection. The difference in CMV viral load values between whole blood and plasma can be as much as 2 $\log_{10}$ copies/mL (100-fold).

Analytical considerations

Until recently, the leading impediment to establishing universally accepted quantitative CMV DNA thresholds that can be used for clinical decision making, has been the **lack of agreement** between different laboratories using a diverse assortment of commercial and laboratory-developed assays. Factors that may contribute to this lack of uniformity include nucleic acid extraction method, CMV gene target selected, and reagents used. This was best illustrated in a large multi-centre study in 2009 involving 33 laboratories across North America and Europe, where the variability in CMV viral load values for individual samples ranged between 2.0 and 4.3 $\log_{10}$ copies/mL[4]. This led to the development of the First WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques (NIBSC 09/162) in 2010. Commercial manufacturers and laboratories can now recalibrate their assays against the WHO International Standard and report their results in International Units per millilitre (IU/mL), as is already done for Hepatitis B and C viral load testing, thus allowing for easier comparison between different laboratories and the establishment of universally accepted quantitative CMV DNA thresholds.

Three further performance characteristics of CMV DNA viral load assays that need to be considered, are the **limit of detection** (LOD), the upper and lower **limit of quantification** (LOQ), and the **precision** or reproducibility of the assay.

- The LOD is defined as the lowest concentration of DNA that can be *detected* in ≥ 95% of samples.
- The upper and lower LOQ are the highest and lowest concentrations of DNA that can be *quantified* with acceptable precision, and form the limits of the assay's linear or quantifiable range. The LOD may equal, but is often lower than the lower LOQ. In the latter situation the assay is able to detect CMV DNA, but is unable to accurately quantify the concentration. The clinical relevance of these very low concentrations of CMV DNA has not been established.
- The precision or reproducibility of an assay refers to the agreement between the different DNA concentrations obtained by repeatedly testing a single homogenous sample. An understanding of the reproducibility of an assay is needed when changes in viral load concentrations over time in an individual are evaluated. Usually the greatest level of variability of viral load tests are found near the upper and lower LOQ. Therefore minor changes in low viral load results may not be clinically relevant. Our experience with HIV-1 has provided the most useful data regarding clinically important changes in viral load results. For HIV-1, changes in viral loads must be at least 0.5 $\log_{10}$ copies/mL to represent biologically important (and therefore clinically relevant) changes in viral replication. This value has been derived from the biological variability observed in untreated, chronically infected individuals (0.3 $\log_{10}$ copies/mL), and the variability of HIV-1 viral load assays (0.1 – 0.2 $\log_{10}$ copies/mL). This threshold may be too narrow for CMV as the biological variability of CMV is currently unknown, and assay variability can be significantly larger, as mentioned above. Therefore, it has been suggested that for CMV DNA viral load values < 1 000 copies/mL (< 910 IU/mL) changes have to be at least 5-fold (0.7 $\log_{10}$ IU/mL) to be of clinical significance, and at least 3-fold (0.5 $\log_{10}$ IU/mL) for viral load values > 1 000 copies/mL (> 910 IU/mL)[1].

Lancet Laboratories uses the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test for CMV viral load testing of plasma samples. This assay has been calibrated against the First WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques, and one copy of CMV DNA (as defined by the COBAS® AmpliPrep/COBAS®TaqMan®CMV Test) is equivalent to 0.91 International Unit (IU). The LOD of the assay is 56 IU/mL (61 copies/mL), and the linear range is 137 – 9 100 000 IU/mL (150 – 10 000 000 copies/mL)[5].

Clinical Interpretation

- When monitoring a patient for the development of CMV disease or response to therapy, a single assay and sample type must be used to be able to compare results.
- CMV viral load results must be interpreted in conjunction with the patient's clinical condition and other special investigations.
- Some patients with CMV end-organ disease may not have detectable levels of CMV DNA in peripheral blood. In such cases, CMV detection via qualitative PCR in clinical samples taken from the organ system in question (e.g. urine, CSF or respiratory tract sample) or immunohistochemical staining of tissue biopsy specimens may prove useful.
- Low CMV DNA viral load values are relatively common and it can be difficult to assess their clinical significance. The following factors must be taken into account:
  - Specimen type
  - Degree of immunosuppression
  - If the patient is a transplant recipient – donor and recipient CMV serostatus and type of transplant
  - LOD, linear range and precision of the assay used
- When predicting the risk of developing CMV disease, both the absolute viral load and the rate of change in the CMV viral load should be considered.
  - In general, viral load changes > $\log^{10}$ 0.5 are indicative of a significant change in viral replication, provided the CMV viral load is more than 1 000 copies/mL (910 IU/mL). If the CMV viral load is less than 1 000 copies/mL (910 IU/mL), a change of more than $\log^{10}$ 0.7 is considered to be significant.
  - In a prospective study by Emery et al. (2000) in a heterogeneous group of bone marrow and solid organ transplant recipients, the rate of increase in CMV DNA viral load was significantly higher in patients who developed CMV disease than those who did not (0.33 vs. 0.19 $\log^{10}$ genome copies/mL)[6]. In another study amongst allogeneic stem cell transplant recipients, a CMV viral load doubling time of ≥ 2.0 days predicted the need for antiviral therapy with 100% sensitivity, but a specificity of only 51%[7].
  - In a prospective study by Forner et al. (2014) a CMV DNA viral load > 12 000 copies/mL (10 920 IU/mL [$\log^{10}$ 4.0]) at birth was associated with an increased risk of late-onset CMV disease in infants with asymptomatic congenital CMV infection[8]. In the same population, a birth CMV DNA viral load > 17 000 copies/mL (15 470 IU/mL [$\log^{10}$ 4.2]) was associated with an increased risk of sensorineural hearing loss.
- Once it has been decided to start antiviral therapy, a baseline CMV viral load should be obtained on the day therapy is begun, even if a result had been obtained a few days earlier, as the CMV viral load may increase exponentially in patients with active disease.

- The suggested CMV DNA viral load thresholds for the initiation of pre-emptive therapy in the table below are based on current evidence from the literature, as universally accepted treatment thresholds have not been established.

Patient Population	Copies/mL	IU/mL	Log_{10}
Haematopoetic stem cell recipients[7]	1 000	910	3.0
Solid-organ transplant recipients[10]	2 500	2 275	3.4
Infants with suspected CMV pneumonia[12]	13 000	11 830	4.1
HIV-positive patients (CD4 < 200 cells/µL) in ICU[11]	1 000	910	3.0

- Follow-up testing to monitor response to therapy should be done at 5 to 7 day intervals, as the half-life of CMV DNA in plasma is 3 – 8 days. Furthermore, keep in that CMV DNA viral load concentrations may temporarily increase for a few days after initiation of treatment, and should not be misinterpreted as treatment failure.
  - In a study amongst 267 solid organ transplant recipients, a baseline CMV viral load of < 20 000 copies/mL (18 200 IU/mL [log_{10} 4.3]) and a CMV viral load below the lower limit of quantification (< 137 IU/mL [log_{10} 2.1]) on day 7, 14 and 21 of treatment have been associated with earlier disease resolution[9].
- Treatment should continue until at least one or preferably two undetectable CMV viral load results, after a minimum of 2 weeks of adequate therapy, have been obtained.
- Intravenous ganciclovir, its oral prodrug valganciclovir, foscarnet and cidofovir have been approved for the treatment of CMV disease. Foscarnet and cidofovir are available on special MCC section 21 request only. Reducing or even stopping the patient's immunosuppression should also be considered.
- Resistance against antiviral therapy should be considered when the CMV viral load does not decrease, plateaus, or increases after an initial decline after at least 2 weeks of uninterrupted, full dose therapy.

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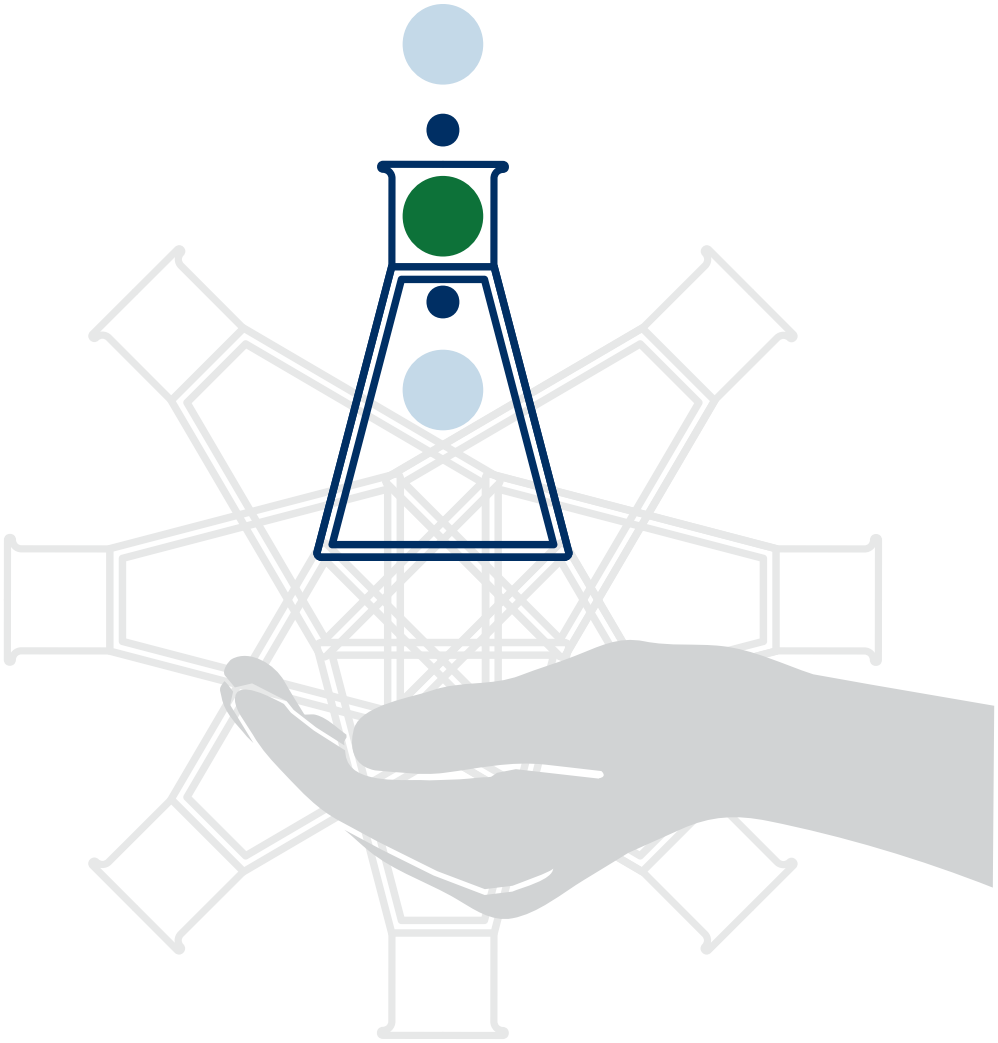

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