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Molecular Tests for Detection of Cytomegalovirus Infection, Useful Guidance or Misleading Advertisement?

Abdollah Karimi[1] and Ali Amanati[2,*]

[1]Pediatric Infections Research Center, Mofid Children's Hospital, Shahid Beheshti University of Medical Sciences, Tehran, IR Iran
[2]Professor Alborzi Clinical Microbiology Research Center, Amir Oncology Hospital, Shiraz University of Medical Sciences, Shiraz, IR Iran

*Corresponding author: Ali Amanati, Professor Alborzi Clinical Microbiology Research Center, Amir Oncology Hospital, Shiraz University of Medical Sciences, Shiraz, IR Iran. Tel: +98-7187915998, Fax: +98-7136325655, E-mail: ali_amanati_1356@yahoo.com

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Human Cytomegalovirus (HCMV) is one of the problematic viral infections in solid organ, Hematopoietic stem cell transplant (HSCT) recipients and other immunocompromised hosts such as patients with HIV infection. Treatment of CMV infection often requires multidisciplinary specialties and a team work management. Every physician who works on CMV infection will inevitably encounter a diagnostic challenge in his routine practices.

Currently, molecular tests are very popular among physicians. The accuracy and precision (reproducibility) of the test, high sensitivity, the ability to detect the lowest concentration of virus (DNA), the lower risk of contamination and shorter turnaround time (1) are advantages of these tests over serologic and culture methods. Along with the increased usage and more access to these tests, physicians should be well familiar with the strengths and weaknesses of the test. In the absence of adequate knowledge about these tests, interpretation may mislead to inaccurate diagnosis and physicians involve in diagnostic challenges. Inaccurate timing for the right diagnosis of congenital cytomegalovirus infection is one of the well-known examples. As it is known, delay in application of the molecular DNA testing in this situation (i e, after three weeks of birthday) had a little significance and the positive results should be interpreted with caution (2).

Interpretation of CMV viral load is one of the common diagnostic challenges of practice. Common pitfalls could be categorized in pre-analytical, analytical and post- analytical errors.

Important pre-analytical errors

- Choosing different specimen type for quantitative-polymerase chain reaction (Q-PCR), which is one of the most common pitfalls. It is better to follow and monitor patients with only one sample type in blood. Although, detection of CMV DNAemia is acceptable both in whole blood and plasma but each specimen has advantages and limitations. Estimated differences between plasma and whole blood may be as great as 100 folds. Whole blood detects both cell-free and intracellular viruses, which means CMV DNA is detected more frequently and often higher in whole blood. On the other hand, detection of CMV DNA in plasma is thought to be more likely correlated with active infection. Thus, plasma samples may be more specific and whole blood samples may be more sensitive to detect CMV DNAemia (3). Regardless of these advantages, no (or low level of) HCMV-DNA copies may be detectable in plasma during the early phase of replication or in the case of latent infection reactivation. Despite all of the aforementioned notes, it is shown that HCMV replication in vivo is a highly dynamic process with a doubling time of approximately one day (4).
- Inappropriate sample transport or storage (5)
- Improper sample processing (6)

Important analytical errors (3, 7-10)

- Differences in DNA extraction methods
- Selection of primers and probes targeting with different genes
- Different ability to limit the detection
- Low sensitivity
- Poor precision and accuracy of the test

Important post-analytical errors

Wrong reporting and interpretation of results that may come from

- Biologically non-significant variability in CMV seropositive patients (less than 0.5 log10 copies/mL changes in viral load). This variability is also known as biological fluctuations (11-13).
- When detecting CMV viral load values of less than1000 copies/mL, changes of less than five-fold rarely reflect clinically important changes in viral replication. For values higher than 1000 copies/mL, at least three-fold changes in viral load may be significant (14).

- Comparison of different values (copies/mL with IU/mL) which may lead to misinterpretation of increment or decrement in viral load (3, 9).

In 2010, the world health organization (WHO) established an international standard to achieve an agreement on viral load values among laboratories (15). Additionally, WHO introduced standard sample to allow laboratories to assess the accuracy of viral load values and the estimation of converting factor. When laboratory use conversion factor, logarithmic value (copy/mL) could be report as IU/ml.

Recently, the conversion factors provided by some manufacturers were validated by some researchers, which showed significant differences with dilutions of the WHO standard samples (16, 17). This fact reemphasized that the standardization efforts must be made within each laboratory to reduce these variability.

Some useful points regarding the interpretation of CMV quantitative DNA testing include: not to compare test results from two laboratories and apply different cutoffs (from different laboratory) to read and interpret test results; use a single specimen type for monitoring, request conversion factor of each laboratory to reach identical unit of viral load counting and omit inter-assay variability; ignoring small changes in viral loads specially in the lower limit of quantification and address longitudinal changes (rapid and great increases in viral loads); interpretation of test results in the view of clinical setting (avoid treating laboratory test results instead of patients).

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