Cytomegalovirus (CMV), Quantitative- PCR / realtime PCR

Use: For detection of cytomegalovirus DNA; early detection and management of CMV infections. Quantitative CMV DNA PCR testing provides a "viral load" value useful for monitoring antiviral therapy and possible identifying patients at risk for CMV disease.

Clinical Significance: Human Cytomegalovirus (CMV) is a member of the Herpes Virus Family and may cause a wide variety of disease manifestations depending on the patient's age and immune status. In an immunosuppressed adult, the lungs, gastrointestinal tract and /or the retina can be infected. Transplant patients are at a high risk for pulmonary infections (Pneumonitis) that can progress to full blown Adult Respiratory Distress Syndrome (ARDS), intestinal necrosis and ulceration leading to debilitating diarrhea.

Assay Availability: Batch testing on every Monday and Thursday

Results Reported (Turn around time): 3-7 days (could be longer due to staff shortage).

Specimen: Whole blood: (EDTA K3) **commercial tube** (in-house tubes not accepted)
Urine in a neonate – in a screwcap plastic bottle
CSF in plastic tube
Bronchoalveolar lavage in Viral transport medium (VTM tube) in ice
Vitreous humor in sterile1.5ml microcentrifuge tube (**Do Not** send in syringes)

Volume: 2 ml (up to marked level of the EDTA tube)
5 ml urine in a sterile bottle

Detail clinical history and CMV specific form is **MANDATORY** for interpretation and selecting for the test.
Incomplete forms or forms that does not accompany history will be rejected.

Specimens should be labeled with patient name, BHT number, date and time of collection, and then sent at 4°C temperature to the laboratory.
Specimens used for testing in other departments/labs are unacceptable due to possible contamination.

Storage: If cannot transport immediately, store whole blood refrigerated for 72 hours.

Causes for Rejection: Heparinized specimen
Specimens in glass **'penicillin bottle'**
Quantity not sufficient for analysis
Specimen grossly contaminated; specimen too old
Frozen whole blood specimen
Specimen leaky or tube broken
Incomplete forms
Forms that does not accompany will be rejected

Methodology:
Extraction of CMV DNA from serum is followed by amplification and detection using polymerase chain reaction (PCR). The assay detects the presence of CMV DNA by amplifying viral genomic DNA.
The amplified target DNA sequence, located within the CMV DNA polymerase gene, is specific for CMV and is not homologous to other members of the Herpesvirus family. The use of plasma avoids potential detection of latent virus in blood.
An internal control is added to each sample to assure that the extraction was performed correctly and to insure that the PCR reaction was not inhibited.

Four standards (Quantification Standards- 10E to 10 4E) which are calibrated against the 1st WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques (NIBSC code: 09/162) are run in each test.
Absolute Quantification: is determined by plotting a standard curve using above standards.
Verification: is performed using below mentioned Control Parameters of the standard curve

Results are expressed in CMV DNA IU/ml plasma.

Platform: ABI Realtime

Assay: Current assay is an in-vitro diagnostic (IVD) and European Union (CE) certificated commercial assay manufactured in Germany (GmBh)

Normal Range: CMV is usually undetectable in blood from healthy persons even if they were previously exposed to the virus. Immunosuppressed patients may have stable low viral loads in the absence of disease. Increasing viral load over time suggests progression of active disease.

Reportable Range:
1.21 IU/µl to 1.21 x 10^9 IU/µl

Interpretative Data: As with any diagnostic test, this result should be interpreted in consideration of all clinical findings and laboratory investigations.
Level of significance in Sri Lankan patient population is yet to be determined.

Serial viral load measurements (one log value rise or decrease) give more reliable information rather than single point testing.

Result: No DNA detected.

Laboratory Contact: For further information, please call the Department of Virology CMV Laboratory at 011-2693532-4 Ext 452 (virology reception) 463 (Medical Officer)

Please log on to MRI website