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## LETTER TO THE EDITOR

# Comparison of PCRs for IE-1 and gB genes with ELISA of IgM antibodies for diagnosis of human cytomegalovirus disease

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Human cytomegalovirus (HCMV) is most frequently associated virus with congenital infections in humans and is found in 0.3–2.4% of live births throughout the world *(1, 2)*. Approximately 10% of HCMV-infected infants develop cytomegalic inclusion disease with a mortality rate of 20–30% *(3, 4)*. In India, 1–20% of infants with suspected congenital infections are positive for the presence of HCMV antibodies in serum *(5, 6, 7)*. Virus isolation, exfoliated cytology, and serology are conventional laboratory tests used for the HCMV diagnosis. However, limited sensitivity, poor specificity of cytology, and absence or delay in IgM antibodies production are the limitations associated with these techniques. The molecular diagnostic methods for the detection of viral nucleic acids by PCR have been a recent accomplishment for the diagnosis of HCMV infections with enhanced sensitivity and specificity *(8, 9)*. A variety of genes coding the immediate-early antigen 1 (IE-1), major immediate-early antigen, glycoproteins B and H (gB, gH), *Eco*R1 D fragment, *Hin*d III X fragment, pp65, pp67, and major capsid protein gene fragments have been targeted for diagnosis, but their suitability has not been evaluated properly *(10)*. The present study was therefore conducted to compare the suitability of two of these commonly used genes, i.e. IE-1 and gB genes for the detection of HCMV DNA in urine along with IgM antibody detection to find out a better alternative for the diagnosis of congenital HCMV infection.

Seventy one infants with clinically suspected HCMV disease were included in the study based on the presence of intrauterine growth retardation, hepatosplenomegaly, thrombocytopenic purpura, jaundice, microcephaly, sensorineural hearing loss, mental retardation, motor deficits, seizures, chorioretinitis *(11)*. The study was approved by the institutional ethics committee and a written informed consent was obtained from the parents.

Fresh urine was collected in sterile vials and processed for PCR assay as described before *(12)*. Also, serum from the venous blood was collected and stored at -20°C until tested. HCMV-specific IgM antibodies were tested by immunoenzymatic μ-capture method using commercially available kit following the manufacturer's instructions (Adaltis Italia).

The processed urine was subjected to PCR for the detection of HCMV DNA by targeting IE-1 and gB genes separately. Nested PCR was carried out for IE-1 gene amplification using 2 sets of primers, which amplified 147 bp from the 4$^{th}$ exon of the IE-1 gene of HCMV following the method described before *(13)*. The amplification for gB gene was done by PCR using a specific primer as described earlier *(12)*. The amplified products were separated by 2% agarose gel electrophoresis, stained with 0.15% ethidium bromide, and visualized under UV transilluminator (Alpha Innotech). HCMV clone and PCR reagents without DNA were used as positive and negative control, respectively.

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**Abbreviations:** HCMV = Human cytomegalovirus; IE-1 = immediate-early antigen 1; gB = glycoprotein B

**Table 1. Comparison of three different assays for HCMV diagnosis**

| Assay | | | No. of positive cases of total 71 (%) |
|---|---|---|---|
| IgM ELISA | IE-1 nested PCR | gB PCR | |
| | | | By one assay only |
| + | – | – | 4 (5.6 %) |
| – | + | – | 6 (8.5 %) |
| – | – | + | 3 (4.2 %) |
| | | | By any two of the three assay |
| + | + | – | 10 (14 %) |
| + | – | + | 7 (9.8 %) |
| – | + | + | 9 (12.7 %) |
| | | | By all the three assays |
| + | + | + | 7 (9.8 %) |

Of 71 infants suspected for HCMV infection, 23 cases (32.4%) were positive by the IgM ELISA or any of the PCRs. The IE-1 nested PCR was found to be the most sensitive method (16 positive cases) followed by IgM ELISA (14 positive cases) and gB PCR (12 positive cases). These results indicated that the highest HCMV positivity could be obtained by the use of combination of all three methods (23/71). When two assays were combined the maximum positivity was observed with IgM ELISA and IE-1 nested PCR (20/23), followed by the combination of IgM ELISA and gB PCR (19/23), and both the PCRs (19/23). The inter-assay comparison has been depicted in Table 1.

The major scope of this study was to assess the different diagnostic methods for the detection of HCMV infection in the infants and newborns. HCMV DNA detection by PCR targeting gB gene and nested PCR targeting IE-1 gene was compared with HCMV-specific IgM antibodies detection. In our study the conventional indirect ELISA was replaced by IgM ELISA that had a higher sensitivity and specificity. The IE-1 nested PCR detected maximum number of cases (16/71; 22.5%), followed by IgM antibodies detection (14/71; 20%), and gB PCR (12/71; 17%). PCR has been shown to be a rapid and sensitive method for the detection of HCMV DNA in urine and has been used to diagnose congenital HCMV infection with a sensitivity of 95% and specificity of 100% *(8, 9)*. IgM antibody response in infants may be delayed or weak and consequently, this technique may pass up some cases *(14)*. However, 4 infants were found to be positive for IgM antibodies, but HCMV DNA could not be detected by any of the PCRs used. This could be possibly due to the presence of inhibitory factors in collected samples *(7)*.

A higher positivity rate for the nested PCR targeting the IE-1 gene than for PCR targeting gB gene was observed (22.5% vs. 17%). A lower sensitivity of PCR targeting gB gene compared to the PCR targeting mtr II gene in clinical samples has been reported earlier *(15)*. On the other hand, Distefano and coworkers have reported higher sensitivity of gB gene PCR using a nested PCR protocol as compared to the simple PCR targeting IE-1 and late antigen gene *(9)*. Thus, the higher positivity of IE-1 nested PCR in our study could be due to the use of nested PCR protocol.

The present study illustrates the potential role of the nested PCR technique targeting IE-1 gene in the urine as a more sensitive and convenient screening method for the diagnosis of HCMV infection in infants suspected of HCMV disease. However, when PCR facilities and expertise are not available, the HCMV IgM (μ-capture) ELISA could be a suitable alternative as it provides a substantial sensitivity.

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