Evaluation of New Commercial Real-Time PCR Quantification Assay for Prenatal Diagnosis of Cytomegalovirus Congenital Infection

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A new commercial real-time human cytomegalovirus (HCMV) PCR kit was evaluated after automated DNA extraction of 153 amniotic fluids in parallel with an in-house real-time PCR assay. The commercial kit displayed 100% sensitivity/specificity compared to the “in-house” assay and was suitable for prenatal diagnosis of HCMV congenital infection.

The diagnosis of fetal human cytomegalovirus (HCMV) infection is usually made by amplification of HCMV genome in amniotic fluid (AF) sampled by amniocentesis. The overall sensitivity of HCMV DNA detection for prenatal diagnosis in AF by PCR ranges from 70 to 100% (13). However, the sensitivity of HCMV PCR in AF is close to 100% when using a PCR test and appropriate timing for amniocentesis (e.g., after 20 weeks of gestation and at least 6 weeks after maternal infection) (4). Nevertheless, false-negative HCMV PCR results have been reported in AF samples even under these optimal diagnostic conditions (5, 7, 13). These false-negative results were related to DNA amplification inhibition by inhibitory properties of AF (2). Moreover, although 100% specificity was reported for HCMV detection by PCR in AF (1, 3, 11, 13), rare cases of false-positive prenatal diagnosis have also been published (5, 7, 9). In these cases, although AF tested PCR positive, the AF culture was negative and the child was not infected at birth. False-positive results could be due to contamination occurring during PCR testing. This risk is higher with nested PCR, a technology that is very sensitive but exposed to contamination. Generalization of semiautomated real-time PCR methodology might help to overcome the risk of contamination and to achieve absolute specificity for HCMV prenatal diagnosis.

In this study, we evaluated the performance of an automated DNA extraction system from AF samples to avoid cross-contamination between samples and to remove PCR inhibitors. We also compared the sensitivity, specificity, and quantification performance for amplification of HCMV in AF of a commercial real-time HCMV PCR assay including a PCR inhibitor detection system with our in-house real-time HCMV PCR assay (10).

We tested 153 AF samples obtained from 153 women who presented with HCMV seroconversion in pregnancy and/or whose fetuses had ultrasound features compatible with HCMV infection. The samples were collected between 2002 and 2007 in two prenatal diagnosis centers in Poissy Hospital and in Necker Hospital. Among these 153 samples, 115 tested HCMV DNA negative and 38 tested HCMV DNA positive with our in-house HCMV PCR assay (10). According to French law, all women gave written consent for CMV detection by PCR in their AF.

In this retrospective study, DNA was extracted from 200 μl of the 153 AF samples on the Magna Pure LC Instrument (RocheMolecular Biochemicals, Meylan, France) using the Total NA serum-plasma kit (Roche Diagnostic), with extracted DNA eluted in 100 μl of the kit elution buffer. An extraction control was included in each batch. Each known positive sample was extracted between two negative samples to check for potential cross-contamination during extraction. DNA extracts from known positive samples were amplified undiluted and 1:10 diluted.

CMV DNA was amplified from these extracted DNA samples by two methods. For method 1, 5 μl of extracted DNA was amplified using an “in-house” CMV PCR test targeting the UL123 gene in 25 μl of mixture containing 1× Platinum qPCR superMix-UDG (Invitrogen, Cergy-Pontoise, France), 400 nM reverse and forward primers, and 200 nM probe (10). Amplification was performed in a DNA Engine Opticon (Bio-Rad, Marne La Coquette, France). Quantification was achieved with a commercial standard diluted to obtain a standard curve of 1,000 to 1,000,000 copies/ml (AD 169 DNA; Tebu-Bio, Perray-en-Yvelines, France). The 95% sensitivity of this in-house quantitative CMV PCR test was 500 copies/ml (10). For method 2, 10 μl of DNA was amplified in 25 μl PCR mixture with the CMV R-gene kit according to the manufacturer (Argene, Varilhes, France). This kit was based on a duplex PCR which allowed in a single tube amplification of CMV DNA in the UL83 gene with a 6-carboxyfluorescein-labeled probe and of an inhibitor control with a VIC fluorochrome-labeled probe. Amplification was performed in an ABI PRISM 7300 (Applied Biosystems, Courtaboeuf, France). Four quantification stan-
those three samples were less than 10^5 copies/ml) and for nine
tracted DNAs were amplified undiluted (the viral loads in
CT
obtain an accurate quantification of the viral load.
1:1,000 dilution of the extracted DNA was recommended to
viral load was considered as accurate. (iv) When CMV DNA
was correct, the sample was validated as CMV positive and the
value incorrect, the sample had to be retested. (iii) When
CMV DNA amplification was negative and the IC
value obtained with the smaller DNA extract dilution in which
with the commercial kit were compared. We used the viral load
1:10-diluted and undiluted DNA extracts were amplified: in all
these 26 samples, the viral load was over 10^6 copies/ml.
Among these 26 samples, 19 were submitted to a second ex-
duction was negative and the value of the cycle threshold (C_T)
was shown in four studies that the median levels of HCMV
AF was highly correlated with both quantitative assays used.
Quantification of DNA loads in
AF was highly correlated with both quantitative assays used.
Quantification of HCMV DNA in AF could be of interest as it
was shown in four studies that the median levels of HCMV
DNA in AF were higher in symptomatic fetuses than in fetuses
with subclinical infection, even if this difference did not always
reach significance (6, 8, 12, 14). To our knowledge, no eval-
uation of a CMV commercial quantitative real-time PCR assay
in AF has ever been reported before. We have demonstrated
that the CMV R-gene is suitable for standardized prenatal
diagnosis of CMV congenital infection.

As viral loads are generally very high in AF, extracted DNA
had to be diluted to 1:100, or even to 1:1,000 for some samples,
to obtain a correct IC value and therefore an accurate quan-
tification of CMV DNA loads. Quantification of DNA loads in
AF was highly correlated with both quantitative assays used.

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