# HCMV DNA load in maternal blood, amniotic fluid and newborn blood in pregnancies complicated by primary HCMV infection

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## Background

Conflicting results have been reported concerning the presence of human cytomegalovirus (HCMV) DNA in the blood of immunocompetent individuals (1,2). Standardization of molecular assays and expression of results in UI/ml can be useful to compare results obtained by different authors and improve the diagnosis and monitoring of primary HCMV infection in pregnancy.

## Aims of the study

- To evaluate the QS-RGQ system in combination with the *artus* CMV QS-RGQ kit (Qiagen assay) for quantification of HCMV DNA load in maternal whole blood (WB), amniotic fluid (AF) and newborn WB in pregnancies complicated by primary HCMV infection.
- To establish a conversion factor from HCMV DNA copy number to WHO international units (IU) in WB and AF matrices.

## Patients and Methods

In order to express HCMV DNA results in international units (IU), HCMV WHO Standard (NIBSC, code: 09/162, version 3.0, 30/11/2010) was tested with the Qiagen assay according to manufacturer's instructions. After reconstitution in 1 ml $H_2O$, the WHO Standard contains $5x10^6$ IU/ml HCMV DNA to be spiked in the biologic matrices of interest (WB and AF).

Among 350 pregnant women with primary HCMV infection monitored at our Institution during the period 2008-2012 a subgroup was selected for this retrospective study according to the following: i) defined onset of primary infection, ii) at least 1 available maternal WB, iii) available AF samples obtained at 20th week of gestation (WOG) for prenatal diagnosis, and iv) known virologic outcome of pregnancy. Available neonatal WB samples, were included in the study. In addition, 10 WB samples from 10 HCMV-seronegative pregnant women and 30 WB samples from 30 HCMV-seropositive pregnant women with remote virus infection were included as controls.

To perform the Qiagen assay, 200µl WB and 800 µl AF were extracted using the QIAsymphony® DNA Mini kit and the QIAsymphony® DSP Virus/Pathogen Midi kit, version1, respectively, using the QiaSymphony instrument.

## Results

**Relationship between copy number and international unit (IU) in WB and AF.** The regression equation (Fig 1) indicated that 1 HCMV DNA IU/ml corresponded to 0.9074 and 1.0685 HCMV DNA copies/ml WB (Fig 1A) and AF (Fig 1B), respectively, when using the Qiagen assay. The intra and inter assay reproducibility for the WHO DNA standard was better for AF than WB (Table 1).

**Patients and samples.** Overall, 194 sequential maternal WB samples from 65 mothers (median 3, range 1-7 per woman), 67 AF (2 twin pregnancies), and 19 neonatal WB from as many as 11 congenitally infected and 6 non congenitally infected newborns were available for the present study.

**HCMV DNA quantification in maternal WB.** HCMV DNAemia was negative both in HCMV seronegative patients and in seropositive patients with remote HCMV infection. In the first mos of infection, HCMV DNAemia was positive in 12/15 (80.0%) women (<30 days, Fig 2A) and the presence of HCMV DNA in maternal WB samples decreased over time. After 6 mos from the onset of infection, 4/17(23%) were still positive (>180 days, Fig 2 A). Similarly, levels of HCMV DNA decreased progressively from a median value of 312 IU/ml (range 0-755) within the first month to 0 IU/ml (0-1,000) in the 5th mos of infection (>30 and 121-150 days, respectively, Fig 2B).

**HCMV DNA quantification in AF and neonatal WB .** Thirty-six out of 67 AF (53.7%) tested positive for HCMV (median 267502 IU/ml, range 8-13522505) whereas 31/67 (46.3%) AF were negative. Intrauterine HCMV infection was confirmed at birth in 23/23 (100.0%) newborns, 9 symptomatic and 14 asymptomatic. Nine women underwent a termination of pregnancy and 3 women were lost at follow-up. Overall, 28/31 (90.3%) negative AF results were confirmed at birth while 3/31 (9.7%) negative AF results were not confirmed at birth. In these cases, HCMV transmission likely occurred after the time of prenatal diagnosis and the three babies were asymptomatic at birth. Eleven out of 11 (100%) congenitally infected newborns were HCMV DNA positive (median 2379 IU/ml, range 57-7182). Six WB samples from as many non infected newborns were HCMV DNA negative.

## Conclusions

- Quantification of HCMV DNA in maternal WB, AF, and newborn WB is an important tool for improving diagnosis and monitoring of primary HCMV infections in pregnancy.
- DNAemia was detected in case of primary infection but not in in seropositive HCMV pregnant women with a remote HCMV infection, confirming previous results showing that presence of HCMV DNA in blood is a marker of primary infection.
- The use of widely available assays and expression of HCMV DNA load in IU/ml is a step forward in standardization of molecular assays.

**Table 1**

| Matrix | Sample | IU/ml | Aliquots | Number of replicates | Intra assay (copies/ml) | | | Inter assay (copies/ml) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Mean | ±SD | CV(%) | Mean | ±SD | CV(%) |
| Whole blood | 1 | 250000 | A | 3 | 273260 | 22708 | 8.3 | 251092 | 47701 | 19.0% |
| | | | B | 3 | 283674 | 75505 | 26.6 | | | |
| | | | C | 3 | 196341 | 47552 | 24.2 | | | |
| | 2 | 25000 | A | 3 | 34573 | 5688 | 16.4 | 30930 | 3866 | 12.5% |
| | | | B | 3 | 31342 | 5700 | 18.2 | | | |
| | | | C | 3 | 26875 | 5937 | 22.1 | | | |
| | 3 | 2500 | A | 3 | 3571 | 695.8 | 19.5 | 3018 | 489.5 | 16.2% |
| | | | B | 3 | 2841 | 492.2 | 17.3 | | | |
| | | | C | 3 | 2641 | 829.9 | 31.4 | | | |
| | 4 | 250 | A | 3 | 195 | 160 | 81.8 | 253.9 | 109.9 | 43.3% |
| | | | B | 3 | 381 | 177 | 46.5 | | | |
| | | | C | 3 | 186 | 153 | 82.2 | | | |
| | 5 | 25 | A | 3 | ND | ND | ND | ND | ND | ND |
| | | | B | 3 | ND | ND | ND | | | |
| | | | C | 3 | ND | ND | ND | | | |
| Amniotic fluid | 1 | 250000 | A | 3 | 254850 | 16373 | 6.4 | 275525 | 34870 | 12.7% |
| | | | B | 3 | 315784 | 38619 | 12.2 | | | |
| | | | C | 3 | 255940 | 18631 | 7.3 | | | |
| | 2 | 25000 | A | 3 | 25966 | 2984 | 11.4 | 23327 | 3863 | 16.6% |
| | | | B | 3 | 25122 | 2589 | 10.3 | | | |
| | | | C | 3 | 18894 | 2079 | 11.0 | | | |
| | 3 | 2500 | A | 3 | 2310 | 252 | 10.9 | 2212 | 87 | 3.9% |
| | | | B | 3 | 2182 | 324 | 14.8 | | | |
| | | | C | 3 | 2144 | 140 | 6.5 | | | |
| | 4 | 250 | A | 3 | 128 | 15 | 12.0 | 184.1 | 48.9 | 26.6% |
| | | | B | 3 | 211 | 16 | 7.4 | | | |
| | | | C | 3 | 214 | 22.5 | 10.5 | | | |
| | 5 | 25 | A | 3 | 15 | 6.2 | 41.6 | 19.1 | 7.7 | 40.3% |
| | | | B | 3 | 28 | 8.8 | 31.7 | | | |
| | | | C | 3 | 14 | 4.5 | 31.5 | | | |

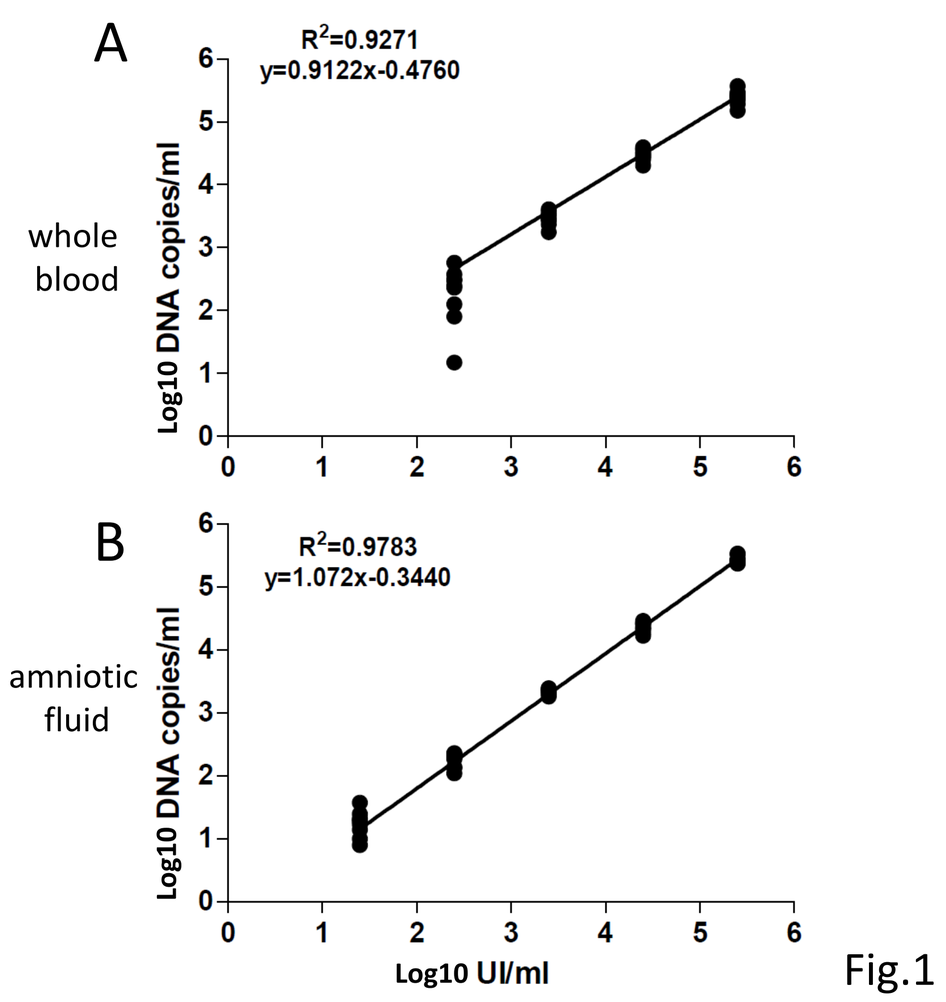


Fig.1

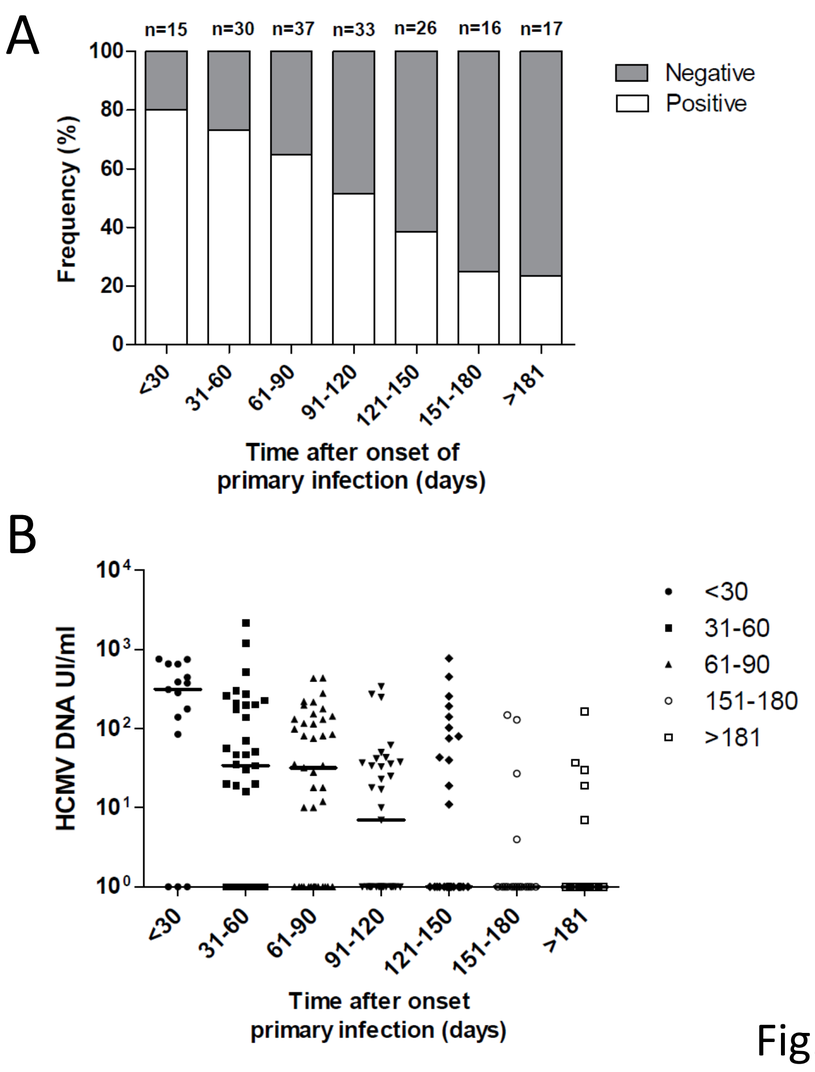


Fig.2

## References

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