CMV-DNA quantification in whole blood with a highly automated system

Mengelle C.[1#], Mansuy J-M.[1], Haslé C.[1], Sandres-Saune K.[1-2], Izopet J[1-2].
[1]Department of Virology, Toulouse University Hospital, [2]Department of Physiopathology, Toulouse Purpan, Unité Inserm U563, Toulouse, France.

Introduction

We have already validated the use of the VERIS™/MDx System CMV Assay® on **plasma** samples {Mengelle, 2016 #4} and we have shown in particular that this highly automated system is very sensitive (14,6 IU/ml).

Aim

The aim of the study was to evaluate the performances of the VERIS™/MDx System CMV Assay® for monitoring CMV-DNA in **whole blood samples.**

Material and methods

Analytical performances (sensitivity, intra and inter-assay variation, linearity) were assessed using quantified quality controls.

Specificity was evaluated on 28 negative whole blood samples stored at -20° C, tested in three separate runs (total of 84 tests).

Clinical performances were evaluated from whole blood potassium-EDTA specimens. Samples were tested prospectively with the in-house technique targeting the UL83 gene (input/output extraction volumes 200/100µl, input PCR volume 5µl / limit of detection 74 IU/ml {Mengelle, 2003 #1}) and 112 positive samples were selected and stored at -20° C pending batch analysis.

Patient's monitoring was assessed on serial specimens collected from three patients undergoing anti-CMV therapy (n=22).

After storage whole blood samples were retested with the in-house method and were tested with the Veris™/MDx System CMV Assay® (minimum volume 650 µl), input/output extraction volumes 250/30 µl, input PCR volume 20 µl.

Results

Analytical performances

Sensitivity was excellent: 57 IU/ml (95%: 47-75 IU/ml).

Intra-assay variation: Standard deviations were **0.15, 0.11 and 0.12** respectively for expected values of 4.70, 3.70 and 2.70 $\log_{10}$ IU /ml respectively (each concentration tested 4 times).

Inter-assay variation: Standard deviations were **0.02 and 0.20** for expected values of 5.90 and 3 $\log_{10}$ UI/ml. The negative sample always tested negative (each sample tested 10 times).

The assay was **linear** over the range 3.83 to 6.83 $\log_{10}$ IU/ml (r^2 = 0.99).

Specificity

The **specificity** was evaluated on 84 negative tests: it was 98.8 % [CI 95%: 93.5– 99.9].

Clinical performances

Veris™/MDx System CMV Assay®: mean viral load **3,38 $\log_{10}$ IU/mL**
In-house method: mean viral load **3,68 $\log_{10}$ IU/mL**

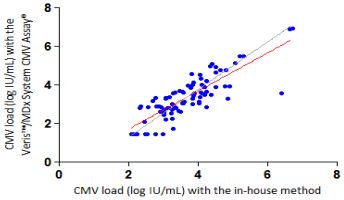


Fig.1: Correlation and linear regression analysis of CMV loads obtained for all positive (n=87) specimens by the in-house method and the VERIS™/MDx System CMV Assay®.
r= 0.83, *p*<0.0001, slope of Deming regression 1.23 [CI 95%: 1.05 – 1.41] and y-intercept -1.159 [CI 95%: -1.839– -0.4788].

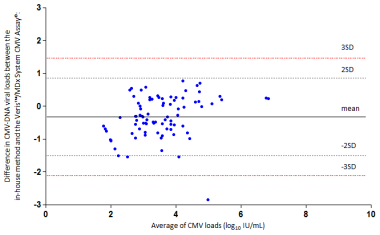
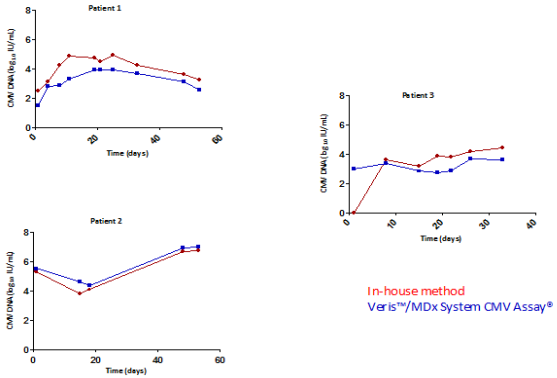


Fig.2: Bland–Altman representation of CMV loads after the in-house method and the VERIS™/MDx System CMV Assay®.
Average deviation: -0.30 $\log_{10}$ IU/ml
Standard deviation: 0.621

Patient's monitoring



Patients' monitoring showed the high similarity between the techniques.

Conclusion

The VERIS™/MDx System CMV Assay® provides **precise, sensitive and reproducible** results on **whole blood**.

It is a completely automated system directly from primary patient sample to automatically integrated results into the laboratory information system.

It thus satisfies quality requirements for routine monitoring of DNA-CMV in whole blood samples.

Mengelle, C., et al. (2003) Automated extraction and quantification of human cytomegalovirus DNA in whole blood by real-time PCR assay. J Clin Microbiol **41**(8): 3840-3845.
Mengelle, C., et al, (2016) Performance of a completely automated system for monitoring CMV DNA in plasma. J Clin Virol. 79:25-31..

Hôpitaux de Toulouse