Standardization of CMV, EBV, B19V, BKV and HHV6 quantification results using conversion factors considering the matrix effect

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INTRODUCTION

One goal of laboratory medicine is that results for patient samples be comparable whatever the medical laboratories or methods used. In order to improve the comparability of quantitative results from different nucleic acid amplification technique (NAT)-based assays some World Health Organization International Standards (WHO IS) were developed. Their assigned concentration, based on the results of worldwide collaborative studies, is expressed in international units/mL (IU/mL).

As of today, the bioMerieux ARGENE® transplant products range, dedicated to the management of viral infections in immunocompromised patients, includes 5 kits for which WHO IS are available: CMV R-GENE® (ref. 69-003B), EBV R-GENE® (ref. 69-002B), Parvovirus B19 R-GENE® (ref. 69-019B), BK Virus R-GENE® (ref. 69-013B) and HHV6 R-GENE® (69-006B)[3] assays. For each assay, a quantification standards range expressed in copies/mL is included, to be amplified within the run to establish a calibration curve.

Conversion factors (CF) allow to translate results from copies/mL into IU/mL for standardization purpose allowing comparison of biological measurements worldwide.

Conversion factor determination depends on the combination of specimen type, extraction and amplification systems used. The CF were determined for those 5 assays using EMAG® (bioMérieux) as extraction system, and ABI 7500 Fast (Applied Biosystems), Rotor-Gene® Q (Qiagen), LightCycler 480 (system II, Roche) and CFX96 (BioRad) as amplification systems. Whole blood and plasma were used for all assays. In addition, conversion factors were determined in amniotic fluid for CMV, cerebrospinal fluid (CSF) for EBV, bone marrow for Parvovirus B19 (B19V) and broncho-alveolar lavage (BAL) for HHV6.

MATERIALS AND METHODS

✓ Study design:

The calculation of conversion factors was performed using 4 dilution series of 3 concentrations of WHO IS in a negative matrix on 4 different days (1 dilution per day). The tested concentrations corresponding to three 10-fold serial dilutions to obtain concentrations from 5.0 to 3.0 $\log_{10}$ IU/mL for CMV 1st WHO IS (#09/162), EBV 1st WHO IS (#09/260) and Parvovirus B19 3rd WHO IS (#12/208) or from 6.0 to 4.0 $\log_{10}$ IU/mL for BKV 1st WHO IS (#14/212) and HHV6 1st WHO IS (#15/266).
Each day, 2 extractions were done per concentration and amplified in simplicate on the 4 amplification platforms tested to reach a total of 8 quantifications per concentration. Variability was introduced at reagent level (2 lots of amplification reagent used for each parameter) and at matrix level (using 2 pools of samples, each made of 3 donors).

✓ Analysis:

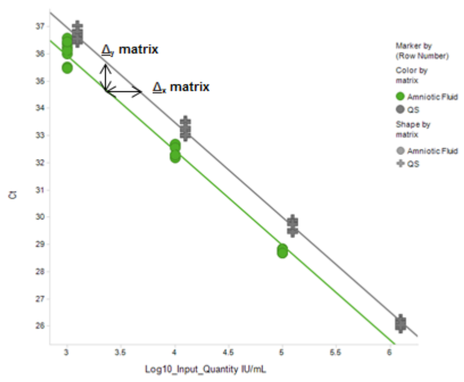

Figure 1: Δx corresponds to the difference, on the x-axis, between the regression line of the QS and the regression line of the matrix (the regression lines are calculated with a common slope).

In a first step, the regression lines of Ct versus theoretical concentration were established for each specimen type and for the quantification standards (QS) range (included in each ARGENE® kit).
The parallelism between the regression lines (specimen type and QS) was checked using a covariance analysis (test of equality of slopes, significance level of 5%). The conversion factor was then calculated as the inverse $\log_{10}$ of the difference between the regression lines on the horizontal axis, that is as the ratio between the difference in intercepts and the common slope (Δx=Δy/slope).
Conversion factors are finally expressed as a multiplication factor on copies/mL to obtain results in IU/mL.

RESULTS

The following figures summarize the results obtained for the different parameters and show the regression fits for all matrices tested and the QS for the platforms Applied Biosystems® 7500 Fast and Rotor-Gene® Q as examples.

Conversion factors determined for CMV

	ABI 7500 Fast	CFX96™	LC480	Rotor-Gene® Q
Amniotic fluid	0.4	0.5	0.5	0.5
Plasma	0.4	0.5	0.5	0.4
Whole blood	1.2	1.5	1.3	1.5

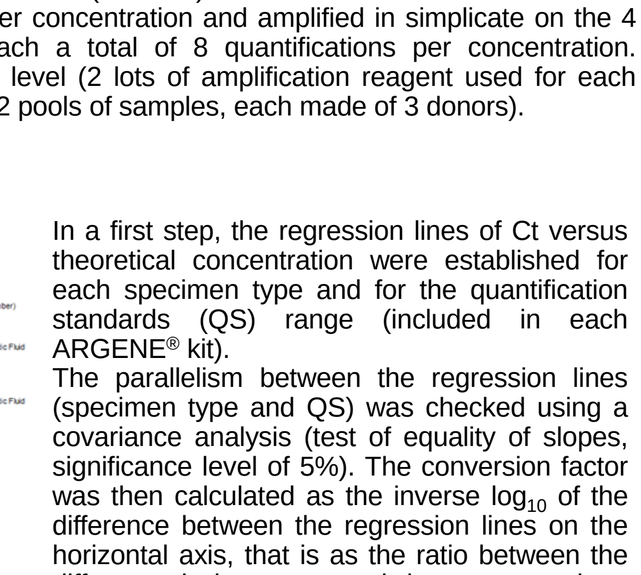

Figure 2: Conversion factor obtained for CMV for all amplification platforms tested and example of regression fits for the 3 concentrations tested in the 3 matrices tested and Quantification standards for ABI® 7500 Fast and Rotor-Gene® Q amplification platforms.

[3] Product under development intended to be CE marked in November 2018

Conversion factors determined for EBV

	ABI 7500 Fast	CFX96™	LC480	Rotor-Gene® Q
CSF	0.6	0.6	0.7	0.4
Plasma	0.5	0.6	0.6	0.4
Whole blood	1.8	1.9	2.0	1.4

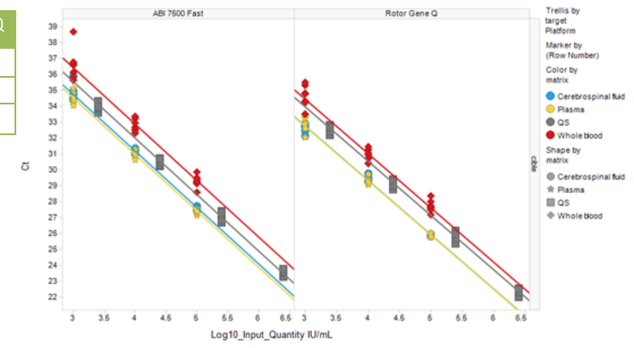

Figure 3: Conversion factor obtained for EBV for all amplification platforms tested and example of regression fits for the 3 concentrations tested in the 3 matrices tested and Quantification standards for ABI® 7500 Fast and Rotor-Gene® Q amplification platforms.

Conversion factors determined for BKV

	ABI 7500 Fast	CFX96™	LC480	Rotor-Gene® Q
Plasma	0.6	0.7	0.8	0.7
Whole blood	3.3	4.0	4.3	3.6

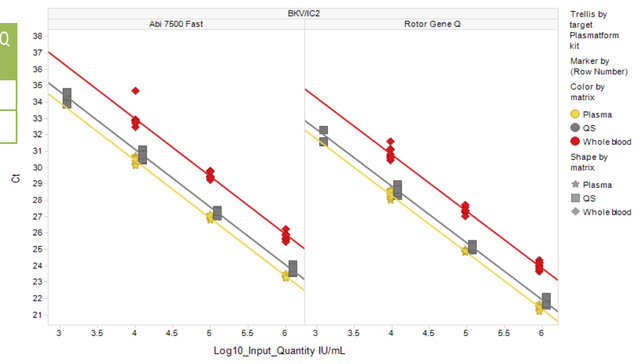

Figure 4: Conversion factor obtained for BK virus for all amplification platforms tested and example of regression fits for the 3 concentrations tested in the 2 matrices tested and Quantification standards for ABI® 7500 Fast and Rotor-Gene® Q amplification platforms.

Conversion factors determined for B19V

	ABI 7500 Fast	CFX96™	LC480	Rotor-Gene® Q
Bone marrow*	1.3	1.5	1.9	1.2
Plasma	0.5	0.5	0.6	0.4
Whole blood	1.4	1.5	1.8	1.1

**Taken into account the 1/3 dilution in PBS for bone marrow samples prior to extraction as indicated in the Parvovirus B19 R-gene® instructions for use.*

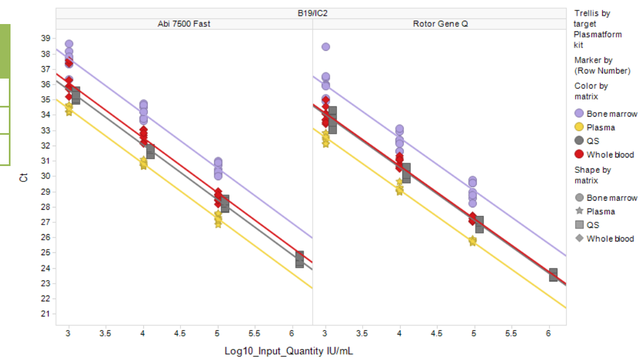

Figure 5: Conversion factor obtained for Parvovirus B19 for all amplification platforms tested and example of regression fits for the 3 concentrations tested in the 3 matrices tested and Quantification standards for ABI® 7500 Fast and Rotor-Gene® Q amplification platforms.

Conversion factors determined for HHV6

	ABI 7500 Fast	CFX96™	LC480	Rotor-Gene® Q
BAL	1.1	0.9	1.2	1.0
Plasma	0.7	0.6	0.7	0.6
Whole blood	1.5	1.2	1.6	1.4

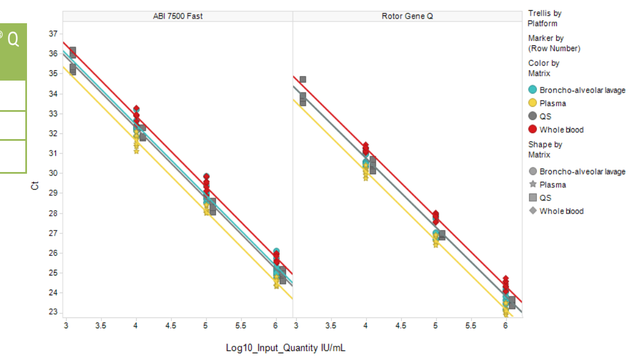

Figure 6: Conversion factor obtained for HHV6 for all amplification platforms tested and example of regression fits for the 3 concentrations tested in the 3 matrices tested and Quantification standards for ABI® 7500 Fast and Rotor-Gene® Q amplification platforms.

CONCLUSION

Overall, differences between obtained conversion factors remain in general weak for a given specimen type amongst the different amplification platforms (e.g. 0.6-0.8 for BKV in plasma). On the contrary, the specimen type dramatically impacts the conversion factor as shown on whole blood (with a ratio up to 5.5 when compared to plasma for BKV), as well as on matrixes known to be complex such as bone marrow or, interestingly BAL samples.
Those results bring additional evidence of the importance for lab to consider the matrix effect in order to improve standardization across different detection methods.