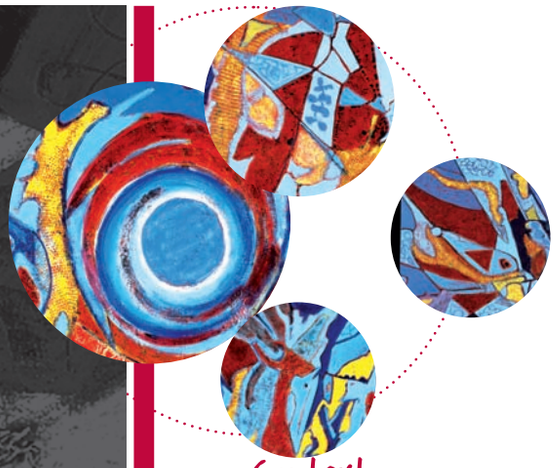


# Standardisation of CMV quantification with the CMV R-gene™ kit and the WHO International Standard

## Context

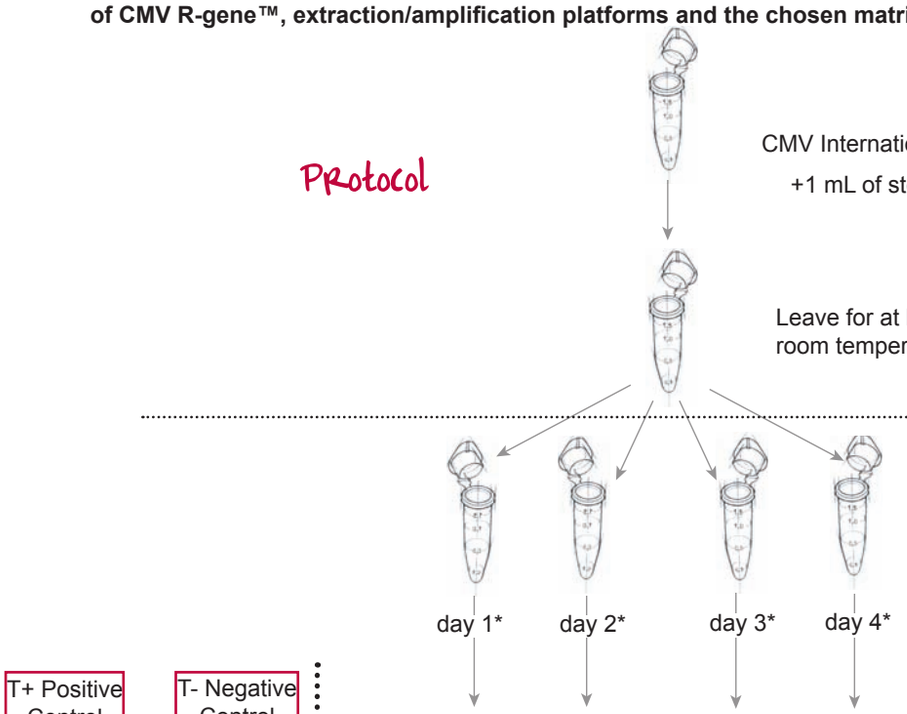
The first WHO (World Health Organisation) International Standard for human CMV is now available.
This product is available via the National Institute for Biological Standards and Control (NIBSC product code: 09/162). This international standard is intended to be used for the **standardisation of quantifications** obtained with nucleic acid amplification assays.

This International Standard has been assigned a value of $5x10^6$ International Units/mL (IU/mL) by the NIBSC.

The results obtained with **CMV** R-gene™ (bioMérieux ref. : 69-003B) and **CMV** HHV6,7,8 R-gene™ (bioMérieux ref.: 69-100B), which are reported in copies/mL, can be converted into International Units/mL (IU/mL) by using a **conversion factor** that is **specific to the matrix** (whole blood, plasma, etc...) **and the combination of extraction/amplification platforms.**

In this leaflet, we supply a **protocol** to define the conversion factor for the **combination of CMV R-gene™, extraction/amplification platforms and the chosen matrix.**

## Protocol




**Note : it is imperative to dilute the International Standard in the **same batch of matrix** for the four independent experiments.

Preparation of the WHO International Standard

Protocol for the determination of the conversion factor

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# Data analysis and calculation of the conversion factor :

ARGENE provides an excel file to calculate the conversion factor.

The mean value is determined with the 12 results (corresponding to the 3 quantifications performed on 4 consecutive days), obtained with **CMV** R-gene™ or **CMV** HHV6,7,8 R-gene™.

## Glossary

**Conversion factor :** Factor to convert quantification results from copies/mL to International Units/mL.

The Conversion Factor can only be validated if the deviation of the 12 individually measured values is less than 0,5 log compared to the mean value.
If 2 or more values do not meet these criteria, we recommand to repeat the experiment.

In this case, the conversion factor is calculated as follows :

**Conversion Factor = value of International Standard (IU/mL)* / Mean value of Argene quantifications (cp/mL)**

* Take into account the 1:10 dilution factor applied to the international standard before extraction

This conversion factor allows the conversion of results obtained with **CMV** R-gene™ and **CMV** HHV6,7,8 R-gene™ kits (copies/mL) into International Units/mL.

## Example

**Kit** : CMV R-gene™
**Matrix** : whole blood
**Extraction** : NucliSENS® easyMAG® whole blood specific B Protocol 200/100
**Amplification** : ABI 7500 Fast

**CMV R-gene™** **Ref. : 69-003B**
**CMV HHV6,7,8 R-gene™** **Ref. : 69-100B**

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**Calculation of a conversion factor to express a CMV viral load in International Units (IU/mL)**

Grey cells must be modified

| | |
|---|---|
| Kit : | CMV R-gene™ |
| Sample type : | Whole Blood |
| Extraction : | easyMAG whole blood Specific B 200/100 |
| Amplification : | ABI 7500Fast |

International Standard (5E+06 UI/mL) diluted 1:10 (5E+05 UI/mL)

| | Argene CMV Quantification (copies/mL) | log | |
|---|---|---|---|
| Day 1 | 4,50E+05 | 5,65 | VALIDATED |
| | 3,29E+05 | 5,52 | VALIDATED |
| | 3,22E+05 | 5,51 | VALIDATED |
| Day 2 | 2,88E+05 | 5,46 | VALIDATED |
| | 2,56E+05 | 5,41 | VALIDATED |
| | 3,44E+05 | 5,54 | VALIDATED |
| Day 3 | 2,75E+05 | 5,44 | VALIDATED |
| | 3,37E+05 | 5,53 | VALIDATED |
| | 3,56E+05 | 5,55 | VALIDATED |
| Day 4 | 2,23E+05 | 5,35 | VALIDATED |
| | 2,26E+05 | 5,35 | VALIDATED |
| | 2,94E+05 | 5,47 | VALIDATED |
| Mean value | 3,08E+05 | 5,49 | |

| | | |
|---|---|---|
| Conversion factor | Theoratical value for the International Standard / Average Argene of quantifications | 1,622 |

International standard diluted 1:10 is quantified at **3.08x10$^5$ copies/mL** with **CMV R-gene™** for a theorical value of 5x10$^5$ (dilution 1:10 of a commercial stock solution determined by the NIBSC at 5x10$^6$ UI/mL).
The conversion factor equals **(5x10$^5$)/(3.08x10$^5$) = 1.622** for the combination whole blood / easyMAG/ ABI7500Fast / CMV R-gene.

Practical example: A whole blood sample from a patient has been extracted on NucliSENS® easyMAG® and amplified on ABI7500Fast.
The CMV viral load, determined with **CMV** R-gene™, is quantified at 20 000 copies/mL. Hence, this quantification is equivalent to **1.622** x 20 000 = 32 440 UI/mL.

## Conclusion

Thanks to the conversion factor, determined for each combination and matrix, the results obtained with nucleic acid amplification techniques can be **standardised**. The use of an internationally recognized standard allows to **define the limits of sensitivity** between different techniques and to **compare results of one patient** during follow-up at 2 different sites. Finally, this tool is a reference that may contribute to the development of quantification standards.

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