

CMV Run Control r-gene® (ARGENE® range, bioMérieux): a tool to ensure the reliability of human cytomegalovirus nucleic acid amplification technique results

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INTRODUCTION

The primary aim of any clinical laboratory is the timely delivery of reliable results with a minimum of errors, maintaining confidence in the results for all stakeholders. The use of an independent quality control (IQC) provides constant and consistent monitoring of an assay results in a systematic manner so that variation in the assay system can be monitored over time (day-to-day test variation, lot-to-lot performance of test kits, operator variation...). An IQC material must be robust, stable and well characterized. Its properties should be as close as practically possible to patient specimens and it should be processed in the same way as the clinical sample.

bioMérieux has developed an IQC named CMV Run Control r-gene® (ARGENE® range, bioMérieux, ref: 68-015). Its routine use enables monitoring run-to-run performance of human cytomegalovirus nucleic acid amplification technique (NAT) assays for human clinical samples. CMV Run Control r-gene® is intended for health care professional and for *in vitro* use only.

This CMV Run Control r-gene® consists on a non-inactivated whole CMV strain (AD169) spiked in pooled human plasma tested negative for CMV, HIV, HCV, HBV, Parvovirus B19, and EBV. This formulation allows to mimic naturally occurring specimens containing CMV DNA. CMV Run control r-gene® has no assigned concentration value but it is defined in order to be within the dynamic range of most molecular assays. This control should therefore be validated for use as a run control and the expected results determined by the end user for their particular CMV NAT assay, extraction and instrument combination.

Precision studies performed internally on two combinations platforms (NucliSENS® easyMAG®/ABI 7500 Fast and eMAG™/ABI 7500 Fast) with the use of CMV R-gene® (1) (ARGENE® range, bioMérieux, ref: 69-003B) as PCR assays are presented. The in-use stabilities results are also described.

MATERIAL AND METHODS

Two precision studies were performed. A first precision study was performed in order to determine the within-lot and between-lot precision of the CMV Run Control r-gene® on the NucliSENS® easyMAG®/ABI 7500 Fast platform combination. A second study was performed on the eMAG™/ABI 7500 Fast combination. In-use stabilities were established.

Extraction Part :

Extraction of samples was performed on NucliSENS® easyMAG® system according to Specific B protocol with 200µL of sample and an elution in 50µL.

Extraction of samples was performed on eMAG™ system according to the B01t_On_Tub_SB_IC2 extraction protocol with 200µL of sample and an elution in 50µL.

Amplification Part :

10µL of eluted samples were added to 15µL of ready-to-use CMV/IC2 amplification premix (reagent R5, #69-003B). CMV and IC2 were respectively detected at 530nm & 560nm on Applied Biosystems® 7500 Fast Dx or Applied Biosystems® 7500 Fast.

Analytical performance of the CMV Run control r-gene® were established.

✓ Precision studies :

In the first precision study, 3 independent lots of CMV Run Control r-gene® were tested each day by 2 different operators, each testing 2 vials per CMV Run Control r-gene® lot, and performing 2 extractions per tube, which were then amplified in duplicate. This protocol was repeated on a total of 12 days, on different systems (2 NucliSENS® easyMAG® and 2 ABI 7500 Fast), using 2 different lots of CMV R-gene® assay. This represents 192 replicates per CMV Run Control r-gene® lot.

In the second precision study, only one lot of CMV Run Control r-gene® was included. The study was conducted using 6 different sections of eMAG™, 3 operators and on 5 non-consecutive days. Each day, 6 extraction runs were performed by 3 operators. In each extraction run, 3 replicates per section of eMAG™ instruments were tested. This represents 90 replicates for CMV Run Control r-gene®.

✓ In-use stabilities :

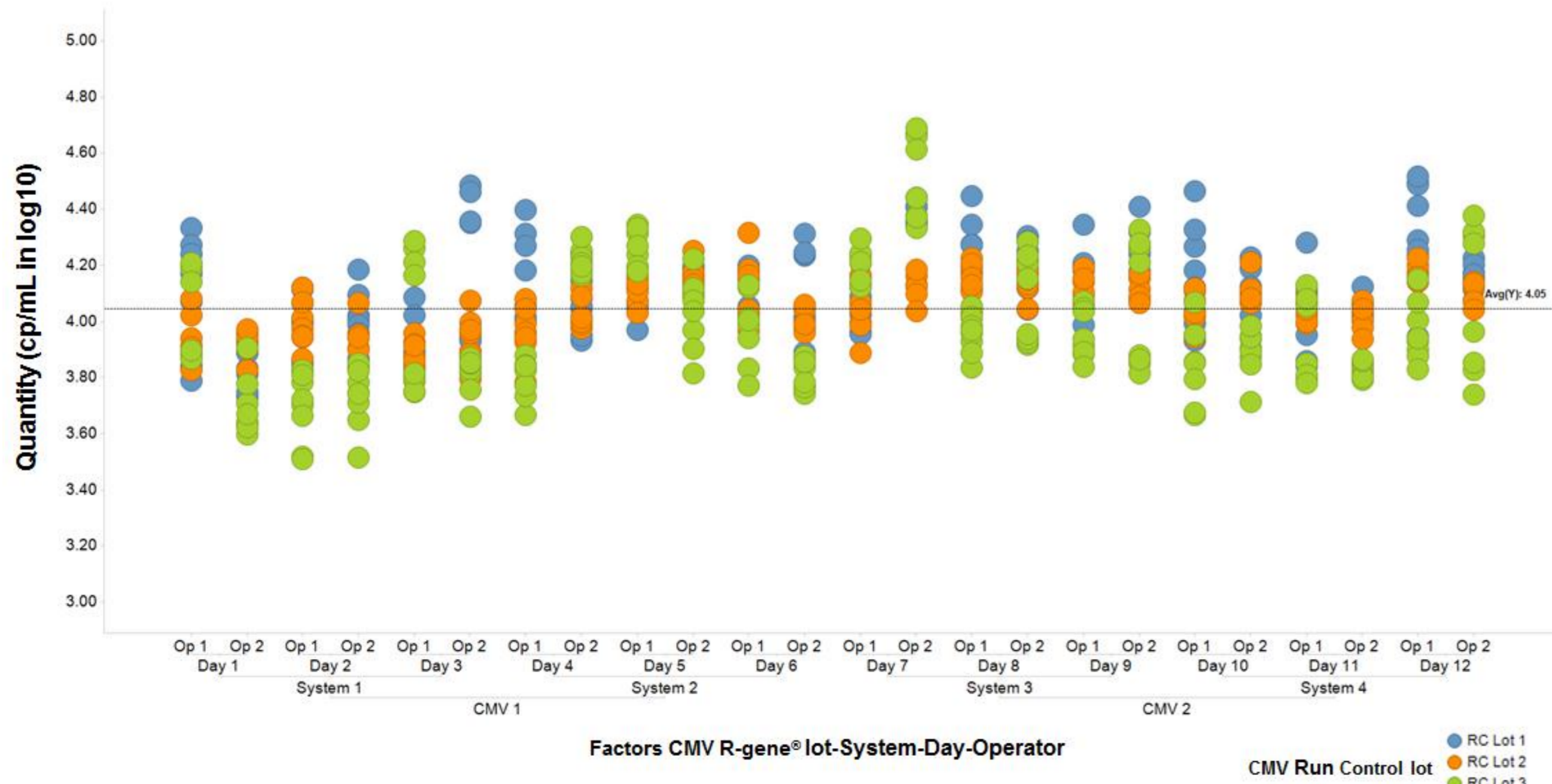
To facilitate the usability of this product and answer to the need of customers (use of the product on a working week (5 days) with storage at +2/+8° C after the first thawing), in-use stabilities were performed. To simulate the in-use in worst case conditions, CMV Run Control r-gene® was left at +37° C for 2 hours each day (with opened cap) in a period of 5 consecutive days. At day 1, 3 and 5, a vial stored at +2/+8° C and a reference tube (stored at -15/-31° C) were extracted 2 times on NucliSENS® easyMAG® and each extract was amplified in triplicates on ABI 7500 Fast.

RESULTS

✓ Precision studies:

☐ Combination Platforms tested: NucliSENS® easyMAG® / ABI 7500 Fast

The results of the first precision study performed on 3 independent lots of CMV Run Control r-gene® on the combination platforms NucliSENS® easyMAG®/ ABI 7500 Fast are presented in the following figure:



(1) eMAG™ will be validated soon with CMV R-gene® and CMV HHV6,7,8 R-gene® kits

The mean quantification value is 4.05 log₁₀ cp/mL.

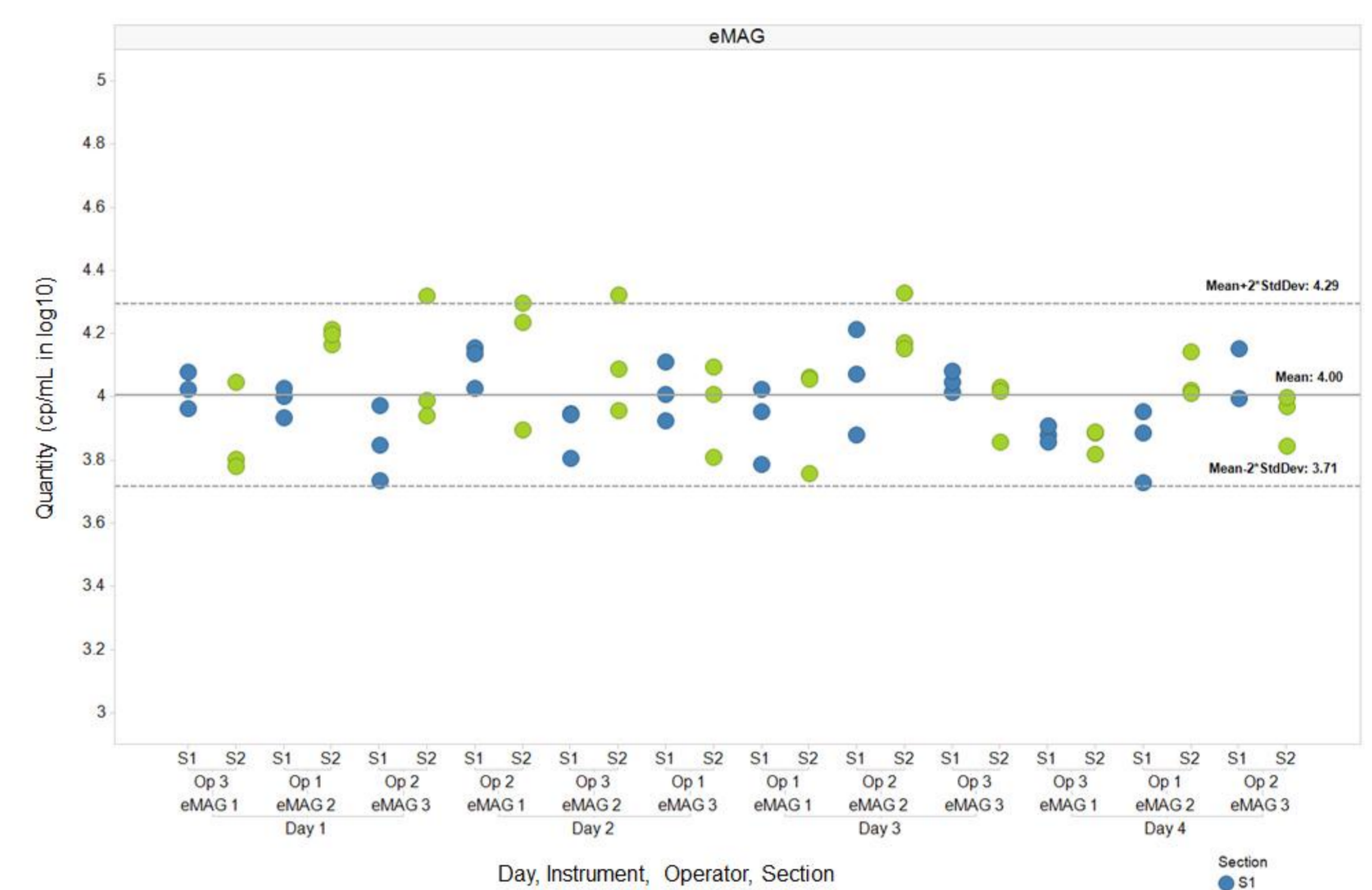
The mean standard deviation associated to the within-lots variability is 0.18 log₁₀ cp/mL, that allows to ensure that 2 repeats with a same CMV Run Control r-gene® lot cannot lead to a difference in results higher than 0.5 log₁₀ cp/mL.

The between-lots intermediate precision is 0.09 log₁₀ cp/mL in addition to the variability linked to the tubes, the extraction and the amplification (respective standard deviations of 0.11, 0.01 and 0.07), leading to a total variability of 0.16 log₁₀ cp/mL. This means that 95% of the quantification results will be ranged between the mean value +/- 0.32 log₁₀ cp/mL. This result ensures that the difference between 2 results from 2 different CMV Run Control r-gene® lots will not be greater than 0.45 log₁₀ cp/mL (95% confidence interval).

CMV Run Control r-gene® lot	Number of replicates	Mean log ₁₀ copies/mL	Within-lot precision (Std Dev)	Between-vials precision (Std Dev)
1	192	4.12	0.18	0.13
2	192	4.05	0.11	0.04
3	192	3.97	0.24	0.14
Mean		4.05	0.18	0.11

☐ Combination Platforms tested: eMAG™ / ABI 7500 Fast

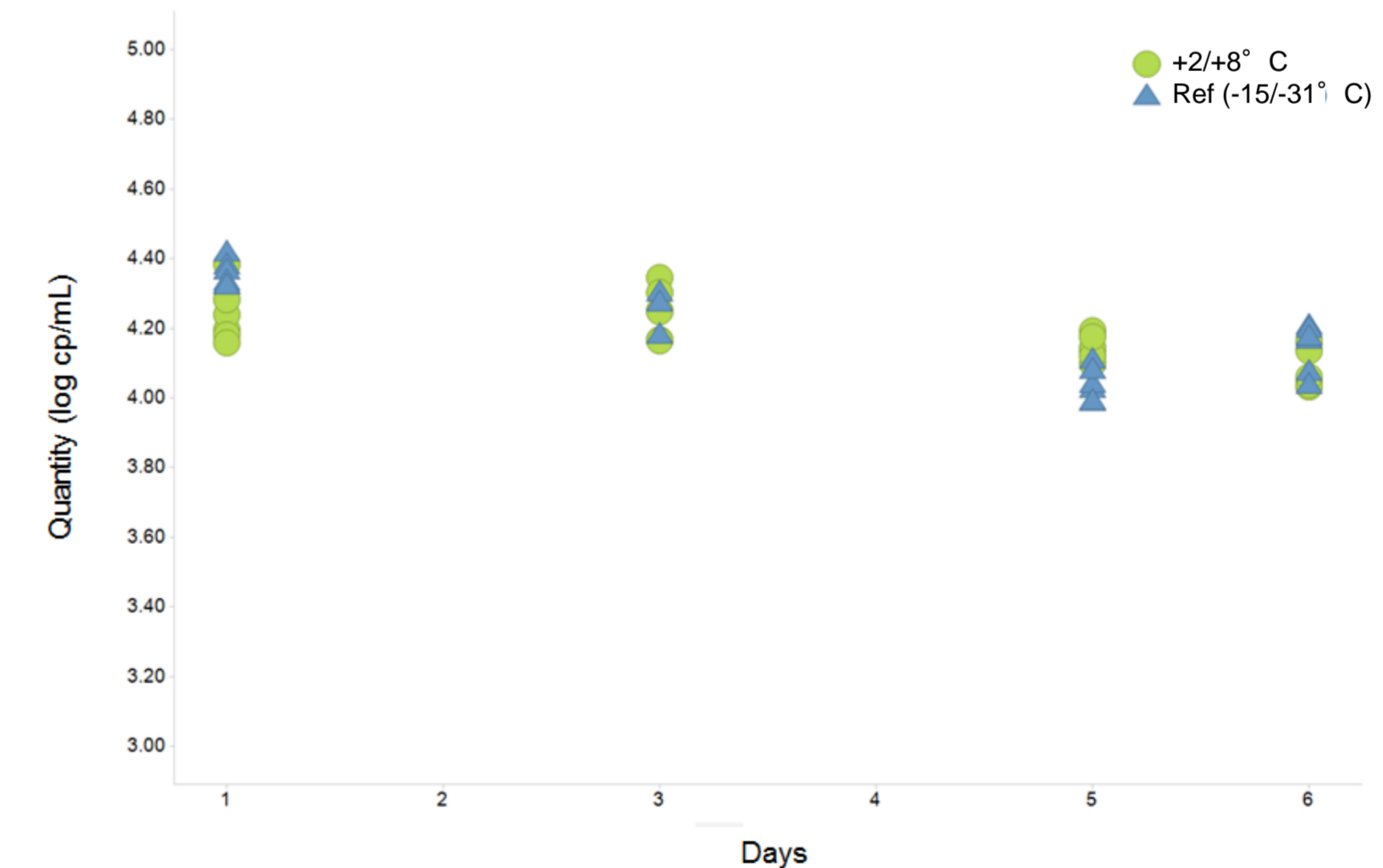
The quantity values (in log₁₀ cp/mL) of CMV Run Control by Operator, Instrument and Section on eMAG™ is represented in the following figure:



The mean quantification value is 4.00 log₁₀ cp/mL with a standard deviation of 0.14 log₁₀ cp/mL. These results are comparable to the results obtained during the first precision study obtained from a NucliSENS® easyMAG® extraction.

☐ In-use Stabilities :

The following figure shows the quantification values in log₁₀ cp/mL obtained after 1, 3 or 5 days at +2/8° C (with an incubation at +37° C for 2 hours each day). Each day a reference vial was tested in parallel (stored at -15/-31° C without freeze and thaw cycles).



These results show that the CMV Run Control r-gene® is stable up to 5 days at +2/+8° C after the first thawing.

CONCLUSIONS

Molecular diagnostic methods are used routinely to make clinical decisions on how to treat and manage patients. The CMV Run Control r-gene® is a new product of the ARGENE® products range that will contribute to ensure the quality and the reliability of the laboratory method.

Its routine use allows to assess the performance over time of nucleic acids test procedures in the detection of CMV DNA. CMV Run Control r-gene®, CE IVD marked, is a tool to support laboratory's accreditation process, in-line with ISO 15189.

The formulation of this product (non-inactivated strain of CMV (AD169) diluted in human plasma matrix) aims at mimicking a CMV positive clinical sample. This control should be treated as a patient sample within each assay run.

The results presented show a good reproducibility and repeatability of the CMV Run Control r-gene®. The results of in-use stabilities demonstrate that this product can be conveniently stored at +2/+8° C for up to 5 days without impact on its performance.