

BIOMERIEUX MOLECULAR WORKFLOW : SOLUTION FOR ARGENE® PRODUCT RANGE AUTOMATION

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INTRODUCTION

Interest in automation of Molecular workflow is increasing in clinical diagnostic laboratories, as it saves time in routine procedures, reduces tedious manual pipetting steps, and improves throughput and traceability.

BioMérieux solution targets the integration of the main steps of molecular diagnostics from sample to result (Extraction, PCR set up, and PCR reaction). The organization by modules (e.g. easyMAG®, easySTREAM™, amplification/detection platforms) enables to maintain and develop the required flexibility, both in terms of configuration and of compatible applications. The current NucliSENtral® V2 is able to connect the easyMAG® extraction platform, the easySTREAM™ and different PCR platforms to transfer relevant data from samples and test requests to the PCR run including the plate layout. In the clinical domain, this solution facilitates and supports the ARGENE product range by using dedicated assay protocols, but it is also compatible for Lab Developed Test (LDT), also called “Homebrew”. In the marketplace, the presented solution aims to answer to the needs of Molecular Laboratories with medium/high throughput which need to improve and develop their connectivity and automation.

The objective of this study was to verify the performances of the bioMérieux molecular automated solution, compared to manual process. The easySTREAM™ volumetric results, carry-over experiment, CMV R-gene® and EBV R-gene® on clinical samples quantification and Influenza A/B r-gene® on QCMD (Quality Control for Molecular Diagnostics) panel results are presented.

MATERIAL AND METHODS

Negative and positive clinical samples (EBV, CMV, FluA and FluB) were collected from different hospital centers or from QCMD panel. Samples were extracted on NucliSENS® easyMAG® (bioMérieux) using the recommended protocol for these type of matrices (whole blood and reconstituted matrix): New improved whole blood extraction protocol (Specific B, 200/50) for whole blood and classical Specific B, 200/50 for the other matrices. 200µL of each sample (and 10µL of internal control when it applies) were placed in the easyMAG® vessel and the vessel was loaded onto the extractor. After the run, the easyMAG® vessels containing the eluates were loaded directly on a specific holder into easySTREAM™ to prepare the PCR reactions. PCR reactions were also manually prepared with the same eluates to compare performance. For both methods, PCR set up was performed as follows: 10 µL of purified nucleic acids were added to 15 µL of ready-to-use amplification/detection premix. The amplifications were performed on Biorad Dx Real Time System using the recommended protocols by bioMérieux.

All the data (samples data, PCR test requests and plate layouts) were transferred through the various elements of the automated platform using NucliSENtral® V2 software. An estimation of the time from samples to results has been done in parallel (data shown on the figure).

easySTREAM™ PERFORMANCE

easySTREAM™ volumetric performance

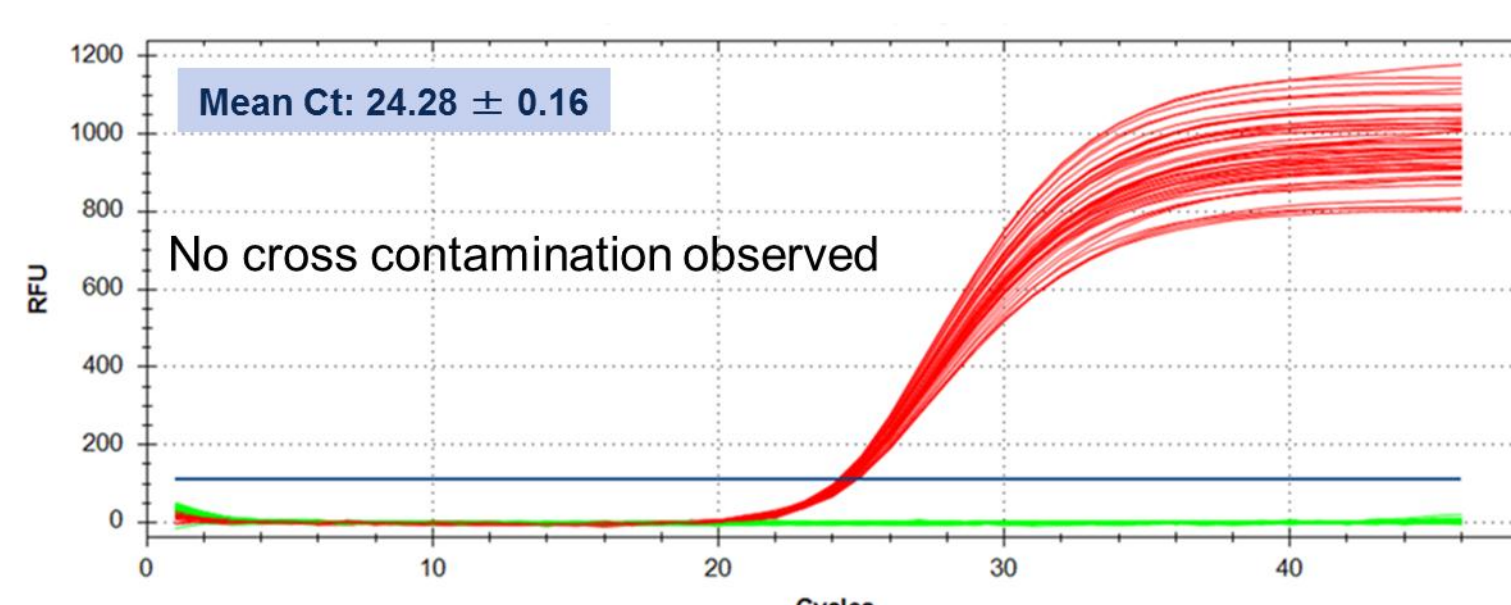
Type of dispense	Volume	Mean	Sd Dev	Accuracy	CV
Single dispense (50µl tips; dry well transfer) from 1,5mL microtubes	5µL	5.25µL	0.30	5.00%	5.80%
	10µL	10.42µL	0.31	4.20%	2.96%
Single dispense (50µl tips; dry well transfer) from easyMAG® vessels	10µL	9.97µL	0.29	0.32%	2.86%
Single dispense (200µl tips; dry well transfer) from 1,5mL microtubes	180µL	180.22µL	1.18	0.12%	0.66%
Multi dispenses (200µl tips; dry well transfer) from 0,5mL Simport microtubes	15µL	14.87µL	0.44	0.87%	2.98%

As expected, the volumetric performance were in the required specifications:

For volume of 5µL : 10% accuracy and 10% CV

For volumes between 5µL and 180µL: 5% accuracy and 5% CV

easySTREAM™ Carry-over (contamination test)



As expected, the 48 positive replicates of EBV samples (plasmid at 5x10⁴ copies/reaction) were correctly amplified and detected (red curves). The 48 negative replicates (green curves) have remained non-detected. The mean of Ct of 48 positive replicates was 24.28 cycles with a standard deviation of 0.16 cycles.

25µL reaction volume, EBV R-gene® amplification kit.

COMPARISON OF BIOMERIEUX WORKFLOW VS MANUAL RESULTS FOR INFLUENZA A/B QCMD PANEL

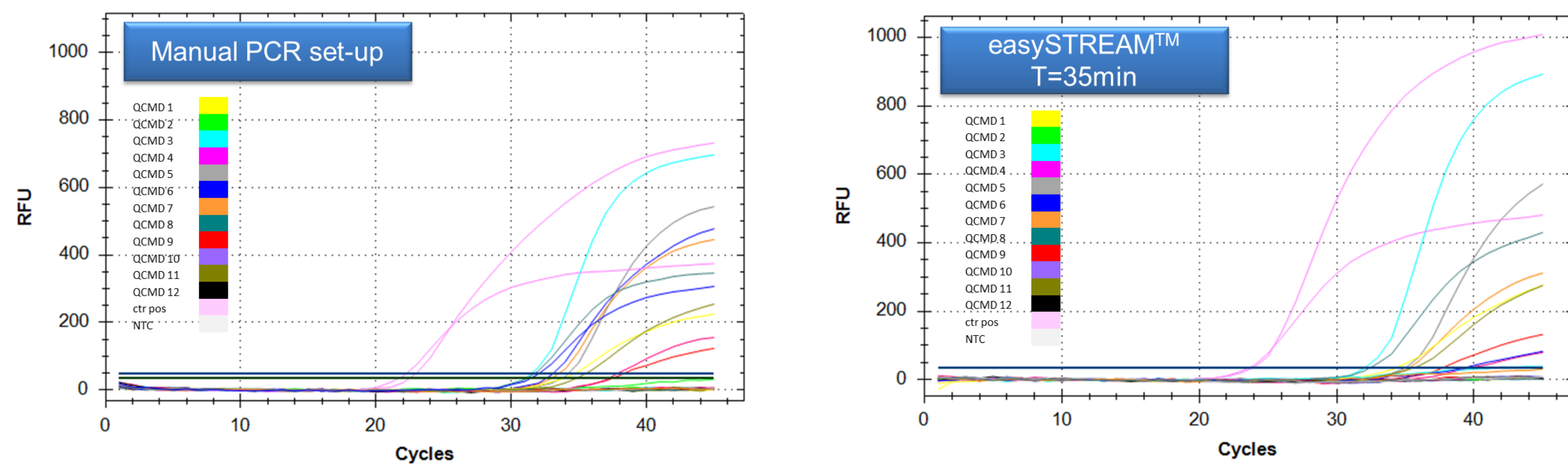
Comparison of qualitative results

INFRNA12 QCMD Panel 2012	Samples Status	Manual		
			NEG	POS
easySTREAM™	Core	NEG	2	0
		POS	0	4
	Educational - Detected or Frequently Detected	NEG	2	0
		POS	0	1
	Educational – Infrequently Detected	NEG	2	0
		POS	0	0

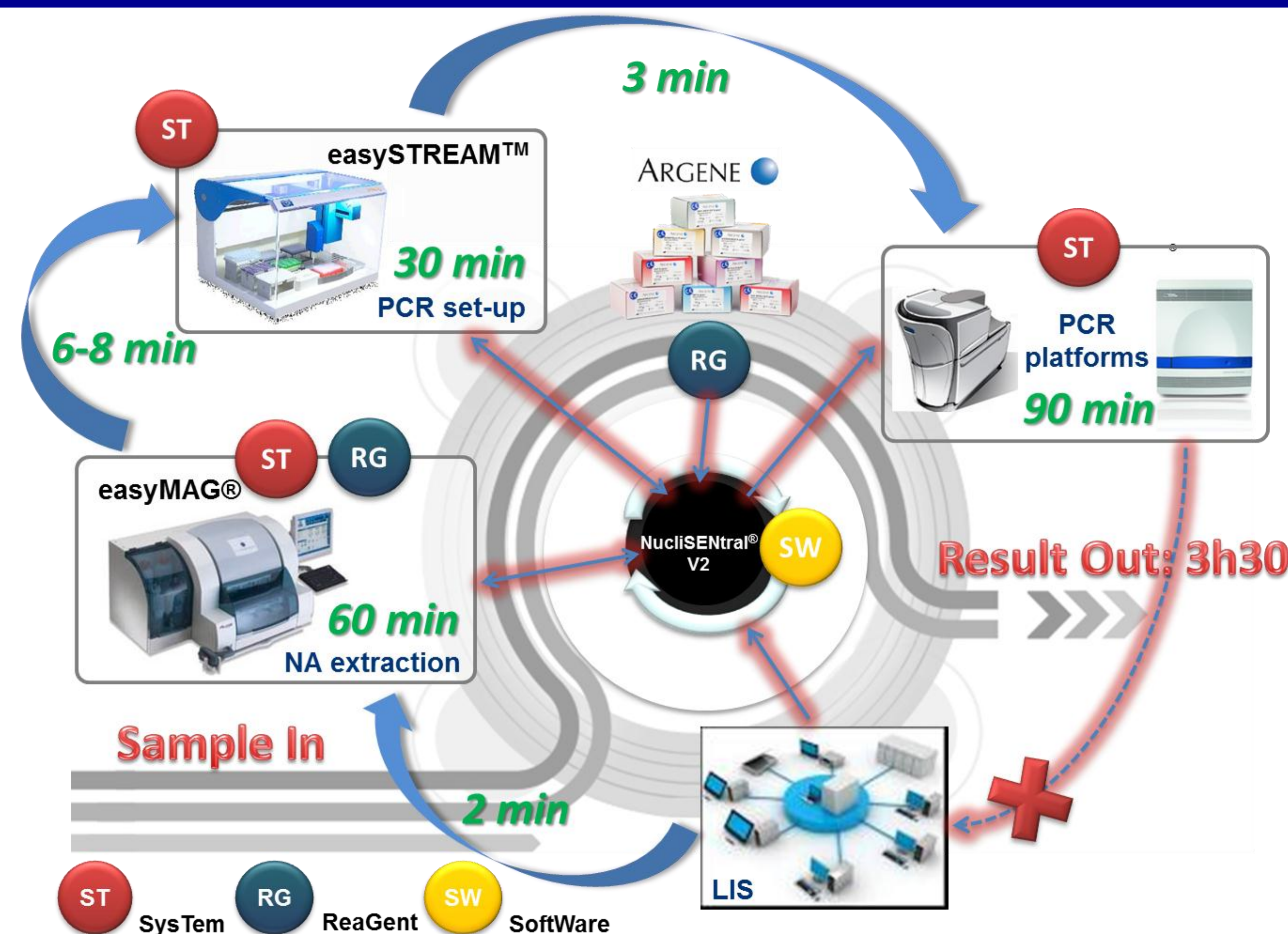
No impact on qualitative results has been identified using the easySTREAM™ instrument compared to manual PCR set-up. Nevertheless, for some samples, a slight impact on Ct values or amplification profiles was observed in function of the time spent in the easySTREAM™ instrument; e.g. t=35min for a full run of 96 wells, without any impact on qualitative clinical results.

Note: the QCMD Core negative sample was not detected in each tested condition as expected.

Comparison of amplification profiles

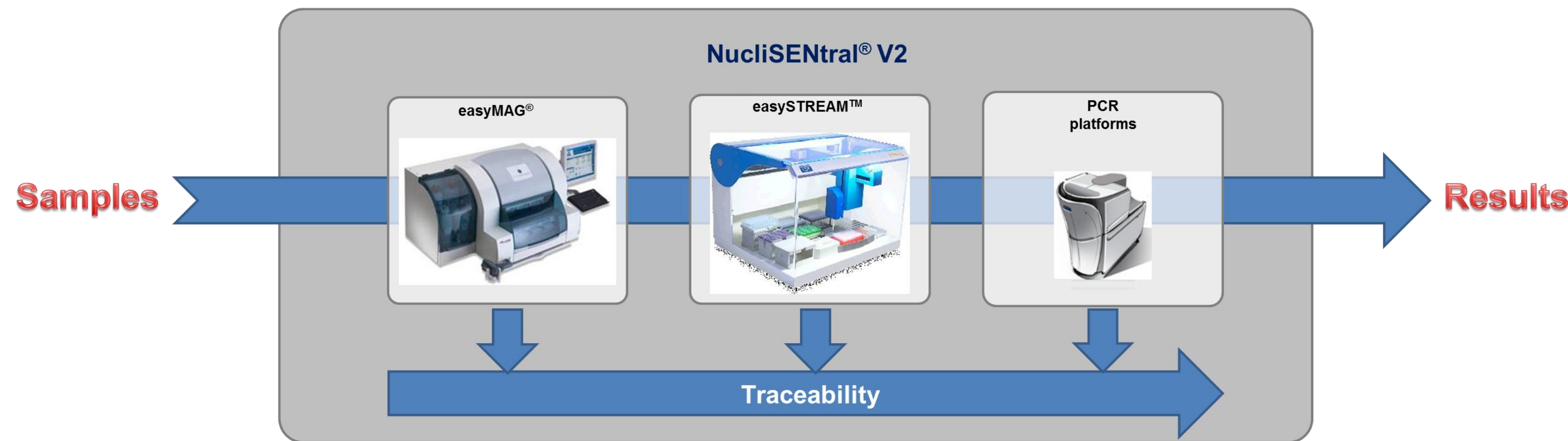


BIOMERIEUX AUTOMATED MOLECULAR WORKFLOW SOLUTION



COMPARISON OF BIOMERIEUX WORKFLOW VS MANUAL RESULTS FOR CMV AND EBV CLINICAL SAMPLES

Benefits of the bioMérieux automated molecular workflow



Comparison of qualitative results

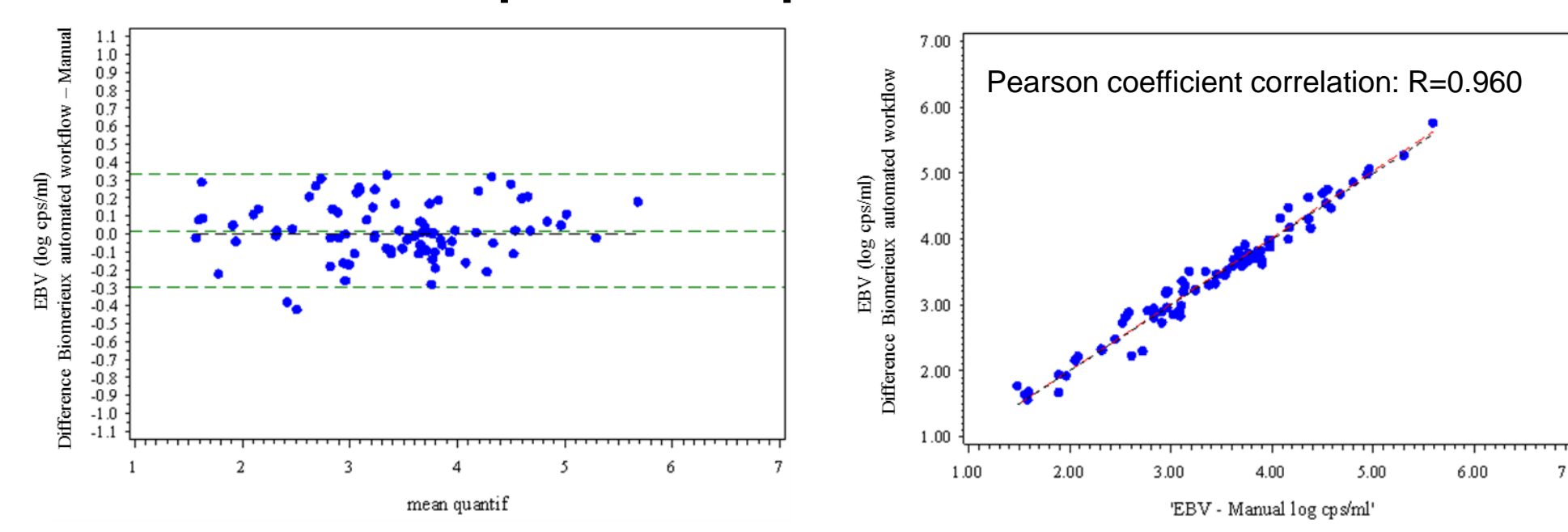
EBV		Manual sample status			
		INV	NEG	POS	Total
bioMerieux automated workflow sample status	INV	3	0	0	3
	NEG	0	24	2	26
	POS	1	0	84	85
	Total	4	24	86	114

Positive percent agreement = 84/86 = 97.7 % [92.0 ; 99.7]%

Negative percent agreement = 24/24 = 100.0 % [85.8 ; 100.0]%

The samples (in red) found negative with automated workflow but positive with manual have a viral load less than 2 log for EBV (cps/mL).

Comparison of quantitative results



Mean difference = +0.02 logcps/ml ; SD=0.16
95% dispersion interval (mean ± 2*SD) = [-0.30 ; +0.33] log cps/ml

(black line=line of perfect agreement Y=X;
red line=deming regression line)

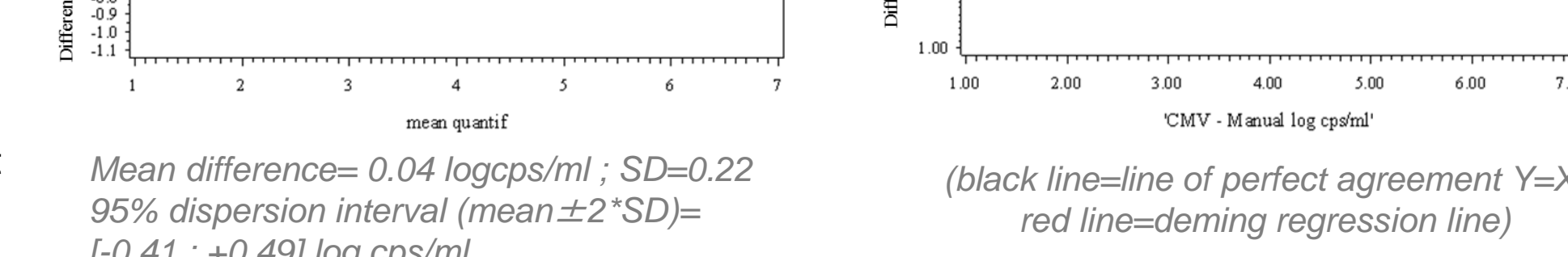
CMV

CMV		Manual sample status			
bioMerieux automated workflow sample status	INV	2	0	0	2
	NEG	0	39	3	42
	POS	1	1	72	74
	Total	3	40	75	118

Positive percent agreement = 39/40 = 97.5 % [86.8; 99.9]%

Negative percent agreement = 72/75 = 96.0 % [88.8; 99.2]%

The samples (in red) found negative with automated workflow but positive with manual have a viral load less than 3 log for CMV (cps/mL).



Mean difference = 0.04 logcps/ml ; SD=0.22
95% dispersion interval (mean ± 2*SD) = [-0.41 ; +0.49] log cps/ml

(black line=line of perfect agreement Y=X;
red line=deming regression line)

Data clearly show the perfect comparison of the bioMérieux automated molecular workflow to the manual process in terms of quantification performances, accompanied also by a drastic improvement of traceability, hand-on time, risk of human error and time to results.

CONCLUSIONS

The presented data demonstrate the key features necessary to provides to clinicians high-level capabilities of the “samples to results” automated bioMérieux solution:

- Automatic transfer of information between steps of the sample workflow,
- Facilitate transfer of disposables from one system to another,

The versatile and compact easySTREAM™ Liquid Handling System, combined with the dedicated NucliSENtral® V2, allows a complete sample workflow from the extraction using easyMAG® instrument through automation of PCR set-up using easySTREAM™ to different PCR platforms. This solution improves throughput, traceability, reproducibility and reliability and ensures the expect biological performance. It can easily be integrated in any laboratory workflow to link test requests, extraction and amplification steps, even for customers using home-brew PCR tests.