BIOMERIEUX ARGENE RANGE VERIFICATIONS ON THE ViiA7™ (FAST AND STANDARD BLOCKS) AND ABi 7500 FAST DX REAL TIME PCR PLATFORMS

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INTRODUCTION

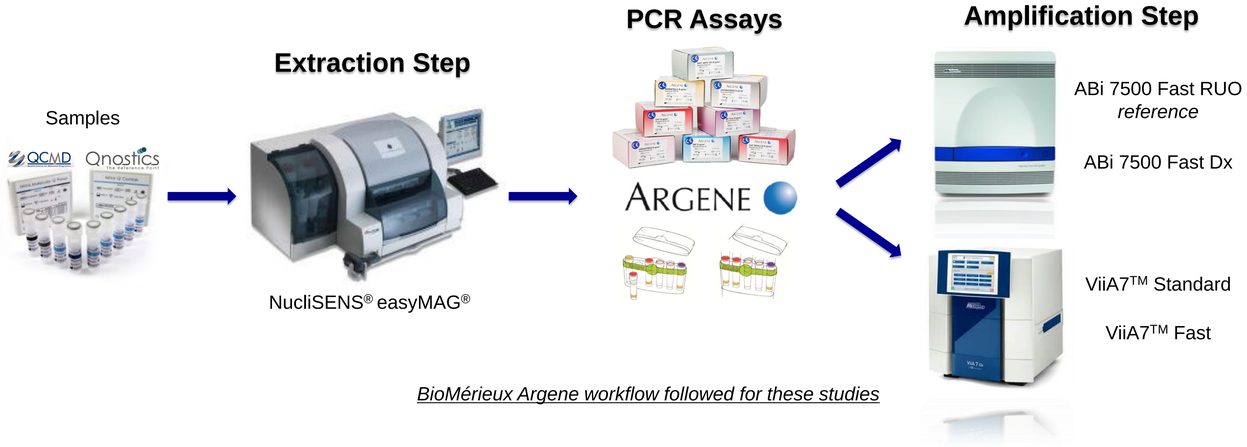
The bioMérieux Argene range is a complete solution, based on the real time PCR 5'nuclease technology, for the management of infections in the immunocompromised patients, respiratory and central nervous system infections. This range provides standardized assays validated with the major real time PCR platforms.

The objective of this study was to verify the performances of R-gene® and M.W.S. r-gene® CE-IVD assays on three Applied Biosystems real time PCR platforms: ViiA™ 7 Fast and Standard blocks and ABi 7500 Fast Dx, compared to the ABi 7500 Fast RUO version (reference). The CMV R-gene® and Influenza A/B r-gene® analytical sensitivities, the CMV R-gene® linearity and precision, the Influenza A/B r-gene® precision, and QCMD or home-made panels testing for the Argene range are presented.

MATERIAL AND METHODS

Samples were extracted on NucliSENS® easyMAG® (bioMérieux) with the specific B protocol, 400/100 and 200/50. 10 µL of purified nucleic acids were added to 15 µL of ready-to-use amplification/detection premix. RNA assays needed a dilution of the reverse transcriptase.

For the 4 studies (i.e. analytical sensitivity, linearity, precision and panels testing), amplifications were performed on ViiA7™ Fast and Standard, ABi 7500 Fast Dx and the ABi 7500 Fast RUO (Life Technologies™), used as reference.



BioMérieux Argene workflow followed for these studies

Limit of Detection (LoD) of the CMV R-gene® and Influenza A/B r-gene® assays were assessed by Probit analysis (SAS 9.2 software) on 15 replicates, of 4 points of CMV, Influenza A and B, respectively diluted in a negative whole blood or nasopharyngeal matrix.

The **precision** study of the CMV R-gene® assays was performed on 2 dilutions, corresponding to QS1+1Log (Log 7) and lower limit of quantification (LLoQ). For the MWS Influenza A/B r-gene® kit, both targets were tested at 5 times the limit of detection. For both assays, each point was extracted 3 times on 2 different easyMAG™ and amplified with 2 reagent batches by 2 operators, 24 replicates in total.

The **linearity** of CMV R-gene® was established on a dilution series of CMV positive sample in negative whole blood, on the 3 new platforms and compared to the reference ABi RUO.

All R-gene® and MWS r-gene® kits were tested with **QCMD Past Panels** (Qnostics) or **home-made panels**. 20 QCMD panels (2012 or 2013) were tested for the kits when a corresponding panel existed. For the other kits, contrived panels were constituted. Each sample was tested in duplicate.

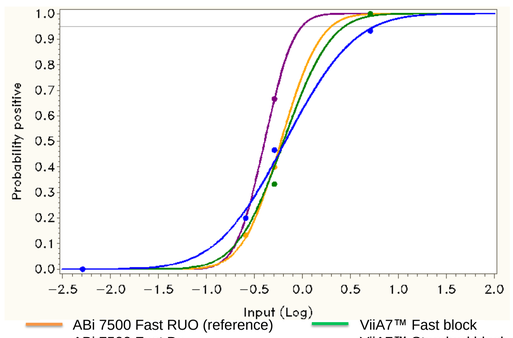
ARGENE

69-002 EBV R-gene®	71-004 JC Virus r-gene® Primers/Probe	71-040 Influenza A/B r-gene®	71-100 DICO Ampli r-gene®
69-003 CMV R-gene®	71-010 Adenovirus r-gene® Primers/Probes	71-041 RSV/hMPV r-gene®	71-101 DICO Extra r-gene®
69-100 CMV HHV6,7,8 R-gene®	71-015 HSV1 r-gene®	71-042 Rhino&EV/Cc r-gene®	71-105 RICO Extra r-gene®
69-004 HSV1 HSV2 VZVZ R-gene®	71-016 HSV2 r-gene®	71-043 AdV/hBoV r-gene®	71-106 CELL Control r-gene®
69-005 Enterovirus R-gene®	71-017 VZV r-gene®	71-044 Chla/Myco pneumo r-gene®	71-300 *Influenza* $A_{(M)}$ Group & H1N1 2009 r-gene®
69-010 Adenovirus R-gene®	71-020 Parechovirus r-gene®	71-045 HCoV/PIV r-gene®	68-006 Quanti Flu A QS r-gene®
69-011 BK Virus R-gene®	71-012 Bordetella parapertussis r-gene®		
69-019 Parvovirus R-gene®	69-011 Bordetella R-gene®		

BioMérieux Argene range used for the verifications studies

RESULTS

Analytical sensitivity



Graphical representation of limit of detection of CMV on the 4 platforms determined by probit analysis

Real Time PCR Platforms	Limit of Detection estimations by Probit Analysis Log_{10} DI50/mL		
	CMV R-gene® 69-003	MWS Influenza A/B r-gene® 71-040	
		Flu A	Flu B
ABi 7500 Fast RUO (reference)	0.302	2.143	-0.440
ABi 7500 Fast Dx	-0.003	1.529	-0.329
ViiA7™ Fast Block	0.440	2.559	-0.454
ViiA7™ Standard Block	0.754	1.529	-0.297

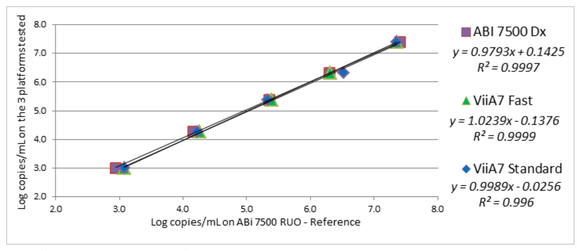
The Limits of Detection of CMV R-gene® and MWS Influenza A/B r-gene®, for the 3 viruses, on the 4 real time PCR platforms tested, show not significantly different (or better) results and are respectively ranged between -0.003 and 0.754 for CMV, 1.529 and 2.559 for Influenza A, and -0.454 and -0.297 for Influenza B (expressed in Log_{10} DI50/mL).

Precision study

Argene Kits	Sample / Viral Loads	Coefficients of Variation (%)			
		ABi 7500 Fast RUO (reference)	ABi 7500 Fast Dx	ViiA7™ Fast Block	ViiA7™ Standard Block
CMV R-gene®	Positive Sample at Log 7	1.2 %	1.8 %	2.1 %	1.3 %
	Positive Sample at LLoQ	8.5 %	4.1 %	4.0 %	5.9 %
MWS Influenza A/B r-gene®	Flu A at 5 x LoD 95%	2.1 %	3.1 %	2.6 %	2.7 %
	Flu B at 5 x LoD 95%	3.4 %	3.3 %	3.1 %	2.6 %

The coefficients of variation are below 2.1% for CMV high viral loads and ranged from 2.1 to 8.5% for CMV and Influenza low positive samples. For CMV, the variability observed for each tested platform is not significantly higher from a statistical point of view than the variability of the reference platform (Fisher test). For Influenza, differences observed are considered acceptable.

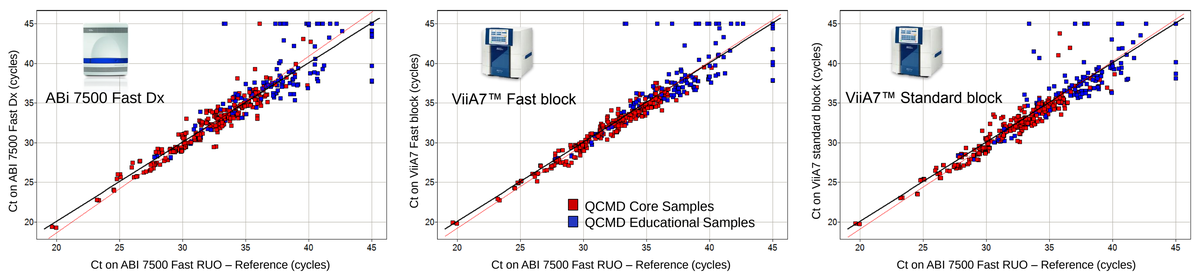
Linearity



Comparison of the 3 linear ranges obtained on the platforms tested against the linear range of the ABi 7500 Fast RUO as reference

The linearity ranges tested on the new platforms, between 3.32 and 7.32 log of CMV, are equal to the limit of the linearity range obtained on the reference ABi 7500 Fast RUO.

QCMD and home-made Panels testing



Crossing threshold (Ct) comparison between ABi 7500 Fast RUO and each of the three platforms tested for QCMD Samples

For the ViiA7™ standard block, 98.5% (382/388) of the core samples were correctly identified versus the QCMD reference (Log_{10} analysis for quantitative assays). For the ViiA7™ Fast block and the ABi 7500 Fast Dx, the % of core samples correctly identified reached 99% or more [i.e. 99% (384/388) for the ABi 7500 Fast Dx and 99.7% (387/388) for the ViiA7™ Fast Block].

For the educational samples, with low pathogen loads, tested for informational purposes, more than 91% of the samples were correctly identified versus the QCMD reference using both ViiA7™ platforms, i.e 91.4% (170/186) for the Fast block and 93% (173/186) for the Standard block. 85.5% (159/186) were correctly identified with the ABi 7500 Fast Dx.

For non-QCMD samples, 97% to 100% of the samples were correctly identified depending on the platform. 100% of the results obtained for DICO/RICO kits on all three platforms were in agreement.

CONCLUSIONS

The performances of the R-gene® and M.W.S. r-gene® assays on the ViiA™ 7 Real-Time PCR systems (Fast and Standard blocks) and on the ABi 7500 Fast Dx are comparable to the ones on the ABi 7500 Fast RUO.

These studies allow to extend the use of the CE-IVD bioMérieux Argene real time PCR range to three additional real time PCR platforms.