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easySTREAM™: A Liquid Handling System to automate ARGENE® assays set-up from extracted samples

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INTRODUCTION

The 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.
We are introducing a flexible liquid handling system (L.H.S), called easySTREAM™ dedicated to Real Time PCR set-up that allows automatic handling of 48 extracted samples from easyMAG® vessels or up to 90 extracted samples from secondary tubes with up to 3 quantitative or 6 qualitative ARGENE® PCR assays within 30 minutes. PCR plates from the common qPCR instruments are available in dedicated library plates.

MATERIAL AND METHODS

Nucleic acids were extracted from various type of clinical samples (Whole Blood, CSF, respiratory specimen….) according to the package insert of ARGENE® kits by using NucliSENS® easyMAG® system. Elution was performed in 50µL or 100µL depending on sample volume input.
easyMAG® vessels, containing fresh eluted nucleic acids, and all PCR reagents are loaded on dedicated holders and transported to the deck of the L.H.S.
Up to 6 vessels can be directly loaded using 16-Well easyMAG® vessel holders on the easySTREAM™. Specific holders for ARGENE® assays are provided to load up to 3 Quantitative assays or up to 6 Qualitative assays. Each Quantitative assay is composed of 2 or 3 ready-to-use Mixes, 4 Quantification Standards (QS) and 1 Sensitivity Control (SC). Qualitative assay is composed of 2 or 3 ready-to-use Mixes and 1 Positive Control (PC). Then for a same extracted sample, both Quantitative and Qualitative assays can be managed at the same time, on the same experiment with easySTREAM™.
Assay setup is performed using a user-friendly software. Pre-stored run templates including positions, volumes and transports of mixes, standards/controls and samples allow to launch a run within 5 min after samples data import. In less than 30 minutes a full 96-wells plate can be set up and is ready to amplify on qPCR instrument.

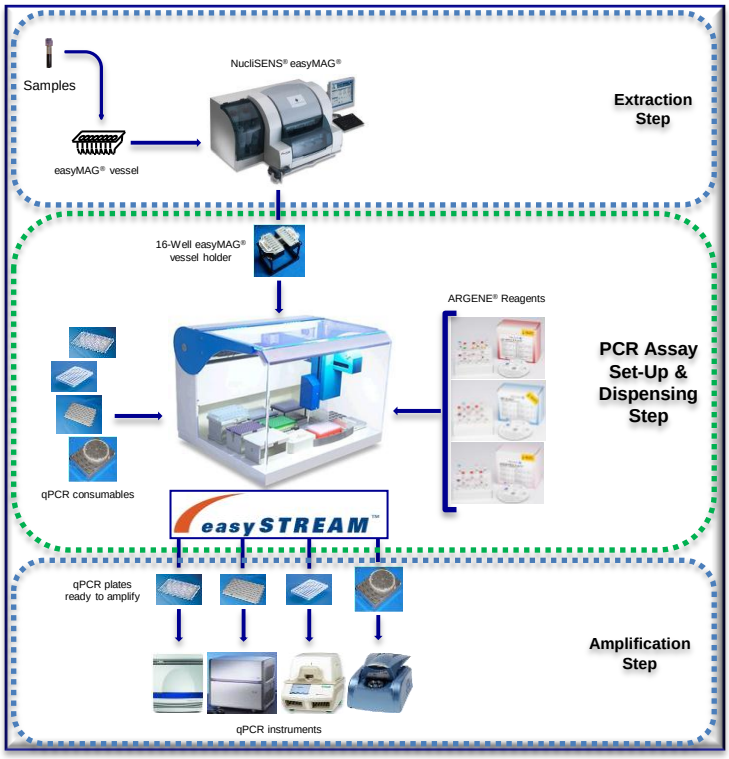


Figure 1: Integration of easySTREAM™ in a workflow laboratory

Technical validation of easySTREAM™ was performed:
- Manual dispensing against easySTREAM™ dispensing on characterized samples.
- Carry over study.

Specific ready to use template have been created:
- 3 Quantitative ARGENE® assays.
- 6 Qualitative ARGENE® assays.

✓Comparison of manual dispensing and easySTREAM™ dispensing on EQA Panels:
EBV DNA EQA 2011 and CMV DNA EQA 2012 Panels were obtained from Quality Control for Molecular Diagnostics (QCMD), an independent international organisation. The 10 samples of EBV panel and the 10 samples of CMV panel were extracted on easyMAG® following the Viral Whole Blood protocol. 200µL of each sample and negative extraction and amplification control (W0) spiked with IC2 (W0_IC2), to check contamination and/or inhibition during the whole process of extraction and amplification, were extracted and eluted in 50µL.
Then 10µL of eluted samples, QS, SC and W0_IC2 were added into 15µL of ready-to-use premix. The dispensing of all reagents, samples, QS, SC, control were performed manually by a qualified user and compared to the dispensing performed by easySTREAM™. The amplification was performed on Applied Biosystems 7500Fast.

✓Carry over:
easySTREAM™ dispensed 15µL of EBV premix (R5) in a full 96-wells plate mix, then 48 replicates of 10µL of positive sample of EBV (1.10^5cp/PCR) or 48 replicates of 10µL of negative sample were dispensed following a checkerboard dispensing.

✓Specific ready to use templates:
easySTREAM™ software is user-friendly and intuitive. It allows the creation and the use of templates dedicated to specific laboratory wokflow, according to diagnosis routine.
We will provide various templates adapted to each laboratory routine and its qPCR equipment. To illustrate this flexibility we created a template with 3 Quantitative tests with 48 samples coming from easyMAG® vessels dispensed on CFX Dx RTS plate (Bio-Rad) then the left over of each eluate were transferred in storage tubes. Another template with 6 Qualitative tests with 80 samples coming from easyMAG® vessels or storage tubes, dispensed on ABI 7500 plate (Applied Biosystems) is also showed.

RESULTS

Comparison of manual dispensing vs easySTREAM™ dispensing on EBV & CMV EQA Panels:

EBV DNA 2011 EQA Panel		Quantity (log_{10} cp/mL)		Delta log_{10}
Sample Name	Status	easySTREAM™	Manual	easySTREAM™ - Manual
EBV2011-01	Positive Educational	2.08	2.20	-0.12
EBV2011-02	Positive Core	4.51	4.50	0.01
EBV2011-03	Positive Core	3.88	3.84	0.04
EBV2011-04	Positive Core	4.81	4.80	0.01
EBV2011-05	Positive Core	3.33	3.35	-0.02
EBV2011-06	Positive Core	3.78	3.86	-0.09
EBV2011-07	Positive Core	5.34	5.27	0.07
EBV2011-08	Positive Core	4.83	4.84	-0.02
EBV2011-09	Negative Core	N.A	N.A	N.A
EBV2011-10	Positive Core	3.94	3.96	-0.02

CMV DNA 2012 EQA Panel		Quantity (log_{10} cp/mL)		Delta log_{10}
Sample Name	Status	easySTREAM™	Manual	easySTREAM™ - Manual
CMV2012-01	Positive Core	4.63	4.66	-0.03
CMV2012-02	Positive Core	4.14	4.15	-0.01
CMV2012-03	Positive Educational	2.37	2.58	-0.21
CMV2012-04	Positive Educational	2.71	2.50	0.21
CMV2012-05	Positive Core	2.82	2.95	-0.13
CMV2012-06	Positive Core	3.66	3.41	0.25
CMV2012-07	Positive Core	3.56	3.68	-0.12
CMV2012-08	Negative Core	N.A	N.A	N.A
CMV2012-09	Positive Core	4.13	4.21	-0.08
CMV2012-10	Positive Core	2.66	3.06	-0.41

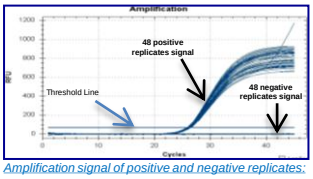
All positive samples were correctly amplified and the negative samples remained negative as expected. The maximums delta log_{10} quantification between easySTREAM™ and Manual dispensing is -0.12log_{10} for EBV and -0.41log_{10} for CMV. These values are still under the significant absolute value of 0.5log_{10}.

QS EBV ARGENE®		Ct (Cycles)		Delta Ct (Cycles)
Sample Name	Status	easySTREAM™	Manual	easySTREAM™ - Manual
QS1 EBV	Positive	24.38	24.14	0.23
QS2 EBV	Positive	27.66	27.75	-0.10
QS3 EBV	Positive	31.43	31.03	0.40
QS4 EBV	Positive	34.55	34.43	0.12
SC EBV	Sensitivity control	38.06	39.80	-1.74
Efficiency	80.00% to 110.00%	97.44%	90.81%	

QS CMV ARGENE®		Ct (Cycles)		Delta Ct (Cycles)
Sample Name	Status	easySTREAM™	Manual	easySTREAM™ - Manual
QS1 CMV	Positive	26.95	26.85	0.10
QS2 CMV	Positive	30.73	30.99	-0.26
QS3 CMV	Positive	34.18	34.54	-0.36
QS4 CMV	Positive	37.86	37.91	-0.06
SC CMV	Sensitivity control	39.26	39.47	-0.22
Efficiency	80.00% to 110.00%	89.26%	90.19%	

All expected positive samples were correctly amplified. The maximum delta Ct between easySTREAM™ and Manual dispensing is 0.40 cycles for EBV and -0.36 cycles for CMV. These values are still under the significant absolute value of 1.67 cycles. The obtained efficiencies values meet the specifications (80% - 110%).

Carry Over:
As expected, the 48 positive replicates of EBV samples were correctly amplified and detected. The 48 negative replicates have remained non-detected. The mean of Ct of 48 positive replicates was 25.86 cycles with a standard deviation of 0.206 cycles.



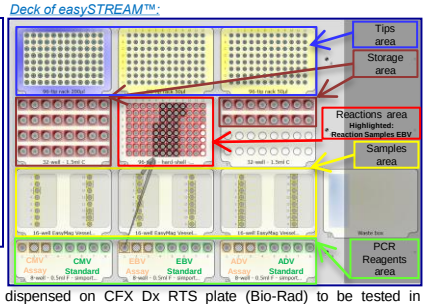
Amplification signal of positive and negative replicates:

easySTREAM™ run templates:
A) 3 Quantitative tests + Storage function:

The layout and the functions of the project are displayed in the feature tree of easySTREAM™.
Each area i.e each function is clearly identified by a specific color.
Different functions are created such as:
- Consumables
- Samples
- Mixes
- Standards
- Reactions
- Storage
- Transports

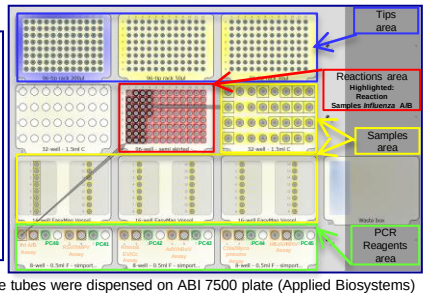
Feature tree of easySTREAM™

Deck of easySTREAM™:



48 samples from easyMAG® vessels were dispensed on CFX Dx RTS plate (Bio-Rad) to be tested in combination with 3 Quantitative Assays. Some samples were tested on 1, 2 or 3 parameters. 25/48 samples were tested on CMV, 31/48 samples on EBV and 20/48 samples on ADV. Then the left over of each eluate was transferred in appropriate storage tubes.

B) 6 Qualitative tests:
The layout and the functions of the project are displayed in the feature tree.
6 Sample groups were created, one for each mix targeted.
Each specific PCR reagents holder can manage 2 Qualitative ARGENE® Assays.



80 samples from easyMAG® vessels and storage tubes were dispensed on ABI 7500 plate (Applied Biosystems) to be tested in combination with 6 Qualitative Assays. Samples were tested on 1 up to 6 parameters. 27/80 samples were tested on Influenza A/B, 20/80 samples were tested on RSV/hMPV, 16/80 samples were tested on Rhino&EV/Cc, 8/80 samples were tested on AdV/hBoV, 10/80 samples were tested on Chla/Myco pneumo and 9/80 samples on HCoV/HPIV.

CONCLUSIONS

easySTREAM™ is a versatile and compact LHS which allows automation of PCR set-up, improves throughput and reliability, while reducing the hands on time. It is compatible with ARGENE® assays and with the major thermocyclers platforms (ABI7500, ABI7500Fast, LC480, Dx RTS and Rotorgene Q). PCR is directly set up from easyMAG® vessels or from secondary storage tubes. A user-friendly software and dedicated templates allow an easy and immediate integration in any laboratory workflow to link extraction and amplification steps.