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Efficient qPCR Setup Without Cross Contamination Using the epMotion® Family* of Automated Liquid Handling Systems

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Abstract

This application note shows that the epMotion family of automated liquid handling systems is ideal for reliable and reproducible setup of sensitive real-time quantitative PCR (qPCR) assays in daily routine, without the risk of cross contamination. The complete workflow from Master Mix preparation to 96-well PCR plate setup was done with the automation system. Cross contamination assay in chess board pattern was performed according to FDA guidance. Runs on two independent epMotion systems with 2 plates each on 3 days are compared in this document.
The assay reproducibility was excellent with low coefficients of variation for both intra and inter-plate comparisons of C_t values. Automated assay setups were carried with the ep*Motion* 5073 and the 5075 systems.

Introduction

Based on the standard PCR combined with a fluorescent reporter monitoring, the real-time quantitative PCR (qPCR) allows to amplify, detect and quantify the targeted molecule in a single step. Over the last few decades, this molecular biology technology has progressively become a reference method commonly used in research for a wide range of applications including gene expression studies, DNA damage measurement or SNP genotyping. By combining excellent sensitivity, great specificity, low contamination risk, performance and speed, the real-time PCR also rapidly appeared as the perfect alternative to conventional culture-based or immunoassay-based methods used for diagnosing infectious diseases [1]. Moreover, because it is compatible with automated liquid handling systems, qPCR can be regarded as a high throughput method. Indeed, manual qPCR setup is time-consuming, tedious and requires lots of practice. In theory, PCR is quite robust, but in reality, minor variations can lead to large changes in the overall amount of amplified product. The most probable source of imprecision is the operator itself. Three different individuals used same pipettes, Master Mix, template, and machine to quantify the same target and found initial copy numbers ranging from 2.7×10^3 to 8.7×10^5 [2]. When a large number of samples has to be processed in a short time, automating qPCR setup can be the solution to increase the throughput while the quality of results is maintained.

Besides issues with reproducibility, as with traditional PCR, real-time PCR reactions can also be affected by nucleic acid contamination, leading to false positive results. The three possible sources of contamination are: cross-contamination between samples, contamination from laboratory equipment and carryover contamination by amplified products from previous qPCRs. Precautions can be taken to reduce the risk of contamination: uracil DNA glycosylase (UDG) can be used to prevent DNA carryover contamination between reactions, materials can be systematically decontaminated, and separate workstations for each step of the qPCR process can be designated to create an efficient workflow. Those measures do not eliminate the major cause of false positive results: the accidental contamination with positive samples during liquid handling. By automating qPCR setups, the challenge is consequently to guarantee a reproducible qPCR setup as well as the absence of cross-contamination. This application note shows the possibility to fully automate a real-time qPCR setup either on the Eppendorf epMotion 5073 or on the epMotion 5075 automated liquid handling systems.

Materials and Methods

qPCR Assay

Real-time qPCR assay developed for *Acinetobacter baumannii (genomic DNA, extracted from ATCC strain 17978, A. baumannii)* was carried out in 96-well twin.tec real-time PCR plates. Each reaction was carried out in a total volume of 25 µL containing 12.5µl of qPCR Master Mix Plus*, 50 nM of each specific primer*, 50 nM of the TaqMan probe* (*Eurogentec), water and sample. The mix was subjected to the following thermal conditions: 2 minutes at 50 °C (UNG incubation), 95 °C for 10 minutes, followed by 40 cycles of 95 °C for 15 seconds and 60 °C for 1 minute.

Automation

For each epMotion instrument used, two methods have been programmed. The goal of the first method is to generate the final Master Mix containing forward and reverse primers, TaqMan probe, qPCR Master Mix Plus and water. The second method is dedicated to the qPCR reaction setup: 20 µL of the final Master Mix is firstly dispensed into the real-time PCR 96-well plates and followed by the sample addition (5 µL). For both methods, epMotion surfaces and tools are cleaned using a DNA decontamination solution. Both UV-lights and air filters are started 15 minutes before using the automate, whereas the UV stopped automatically after this time span. Worktables of both epMotion instruments are equipped as shown in figures 1 and 2.

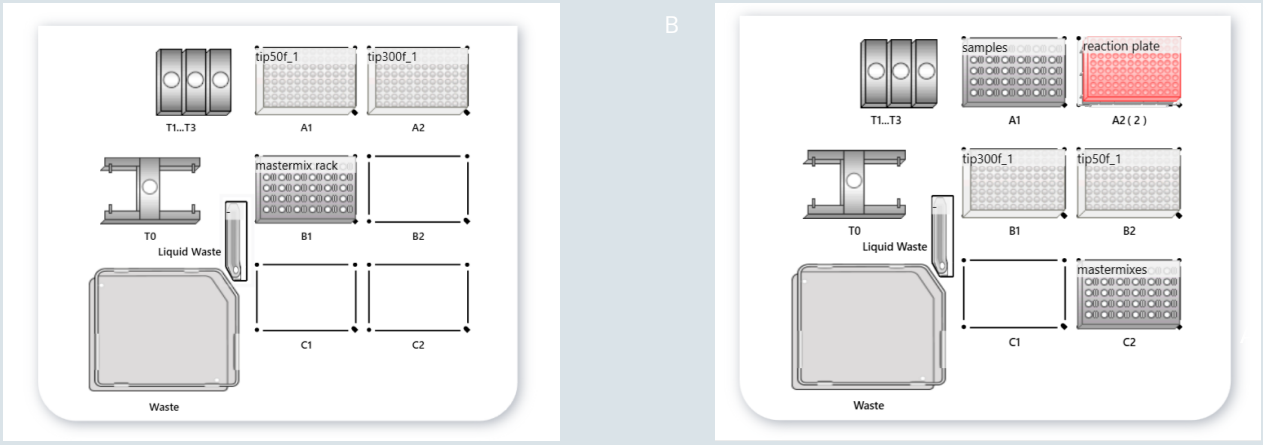


Figure 1: epMotion 5073l worktable for Master Mix preparation (A) and for qPCR setup (B)

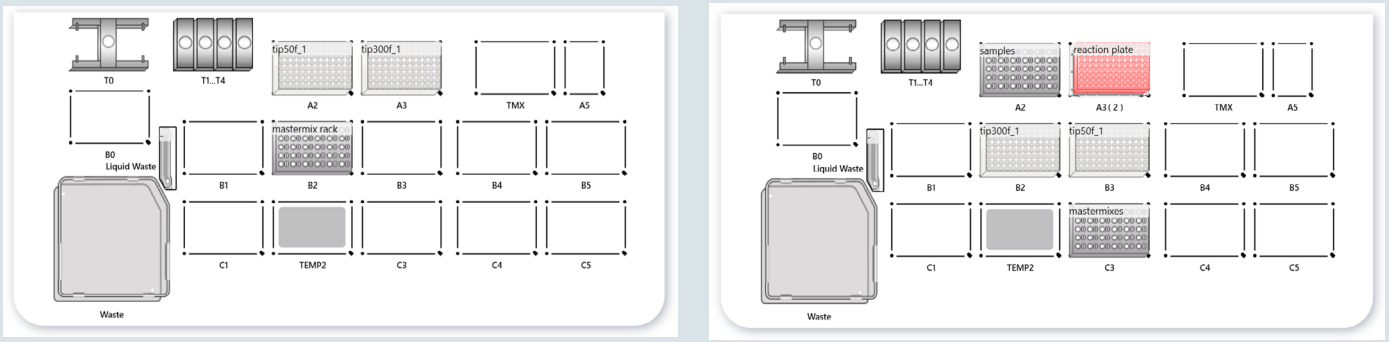
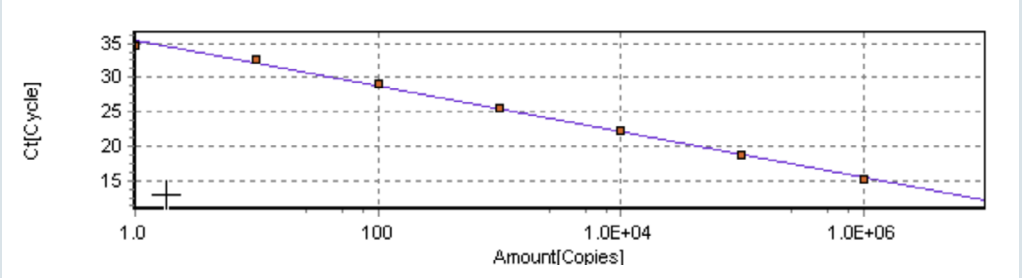


Figure 2: epMotion 5075l worktable for Master Mix preparation (A) and for qPCR setup (B)

Results and Discussion

To evalute the risk of cross contamination when an epMotion liquid handling system is used, a very sensitive qPCR assay developed for *Acinetobacter baumannii (A. baumannii)* has been selected. Performances of this assay are demonstrated by generating a standard concentration curve of *A. baumannii* genomic DNA (gDNA). This curve shown in figure 3A corresponds to a series of 10-fold dilutions of target tested from 10^6 copies to 1 copy including a negative control using water.

Parameters used to determine the assay efficiency are the detection limit, the dynamic range (indicated by the slope value) and the R^2 value (figure 3B). A slope of -3.3 reflects an efficiency of 100 % while an R^2 value above 0.99 indicates the good curve linearity and provides confidence in correlating two values. Finally, the assay sensitivity is ensured as 1 copy of genomic DNA can be detected.



Parameter	Value
Slope	-3,316
R^2 value	0.996
Detection limit	1 copy

Figure 3: Standard curve generated by using a 10-fold dilution series of *A. baumannii* genomic DNA (A) and efficiency parameters evaluated (B)

Cross contamination

Even if the risk of contamination is considerably reduced with real-time PCR compared to conventional PCR, contamination remains a concern - especially for forensic laboratories. The main causes of false positive results are the accidental contamination of samples or reagents with positive samples (cross-contamination). The sample transfer to the PCR plate represents one of the most critical steps. Care must be taken to avoid the creation of aerosol or droplet. The FDA provides information to the industry to establish the performance of devices intended for pathogen detection. In order to demonstrate that cross-contamination will not occur with the device, this guideline proposes to use high positive samples in series alternating with negative samples. The FDA also suggests that at least five runs with alternating high positive and negative samples should be performed [3]. Based on those recommendations and in order to demonstrate that epMotion systems can be used for a complete qPCR set-up without cross-contamination, 48 high positive samples (containing 10^5 copies of *A. baumanii* gDNA) and 48 negative samples (containing water) have been dispensed in the 96-well PCR plate in a chessboard pattern (figure 4). One plate was processed per run. Two runs were performed per day and the complete experiment has been carried out during three different days. The complete procedure (Master Mix preparation and qPCR setup) was executed on two epMotion models: either on epMotion 5073l or on epMotion 5075l.

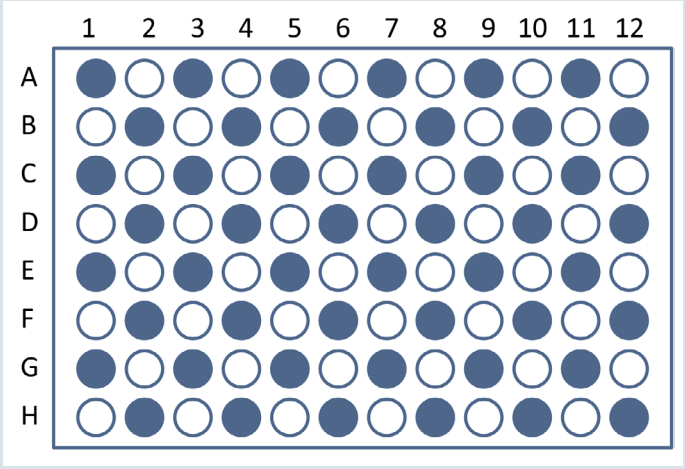


Figure 4: 96-well PCR plate containing 10^5 copies of *A. baumannii* gDNA (gray wells) and water (white wells)

Table 1: Number of negative samples in which *A. baumannii* gDNA is detected when the complete PCR setup is performed with epMotion 5073l (A) and epMotion 5075l (B)

epMotion **5073l**					
Day 1		**Day 2**		**Day 3**	
Plate 1	Plate 2	Plate 1	Plate 2	Plate 1	Plate 2
0/48	0/48	0/48	0/48	0/48	0/48

epMotion **5075l**					
Day 1		**Day 2**		**Day 3**	
Plate 1	Plate 2	Plate 1	Plate 2	Plate 1	Plate 2
0/48	0/48	0/48	0/48	0/48	0/48

Reproducibility

Reproducibility is a key component of real-time PCR assay reliability. When a large number of samples are analyzed in 96-well format, the assay must be reproducible enough to allow the data comparison between samples, plates and separate runs. The assay reproducibility when an epMotion automate is used for qPCR setup was assessed by comparing results generated from 48 positive samples containing a known gDNA amount. As illustrated on figure 5, the complete process is highly reproducible. The mean intra-plate C_t value is very consistent with a coefficient of variation never exceeding 1.5 %.

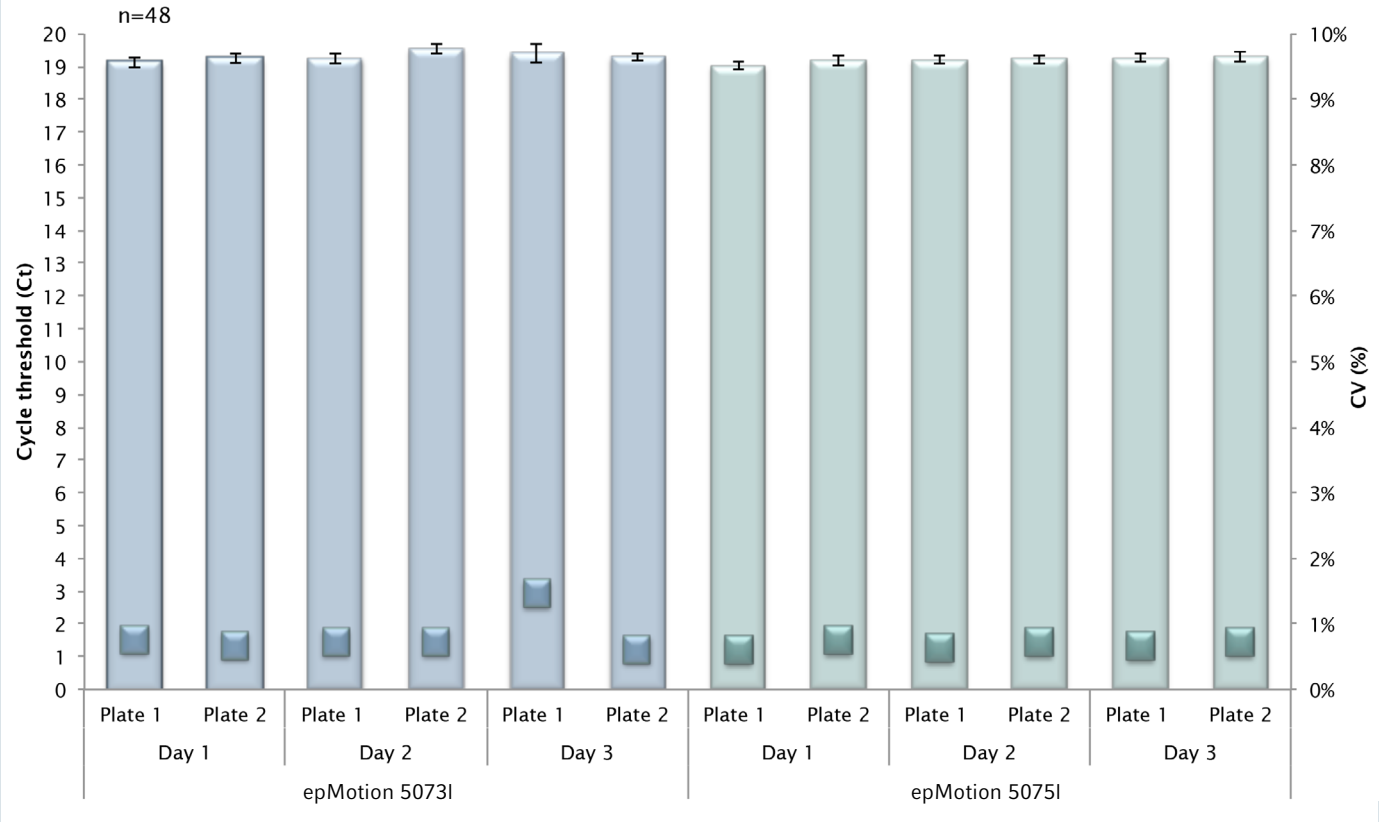


Figure 5: *A. baumannii* qPCR results. Mean intra-plate C_t value, standard deviation and coefficient of variation (CV) are calculated for each plate containing 48 positive samples.

Both epMotion models offer comparable reproducibility as the epMotion 5073l gives an average inter-plate C_t value of 19.31 ± 1.11 % while a mean inter-plate C_t value of 19.19 ± 0.80 % is obtained with the epMotion 5075l.

Conclusion

In the present Application Note, we demonstrate the capability of the epMotion liquid handling system to automate a complete real-time quantitative PCR assay from Master Mix preparation to 96-well PCR plate setup. A cross contamination study was carried out and showed that qPCR setup can be completed by the epMotion without contamination. The assay reproducibility was also assessed by comparing results generated from positive samples containing a known gDNA amount and concluded to a highly consistent amplification with a CV lower than 1.5 %. The qPCR automation was equally successfully performed on two epMotion models: epMotion 5073l and epMotion 5075l allowing customer to select the most suitable automated solution to his needs. Those results clearly indicate that for scientists interested in a low to a medium-throughput analysis, epMotion workstations can represent a perfect solution. By reducing human intervention and thanks to an accurate pipetting system, epMotion systems provide superior assay reproducibility without cross contamination, ensuring reliable results.

Literature

[1] Espy M.J., Uhl J.R., Sloan M., Buckwalter S. P., Jones M.F., Vetter E.A., Yao J.D.C., Wengenack N.L., Rosenblatt J.E., Cockerill III F.R. and Smith T.F. Real-Time PCR in Clinical Microbiology: Applications for Routine Laboratory Testing. Clinical Microbiology Reviews 2006; 19:165-256.

[2] Bustin S.A. Quantification of mRNA using real-time reverse transcription PCR (RT-PCR): trends and problems Journal of Molecular Endocrinology 2002; 29:23-39.

[3] Establishing the Performance Characteristics of In Vitro Diagnostic Devices for the Detection or Detection and Differentiation of Human Papillomaviruses. *Guidance for Industry and Food and Drug Administration Staff. 2011.*

Ordering information

Description	Order no. international	Order no. North America
epMotion® 5073l	5073000110	5073000110
CleanCap Upgrade Set for epMotion® 5073	5073001333	5073001333
Waste bags	5075751780	5075751780
epMotion® 5075l	5075000041	5075000041
CleanCap Upgrade Set for epMotion® 5075	5075001888	5075001888
Waste bags	5075752034	5075752034
TS50 single-channel Dispensing tool	5280000010	960001010
TS300 single-channel Dispensing tool	5280000037	960001028
Thermorack for 24 x 1.5/2.0 mL Safe-lock tubes	5075771004	960002075
Height adapter, 40 mm	5075755009	960002121
epT.I.P.S.® Motion 50 µL Filter, sterile	0030015215	0030015215
epT.I.P.S.® Motion 300 µL Filter, sterile	0030015231	0030015231
DNA LoBind Tubes, 2.0 mL	0030108078	022431048
DNA LoBind Tubes, 1.5 mL	0030108051	022431021
Eppendorf twin.tec® real-time PCR plates 96, semi-skirted	0030132505	951022003
HeatSealing PCR Film	0030127838	0030127838
HeatSealer S100	5391000001	5391000010

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*Developed on a predecessor model, but thanks to the migration feature, this method can easily be transferred to the newest generation of epMotion®.