Using **PCRMix™ QualAssure™** for assessing liquid transfer performance with the **MVS®**

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Summary

When assessing the accuracy and precision of a liquid transfer with the Multichannel Verification System (MVS), specially formulated dye-containing solutions are used as a proxy for the liquid being transferred. Liquid handling is affected by the physical properties of the liquid, therefore, the specific MVS dye-containing solutions must possess similar physical and fluidic properties as the actual liquid used in the transfer, i.e. the standard MVS Aqueous QualAssure solutions are used for assessing aqueous liquid transfers whereas the DMSO QualAssure solutions are used for assessing DMSO-containing liquid transfers. Here we show how the PCRMix QualAssure solutions replicate the liquid transfer properties of several commercially available PCR master mixes, making them reliable and ready-to-use reagents for determining liquid transfer performance using the MVS.

Introduction

Determining the accuracy and precision of liquid transfers is an essential activity for any life science lab, whether it is for assay optimization, routine calibration, or as-needed volume verification. Gravimetry is a common approach for evaluating the performance of liquid handling devices, and typically involves measuring a volume of solution (usually water) into a container whose weight is measured before and after dispensing the solution. Working conditions (e.g. temperature, pressure, humidity, etc.) must be carefully controlled and monitored to obtain accurate results with gravimetric methods; control of the working conditions is particularly important when measuring small volumes in order to reduce the uncertainty introduced by electrostatic effects and evaporation. A more efficient method is to use an MVS, which leverages a dual-dye, dual wavelength ratiometric absorbance-based method[1] to quickly and easily calculate the volume of sample dispensed into the well of a microtiter plate. The system provides highly accurate measurements of small volumes of liquid traceable to national and international standards.

When using the MVS to evaluate the performance of a liquid transfer, a specially formulated, dye-containing QualAssure solution is used in the place of the liquid whose transfer is being evaluated (the target liquid). Because physical properties like viscosity can affect the behavior of the target liquid during pipetting, resulting in over- or under-dispensing, the formulation of the sample solution is critical — if the sample solution does not possess physical properties similar to the target liquid, it will not adequately replicate the behavior of the target liquid during pipetting. To address this issue and provide accurate assessments of liquid transfers, Artel manufactures different types of sample solutions that can be used to evaluate the transfer of different types of target liquids.

In this white paper, we demonstrate the ability of PCRMix QualAssure to replicate the liquid transfer behavior of commercially available master mixes, enabling fast and reliable evaluation of pipetting steps involving master mix, such as during the setup of PCR assays, qPCR assays, and NGS library preparation.

Materials and Methods

To show the importance of using like-solutions in determining accuracy and precision for liquid transfers we dispensed Aqueous QualAssure C with an aqueous liquid class, and again with a master mix liquid class. Alternatively, we repeated this process with PCRMix QualAssure C using new tips for each liquid transfer. These liquid classes were preset methods, specific for aqueous and master mix liquid transfers. Data for these tests were collected with the MVS using 96-well Verification Plates.

To understand how well PCRMix QualAssure replicates the behavior of commercial master mixes during pipetting, we set up a study comparing the accuracy of liquid transfers of four commercially available master mixes (Table 1), Aqueous QualAssure, PCRMix QualAssure, and an alternative solution prepared using aqueous dye-based QualAssure Stock Solution in 20% Glycerol. Commercial master mixes were selected to include the major mix components - sucrose, glycerol, enhancers, stabilizers, proteins, enzymes – that contribute to viscosity.

Table 1. Commercially available master mixes used in this study

Master Mix	Source	Assay
EconoTaq® PLUS 2x Master Mix	Lucigen	qPCR
Quick-Load® Taq 2X Master Mix	New England Biolabs (NEB)	qPCR
TaqMan Fast Advanced Master Mix	ThermoFisher	qPCR
PCR Master Mix (2X)	ThermoFisher	PCR

For conducting the liquid transfer, we used an epMotion (Eppendorf) automated liquid handler (ALH) with either the TM10 or TM50 8-channel dispensing tool, and optimized the liquid class settings for pipetting using PCRMix QualAssure C with the MVS (Table 2). Commercial master mixes were thawed on ice and kept on ice until testing (see boxed section), while the MVS solutions were refrigerated and kept on ice until testing. However, once on the deck of the epMotion, all solutions remained at ambient temperature (21-22°C) throughout the testing procedure. The average time each solution remained at room temperature until pipetting was 5 to 10 minutes. Environmental measurements of temperature, humidity and barometric pressure were used in our calculations to account for evaporation.

Table 2. Liquid class settings used in this study

For all three volumes tested, these liquid class settings were the same:

Solution:	PCRMix QualAssure C
Aspirate speed:	3 mm/s
Dispense speed:	3 mm/s
Delay blowout:	3,000 ms
Speed blowout:	66 mm/s
Movement blowout:	0%

Target volume (µL)	Dispense height from well bottom (mm)	Volume offset (µL)	Relative inaccuracy (%)	CV (%)
9.9 (TM50)	0	+0.1 µL = 10.0 µL total	0.84	0.25
5 (TM50)	0	-0.1 µL = 5.0 µL total	0.23	0.46
2 (TM10)	1	-0.2 µL = 1.8 µL total	-1.17	0.54

Liquid transfers were tested at three different volumes (2 µL, 5 µL, and 9.9 µL) in triplicate using new pipette tips for each liquid transfer. The transferred liquid was measured using a 4-place Sartorius balance in our ISO 17025 accredited calibration laboratory, equipped for low and ultra-low gravimetric measurements. We originally conducted this study in our Applications lab, but found it difficult to obtain consistent results without the additional technology found in the QC lab, which include a vibrationally-isolated table for the balance, electrostatic ground devices, and ability to effectively control environmental conditions such as air flow, temperature, and humidity.

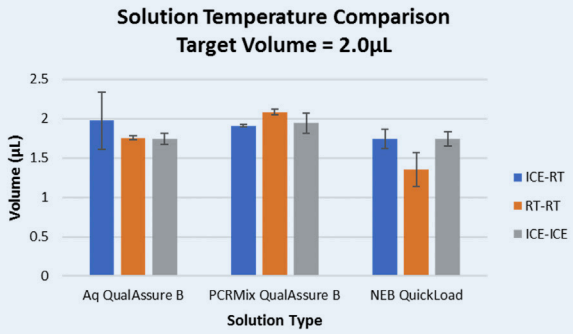
Why we chose "thaw on ice/keep on ice" for master mix handling

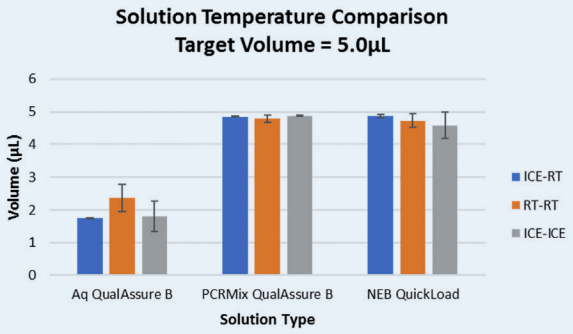
One potential source of lab-to-lab or operator-to-operator variability in the volume of liquid that is transferred during pipetting is how reagents are handled prior to aliquoting. Both the temperature of the solution and the environment can affect pipetting performance[2].

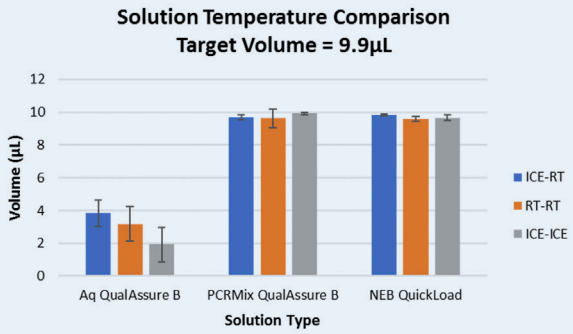
With these concerns in mind, we compared the effect of three different temperature handling conditions on the pipetting performance of our Aqueous QualAssure, PCRMix QualAssure, and NEB's Quick-Load Taq 2X Master Mix:

- ICE-RT— master mix was thawed on ice and kept on ice until placement on the deck of the automated liquid handler, where it was kept at room temperature (21-22 ˚C) for ~5-10 minutes until pipetting.
- RT-RT— master mix was thawed at room temperature (21-22 ˚C) and kept at room temperature until pipetting.
- ICE-ICE — master mix was thawed on ice and kept cold via thermoblock on deck of automated liquid handler throughout pipetting.

The most accurate and consistent results were found using the ICE-RT testing conditions. In addition, we believe the ICE-RT settings are the most widely used conditions for these assays.







It is important to note that because we used a liquid class method optimized for PCRMix QualAssure, we anticipated lower volume transfers with Aqueous QualAssure across all volumes but more accurate and comparable volume transfers with NEB QuickLoad. This observation is most noticeable at the 5.0 µL and 9.9 µL volumes.

ICE-RT master mix handling conditions led to accurate pipetting at all tested volumes (n=3 for each measurement), as shown by measurement of liquid transfer accuracy.

Results and Discussion

To show the impact of what different liquid classes can have on the same solution and why it's essential to use like-solutions for determining volume accuracy and precision we transferred 9.9 µL Aqueous QualAssure C with the aqueous liquid class and again with a master mix liquid class. Figure 1 demonstrates that liquid class selection appears to have little effect on the aqueous solution, where the transfer was both accurate and precise. This process was repeated with PCRMix QualAssure where the data revealed a significant under delivery when using the aqueous liquid class and only slight improvement when using the master mix liquid class. Further modifications to the existing master mix liquid class were needed to achieve accurate and precise results. This was repeated at 2 µL and 5 µL (data not shown) with similar results.

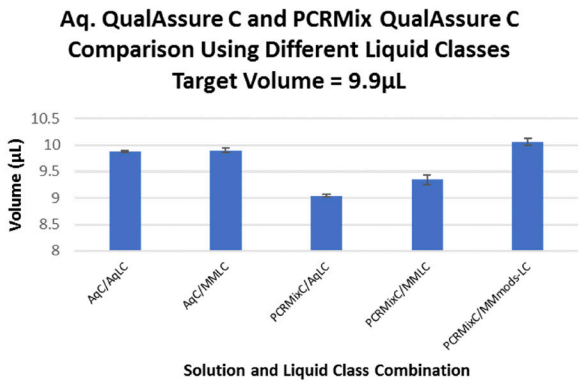


Figure 1. *Identifying the importance of using the appropriate liquid class method with the appropriate QualAssure solution (n=3 for each measurement).*

For the purpose of this figure, "AqLC" refers to the aqueous liquid class, "MMLC" refers to the master mix liquid class and "MMmods-LC" refers to the modified version of the master mix liquid class.

Equivalency testing was performed to show the similar pipetting behaviors between PCRMix QualAssure and four commercially available master mixes. Figure 2 shows the difference in transferred volume between PCRMix QualAssure and the commercially available master mixes. The master mixes were consistent within 0.8 µL for the 9.9 µL target volume and within 0.9 µL for the 5 µL target volume for all four master mixes. The 2 µL target volume was within 0.5 µL for three out of four master mixes.

In contrast, Aqueous QualAssure C and 20% glycerol solutions are not suitable substitutes for accurate representation of master mix reagents. When Aqueous QualAssure C and 20% glycerol solutions were measured, we saw a significant decline in volume, comparable to one another but not to PCRMix QualAssure or any of the commercial master mixes.

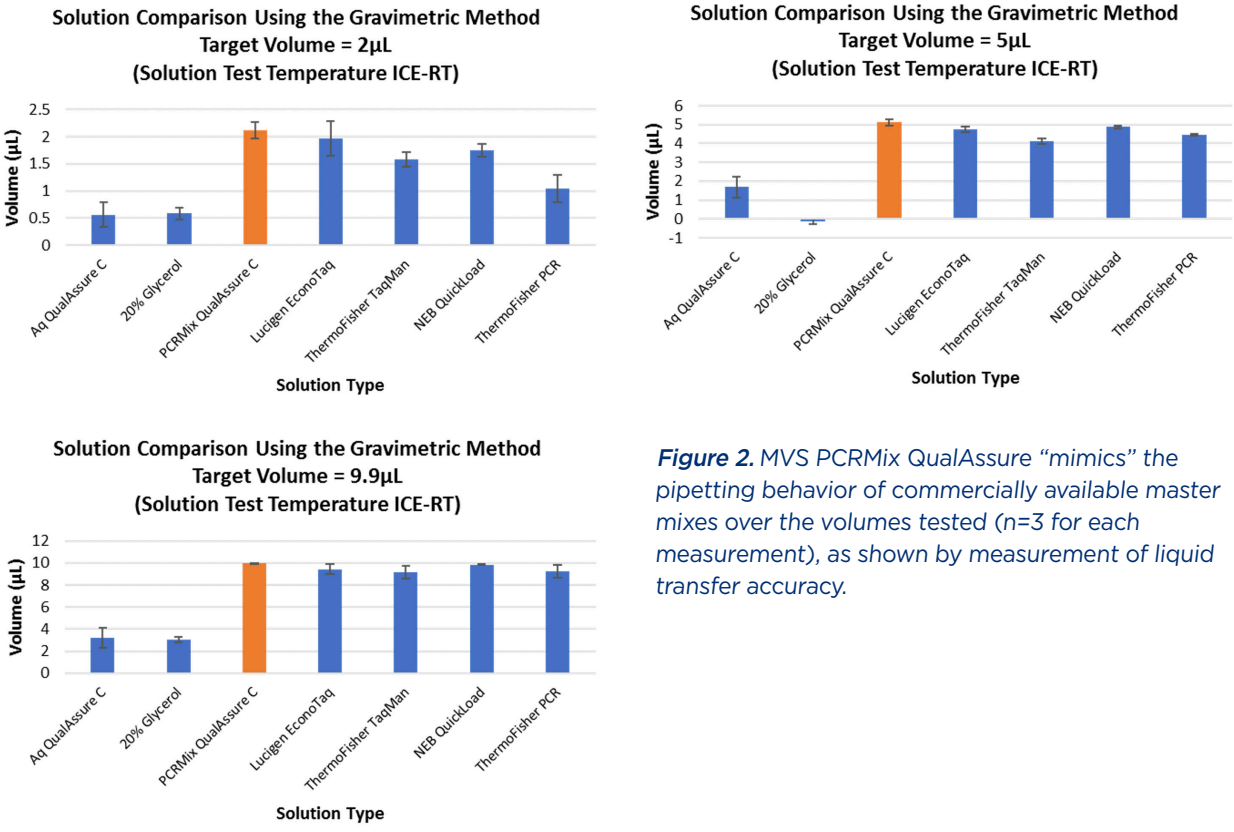


Figure 2. *MVS PCRMix QualAssure "mimics" the pipetting behavior of commercially available master mixes over the volumes tested (n=3 for each measurement), as shown by measurement of liquid transfer accuracy.*

In addition, the difference between PCRMix QualAssure and each specific master mix was minimal across all volumes (Figure 3). We observed volume measurements within 20% of PCRMix QualAssure average for both 5.0 µL and 9.9 µL volumes. At 2.0 µL, we see increased inaccuracy, but remaining within 33% of the PCRMix QualAssure average. This increased variability between commercial master mixes and PCRMix QualAssure solutions are likely attributed to the difficulties of consistently pipetting master mixes with varying fluidic compositions at low volumes. Moreover, the data show that PCRMix QualAssure is engineered to be compatible with all commercially available master mixes and is a significantly better alternative than Aqueous QualAssure, 20% glycerol, or any other substitutes.

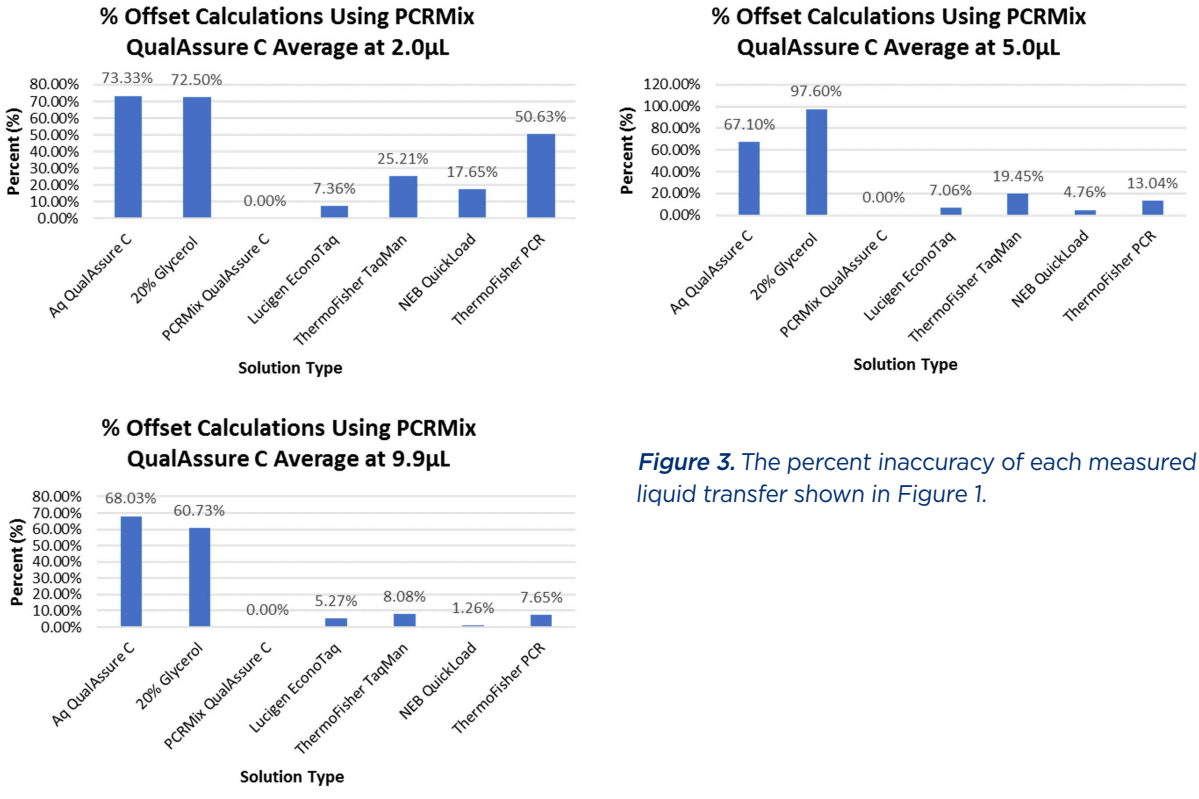


Figure 3. *The percent inaccuracy of each measured liquid transfer shown in Figure 1.*

Conclusion

The rheological properties of any liquid can have a significant effect on the accuracy of a liquid transfer. Aqueous solutions perform very differently than solutions like master mix which are difficult to pipette due to various properties including viscosity. We have shown that the PCRMix QualAssure solutions for MVS are accurate proxies for a variety of commercially available master mixes and can be used to evaluate the liquid transfer properties of these viscous solutions. In addition, PCRMix QualAssure can be used as a tool to create new liquid class methods efficiently and accurately as well as optimize existing liquid class methods for any PCR/qPCR/RT-PCR assay. We also recommend using PCRMix QualAssure as a quick and efficient way to routinely check liquid class methods, especially when any element of the method has been changed, replaced, or modified. Following these guidelines with PCRMix QualAssure will ensure more reliable and accurate data.

References

1. Learn more about Artel's ratiometric absorbance technology by visiting:
   https://www.artel.co/artel-technology/
2. Learn about the effects of temperature and other parameters on pipetting performance by visiting:
   https://www.artel.co/learning_center/impact-of-pipetting-technique/

   https://www.artel.co/improving-data-quality-and-reproducibility-part-3-temperature-differences-affect-assay-data-quality/

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