

# 1077-B: Turnkey Qualification of Liquid Handlers and Manual Pipettes

Laura Simdon,<sup>1\*</sup> John Coller,<sup>2</sup> Petar Stojadinovic,<sup>3</sup> Daniel Leach<sup>4</sup>

1. IFF, 2. Stanford University, 3. Automation Trainer, 4. Optimize Laboratory Consultants



## Overview

The ability to generate reproducible results in assay development, research and production is a major requirement of automated liquid handlers (ALHs) and pipettes in the modern laboratory environment. Regular testing of such equipment is key to qualify proper operation.

We present data generated using a turn key QC kit that can be implemented by lab personnel using either automated and manual liquid handlers to:

- assess reproducibility of volume delivery and concentration data
- test and validate tips from different suppliers
- characterize assay preparation performance and improve the consistency of quantitative polymerase chain reaction (qPCR) and PCR assays

## Introduction

During the Covid-19 pandemic, liquid handlers have been in high demand whereas the supply of laboratory consumables such as robotic disposable tips has been scarce. Some laboratories faced sharply increased costs associated with consumables as well as a need to qualify new consumables, such as robotic tips, from alternative suppliers. In addition, on site field service was often restricted by pandemic safety protocols, resulting in a need for test methods that can be implemented by lab personnel without the need for a field engineer. To address these challenges, the Good Liquid Handling (GLH) QC Kit from Automation Trainer LLC was developed.

## Methods & Equipment



Fig 1. The Good Liquid Handling (GLH) QC Kit

The GLH QC Kit from Automation Trainer LLC contains materials and reagents for performing validation and verification of manual or automated liquid handling systems. The kit includes tartrazine standards to eliminate the need for dilution (which could introduce errors if dilutions are performed using non-calibrated pipettes or liquid handlers). The system is designed to work with any plate reader and optionally includes a miniaturized plate reader. It also includes a tablet pre-loaded with software for analysis of spectrophotometric data. The GLHTracker software is preconfigured with QC methods and undistruptive robot software with liquid classes.



Fig. 2. Modified single-dye tartrazine quantification method

Included in GLH QC Cal Kit are packets of standardized concentration solutions of modified Tartrazine single dye that follows ISO IWA 15 "Specification and method for the determination of performance of automated liquid handling (ALH) systems." This material can be measured at common wavelengths and therefore works with the Mini-Reader and most off the shelf plate readers.

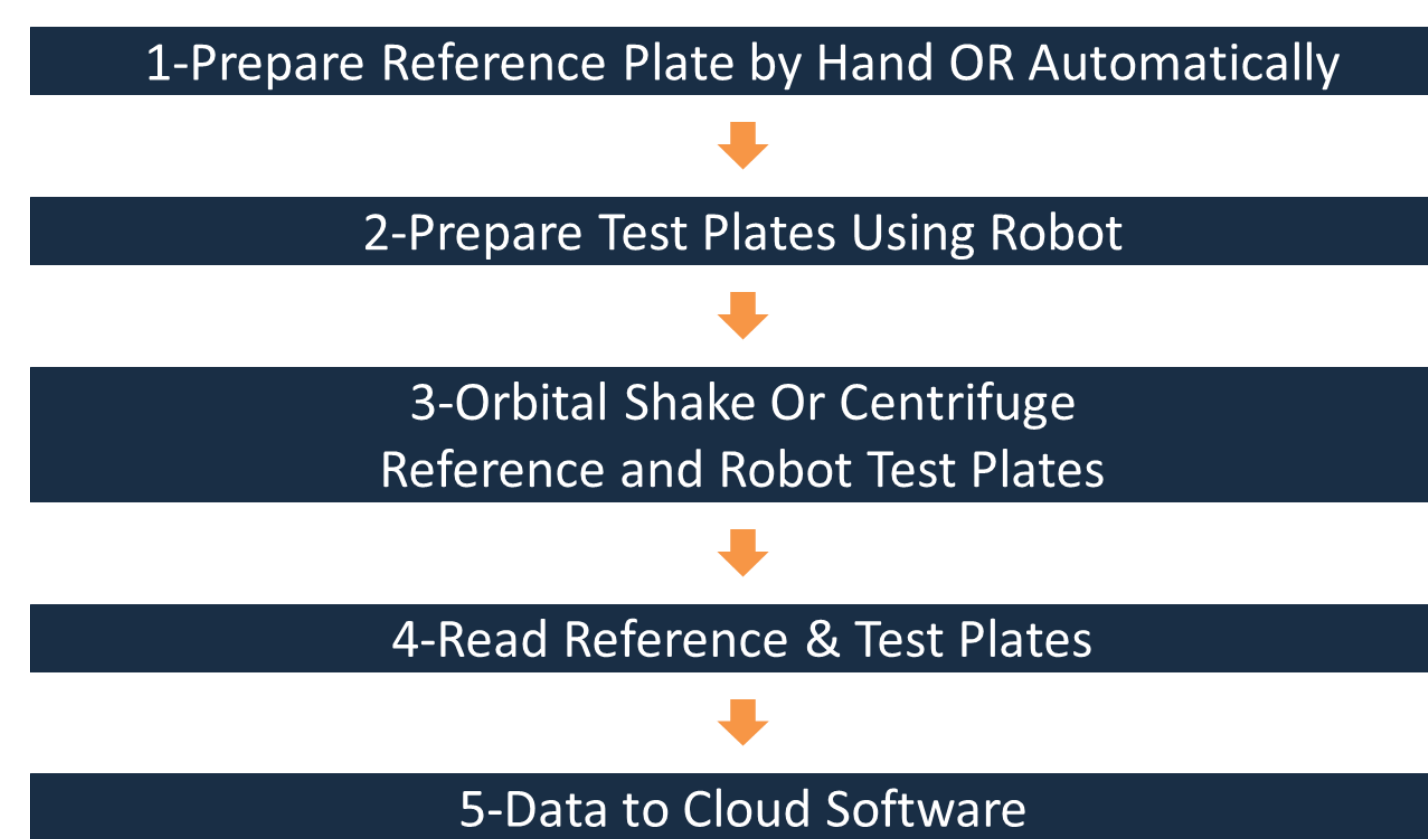


Fig. 3. Overview of GLH QC kit workflow

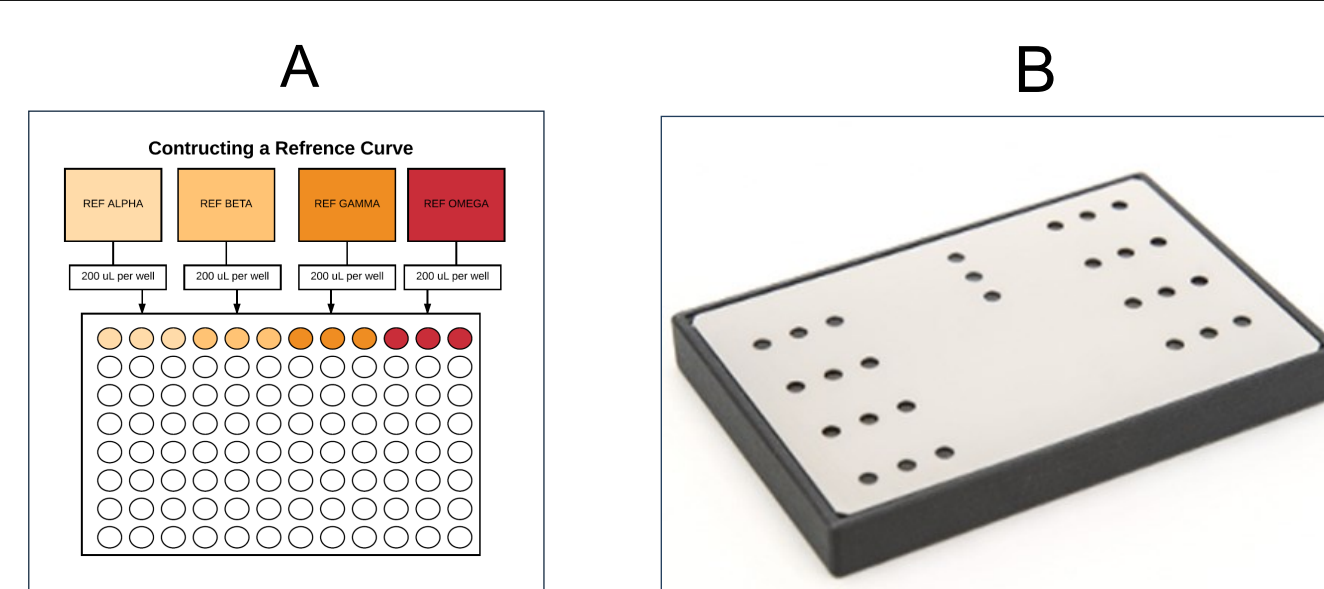


Fig. 4. Standard curves can be created A) each time the validation is performed or B) with a re-usable validation reference plate.

The Validation Reference Plate (VRP) is a NIST- traceable tool for validation of microplate readers and photometric accuracy. The VRP is pathway independent and can be used to generate automatic standard curves for 96, 384, and 1536 well microplates. VRP results has an average internal tolerance of 0.4% and worked effectively on the mini-reader and other readers.



Fig. 5. Solid-state miniaturized absorbance reader

The Automation Trainer Mini-reader is equipped with 96 parallel light sources for absorbance measurements. The instrument provides reliable measurement results and rapid readout of all 96 wells in parallel. The small footprint instrument is USB-powered, easy to transport, and with reagents are TSA-compliant. The plug-n-play instrument is easy to operate, controlled through user-friendly software, and can be integrated with many automated liquid handlers. It is compatible with 96-well plates as well as the VRP.

## Results

Mini-reader		MD Spectromax	
Target Vol (uL)	10.000	Target Vol (uL)	10.000
Precision Criteria	10.000	Precision Criteria	10.000
Accuracy Criteria	10.000	Accuracy Criteria	10.000
Avg Vol (uL) As Found	10.026	Avg Vol (uL) As Found	10.031
%CV	2.010	%CV	1.193
%Err	0.064	%Err	0.310
Precision	PASS	Precision	PASS
Accuracy	PASS	Accuracy	PASS
#Replicates	3	#Replicates	3

Fig 6. Data collected on mini-reader strongly correlate with MD Spectromax

VRP Absorbance 96 & MD Spectromax validation comparison using GLH QC Kits with VRP 10uL verification on a Biomek NX 96 Channel Head. Results show the readers are comparable. The mini-plate reader exhibited greater sensitivity under the conditions tested.

Daily PCR Tests	Type	Volume	Volume Delivery %Err	Volume Delivery %CV
1250	Manual	1-3uL	5.00	5.00
6250	Automated		2.00	2.00
1250	Manual	10uL	5.00	5.00
6250	Automated		2.00	2.00
1250	Manual	200-275uL	5.00	5.00
6250	Automated		2.00	2.00

Table 1. Covid-19 Daily PCR Test Throughput

The GLK QC kit was utilized in a high throughput Covid-19 testing site that scaled up from 1250 manual tests/day to 6250 automated tests/day using Agilent Bravo and Tecan Genesis. The table demonstrates the lab achieved 5 fold scalability with consistent and reliable testing by use of GLH Kit routine daily/weekly testing to ensure proper equipment function.

The GLC QC kit was applied for verification and validation of manual and automated liquid handlers. Data were collected using a microplate reader and analyzed using GLHTracker software. This software is preconfigured with methods for volume verification, compensates for different liquid classes, and is compatible with a variety of robot automation software. The software provides rapid methods for QC Volumetric Calibration, Volume verification, IQ/OQ/PQ of Liquid handlers and pipettes, and Equipment performance tracking. It is user-friendly and enables lab personnel to assess the reproducibility of volume delivery and concentration data for their instrument



Fig. 7. Volume verification within GLH Tracker software.

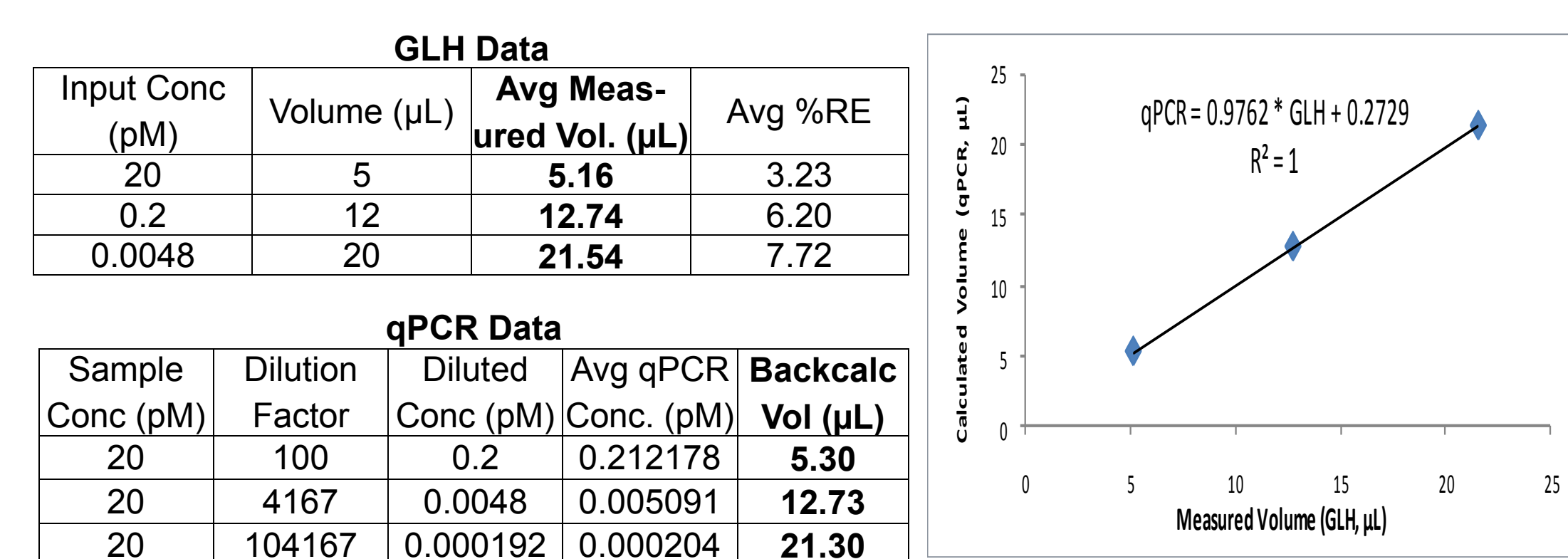


Fig. 8. Comparison data between simulated spike volume (GLH QC data) and actual sample serial dilutions of NGS library samples (qPCR data).

The GLH QC kit was applied to characterize assay preparation performance and improve the consistency of quantitative polymerase chain reaction (qPCR) and PCR assays. Serial dilution performance was compared between simulated spike volume delivery (GLH QC data) and actual serial dilutions of NGS library samples (KAPA LQK Standard 1, 20 pM 452bp amplicon) measured on a Roche LightCycler 480 qPCR instrument. The comparison of determined volumes delivered showed a strong correlation which can be further utilized to simplify calibration activities without costly qPCR execution each cycle.

## Conclusions

In this study, the GLH QC Kit was applied for validation of performance of automated liquid handlers and manual pipettes. The kit can be used by lab personnel to carry out IQ/OQ/PQ methods to confirm proper operation of equipment, thereby minimizing the need for field service. The modified Tartrazine single dye method follows ISO IWA 15.

The kit includes a universal NIST traceable validation plate that can be used with standard plate readers or the miniaturized absorbance reader available with the kit. The GLHTracker software includes pre-loaded methods for data analysis, including automated generation of standard curves.

In addition to use in qualification of automated and manual liquid handlers, this kit can be used to evaluate and improve the consistency of assay preparation methods.

## Acknowledgements

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