Environmental Studies Using the Tecan QC Kit and an Automated Liquid Handling System

George Rodrigues[(a)], Emily Avis[(a)], Alf Price[(b)], Verena Gerber[(b)]

Introduction

Liquid handler performance can be optimized by adjusting system operating parameters, but the effect of environmental conditions on liquid handling quality is often underappreciated.

The study described in this poster illustrates the effect of two environmental factors (air temperature and humidity) on the volumetric performance of a Tecan Freedom EVO ® pipetting robot, equipped with a liquid LiHa (Liquid Handling Arm). Tecan recommends that the EVO be installed in a controlled environment. However, some users choose to operate in less controlled conditions.

This study uses a two factor design (temperature and humidity) to investigate volumetric performance (precision and trueness) at environmental conditions from 15 °C to 30 °C and 30% to 80% relative humidity. Nine environmental conditions were tested, the mid point, four corner cases and four edge cases. Replicate testing was performed at the mid point and corner cases.

The Tecan QC Kit, which is based on Artel's ratiometric photometry, was used to make measurements at each set of environmental conditions. The precision (%CV), and trueness (%SE) is reported for each environmental condition using default liquid classes.

Equipment and Methods

The liquid handling system was a Tecan Freedom EVO ® 150 configured with an 8 channel liquid LiHa. A total of six different volume configurations were tested ranging from 10 µL to 200 µL. Syringe size of 1000 µL and standard tubing were used for all volumes. Deionized water was used as the system fluid.

Volume measurements were made using the Tecan QC Kit with an Infinite® F50 reader. The measurement system principles are as previously described by Artel.[1]

All equipment was installed in an environmentally controlled room and equilibrated overnight after each temperature change. An additional two hours of equilibration was used following changes to relative humidity. After equilibration, aqueous aliquots were pipetted into 96-well microplates.

At each of nine environmental conditions, six different volumes were tested. The well plate data was statistically summarized to measure precision (%CV), and trueness (% systematic error, abbreviated %SE). This measure of trueness is sometimes called "relative inaccuracy", though the term systematic error is preferred.[2,3]

At each environmental condition, at least one 96 well microplate was filled for each volume tested, so that each test result included all 8 channels, each delivering at least n=12 replicates of the same volume.

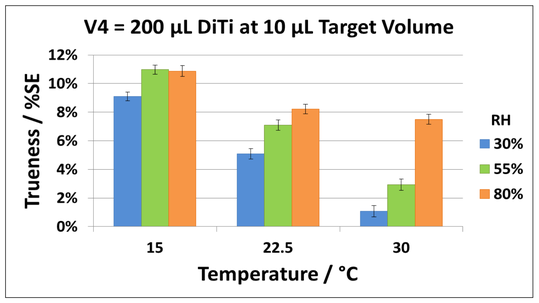
The central point of the study design was 22.5 °C and 55% relative humidity. As a check against instrumental drift, this central environmental test point was tested multiple times, at the beginning, middle and end of the study.

Acknowledgements

Equipment and consumable supplies were provided by Tecan. Testing was performed at Artel in Westbrook, Maine, USA.

Results

Aqueous solutions of Ponceau S dye were pipetted by the Tecan Freedom EVO ® and measured using the Tecan QC Kit.



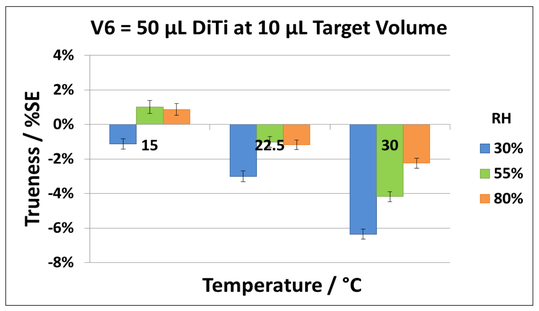
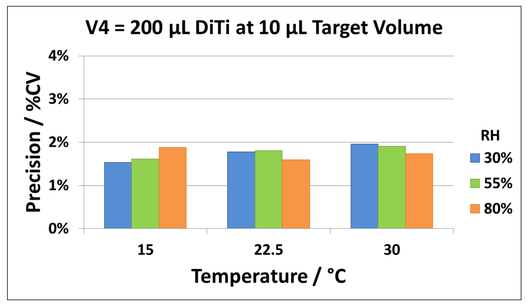


Figure 1: Two examples of the most notable effect; increasing temperature and decreasing humidity (RH) resulted in lower delivered volumes for a given instrument setting.



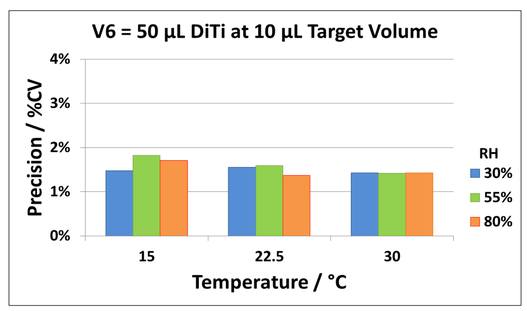


Figure 2: Two examples showing that precision (%CV) was not effected by environmental conditions.

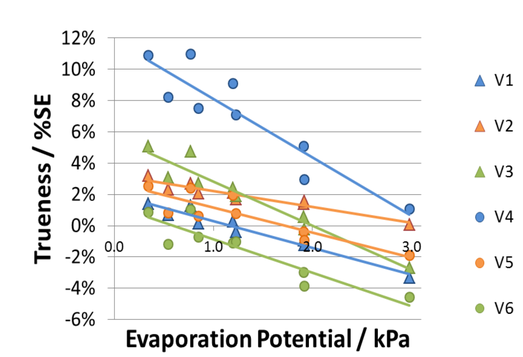


Figure 3: Correlation between trueness of delivered volume and "evaporation potential". Evaporation potential depends on both temperature and relative humidity as described in Figure 4.

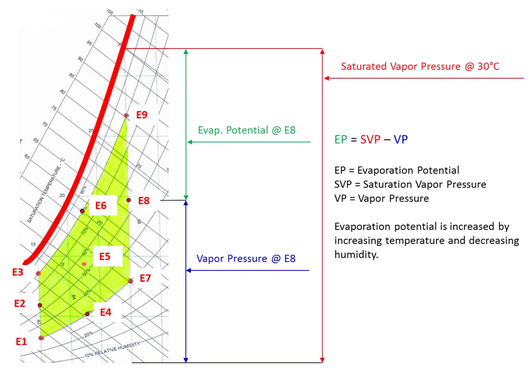


Figure 4: The rate at which evaporation occurs inside pipette tips is proportional to the evaporation potential, which can be calculated as shown in this figure.

Table 1: Instrument configuration for each tested volume

	Instrument Configuration				
V-code	Volume	Tip Size	Tip Detail	Syringe	Tubing
V1	200 µl	1000 µl tips	LiHa DiTi pn 30 000 631 conductive w/filter	1000 µl	Normal
V2	100 µl	200 µl tips	LiHa DiTi pn 30 000 627 conductive no filter	1000 µl	Normal
V3	25 µl	200 µl tips	LiHa DiTi pn 30 000 627 conductive no filter	1000 µl	Normal
V4	10 µl	200 µl tips	LiHa DiTi pn 30 000 627 conductive no filter	1000 µl	Normal
V5	25 µl	50 µl tips	LiHa DiTi pn 30 032 114 conductive w/filter	1000 µl	Normal
V6	10 µl	50 µl tips	LiHa DiTi pn 30 032 114 conductive w/filter	1000 µl	Normal

Table 2: Statistical summary of experimental data

				V1		V2		V3		V4		V5		V6	
E-Code	Humidity	Temp.	EP	Trueness	Precision	Trueness	Precision	Trueness	Precision	Trueness	Precision	Trueness	Precision	Trueness	Precision
	% RH	°c	kPa	%SE	%CV	%SE	%CV	%SE	%CV	%SE	%CV	%SE	%CV	%SE	%CV
E1	30	15	1.20	0.26%	0.36%	2.13%	0.44%	2.42%	0.90%	9.09%	1.54%	1.93%	0.77%	-1.12%	1.47%
E2	55	15	0.77	1.29%	0.42%	2.71%	0.54%	4.74%	0.89%	10.98%	1.61%	2.42%	1.02%	1.01%	1.82%
E3	80	15	0.34	1.41%	0.40%	3.21%	0.37%	5.09%	0.82%	10.87%	1.88%	2.55%	0.84%	0.87%	1.71%
E4	30	22.5	1.91	-0.92%	0.36%	1.42%	0.44%	0.57%	0.85%	5.09%	1.78%	-0.38%	0.78%	-3.01%	1.55%
E5	55	22.5	1.23	-0.40%	0.37%	1.70%	0.43%	1.92%	0.85%	7.09%	1.80%	0.78%	0.85%	-1.02%	1.59%
E6	80	22.5	0.55	0.69%	0.40%	2.33%	0.40%	3.09%	0.71%	8.22%	1.59%	0.82%	0.70%	-1.17%	1.36%
E7	30	30	2.98	-3.32%	1.02%	0.08%	0.44%	-2.69%	0.88%	1.07%	1.96%	-1.91%	1.07%	-6.35%	1.43%
E8	55	30	1.92	-1.23%	0.33%	1.58%	0.49%	-0.21%	0.94%	2.93%	1.91%	-0.92%	0.98%	-4.18%	1.41%
E9	80	30	0.85	0.14%	0.34%	2.08%	0.46%	2.69%	0.87%	7.50%	1.74%	0.59%	1.01%	-2.24%	1.43%

Conclusions

The precision of the Tecan Freedom EVO ® was found to be insensitive to environmental conditions and performed within manufacturer's limits over the entire range tested in this study (15 °C to 30 °C and 30% to 80% relative humidity).[(note 1)]

Over the range of this test, the trueness (systematic error) does depend on environmental conditions. Increased temperature and decreased relative humidity resulted in lower delivered volume. For the greatest accuracy in laboratory work, and particularly when operated outside of recommended temperatures[(note 1)], the automated liquid handling system should be tested and optimized for the local environment.

The environmental effect on trueness exhibited a linear correlation with the "evaporation potential" (the driving force for evaporation of the sample). The evaporation potential is dependent on both temperature and relative humidity. Use of this concept permits the experiment to be reduced from two factors (temperature and relative humidity) to one factor (evaporation potential).

Although the QC Kit is intended to be used for checks such as IQ/OQ, this study shows that the QC Kit can also be used for performance checks under environmental conditions that deviate from the manufacturer's specified operating conditions.

note 1: Tecan specifies the pipetting behavior of the Freedom EVO ® under controlled environment conditions (T = 20 °C to 27 °C; relative humidity at 25 °C between 30% and 60%). Volumetric performance specifications apply to this controlled limit. For operation outside these limits meeting the manufacturer's specifications for volumetric performance is not guaranteed.

Literature Cited

1. Multichannel verification system (MVS): a dual-dye ratiometric photometry system for performance verification of multichannel liquid delivery devices, JALA 10(1): 35-42.

2. ISO IWA 15, Specification and method for the determination of volumetric performance of automated liquid handling systems. (in preparation, expected publication 2015).

3. ISO 8655-1:2002, Piston-operated volumetric apparatus - Terminology, general requirements and user recommendations, clause 3.1.2.

Future Work

A full technical paper providing greater detail on this study is in preparation and intended to be submitted to JALA.

Further Information

The authors welcome inquiries and can be reached directly at the following email addresses:

Dr. George Rodrigues[(a)]: grodrigues@artel-usa.com

Ms. Emily Avis[(a)]: eavis@artel-usa.com

Mr. Alf Price[(b)]: alf.price@tecan.com

Author Affiliations: (a) Artel, (b) Tecan Schweiz AG