Determining the Pipetting Accuracy of the Quadra 96 in the Air Displacement Mode

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INTRODUCTION

This bulletin provides a convenient protocol to experimentally determine the pipetting accuracy of the Tomtec Quadra 96 well pipettor in the air displacement mode, using a colorimetric analysis. In addition, it provides a table of reference, that includes the standards of accuracy performance that all Quadra's must pass in this assay prior to shipment by Tomtec.

An informative measurement of accuracy for any 96 well pipettor is the relative variability of its liquid handling performance among its samples. This is best defined as a measure of dispersion divided by a measure of central tendency. **The coefficient of variability (CV%)** is such and index and in this colorimetric analysis compares the raw **optical density (OD492nm)** data across the microplate after dispensing a specified volume of methyl orange dye solution into all 96 wells. It is calculated from the standard deviation divided by the mean and expressed as a percentage.

MATERIALS AND METHODS

This assay utilizes the following materials:

Clean 96 well flat bottom polystyrene based microplates; carefully handled Tomtec polypropylene tips; deionized distilled water; methyl orange (Sigma#M-0402); microplate vortexer; spectrophotometer (set at measuring optical density at 492nm) interfaced with a computer that has a programmable spreadsheet (to calculate mean, standard deviation and coefficient of variability amongst the OD492 readings of all 96 wells).

Make up the following **fresh** methyl orange solutions (w / v) in **deionized distilled** water:

0.1% methyl orange (0.1 gm methyl orange powder / 100ml water)
0.04% methyl orange (40ml 0.1% methyl orange (w / v) / 60ml water)
0.02% methyl orange (20ml 0.1% methyl orange (w / v) / 80ml water)
0.01% methyl orange (10ml 0.1% methyl orange (w / v) / 90ml water)

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Protocol Method:

The protocols for these tests are summarized in the following steps:

1. Lubricate tip pin o-rings, load Tomtec polypropylene tips.
2. Aspirate 100µL air gap, then designated volume of water.
3. Aspirate 15µL air gap, then designated volume of dye.
4. Dispense all 315µL of aspirate (includes air gaps) into microplate.
5. Mix 100µL of well volume three to five times in Quadra mix cycle (aspirate from bottom of well, dispense into top of well.)
6. Carefully vortex microplates (e.g., orbital vortexer) for ~1 minute.
7. Insure no air bubbles inhibit light path within wells (remove with tapping, scrapping or hot air from a hand held blower).
8. Read OD492, import raw data into spread sheet and calculate them, St. Dev. and CV%.

Briefly, the volume parameters of each test are summarized as follows:

Quality Control Test	1st Air Gap	Water	2nd Air Gap	Methyl Orange (vol, conc.)	Final Well Volume	Final Dye Conc.
10µL Q.C.	100µL	190µL	15µL	10µL, 0.1% (1)	200µL	0.005%
25µL Q.C.	100µL	175µL	15µL	25µL, 0.04%	200µL	0.005%
50µL Q.C.	100µL	150µL	15µL	50µL, 0.02%	200µL	0.005%
100µL Q.C.	100µL	100µL	15µL	100µL, 0.01%	200µL	0.005%

RESULTS AND DISCUSSION

Results:

The following table contains the required Tomtec CV% accuracy for any Quadra 96 by Tomtec Quality Control prior to shipment.

Test	Volume of Dye	Volume of Water	Required CV%
10µL	10µL	190µL	< 4.0%
25µL	25µL	175µL	< 3.0%
50µL	50µL	150µL	< 3.0%
100µL	100µL	100µL	< 2.5%

Discussion:

This colorimetric analysis incorporates specific design elements that influences the accuracy performance of the Quadra 96. It is important that any user appreciate these features so as to increase success in all Quadra liquid handling applications.

The Quadra 96 uses a stepper motor to drive the individual 96 pistons in unison up and down. Whenever the stepper motor reverses direction there is backlash. This backlash is an artefactual result of mechanical windup in the gear train and drive plate of the Quadra 96 when initiating or changing from aspirate or dispense. The Quadra 96 control system automatically adds a backlash factor anytime the pistons are asked to change direction. The consequence of this correction is that better accuracy is obtained by including an aspirate of blow out air (i.e., initial 100µL air gap) so that the backlash correction occurs in the aspirate and subsequent dispense of this air gap and not in any desired liquid volumes.

When dispensing any volume from a small orifice, such as a pipette tip, there will be a certain amount retained at the tip orifice by surface tension and capillary action. This can be minimized by the included 100µL air gap, which serves as a blow out sweep to remove residual liquid from the tip.

The Quadra 96 uses two proprietary polypropylene tips differing by the internal diameter (0.026, 0.036) at the tip orifice. Each tip has a total volume of 450µL. The long narrow the bottom of deep well plates and allows convenient stacking of reagents separated by an air gap (i.e., 15µL air gap between water and dye) without mixing of reagents. In this protocol, thisseparation gap prevents artefactuals upward diffusion of dye into the water layer before aspirating the dye which would result in greater inaccuracy.

The mixing function of the Quadra 96 allows setting different stage heights during the aspirate and dispense steps. Set the stage height so that tips aspirate from the bottom and dispense into the top of the well. This insures that the contents are turned over. Tomtec also strongly **recommends further mixing with an orbital vortex** to insure a heterogeneous solution for OD measurements. Care should be taken to remove any air bubble that can impede light measurements.

REFERENCES

T.W. Astle and A. Akowitz (1996). Accuracy and Tip Carryover Contamination in 96 well Pipetting. Journal of Biomolecular Screening, Vol. 1, Number 4, p.211.