

# Agilent Bravo Liquid Handling Platform: 96 Channel Fixed Tip 50 $\mu$ L Accuracy and Precision

## Technical Overview

### Summary

- Bravo, 96 fixed tip head, 50  $\mu$ L capacity
- 0.1  $\mu$ L: 3.9% CV  $\pm$  4.4% accuracy
- 0.5  $\mu$ L: 2.0% CV  $\pm$  0.9% accuracy
- 1.0  $\mu$ L: 2.1% CV  $\pm$  2.1% accuracy
- >1  $\mu$ L: equal or better than 1  $\mu$ L CV and accuracy

### Introduction

Automated liquid handling devices are used in many processes performed in laboratories today. Liquid handling automation increases sample processing and throughput<sup>(1)</sup> and improves accuracy (desired dispensed volume is equal to the actual dispensed volume) and precision (a narrow distribution of dispensed volume) when compared to a person operating a handheld liquid pipettor. Automated liquid handling devices can also significantly reduce the occurrence of errors in a process<sup>(2)</sup>. To further the effort to automate routine laboratory processes, Agilent Automation Solutions has developed the Bravo liquid handling platform.

### Bravo Features

The Bravo has nine deck positions which accommodate any 96-, 384-, or 1,536-well SBS standard microplates. Deck positions can be configured with heating, cooling, and shaking stations, as well as maintaining open locations for tip boxes, sample microplates and reservoirs.

The Bravo can be used with 8-, 16-, 96-, or 384-channel fixed tip or disposable tip heads. Heads can be interchanged in a matter of seconds. The Bravo is designed to be used either standalone or as part of a robotic laboratory automation system. It is also designed to fit inside some models of laminar flow hoods and retain laminar flow, thereby opening up cell-based plating and cellular assays to an automated liquid handling platform.

### Accuracy and Precision Testing

This technical note describes a method to measure the accuracy and precision of the Bravo in conjunction with a 96 Channel 50  $\mu$ L Fixed Tip Head. The 50  $\mu$ L fixed tips were tested at the lower end of the practical volume range (0.1-1  $\mu$ L). Performance at volumes > 1  $\mu$ L meets or exceeds the 1  $\mu$ L performance. Measurements are determined by dispensing a tartrazine/DMSO solution into dry microplates, filling the plates with water, and measuring tartrazine absorbance. The product's performance meets or exceeds CVs of 5% and accuracy of  $\pm$  10% of the desired volume across the practical volume range.

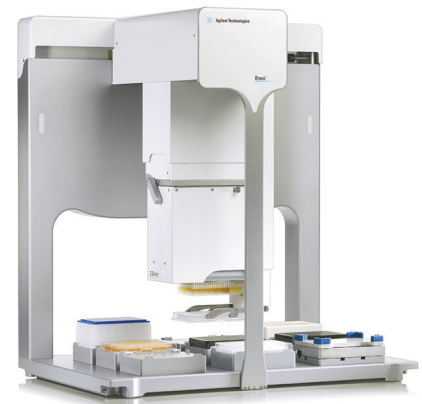
### Materials

- Bravo with a 96 Channel 50  $\mu$ L fixed tip head (product no. G5498B/G#044)
- Agilent 96-well manual fill reservoir (product no. G5498B/G#049)
- 96-well polystyrene, flat bottom plates (Greiner 655101)
- Tartrazine solution, 0.25% (w/v) dissolved in dimethylsulfoxide [DMSO]
- UV/Vis Spectrophotometer with a 405 nm filter (Thermo Multiskan Ascent)

### Method

60 mL of tartrazine solution is poured into a manual fill reservoir. The reservoir is placed on position 2 of the Bravo. A 96-well polystyrene plate is placed on position 5. A VWorks liquid classes for 0.05-1  $\mu$ L dispenses is utilized. A VWorks protocol is created and run in the following manner:

1. Tips are primed by mixing 3  $\mu$ L for 10 cycles in the reservoir.



The Bravo has nine deck positions and can be configured with interchangeable 8-, 16-, 96-, or 384- fixed and disposable tip heads.

2. 0.1, 0.5 or 1.0  $\mu$ L tartrazine solution is aspirated from the manual fill reservoir. Aspirate parameters are 6 mm from the bottom of the plate, and preceded by a 0  $\mu$ L, 0.25  $\mu$ L or 0.5  $\mu$ L pre-aspirate volume, respectively.
3. 0.1, 0.5 or 1.0  $\mu$ L tartrazine solution is dispensed into the 96-well plate. Dispense parameters are 0 mm from the bottom of the plate, and followed by dispensing the pre-aspirate volume of air.

Dispensed volumes are diluted to 50  $\mu$ L by the addition of water (after washing tips in DMSO). Plates are centrifuged at 1,800 rpm for 60 seconds to ensure full mixing and consistent well menisci. Absorbance is read at 405 nm. Accuracy is calculated based on an equation derived from a tartrazine/DMSO calibration curve consisting of data points at 0.05, 0.1, 0.25, 0.5, 1 and 1.5  $\mu$ L, compared to the actual absorbance value in each well.

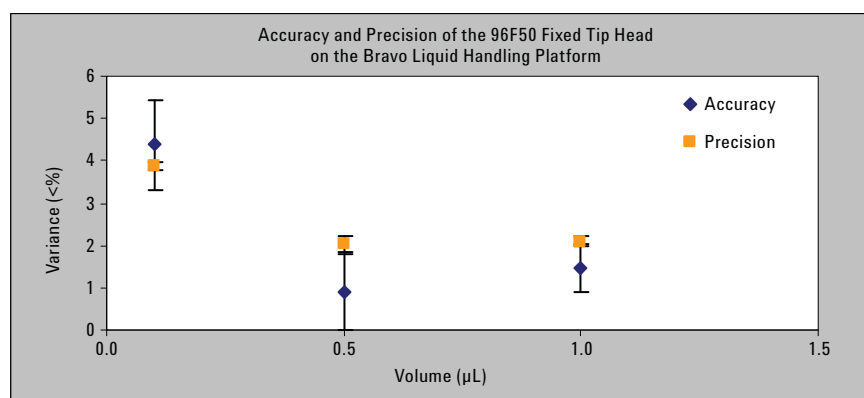


Absorbance values in each well is used to determine the precision of the dispense. Coefficient of Variance (CV) calculations were made by dividing the standard deviation by the mean. The accuracy and precision results are typical, based on

averages of three successive dispenses. Accuracy outliers are an average per plate. Results may vary, depending on individual experimental methods and liquid class optimization.

## Results

Volume	0.1 $\mu\text{L}$	0.5 $\mu\text{L}$	1 $\mu\text{L}$
Precision (% CV)	3.9	2.0	2.1
% Accuracy ( $\pm$ )	4.4	0.9	1.5
Outliers ( $\pm$ 5%)	31	2	2
Outliers ( $\pm$ 10%)	7	0	0
Transfer time (seconds/plate)	36	37	38



## Conclusion

In summary, the liquid handling capabilities of the Bravo liquid handling platform in conjunction with a 96 Channel 50  $\mu\text{L}$  fixed tip head provides precise, accurate and consistent liquid transfers. The 96 channel head can pipette across a broad volume range (0.1-50  $\mu\text{L}$ ), making it ideal for applications requiring precise dispensing at very low volumes. The highly configurable deck of the Bravo liquid handling platform along with its compact footprint makes it ideal for many applications in the laboratory including compound sample transfers, genomic applications and cell-based assays.

### References:

1. Sofia MJ. Leveraging Compound Management Capabilities in Support of Drug Discovery: From Sample Archive to Sample Distribution — Driving Efficiency and Improving Productivity. Laboratory Robotics Interest Group Meeting Oral Presentation, Jan. 2005.
2. Holman JW, Mifflin TE, Felder RA, Demers LM. Evaluation of an automated preanalytical robotic workstation at two academic health centers. Clin Chem. 2002 Mar; 48(3): 540-8.

Please contact your sales representative if you have particular questions regarding your specific application. Supplemental information (protocol files and data analysis spreadsheets) are also available upon request.

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