

# Agilent Bravo Automated Liquid Handling Platform: 384-Channel 70 $\mu$ L Tip Accuracy and Precision



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

## Abstract

Automation helps to scale up sample throughput without sacrificing quality. It improves accuracy (the desired dispensed volume is equal to the actual dispensed volume) and precision (a narrow distribution of dispensed volume). Automated liquid handling devices can also significantly reduce the error recurrence in a workflow.

This technical overview describes a method to measure the accuracy and precision of the Agilent Bravo in conjunction with a 384-channel ST disposable tip head at the lower end of the practical volume range (0.75 to 5  $\mu$ L).

## Introduction

Automated liquid handling devices are used in many processes performed in laboratories today. Liquid handling automation enables larger volumes of samples to be processed, 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>1</sup> To further the effort to automate routine laboratory processes, Agilent Automation Solutions has developed the Agilent Bravo Automated Liquid Handling Platform.

### Bravo features

The Bravo has nine deck positions that accommodate any 96-, 384-, or 1536-well Society for Biomolecular Screening (SBS) standard microplates. Deck positions can be configured with thermal cycling, heating, cooling, and shaking stations, as well as maintaining open locations for tip boxes, sample microplates and reservoirs.

The Bravo can be used with 96- or 384-channel 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 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 overview describes a method to measure the accuracy and precision of the Bravo in conjunction with a 384-channel small transfer (ST) disposable tip head. The 70  $\mu\text{L}$  disposable tips were tested at the lower end of the practical volume range (0.75 to 5  $\mu\text{L}$ ). Performance at volumes >5  $\mu\text{L}$  meets or exceeds the 5  $\mu\text{L}$  performance. Measurements are determined by dispensing a tartrazine/dimethylsulfoxide (DMSO) solution into dry microplates, filling the plates with water, and measuring tartrazine absorbance. The performance of the product meets or exceeds coefficient of variation (CV) of 5% and accuracy of  $\pm 10\%$  of the desired volume across the practical volume range.

## Summary

The method used the Bravo 384ST head, 70  $\mu\text{L}$  tips and provided the following results:

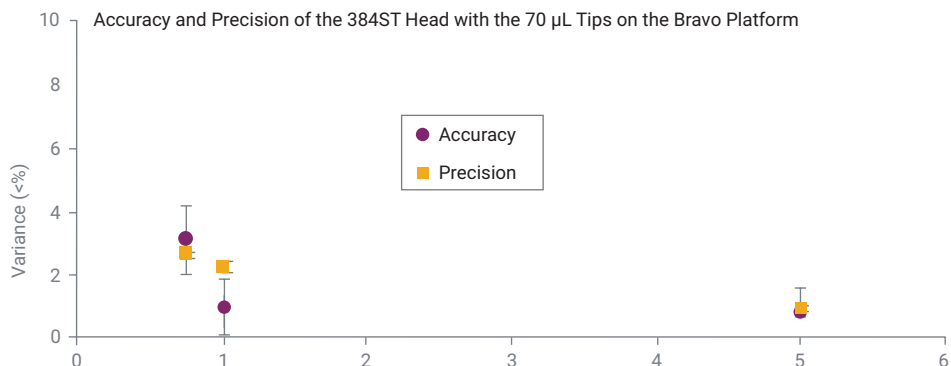
- 0.75  $\mu\text{L}$ : 2.7% CV  $\pm$  2.7% accuracy
- 1.0  $\mu\text{L}$ : 2.3% CV  $\pm$  1.0% accuracy
- 5.0  $\mu\text{L}$ : 0.9% CV  $\pm$  0.8% accuracy
- >5  $\mu\text{L}$ : equal or better than 5  $\mu\text{L}$  CV and accuracy

## Materials

- Bravo with a 384-channel ST disposable tip head
- Agilent 384ST 70  $\mu\text{L}$  tips (part number 19133-102)
- Agilent 384-well manual fill reservoir (part number G5498B/G#050)
- 384-well polystyrene, flat bottom plates (Greiner 781101)
- Tartrazine solution, 0.25% (w/v) dissolved in DMSO
- UV/Vis Spectrophotometer with a 405 nm filter

## Results

| Volume ( $\mu\text{L}$ )      | 0.75 | 1   | 5   |
|-------------------------------|------|-----|-----|
| Precision (% CV)              | 2.7  | 2.3 | 0.9 |
| % Accuracy ( $\pm$ )          | 2.7  | 1.0 | 0.8 |
| Outliers (5%)                 | 54   | 33  | 0   |
| Outliers (10%)                | 3    | 0   | 0   |
| Transfer time (seconds/plate) | 28   | 28  | 40  |



## Plate compatibility

| Tip type               | 96-Well Microplates | 384-Well Microplates | 1536-Well Microplates |
|------------------------|---------------------|----------------------|-----------------------|
| 96LT 250 $\mu\text{L}$ | Yes                 | Yes                  | No                    |
| 384ST 10 $\mu\text{L}$ | Yes                 | Yes                  | Yes                   |
| 384ST 30 $\mu\text{L}$ | Yes                 | Yes                  | Yes                   |
| 384ST 70 $\mu\text{L}$ | Yes                 | Yes                  | No                    |

## Method

60 mL of tartrazine solution is poured into a manual fill reservoir. The reservoir is placed on position 2 of the Bravo. A 70  $\mu\text{L}$  tip box is placed on position 6. A 384-well polystyrene plate is placed on position 5. Agilent VWorks automation control software liquid classes for 0.75 to 1  $\mu\text{L}$  and 1 to 5  $\mu\text{L}$  dispenses are utilized. A VWorks protocol is created and run in the following manner:

1. Tips are pressed onto the head.
2. 0.75, 1.0, or 5.0  $\mu\text{L}$  tartrazine solution is aspirated from the manual fill reservoir. Aspirate parameters are 6 mm from the bottom of the plate, and preceded by a 0.5  $\mu\text{L}$  (for 0.75 and 1  $\mu\text{L}$  dispense) or 2  $\mu\text{L}$  (for 5  $\mu\text{L}$  dispense) preaspirate volume.

3. 0.75, 1.0, or 5.0  $\mu\text{L}$  tartrazine solution is dispensed into a 384-well plate. Dispense parameters are 0 mm from the bottom of the plate with 0.05 mm/ $\mu\text{L}$  (for 0.75 and 1  $\mu\text{L}$  dispense) or 0.1 mm/ $\mu\text{L}$  (for 5  $\mu\text{L}$  dispense) tip retraction, and followed by dispensing a 0.5  $\mu\text{L}$  (for 0.75 and 1  $\mu\text{L}$  dispense) or 2  $\mu\text{L}$  (for 5  $\mu\text{L}$  dispense) blowout volume.

Dispensed volumes are diluted to 50  $\mu\text{L}$  by the addition of water (in this case, with clean tips and a separate VWorks protocol). Plates are centrifuged at 1800 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 0.25, 0.75, 1, 2, 5, 6, and 10  $\mu\text{L}$ , compared to the actual absorbance value in each well. Absorbance values in each well are used to determine the precision of the dispense. 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 plate dispenses and readings. Accuracy outliers are an average per plate. Results may vary, depending on individual experimental methods and liquid class optimization.

## Conclusion

In summary, the liquid handling capabilities of the Agilent Bravo Automated Liquid Handling Platform in conjunction with a 384-Channel ST disposable tip head provides precise, accurate and consistent liquid transfers. The 384-channel head, combined with the Agilent 70  $\mu\text{L}$  disposable tips, can pipette across a low volume range (0.75 to 70  $\mu\text{L}$ ), making it ideal for applications requiring precise dispensing at low volumes. The highly configurable deck of the Bravo Automated Liquid Handling Platform along with the compact footprint makes it ideal for many applications in the laboratory including compound sample transfers, genomic applications, cell plating, and cell-based assays.

## References

1. 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.

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