A Quality Control Procedure for the Agilent Bravo Platform

Technical Overview

Authors

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Abstract

Automated liquid handling systems (ALHS) such as the Agilent Bravo are routinely used in laboratories to reduce the variability of pipetting processes, and alleviate repetitive tasks. The pipetting performance of these devices are characterized by their accuracy (trueness of the delivered volume to the desired volume), and their precision (spread or distribution of dispensed volumes at a desired volume). Applied Proteomics leverages ALHS to reduce the technical noise of their assays, and uses routine performance testing to monitor platform stability prior to and during studies. For the purpose of post-installation performance qualification (PQ), an absorbance-based functional test was designed with commonly available laboratory reagents and equipment. This test was subsequently executed across three Agilent Bravo systems over a 4-year period as a continuing quality control measure.

The quality control dataset for each system contains variability from several sources, including multiple test operators, physical location within the laboratory, reagent lots and preparation, mechanical maintenance, and service events. For each system, the overall precision of all recorded tests at 10 µL is <3.0 %. This exceeds the expected ≤5.0 % CV precision performance metric for a single test instance, as defined in the Agilent Bravo technical specifications[1].

Agilent Technologies

Introduction

This Technical Overview describes a method to measure the accuracy and precision of the Agilent Bravo 96-Channel LT Disposable Tip Head at the lower end of the device working range, 2.0–10.0 µL. The working range of the 96 LT head is 2.0–250.0 µL (Agilent, 2016). Upon receipt of a new Agilent Bravo instrument, a quality control liquid class is established and calibrated to deliver accurate volumes across the test range (2.0–10.0 µL). The test method and calibrated liquid class are used as a longitudinal quality control protocol to confirm system performance after service or before experiments. Running control charts are used to visualize system stability, and to proactively identify performance failures before experimental results are impacted. This volumetric test has not been optimized beyond liquid class calibration, and is only used to benchmark mechanical performance.

Materials

- Agilent Bravo with 96-Channel LT Disposable Tip Head (p/n G5523BA)
- Agilent 96LT 250 µL tips (p/n 19477-002)
- Agilent 96-well manual fill reservoir (p/n G5498B#049)
- Orbital shaker (p/n G5498B#033)
- Vworks version 11.4.0.1233
- 384-well polystyrene, flat bottom plates (Greiner 781101)
- Tartrazine (Sigma-Aldrich T03888) solution, 0.05 % w/v, in dimethylsulfoxide (DMSO, Sigma-Aldrich D8418)
- UV/Vis Spectrophotometer with a 405 nm filter or monochrometer (BioTek Synergy H1M)
- Centrifuge (Eppendorf 5810R)
- Microsoft Excel
- R version 3.3.1 (packages: ggplot2, RColorBrewer, grid, gridExtra)

Method

Volumetric test

A 60-mL amount of 0.05 % tartrazine solution (dye) is poured into a 96-well manual fill reservoir. The dye reservoir is placed on position 8 of the Agilent Bravo. 60 mL of deionized water is poured into a 96-well manual fill reservoir. The water reservoir is placed on position 2 of the Agilent Bravo. Boxes of 250-µL tips are placed on positions 1 and 7. Tips in position 1 are used for water only, and referred to as water tips. Tips in position 7 are used for tartrazine dye solution, and are referred to as dye tips. A 384-well polystyrene plate is plated on position 5. The Agilent Orbital shaker station is configured at position 3. Refer to the deck layout in Figure 1.

Position 1	Position 2	Position 3
250 µL Tips	Water reservoir	Shaker (empty)
Position 4	Position 5	Position 6
Empty	384-Well plate	Empty
Position 7	Position 8	Position 9
250 µL Tips	Dye reservoir	Empty

Figure 1. Agilent Bravo deck layout for dye test.

The following steps are performed for the volumetric test:

1. The test volume (2.0, 5.0, or 10.0 µL) is selected by the user.
2. The water volume is calculated using the following equation: Water volume = 50.0 µL – Test volume.
3. **Dye addition**
    a. Dye tips are pressed onto the head.
    b. The test volume of dye solution is aspirated from the 96-well manual fill reservoir. Aspirate parameters are 1.0 mm from the bottom of the plate with no other changes from the base liquid class.
    c. The test volume of dye solution is dispensed into quadrant 1 of the 384-well plate. Dispense parameters are 0.1 mm from the bottom of the plate with no other changes from the base liquid class.
    d. Dye tips are ejected into the box at position 7.
4. **Water addition**
    a. Water tips are pressed onto the head.
    b. The calculated water volume is aspirated from the 96-well manual fill reservoir. Aspirate parameters are 2.0 mm from the bottom of the plate with no other changes from the base liquid class.
    c. The calculated water volume is dispensed into quadrant 1 of the 384-well plate. Dispense parameters are 4.0 mm from the bottom of the plate with no other changes from the base liquid class.
    d. Water tips are ejected into the box at position 1.
5. Steps 3–4 are repeated for a total of four dispenses of dye and water to the 384-well plate, varying the quadrant of the 384-well plate in each cycle so that all wells are filled.
    a. The dispense pattern for tip 1 (A1) maps to the wells of the 384-well plate as follows:

	1	2
A	Dispense 1	Dispense 2
B	Dispense 3	Dispense 4

    b. For the 5.0 and 10.0 µL test volumes, tips are not replaced between dye or water dispenses.
    c. For the 2.0 µL test volume, the dye tips are replaced in each water cycle.
6. The filled plate is shaken at 1,000 rpm for 60 seconds.
7. Plates are centrifuged at 500 rpm for 2 minutes to ensure full mixing and consistent well menisci.

8. The bottoms of the plates are inspected for dust, droplets, or other debris.

9. Absorbance is measured at 405 nm.

Dispensed volume is calculated based on a linear equation derived from a tartrazine solution/water calibration curve consisting of data points at 0.15, 0.32, 0.63, 1.25, 2.5, 5, 10, and 20 µL of delivered dye solution, compared to the mean absorbance value. For each point of the calibration curve, 50 µL of the corresponding diluted dye solution is plated to each of three wells, and the resulting absorbance values of these three wells are averaged. % Inaccuracy is calculated by:

(Delivered volume – Test volume)/Test volume × 100

Absorbance values in each well are used to determine the precision of the dispense. Coefficient of variance (CV) calculations are made by dividing the standard deviation by the mean. These summary statistics may be calculated and reported at the plate (overall, n = 384), quadrant (dispense order, n = 96) or tip (n = 4) level for the 10.0 and 5.0 µL test volumes. Reporting at the tip level for the 2.0 µL volume may be misleading.

Performance limits of ±10 % inaccuracy (RI) and ≤5 % CV are established for all test volumes per the technical specifications in Agilent Bravo Data Sheet 5990-3480EN[1].

This method was developed in January 2013, and is similar to the Photometric with Orange G method described in Annex B.5 of ISO IWA:15 (ISO, 2015)[2]. IWA:15 discusses volumetric performance in terms of trueness (closeness of agreement between average delivered volume and target volume) and precision (spread or dispersion of delivered volumes at a given target volume), which together define the accuracy of an ALHS. We have chosen to use the terms accuracy and precision in keeping with how they have been described in the Agilent Bravo technical specifications[1].

Calibration of test liquid class

Once precision has been established for a volumetric range, inaccuracy of the delivered volumes can be controlled by calibrating the test liquid class.

The volumetric test in the previous section is performed for each of three volumes (2.0, 5.0, and 10.0 µL) across the lower working volume range of the 96 LT head, using the *96 disposable tip 1-2 µL* liquid class delivered with Vworks (version 11.4.0.1233). For the initial testing, the calibration equation coefficients are set as follows:

X^0 (intercept) = 0, X^1 (slope) = 1.

The test target volume (y-axis) is plotted against the mean delivered volume for each test (x-axis), and a linear regression is fit using Microsoft Excel. The slope and intercept coefficients of this regression are entered into the calibration equation for the test liquid class in Vworks as X^1 (slope) and X^0 (intercept).

The volumetric tests are repeated with the calibrated liquid class to confirm that the inaccuracy of the mean dispensed volume is within the allowable bounds. Once verified, these results become the baseline measurements for longitudinal performance review.

Longitudinal data analysis

Quality control data are collected at 10.0 µL and any other test volume (2.0 µL, 5.0 µL) required to bracket the critical volumes of upcoming experiments. System performance of the current testing interval is evaluated by the Pass/Fail metrics for an individual test, and compared with historical performance of the system through running control charts, generated in R (version 3.3.1)[4]. Control charts are a common statistical process control tool used for monitoring process performance, allowing for the visualization of trends in system performance.

For each testing interval, the overall mean dispensed volume, overall %CV and the number of pipetting channels exceeding the allowable %CV at the per-tip level are charted for the 10.0 µL dispense.

Results and Discussion

Calibration of test liquid class

For each of three systems (Bravo 1, Bravo 2, and Bravo 3), volumetric testing and test liquid class calibration was performed upon system installation. Accuracy of the calibrated liquid class was verified by repeating the volumetric tests with the new liquid class coefficients. Table 1 presents the volumetric test results before and after calibration for each system.

Table 1. Volumetric test results for calibration of test liquid classes. Highlighted cells indicate failing values.

System name	Test volume	Overall %CV	Mean delivered volume	% Inaccuracy	Mean volume after calibration	% Inaccuracy after calibration
Bravo 1	10.0 µL	0.88 %	10.02 µL	0.2 %	10.35 µL	3.5 %
	5.0 µL	1.71 %	5.39 µL	7.8 %	5.24 µL	4.8 %
	2.0 µL	2.61 %	2.30 µL	15.0 %	1.98 µL	-1.0 %
Bravo 2	10.0 µL	1.13 %	10.42 µL	4.2 %	9.92 µL	-0.8 %
	5.0 µL	1.03 %	5.41 µL	8.2 %	5.06 µL	1.2 %
	2.0 µL	1.57 %	2.32 µL	16.0 %	2.07 µL	3.5 %
Bravo 3	10.0 µL	1.50 %	10.71 µL	7.1 %	9.80 µL	-2.0 %
	5.0 µL	1.05 %	5.39 µL	7.8 %	5.03 µL	0.6 %
	2.0 µL	1.62 %	2.31 µL	15.5 %	2.08 µL	4.0 %

For all systems, the % inaccuracy of the mean delivered volume for the uncalibrated 2.0 µL test exceeded the allowable ±10 % boundary. Precision for all systems at all test volumes passed the ≤5 % boundary, indicating that the mechanical performance of the pipetting head was acceptable. After calibration of the test liquid class, the accuracy requirement for all test volumes was met. Table 2 shows the calculated calibration coefficients used for each system.

Table 2. Calibration coefficients.

System name	X^0 (Intercept)	X^1 (slope)
Bravo 1	-0.4748	1.0403
Bravo 2	-0.3119	0.9882
Bravo 3	-0.1803	1.0374

The performance of the test liquid class specific to each pipetting head has been stable since installation and has not been recalibrated following maintenance or service, allowing for longitudinal evaluation of system stability.

Longitudinal data analysis

Volumetric test results for each system have been collected from the date of installation through present, when required to confirm system performance prior to use in critical experiments. In the research laboratory, test frequency is determined by system use rather than a defined interval. At minimum and during idle periods, the test was performed following service interventions and the annual preventative maintenance. In addition to these requested tests, volumetric data were collected at approximately 1 week intervals for several months after the installation of Bravo 1 to establish baseline performance expectations. Data collected with loaner 96 LT heads provided by the Agilent Service Center during Preventative Maintenance (PM) or repair were annotated and analyzed separately from the data collected with dedicated 96 LT head for each system. Control charts were not prepared for loaner devices due to the limited duration of their use at our facility.

The 10.0 µL test data were chosen for the system control charts because it corresponds to the most common low-end critical volume used in our assays on the Agilent Bravo Liquid Handler.

Table 3 presents the:

- Calibration date of the test liquid class following system installation (calibration date)
- Number of 10.0 µL volumetric tests performed on each system (N)
- Grand mean of the mean delivered volumes
- %CV of the mean delivered volumes for all 10.0 µL tests performed on each system

These summary statistics are very similar to those calculated with every individual dispense volume, as opposed to the mean volume for each test. Table 3 also presents the mean and %CV calculated using every individual dispense. The implied N for these calculations is 384 wells × the number of test plates.

Table 3. Summary statistics for 10.0 µL test performance.

System name	Calibration date	N	Grand mean volume	%CV of mean volumes	Mean of all well volumes	%CV of all well volumes
Bravo 1	Jan-2013	41	10.39 µL	2.48 %	10.39 µL	2.65%
Bravo 2	Apr-2013	36	10.03 µL	2.19 %	10.03 µL	2.47 %
Bravo 3	July-2013	21	10.12 µL	2.68 %	10.13 µL	2.90 %
Loaners		3	10.31 µL	2.52 %	10.31 µL	2.29 %

A system control chart was constructed in R (version 3.3.1)[4] by plotting each control metric versus the run date with the allowable ranges demarcated on each plot. The control chart for Bravo 1 is shown in Figure 2, with sub-plots for the mean volume (allowable range 9.0 µL–11.0 µL), overall %CV (5 % CV boundary), and the number of individual channels that fail either mean volume (accuracy) or %CV (precision) criteria. Failing channels are represented by bar charts in the lowest panel, with green bars indicating the number of channels failing the accuracy requirements, and orange bars indicating the number of channels failing the precision requirement. At the 10.0 µL test volume, no failing channels were allowed for either metric.

Separate control charts were prepared for each system. System use is halted, and troubleshooting or root cause analysis is performed whenever a single result for mean volume, overall %CV, or number of failing channels is outside the allowable range.

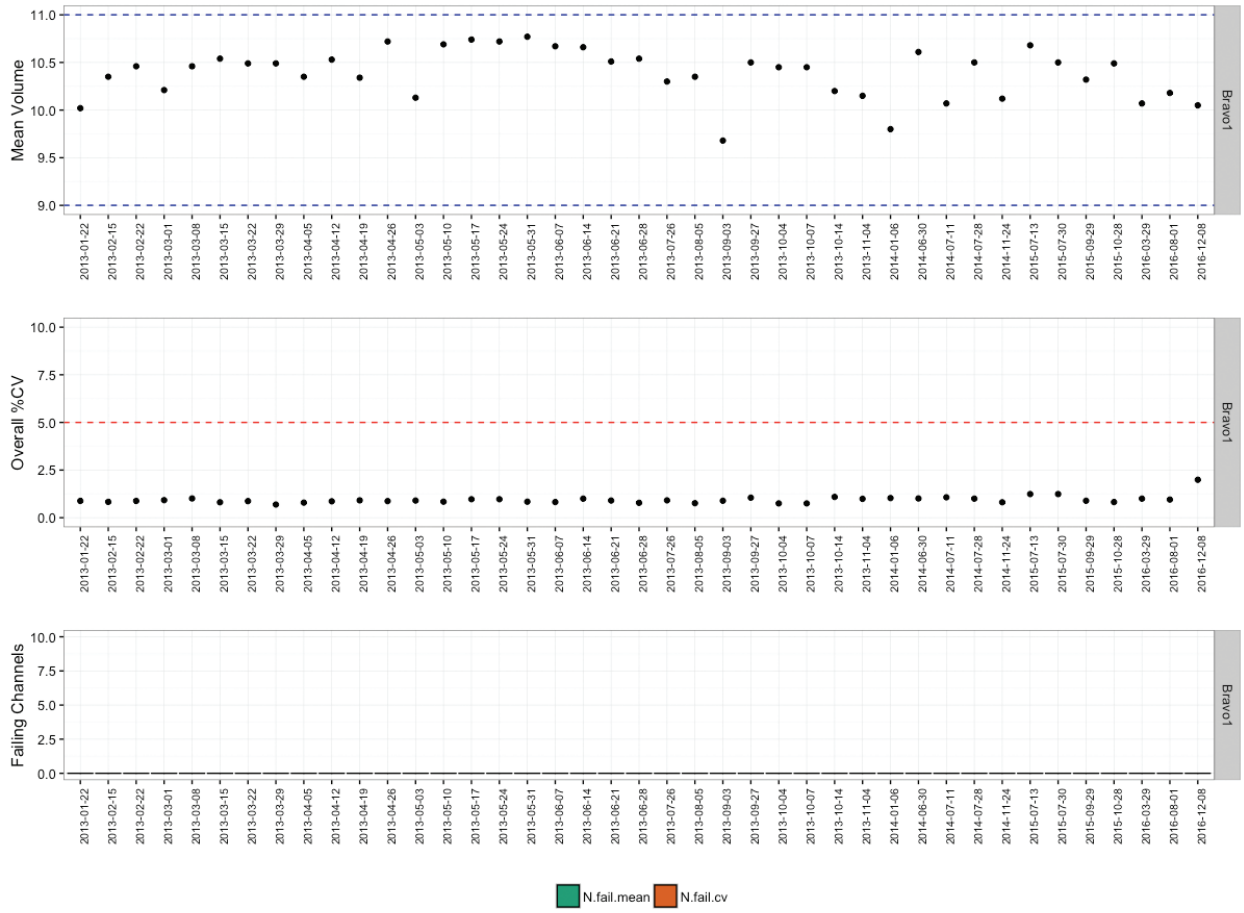

Figure 2. Agilent Bravo 1 control chart. Boundaries of allowable accuracy range per the Agilent Bravo technical specifications (9.0 µL and 11.0 µL) are marked in blue-dashed lines. The precision cutoff of 5 % overall CV is marked in a red-dashed line in the center panel plot.

The Bravo 3 control chart, shown in Figure 3, indicates a head failure on 2013-08-05 due to two barrels failing the precision requirements (N.fail.cv). The failing barrels were exchanged, but the volumetric test performance did not improve. The head was returned to Agilent Service for repair. System use was suspended until a replacement pipette head was qualified for use.

Independent of using the device's technical specifications for system performance, upper and lower control limits for the mean delivered volume can be statistically established using the first 20 observations for each system (Tague, 2005)[3]. Table 4 shows the mean delivered volume and control limits, based on the mean plus or minus three standard deviations.

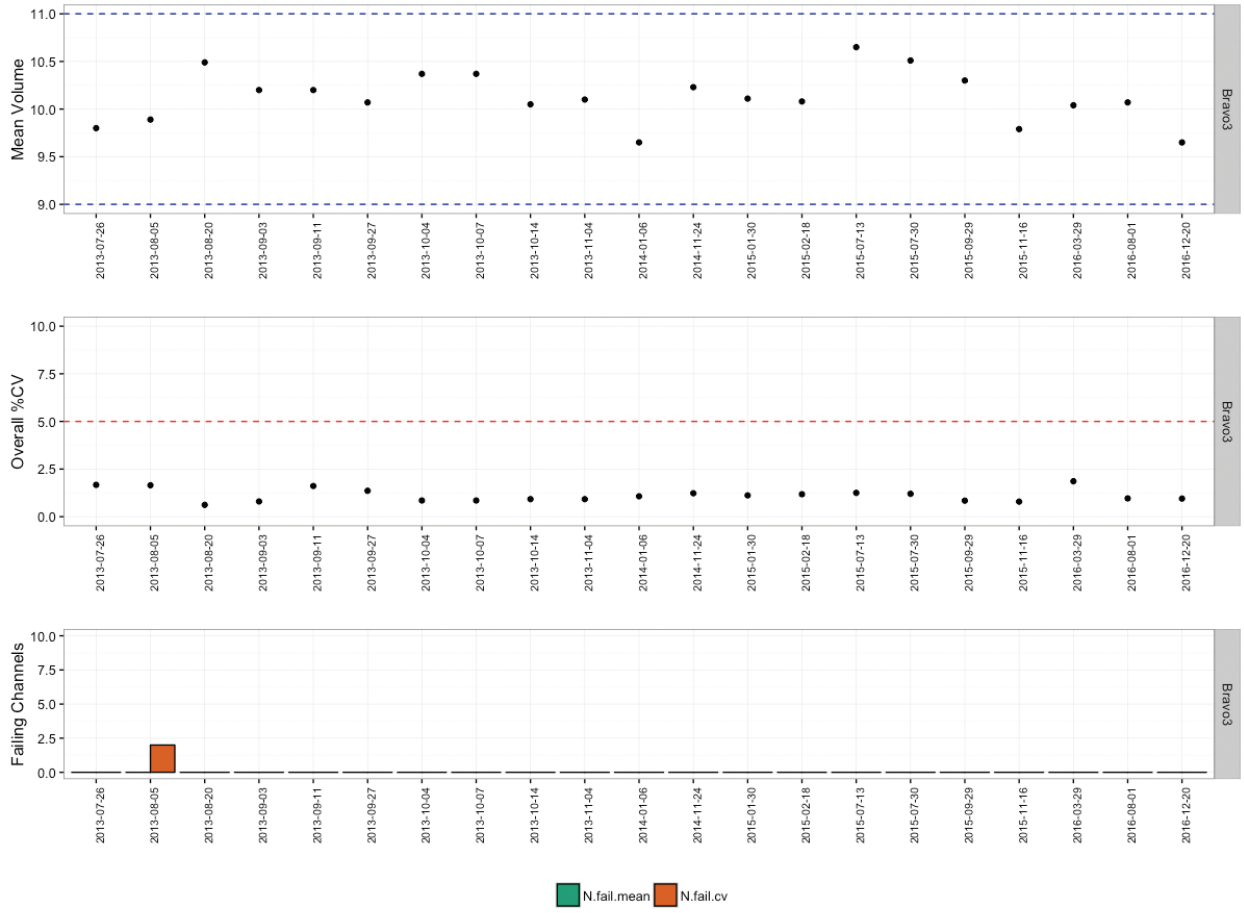

Figure 3. Agilent Bravo 3 control chart. Boundaries of allowable accuracy range per the Agilent Bravo technical specifications (9.0 µL and 11.0 µL) are marked in blue-dashed lines. The precision cutoff of 5 %CV is marked in a red-dashed line in the center panel plot.

Table 4. 3SD Upper and lower control limits.

System name	N	Mean	Standard deviation	Upper control limit (3SD)	Lower control limit (3SD)
Bravo 1	20	10.49 µL	0.21 µL	11.12 µL	9.86 µL
Bravo 2	20	10.05 µL	0.23 µL	10.74 µL	9.36 µL
Bravo 3	20	10.15 µL	0.26 µL	10.93 µL	9.37 µL

These control limits are more restrictive than the device technical specifications for accuracy and precision, indicating that the initial system inter-day performance exceeds expectations. Using the device technical specifications, 1 % of tests (1 of 98) indicate nonconformance, as discussed above, and 3 % of tests (3 of 98) require investigation for nonconformance when the calculated 3SD boundaries are applied. New control charts (Figure 4) can be generated using the calculated boundaries, revealing two additional test dates that would be flagged for investigation (Bravo 1 on 2013-09-03 and 2014-01-06). 2SD boundaries are also shown for reference, in blue-dashed lines, and may be used as warning boundaries.

We have chosen to retain the technical specification of 5% CV as the upper control limit for precision. For applications where characterizing the differences in the spread or dispersion of measurements within a single test is critical, a statistical approach may be used to set an upper and lower control limit for the precision observations. The resulting chart would be similar to the X-bar and s chart variant used in statistical process control (Tague, 2005)[3]. Our process could be further improved by instituting a fixed testing interval, rather than relying on system use to dictate the frequency of performance testing.

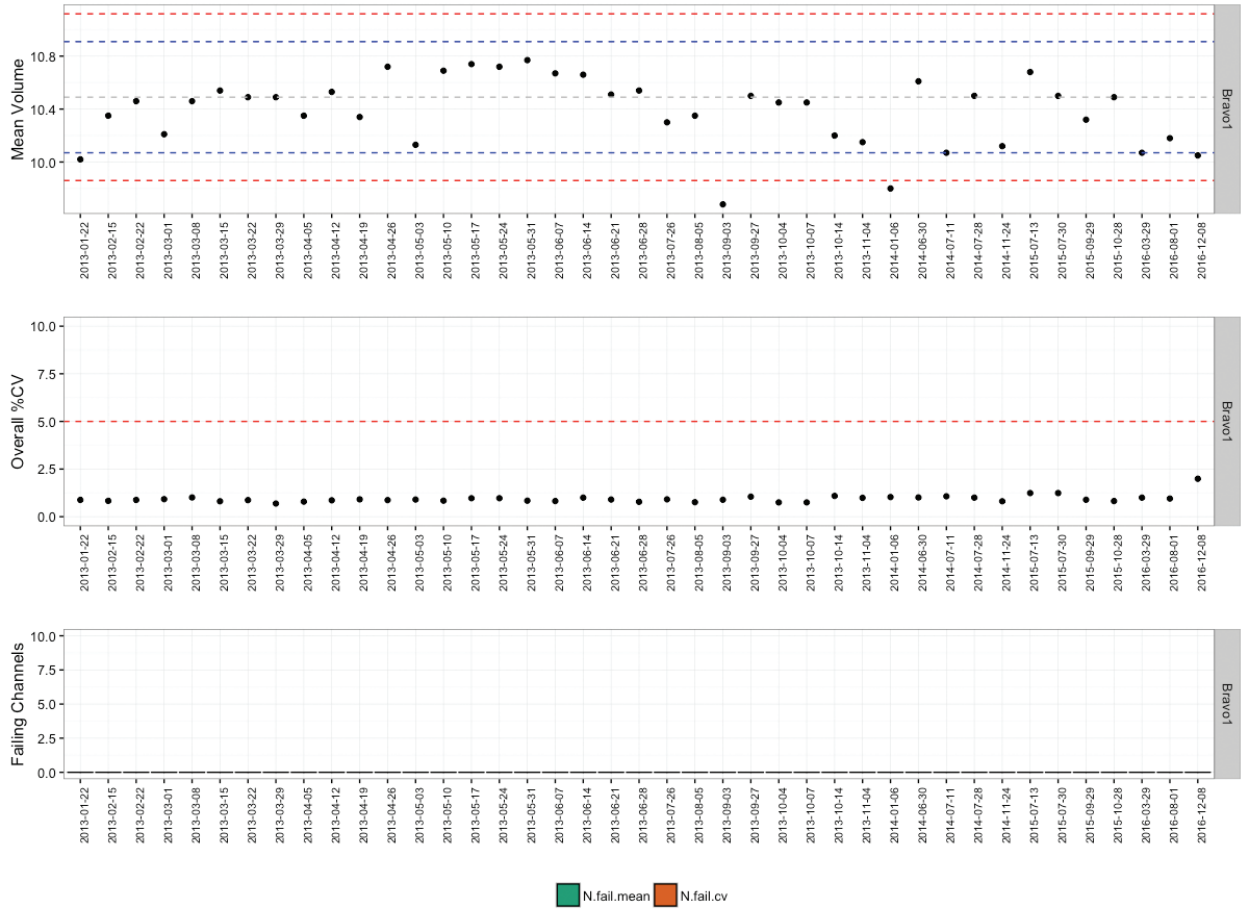

Figure 4. Agilent Bravo 1 control chart with calculated 2SD (blue), 3SD (red) control limits for mean volume. The precision cutoff of 5 % overall CV is marked in a red-dashed line in the center panel plot.

Conclusion

Automated liquid handlers are a necessity for high-throughput laboratories seeking consistency, accuracy, and precision in liquid handling.

In this study, the Agilent Bravo automated liquid handler was evaluated for its ability to address these points by testing the liquid handling at 2.0 µL, 5.0 µL, and 10.0 µL using a 96 LT head with 250 µL pipette tips. The results show that overall performance of the Bravo exceeds the standard instrument release criteria of 5 % CV and 10 % RI. Spanning multiple days with multiple runs by multiple operators, the performance on three separate Bravo units showed an average of <3 % CV and <5 % RI.

The ability to interrogate system performance and evaluate longitudinal stability has given us confidence in deploying our applications to the Agilent Bravo Liquid Handler. The Bravo robustness allows us to execute repeated experiments on the platforms without concerns about changes to the delivery of critical volumes. The volumetric test allows us to requalify a system after service or maintenance, or to quickly rule out instrument errors when troubleshooting assay performance, resulting in lower instrument down-time.

References

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