Integration of Liquid Handler Quality Control Into the HTS Daily Routine

Bernhard Ellinger PhD, Alexander Hochreiner
European ScreeningPort[1]

Stefan Schork PhD, Matt Wilkinson PhD MBA
Artel[2]

June 2012

Abstract
This application note describes how the Artel MVS® Multichannel Verification System can be used to assess the performance of the liquid handlers integrated into a PerkinElmer cell::explorer high throughput screening (HTS) system.

The MVS enables users to measure the performance of all six of the liquid handlers integrated into the cell::explorer system over the entire volume range of 10 nL to 350 µL and provides data that allows direct comparability of instrument performance.

Previous studies[1] have shown that highly accurate and precise liquid handling is critical if the results of HTS experiments are to be reliable. However, liquid handlers are prone to variation and error and need to be verified using a standardized methodology. The need for a standardized verification method becomes increasingly important when multiple liquid handlers are used in the same experiment, or when procedures are transferred between instruments and/or locations.

Studies were conducted to prove the verification process does not interfere with assay results and the performance of the liquid handlers integrated into the screening system was assessed. The MVS enabled the analysis of pre-existing protocols and dramatically reduced the time required for routine quality control processes.

12A6278A

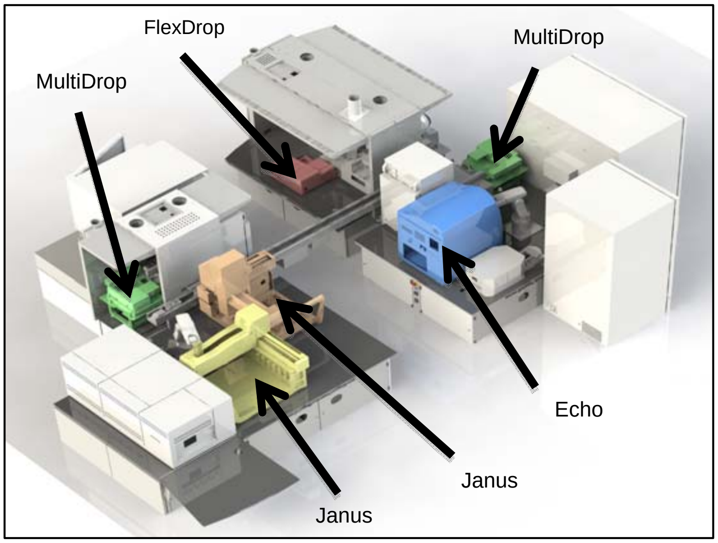

Image 1. cell::explorer™ Screening System

MVS dual-dye Ratiometric Photometry™

The dual-dye ratiometric photometry method employed by the MVS utilizes two dyes with distinct absorbance maxima at 520 nm (red) and 730 nm (blue). Sample solutions containing different concentrations of the red dye are used for testing the performance of automated liquid handlers dispensing into 96- or 384-well plates over the entire volume range of 10 nL to 350 µL.

The concentration of blue dye is constant in all sample solutions and is equal to that of a Diluent buffer. The blue dye is therefore used as an internal standard to calculate solution depth in each well. The liquid handler to be tested is used to dispense sample solution (and Diluent if appropriate) into the wells of a microtiter plate and the absorbance at both wavelengths are measured for every well. By applying the Beer-Lambert law, the MVS automatically calculates both the precision and accuracy of the volume delivered by each pipetting channel.

Image 1. Artel MVS® Multichannel Verification System

The MVS software generates a standardized output or test report which includes information for every channel and every tip of the liquid handler assessed including a “heat map” version for visually identifying patterns in performance (Figure 1). Results can be directly integrated into electronic laboratory notebooks (ELN). The imprecision and inaccuracy of every single pipetting step is automatically displayed. Additionally, performance data can be easily exported. The data provided by the MVS allows direct analysis of manual adjustments as well as of instrument performance over time.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	15.34	15.30	15.21	15.25	15.05	15.14	15.79	15.65	15.41	15.26	15.60	15.20	14.86	15.55	15.22	15.58	15.25	15.69	15.36	15.23	15.32	15.36	15.36	15.42
B	15.10	15.07	15.13	15.27	15.34	15.76	15.83	15.97	15.35	15.87	15.70	15.51	15.15	15.17	15.09	15.60	15.27	15.63	15.57	15.48	15.31	15.60	15.47	15.76
C	15.50	15.08	15.59	15.69	15.65	15.58	15.63	15.73	15.62	15.75	15.85	15.54	14.88	14.95	15.48	15.54	16.27	15.74	15.99	15.40	15.68	15.77	15.70	15.84
D	15.15	15.31	15.30	15.53	15.43	15.65	15.91	16.04	15.97	16.22	15.76	15.40	15.05	15.19	15.38	15.48	15.32	15.44	15.53	15.80	15.57	15.93	16.01	15.56
E	15.29	15.71	15.39	15.75	15.99	15.69	16.37	15.75	16.15	16.00	15.77	15.78	14.86	15.21	15.92	15.47	15.68	15.70	15.84	15.67	15.59	16.00	16.02	15.92
F	15.56	15.32	15.58	15.78	15.39	15.82	15.80	16.84	15.72	16.48	16.14	15.39	14.99	15.33	15.09	16.65	15.88	15.55	15.85	15.84	15.77	15.88	15.88	15.67
G	15.56	15.86	15.81	15.59	15.98	15.72	15.94	16.14	16.06	15.94	15.86	15.61	15.37	15.56	15.55	15.71	15.73	15.80	15.70	15.60	15.65	15.87	15.72	16.08
H	15.34	15.76	15.45	15.91	15.89	15.63	16.19	15.69	15.60	16.45	15.94	15.70	14.90	15.84	15.37	15.80	15.71	15.72	15.85	15.34	15.94	15.84	16.02	16.06
I	15.40	15.56	15.83	15.88	15.86	16.09	15.85	16.21	16.30	15.73	16.17	16.05	15.11	15.05	15.89	15.93	15.47	15.86	15.71	15.29	15.74	15.97	15.78	15.99
J	15.59	15.98	15.50	15.91	15.64	15.78	15.78	16.06	16.04	15.97	15.79	15.86	14.95	15.65	15.47	15.65	15.81	15.56	15.46	15.42	15.85	15.78	15.81	16.08
K	15.27	15.76	15.40	15.86	15.55	15.63	15.63	16.09	15.75	15.79	15.90	15.50	15.48	15.23	15.26	15.79	15.53	15.71	15.74	15.57	15.83	15.78	15.98	15.95
L	15.46	15.80	15.71	15.69	15.65	15.46	15.95	15.87	15.70	15.91	15.89	15.51	15.29	15.70	15.43	15.77	15.32	15.66	15.73	15.35	16.01	15.92	15.91	15.94
M	15.26	15.64	15.36	15.39	15.72	15.86	16.05	15.64	15.83	15.90	16.03	15.52	15.12	15.41	15.51	15.76	15.52	15.66	15.60	15.28	15.90	15.82	15.55	15.97
N	15.39	15.69	15.59	15.81	15.36	15.66	15.92	16.10	15.69	15.71	16.06	15.38	15.66	14.95	15.68	15.77	15.39	15.58	15.35	15.40	15.41	16.16	15.88	15.92
O	15.32	15.72	15.45	15.55	15.80	15.55	15.65	15.91	15.68	15.85	15.49	15.42	14.89	15.20	15.22	15.66	15.40	15.76	15.34	15.53	15.42	16.01	16.07	15.66
P	15.44	15.31	15.46	15.65	15.37	15.29	15.81	15.81	15.43	15.81	15.49	15.60	14.81	15.46	15.24	15.63	15.29	15.19	15.35	15.23	15.43	15.78	15.49	15.88

Figure 1. Heat Map as output by Artel MVS
The detailed results generated by the MVS help in identifying patterns of imprecision and inaccuracy.

Integration of the MVS into the cell::explorer quality control process

The MVS was used to evaluate the performance of all dispensers integrated into the cell:explorer system without adaptations to the dispensers being necessary.

All measurements were conducted in a manner that reflected routine laboratory operations – which includes wet and dry dispenses - starting with the storage of the reagents at room temperature. Dispensing volumes were aligned to volumes commonly used for screenings. After adding the Diluent, the microtiter well plates were mixed at 1000rpm for one minute. Visual checks for bubbles or inhomogeneous mixed wells where conducted prior to measurement.

All measurements were performed using standard aqueous solutions supplied by Artel, except for the Echo measurements which required a DMSO solution. These solutions were prepared from MVS Stock Solution following instructions provided by the Artel Alternative Solutions Helper software package producing a final solution containing 75% DMSO by weight.

ThermoFisher Multidrop verification

The Thermo Fisher Multidrop systems utilize replaceable tubing cassettes which may be subject to material absorption. It was therefore decided to ensure that the MVS dye solutions caused no interference to subsequent HTS luminescence-based results prior to verifying liquid handler performance.

Interference Testing was performed as follows. Following an incubation step in which the tubing cassettes were exposed to the MVS dye solutions and subsequently were rinsed with 50ml of water to remove the dye solution, a standard ADP determination assay using luciferase as a luminescent indicator was conducted using the high, medium and low controls normally included as part of a screening experiment.

Data were collected on two consecutive screening days, one with an MVS performance assessment before the start of the screen (red) and one without the performance assessment (green). Each 384 well plate used in the experiment contained 2 rows for controls, 4 wells for the mid standards, 6 wells for the high standards and 22 wells for the low standards. Data for the medium and low controls were normalized to 100% and are shown in Figure 2.

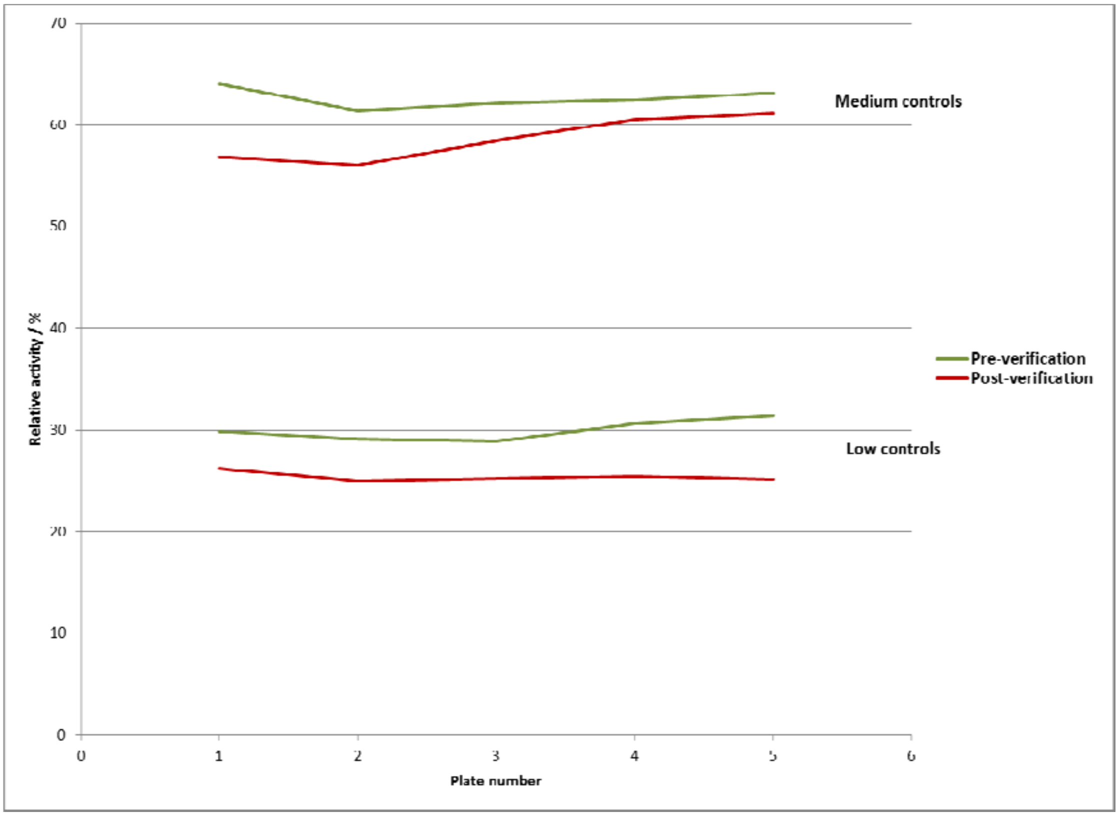

Figure 2. Non-interference study
The relative activity of the medium and low controls normalized to 100% compared to the high controls

The relative activities of the medium and low controls are within the expected experimental error for the luciferase assay. This suggests that the MVS dye solutions were sufficiently removed from the tubing cassettes of the Multidrop by the rinsing step and that the MVS is a suitable system for verifying the performance of this liquid handler.

Long-term studies into the accuracy of the Multidrop system were also conducted, with target volumes of 2.5 µl and 5 µl being dispensed into empty microtiter well-plates before the addition of Diluent and mixing.

The experiment was repeated after a period of 2 weeks and the accuracy and precision data fell well within assay tolerance, with imprecision values all falling within 1.45 %CV. Furthermore, accuracy drift during that period was deemed to be insignificant.

PerkinElmer JANUS

Target volumes of 5 µL of Artel Sample Solution were dispensed by the PerkinElmer JANUS fitted with a 384-channel Modular Dispense Technology™ (MTD) pipetting head into 384-well microtiter Verification Plates that had been pre-filled with Diluent Solution. The plates were then mixed prior to analysis with the MVS. The studies were conducted on consecutive days and the relative volumes transferred are shown in Figure 3.

The relative inaccuracy on day 1 was found to be -2.14% and the imprecision to be 2.28 %CV. On day 2 the inaccuracy was found to be 1.91% and the imprecision 2.61 %CV. This level of inaccuracy and imprecision was deemed to be within the tolerance of the assay protocols.

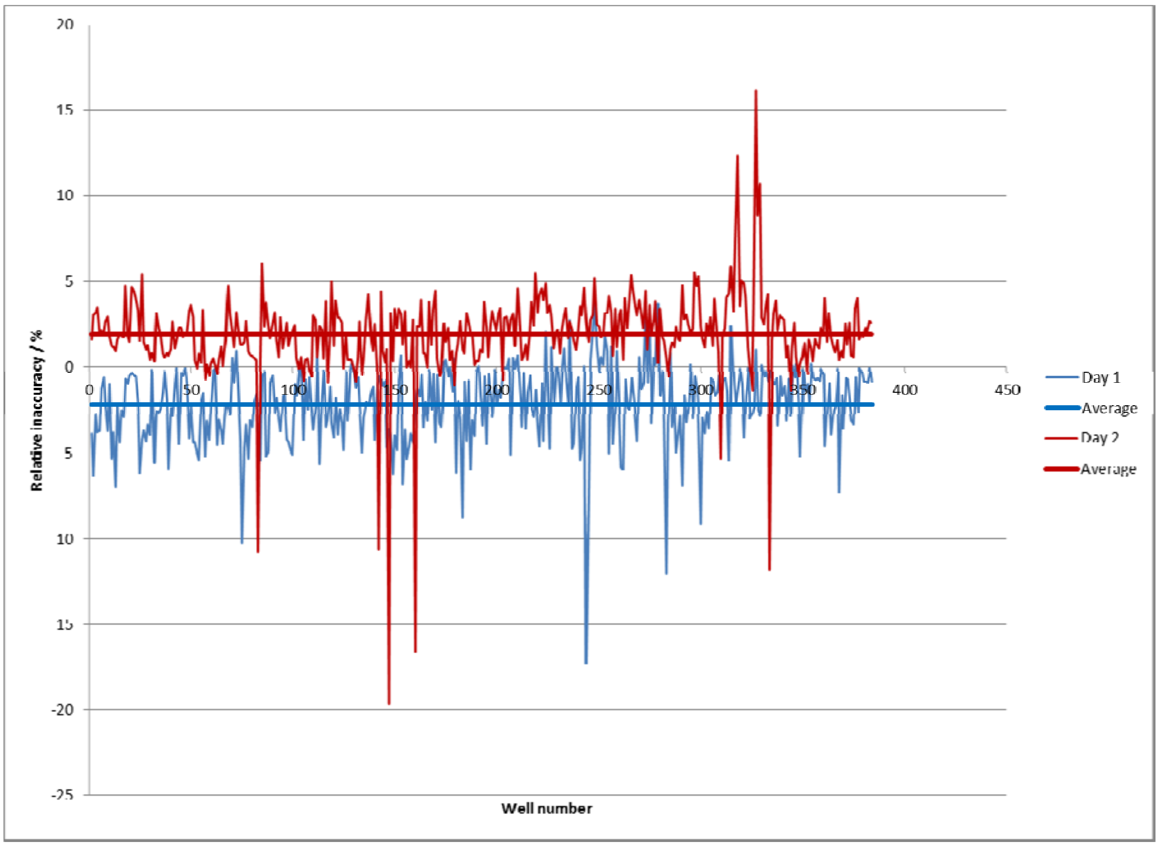

Figure 3. Day-to-day accuracy of Janus MTD
Day 1 shown in red with average relative inaccuracy of -2.14% and imprecision 2.28 %CV Day 2 shown in blue with average relative inaccuracy of 1.91% and imprecision of 2.61%CV.

Labcyte Echo 550

To test the performance of the Echo 550, DMSO solutions were prepared using the MVS Stock Solution following instructions provided by the Artel Alternative Solutions Helper software package producing a final solution containing 75% DMSO by weight.

Target volumes of 100 nL of the DMSO-based solution were then dispensed by the Labcyte Echo into empty 384-well Verification Plates. The plates were then filled with Diluent and centrifuged and mixed prior to analysis.

The mean value for all channels was 106.2 nL, which equates to a relative inaccuracy of 6.2% and an imprecision of 2.26 %CV. These data fall within the manufacturers tolerances, and have been plotted in Figure 4.

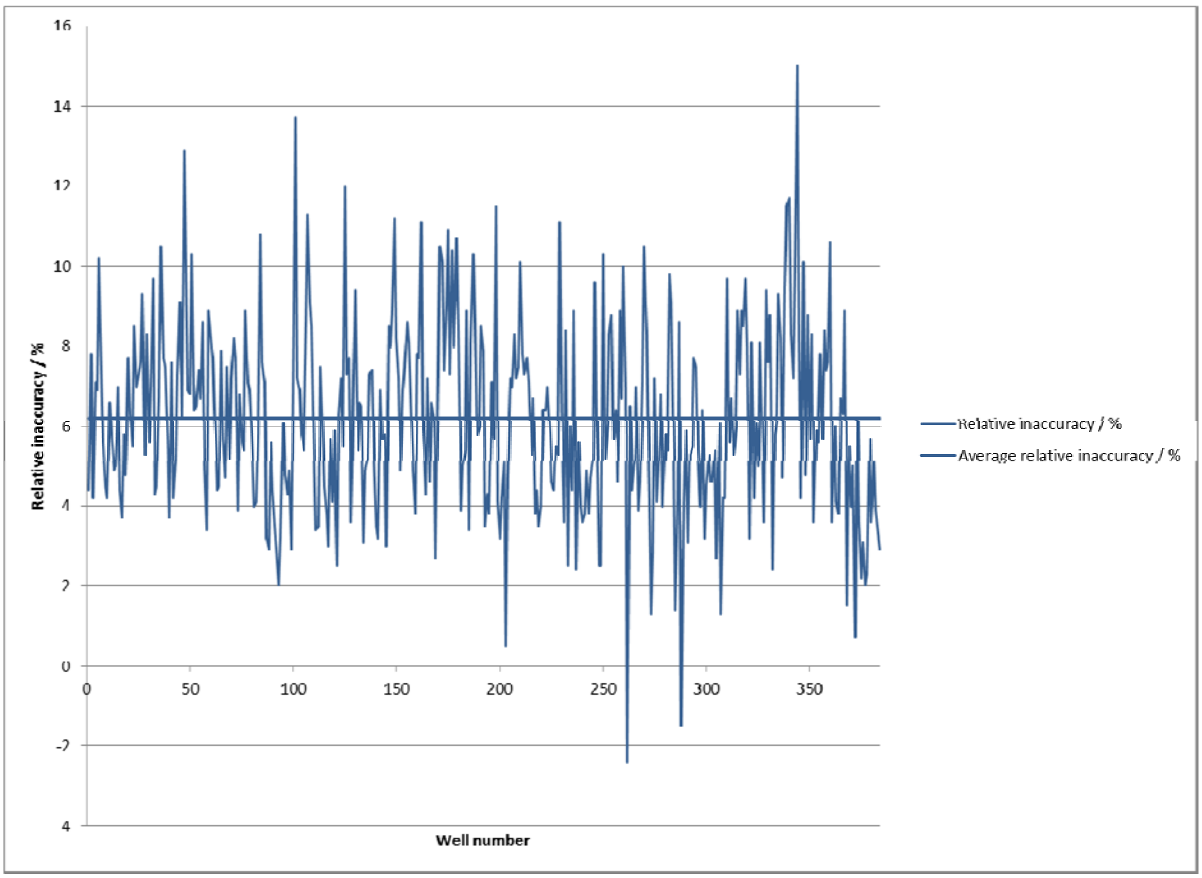

Figure 5. Relative inaccuracy data for a 100 nL DMSO dispense from an Echo 550
Average relative inaccuracy = 6.2% and imprecision = 2.26 %CV.

Summary
The Artel MVS was successfully used with the PerkinElmer cell::explorer HTS system and detected dispensing errors in volume delivery ranging from 10 nL to 350 µL. Using standard MVS solutions, the system was shown to be suitable for verifying the performance of the Thermo Fisher Multidrop and PerkinElmer JANUS MTD liquid handlers without interference with subsequent luminescence based HTS readouts. The data discussed herein also shows how the Artel Alternative Solutions Helper program could be used to create DMSO solutions suitable for verifying the Labcyte Echo 550 acoustic liquid handler.

In addition, Artel technology guarantees traceability to the International System of Units (SI), which enables standardization between liquid handlers, methods and locations.

For the user of the cell::explorer in this study, the implementation of the MVS as a single performance verification method for all dispensing units in the system achieved the following:

- Accelerated quality control and performance measurement procedures for liquid handlers.
- Increased the reliability of the user's quality control process without interfering with luminescence- based HTS readouts.
- Improved existing protocols as a result of the standardized output from the MVS.

[1] European ScreeningPort GmbH, Schnackenburgallee 114, D-22525 Hamburg, Germany
phone: +49 (0) 40.560 81-470 • email: contact@screeningport.com • www.screeningport.com

[2] Artel, 25 Bradley Drive, Westbrook, Maine 04092, USA
phone: 1-888-406-3463 • email: info@artel-usa.com • www.artel-usa.com

References

1. Hentz, N., "The Importance of Liquid Handling Quality Assurance Through the Drug Discovery Process", *Drug Discovery News*, **July 2010** available at http://www.artel-usa.com/nathentz.aspx

ARTEL
25 Bradley Drive
Westbrook, ME 04092-2013
toll-free: 888-406-3463
tel: 207-854-0860
fax: 207-854-0867
email: info@artel-usa.com
web: www.artel-usa.com