

# ATS-100 Acoustic Transfer System calibration using Artel MVS and DMSO Range E dye:

## A practical approach to developing calibration curves

Daniel Scheibe<sup>1</sup>, John Palan<sup>1</sup>, Kevin Khovananth<sup>2</sup>

<sup>1</sup>Takeda California, Inc., 10410 Science Center Dr. San Diego, CA 92121

<sup>2</sup>Artel, 25 Bradley Drive, Westbrook ME, 04092



### BACKGROUND

Takeda California uses the non-contact ATS-100 Acoustic Transfer System (EDC Biosystems, Milpitas, CA, [www.EDCBiosystems.com](http://www.EDCBiosystems.com)) in various compound management liquid handling activities in support of drug discovery processes. It is widely accepted that proper quality assurance is important for accurate and efficient processes -- and proper calibration of automated liquid handling instruments is a key component of such quality assurance.

### HYPOTHESIS

Dispense accuracy of the ATS-100 can be iteratively improved by adjusting the "burst values" in the calibration file by the relative percentage of volumetric inaccuracy as determined by experimental measurement.

### OVERVIEW

The ATS-100 transfers one or more droplets of a given fixed size (e.g. 1nL, 2.5nL, 10nL, etc.) in multiples necessary to attain the desired transfer volume. A fast and efficient calibration process is highly desirable since the ATS-100 is calibrated independently for each drop size. Each calibration consists of a set of numbers that assigns a "burst value" to each of 36 possible liquid levels that correspond to an amount of liquid present in the well of a source plate. At run time, the instrument interrogates the liquid found in a source well, compares it to the calibration values and determines the appropriate burst to use to successfully eject droplets into a target destination.

Here we discuss a process utilizing the Artel MVS Multichannel Verification System (Artel, Westbrook, ME, [www.Artel-USA.com](http://www.Artel-USA.com)) for volumetric measurement. MVS uses dual-dye photometric measurements that are robust against environmental influences, which together with the Artel-certified Calibrator Plate, Verification Plates, Plate Reader, and dyes are said to support an unbroken chain of traceability to national and international standards.

The process here uses the new Artel DMSO Range E dye which closely represents the materials (DMSO compound solutions) and can be used to measure volumes (0.0001-0.2999ul in a 384 well plate and 0.0001-0.9999ul in a 96 well plate) typically transferred by the ATS-100 in sample management operations.

Additionally, this process benefits from the elimination of out-of-process steps and their associated opportunities for error, and closely represents production conditions. Details of the preparation and operation of the Artel MVS system itself are beyond the scope of this discussion. In summary, MVS measures absorbance values and generates individual volume measurements, accuracy and precision for each tip or channel and summary statistics by well, row, and column.

### METHOD

#### Prepare acoustic source plate:

- DMSO Range E dye is transferred to an acoustic source plate using a multichannel pipette (2ul in column 1 with each subsequent column's volume incremented by 0.91ul up to 22ul in column 24.)

#### Add diluent to target plate:

- Diluent Solution is transferred to a 384-well MVS standard verification plate (55ul in each well.)

#### Dispense dye to target plate:

- Load newly-prepared source and destination plates on to EDC ATS-100, select the drop size calibration to be tested, and dispense a constant test volume from each source well to the corresponding target plate well. The calibration file is a plain text file containing "burst values" for specified source liquid levels. At the completion of the run, the ATS-100 produces a plain-text "process file" containing each source well's liquid level and burst value used.
- Spin down the target plate in a plate centrifuge to ensure all liquid is at the bottom of the well.

#### Measure dispensed dye volume:

- Read the target plate on the Artel MVS system following the standard MVS verification protocol, producing the Artel verification report.

#### Analysis and calibration adjustment:

- The dispensed volume is a direct result of a "burst value" used by the ATS-100 relative to the detected source well liquid level.
- For each individual dispense, the variance of the actual volume from the intended target volume is calculated as a correction percentage:

$$\text{Correction \%} = 1 - (\text{Actual Volume} \div \text{Target Volume})$$

- A new "calculated burst" is computed using the correction percentage:

$$\text{Calculated Burst} = \text{Old Burst} + (\text{Old Burst} \times \text{Correction \%})$$

- For each LL, there are 16 "burst values" that are adjusted. The new calculated "bursts values" are then averaged to create one representative new "burst value" for a specific LL:

$$\text{New Burst} = \text{Average of 16 Calculated Bursts}$$

- New "burst values" are calculated for each specific source well liquid level being tested and are then used to replace the prior "burst values" in the calibration file.

#### Lather, rinse and repeat:

- The method is re-executed until the ATS-100 achieves at most 5% inaccuracy. The entire process is repeated for each drop size's calibration file.

#### Calibration Method Schematic:

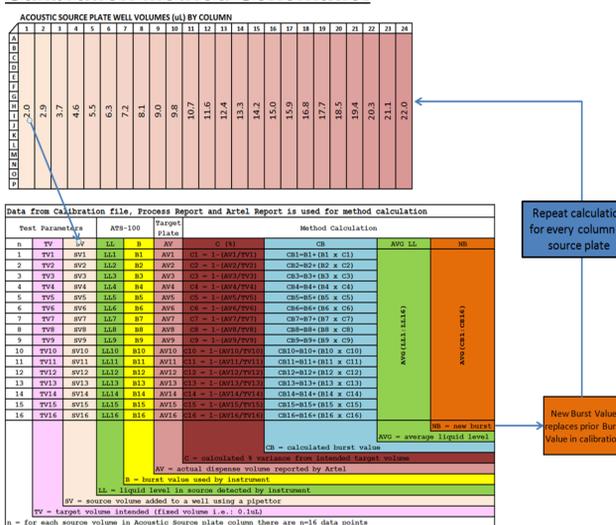


Figure 1. An acoustic source plate is filled with an increasing array of volumes. Each column in the plate represents 16 tests of a particular volume. A fixed volume is dispensed from source to a destination well. The dispense parameters and results are collected. The information is processed to calculate one new "burst value" for each source liquid level in a calibration curve.

### RESULTS

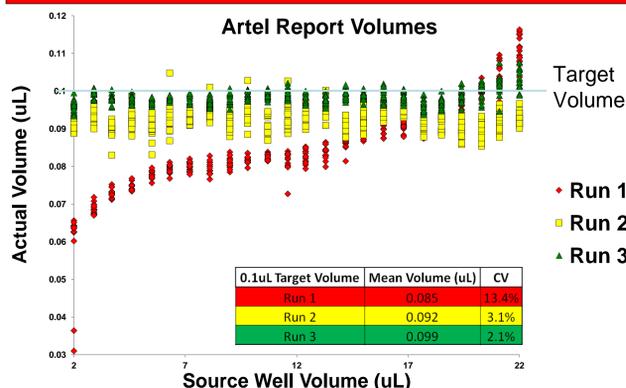


Figure 2. Scatter plot overlays the Artel reported volumes for runs 1-3 of the calibration method. The results show that with each successive run and subsequent calibration value adjustment, the ATS-100's ability to dispense the correct target volume improves dramatically.

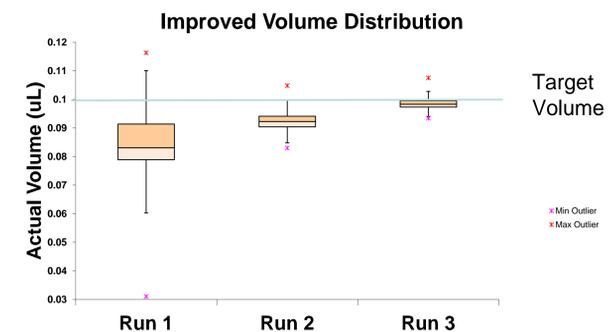


Figure 3. Display of raw data distribution around the intended target volume for each successive run of the calibration method. Each box represents 384 data points.

### DISCUSSION

The experimental data clearly shows that adjustment of "burst values" in a calibration based on volumetric feedback from the Artel report improves the accuracy of the ATS-100.

In addition, the new "burst values" calculated from this method dramatically improve the precision (CV). Variations in source plate bottom thickness and meniscus morphology can affect how the instrument detects the liquid level in a well and therefore the "burst value" used for a dispense. By averaging 16 raw data points per liquid level we produce a single representative burst value that takes these inconsistencies into account. Interestingly, we have found that the calibrations that produce the best results are not linear. Figure 3 demonstrates the pattern of improvement in the target volume distribution around the intended target volume.

### CONCLUSIONS

- Making iterative adjustments to calibration curve "burst values" based on percent variance from expected target volume significantly improves accuracy and precision of a calibration file.
- Using the Artel MVS and DMSO Range E Dye was an extremely effective tool for the collection of quantitative data used to carry out this method for calibrating an ATS-100.

### ACKNOWLEDGEMENTS

Work for this poster would not have been possible without the help from the following:  
Takeda California: Robert Wynands

#### About Takeda California

Takeda California is Takeda's center of excellence for structure-based drug discovery and protein therapeutics. The Company combines protein x-ray crystallography, small molecule, antibody, and antibody drug-conjugate capabilities to generate potential new medicines for treating cancer, inflammatory diseases, metabolic diseases, and cardiovascular disease. Takeda California is a wholly owned subsidiary of Takeda Pharmaceutical Company Limited, Japan's largest pharmaceutical company and one with a 230-year heritage. Together with its worldwide partners in science, Takeda California is committed toward better health for people through leading innovation in medicine. Takeda California also mobilizes frequent company-wide efforts to help local nonprofit organizations, schools and the environment. Additional information about Takeda California is available through its affiliate website, [www.takedacalifornia.com](http://www.takedacalifornia.com)

