Verifying dispensing performance & optimizing accuracy on a Redd&Whyte Preddator nano-bulk reagent dispenser using the Artel MVS®

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ABSTRACT:

This application note discusses an approach to verify and optimize the dispensing accuracy on a Redd & Whyte Preddator[1] dispenser using measurement information from the Artel MVS®. The Preddator is able to dispense any number of liquid types and the MVS supports verifying both tip-to-tip precision and accuracy values for nearly all liquid handlers.[2,3] The focus of this application note is on volume verification and subsequent optimization; it does not speak to the quality of volume transfer performance you should expect from any Preddator. Pipetting accuracy can be improved in multiple ways on the Preddator, such as by adjusting tip-by-tip calibration factors, system pressure settings, varying solenoid size, etc. This application note focuses on optimization of volume transfer accuracy by adjusting the calibration factors for the specific target volume or volume range. In this approach, the other variables and/or individual settings within the liquid handler software remain relatively unimportant to the optimization process. In the examples discussed, a 4-tip Preddator dispenser was employed to transfer Artel dye-based Sample Solutions: aqueous (0.5 – 2.5 µL) and DMSO (100 – 300 nL). In both the aqueous and DMSO methods, the MVS "as found" (pre-optimized) volume values were used to determine new calibration factors and, within minutes, the volume transfer accuracy was dramatically improved for both the aqueous and low-volume DMSO solutions.

INTRODUCTION:

The Preddator software enables each tip to have an independently addressable calibration factor (in units of microsecond per microliter; µs/ µL), which corresponds to the amount of time the solenoid valve remains open to dispense a specific amount of liquid. Reducing the calibration factors in turn reduces the amount of liquid dispensed and vice versa. When trying to determine the optimal calibration factor for a given target volume, the accurate measurement of a transfer is critical to the process. A new calibration factor can be determined using the current calibration, the target (goal) volume, and the measured volume transferred with the MVS, as shown by Equation 1:

$$\text{New Calibration Factor Value } (\mu s/\mu L) = [(\text{Current Calibration Factor } \mu s/\mu L) / (\text{MVS measured volume } \mu L)] * (\text{Target Volume } \mu L) \qquad \text{(Eq 1)}$$

Using Equation 1 and MVS volume measurement results, the calibration factors (one factor per tip) can be recalculated and subsequently retested to improve accuracy. Improving pipetting accuracy via calibration factor adjustment is a simple and effective process: (1) transfer target volumes and measure performance with the MVS; (2) calculate the new calibration factors using Equation 1 and enter them into the software interface; (3) re-test to confirm improved transfer accuracy, and (4) re-peat as needed for specific tolerances and volume transfer goals.

MATERIALS:

- Artel MVS, Sample Solutions, Diluent and Verification Plates
- Excel spreadsheet and/or calculator for determining calibration factor values
- Redd&Whyte Preddator dispenser
- Associated source vessels and tubing
- Thermo WellMate dispenser

PROCEDURE & RESULTS:

To save time and prepare plates for Preddator testing, a WellMate dispenser (Thermo Scientific, Hudson, NH) was first employed to pre-load each 384-w Verification Plate with 50 µL Diluent before positioning the plate on the Preddator deck. The Preddator with loaded 384-w plate is shown in **Figure 1**. The Preddator was employed to dispense aqueous Range C Sample Solution (0.5 and 2.5 µL) and DMSO Range E Sample Solution (100 – 300 nL). In some instances, the optimization process and associated data are shown herein and in other portions, the volume range only showing "as left", or post-optimization, performance is shown. After each transfer, the test plate was measured with the MVS and the overall results were tabulated.

Figure 1. The 4-tip Preddator showing the MVS 384-w Verification Plate loaded on the plate deck before a volume transfer measurement. The reservoir trough, which is seen below the tips, was employed when priming the tips before the initial dispense. Tip ID #4 is the right-most tip and tip ID #1 is the left-most tip.

MVS Setup.
Because the Plate Layout Manager within the MVS system software v3.1 does not support a 4-tip device dispensing into a 384-w plate, a slight work-around was employed that split the 384-w plate into four equal groups for the same test volume. In the examples described, a 1-tip device was defined to dispense four distinct groups (where each group was a unique tip; 96 reps per tip) so that each tip's statistics would be properly calculated (**Figure 2**). When dispensing the DMSO Range E solution between 100 – 300 nL, only 24 reps were acquired per tip (filling one complete row per tip). All aqueous testing was performed with Range C and note, within the MVS Plate Layout the target volume was set to 2.4999 µL (as opposed to 2.5 µL) so that the aqueous testing could be performed with only one dye. Likewise for the DMSO dye, the target volume in the MVS software was set to 299 nL (as opposed to 300 nL).

For this study, the MVS data grouping was coordinated to the actual Preddator tip with the following information, which was critical to making sure the proper tips were each correctly addressed during the optimization process:

- MVS group 1 = Preddator tip 4
- MVS group 2 = Preddator tip 3
- MVS group 3 = Preddator tip 2
- MVS group 4 = Preddator tip 1

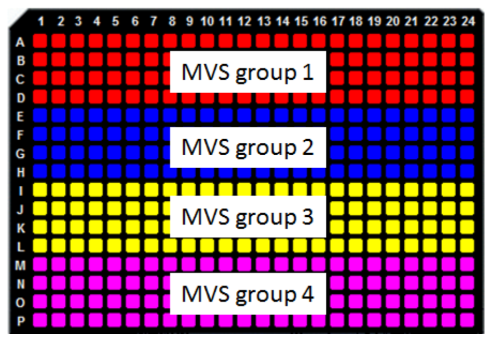

Figure 2. The MVS Plate Layout was coordinated so that each of the four individual tips on the Preddator dispensed into an equal portion of each 384-w Verification Plate.

Preddator Setup
During the preparation before the volume transfer measurements, the Preddator required a "prime" to fill all fluid lines with the test liquid of interest. Typical system dead volume is as low as 100 µL per channel. In the Preddator software, the liquid classes and calibration factors can be determined by opening the Setup tab from the Main Menu and then selecting Liquid Class Setup (**Figure 3**).

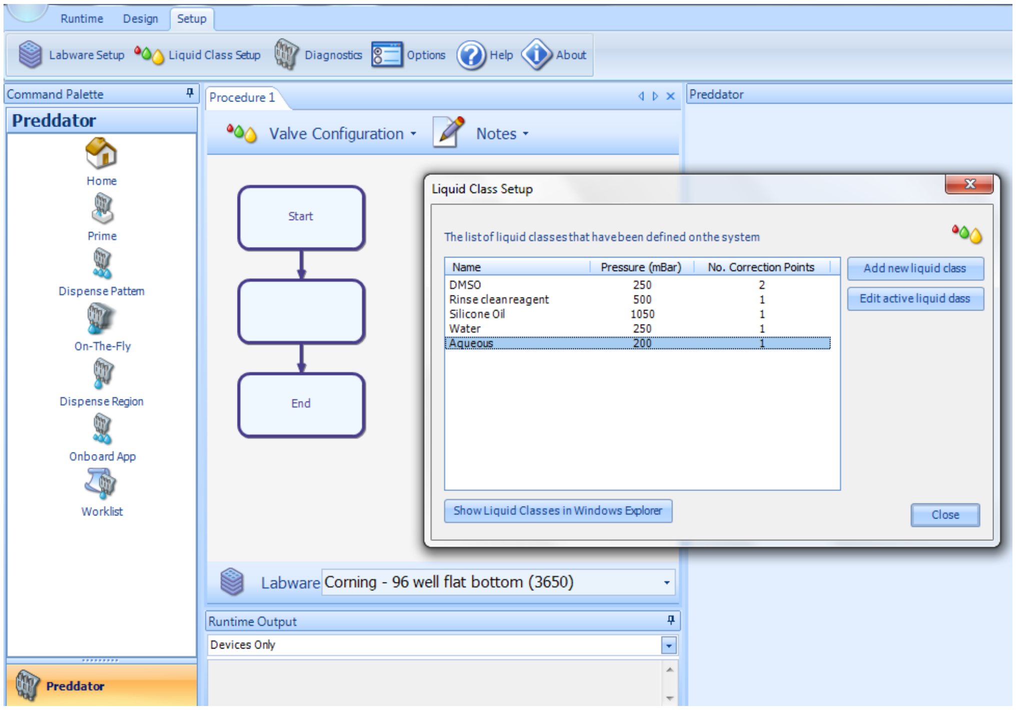

Figure 3. Click Setup | Liquid Class Setup for viewing, adding, or editing a liquid class. With the Aqueous liquid class highlighted, by clicking "Edit active liquid class" button, the image in **Figure 4** is presented to the user.

Within the Liquid Class Setup interface, one can add a new liquid class or edit a previously defined liquid class. In the example shown, five different liquid classes and their defined pressure values and number of correction points are shown. The Aqueous liquid class was employed when dispensing aqueous MVS Range C into the 384-w plate. The DMSO liquid class was employed when dispensing MVS DMSO Range E into the 384-w plate. Calibration points for each individual valve are shown when the specific valve is selected. Initial calibration values for the aqueous testing were set at 20,000 µs/µL (run 1, data not shown). The pre-optimized 2.5-µL calibration factor for tip 1 is shown in the screen image in **Figure 4** (run 2) and the calibration factor for the optimized test for tip 4 is shown in **Figure 5** (also as reflected in **Table 1**, run 4).

Preddator Experiments

Aqueous testing. For the aqueous testing, the Aqueous liquid class was employed with a pressure value set to 200 mbar. In three successive experimental runs, the tip-by-tip calibration factors were sequentially adjusted to show how easy the optimization process works for the aqueous Sample Solution at 2.5 µL. As long as the current calibration factor and the resulting amount of dispensed volume are known from the MVS measurements, the calibration adjustments and re-testing requires only minutes. **Table 1** shows the measurement results, the overall statistics and the calibration factors on a tip-by-tip basis for the 2.5-µL optimization process. Subsequent tests included a two-test optimization for 0.5 µL aqueous dye (data not shown) and then a re-test at 0.5 as well as 2.5 µL are shown in **Table 2**.

DMSO testing. For the DMSO testing, the DMSO liquid class was employed with a pressure value set to 250 mbar. In three successive experimental runs, the Preddator was optimized to transfer 150 nL DMSO Sample Solution (and to avoid repetition of this process, only tip 1 data are shown; see **Table 3**). Subsequent testing including a range of DMSO target volumes between 100 – 300 nL (24 reps at each volume) as shown in **Table 4**.

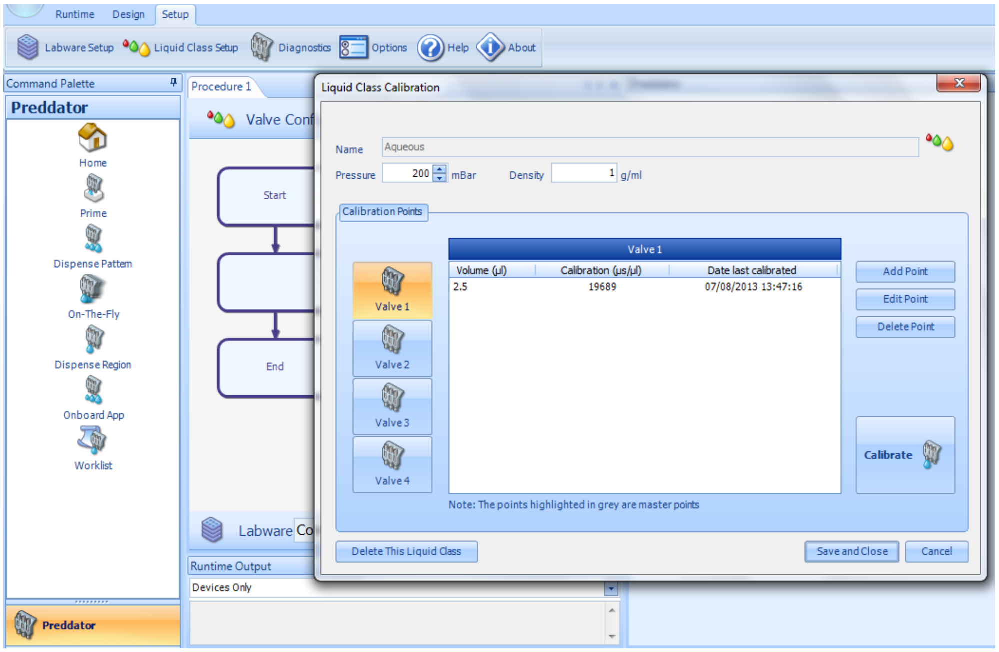

Figure 4. Click Setup | Liquid Class Setup and then **"**Edit active liquid class" to adjust the calibration factors for each of the four valves (tips). As shown, the setting for tip 1 (MVS group 4) is 19689 µs/µL, which is also reflected for the pre-optimized 2.5 µL test shown in **Table 1** (run 2). Other options within this graphical user interface include adding, editing or deleting a calibration point for each valve. Use Equation 1 to determine the new factor then click "Edit Point". To edit the current value, type over the existing calibration value and save changes by hitting "OK" (not shown).

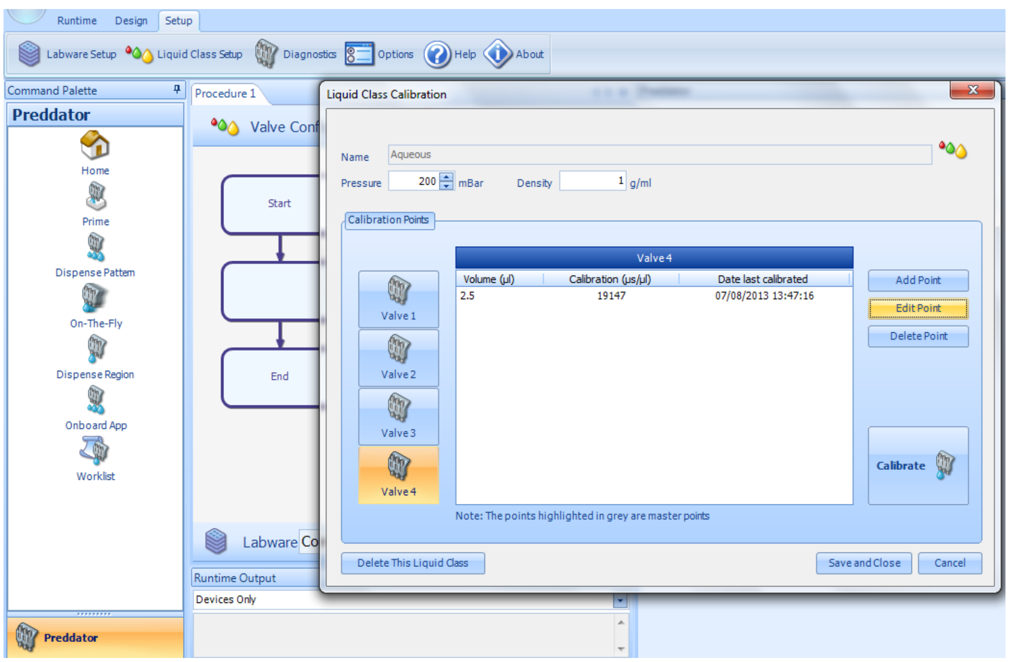

Figure 5. After test run 3, the calibration factor was determined using Equation 1 and the value for tip 4 (MVS group 1) was entered as 19147 µs/µL before re-testing in the final optimized run, which is shown in **Table 1** (run 4).

Table 1. Tip-by-tip aqueous dispense data at 2.5 µL from run 2 (pre-optimized) to run 4 (optimized)

MVS Group ID	Pred Tip ID	Target Volume (µL)	Mean Volume (µL)	Relative Inaccuracy (%)	Stand Dev (µL)	CV %	*Calibration Settings (µs/µL) **
run 2							
1	4	2.5	2.618	4.73	0.0203	0.78	*19772*
2	3	2.5	2.602	4.1	0.0206	0.79	*19893*
3	2	2.5	2.567	2.66	0.0186	0.72	*19943*
4	1	2.5	2.595	3.82	0.0271	1.04	*19689*
run 3							
1	4	2.5	2.465	-1.4	0.0097	0.39	*18879*
2	3	2.5	2.473	-1.07	0.0241	0.97	*19109*
3	2	2.5	2.457	-1.73	0.0151	0.61	*19425*
4	1	2.5	2.470	-1.22	0.0241	0.98	*18965*
run 4							
1	4	2.5	2.493	-0.28	0.0109	0.44	*19147*
2	3	2.5	2.498	-0.08	0.0102	0.41	*19317*
3	2	2.5	2.499	-0.04	0.0065	0.26	*19769*
4	1	2.5	2.499	-0.02	0.0069	0.28	*19199*

*Calibration Settings for Run 2 were based on a starting point from the run 1 data (not shown)
Calibration Settings for run 3 = [(CalFacts from Run 2/Mean Volume Run 2)]*[Target Volume Run 2]
Calibration Settings for run 4 = [(CalFacts from Run 3/Mean Volume Run 3)]*[Target Volume Run 3]

Table 2. Post-optimization data for 0.5 and 2.5 µL Aqueous Dispensing

MVS Group ID	Pred Tip ID	Target Volume (µL)	Mean Volume (µL)	Relative Inaccuracy (%)	Stand Dev (µL)	CV %
4	1	2.5	2.497	-0.105	0.008625	0.3475
4	1	0.5	0.495	-0.96	0.0050275	1.015

Table 3. Optimization at 150 nL of DMSO (only showing tip 1 data)

MVS Group ID	Pred Tip ID	Target Volume (µL)	Mean Volume (µL)	Relative Inaccuracy (%)	Stand Dev (µL)	CV %	*Calibration Settings (µs/µL) **
run 12							
4	1	0.15	0.117	-22.13	0.00103	0.88	*18000*
run 13							
4	1	0.15	0.153	1.79	0.00141	0.92	*23116*
run 14							
4	1	0.15	0.150	0.15	0.00143	0.95	*22710*

*Calibration Settings for Run 12 were used as a starting point
Calibration Settings for run 13 = [(CalFacts from Run 12/Mean Volume Run 12)]*[Target Volume Run 12]
Calibration Settings for run 14 = [(CalFacts from Run 13/Mean Volume Run 13)]*[Target Volume Run 13]

Table 4. Tip 1 post-optimization data for 100-300 nL DMSO

MVS Group ID	Pred Tip ID	Target Volume (µL)	Mean Volume (µL)	Relative Inaccuracy (%)	Stand Dev (µL)	CV %
4	1	0.2999	0.303	1.12	0.00076	0.25
4	1	0.25	0.253	1.29	0.0018	0.71
4	1	0.2	0.200	0.24	0.00132	0.66
4	1	0.15	0.149	-0.34	0.00116	0.78
4	1	0.1	0.095	-4.68	0.00068	0.71

CONCLUSIONS & CONSIDERATIONS:

Immediately upon testing and re-testing with new calibration factors, it was obvious that dispensing accuracy improved dramatically. The MVS overall relative inaccuracy and mean volume values for both the pre- and post-optimization testing are shown in **Tables 1-4** for both aqueous and low-volume DMSO dispensing. In a few quick tests, the methods employed on the Preddator liquid handling system can be quickly improved by calibration adjustment using information garnered from the MVS test results.

There are a few things to consider before, and during, this type of optimization process:

- A more involved iterative testing approach might be required to further reach accuracy goals.
- *As noted above, this application note does not speak to the quality of volume transfer performance you should expect from any Preddator*. This application note focuses on the optimization process only.
- The calibration factors are solenoid dependent. For instance, changing the size of the solenoid after an optimization is performed, may not yield the same volume transfer results.
- One should not adjust or optimize for accuracy without first knowing and ensuring the volume transfer precision is good/repeatable.
- MVS measurement data must be coordinated to the right dispensing tip. In the case presented herein, we manually matched the tips to the dispensing region in the plate. In a more automated process for coordinating MVS test results to Preddator tip calibration factors, the test plate could have been simply rotated 180 degrees so that MVS group 1 = Preddator tip ID 1, and so forth (as opposed to MVS group 1 = Preddator tip ID 4).
- The user may need to establish volume range criteria (tolerance specifications for their assays) and also document the optimization processes when changes are made. Multiple liquid classes for different volume ranges may require multiple calibration factors.
- It is recommended, when possible and/or practical, to separate liquid classes for different critical volume transfers so that one optimization process does not affect another use/application (if multiple applications use the same liquid class).

REFERENCES

(1) www.reddandwhyte.com

(2) Bradshaw *et al*. *J. Assoc. Lab. Autom.*, **2005**, *10*, 35-42.

(3) Albert and Bradshaw. *J. Assoc. Lab. Autom.*, **2007**, *12*, 172-180.