



MVS[®] Volume Verification Using Any Microtiter Plate or Small Volume Container, Such as a Tube or Vial

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Abstract.

This application note discusses methods for measuring target volumes (10 nL - 200 μ L) dispensed into any microtiter plate or small volume container using the Artel MVS[®]. If a target volume is dispensed into a microtiter plate or small container that cannot be directly inserted into the MVS plate reader, that target volume must be transferred to an MVS-compatible microtiter plate to perform volume measurements. By using multiple Diluent wash, mix, and transfer steps, the target volume can be transferred from the original container or plate to an MVS-compatible test plate. Proof-of-concept testing is shown for target volumes dispensed into various microtiter plates using gravimetrically-calibrated syringes.

Additionally, field testing results are shown for a 1- μ L DMSO target volume dispensed into three 384-well v-bottom microtiter plates with a 384-channel Biomek FX automated liquid handler.

Introduction.

The MVS supports volume verification measurements for target volumes between 10 nL and 200 μ L in MVS-compatible microtiter plate types, which are defined as being 96-well or 384-well plates with optically-clear, flat-bottom wells. Flat-bottom, optically-clear microtiter plates are required so that accurate absorbance values can be acquired with the MVS plate reader and subsequently used for volume output calculations. Because the measurements are absorbance-based

and the plate reader is a vertical beam spectrophotometer, there are many types of microtiter plates and other small liquid containers that are incompatible for directly measuring absorbance values with the MVS, i.e., these are referred to herein as MVS-incompatible plates and containers. **Table 1** shows a list of MVS-compatible microtiter plates as well as examples of MVS-incompatible microtiter plates and liquid containers. In some cases, laboratories prefer to measure target volumes in the same plates as employed in their assays, which means that the MVS might not meet all of their volume measurement requirements. This application note describes the simple process required to verify volumes dispensed into MVS-incompatible containers, which are then transferred to an MVS-compatible test plate via multiple wash, mix, and transfer steps with Diluent. Because the addition of Diluent is non-quantitative¹, the Diluent can be used to facilitate the transfer of target volume to the MVS-compatible test plate. The measured volume in the test plate correlates to the target volumes dispensed into the original plate or liquid container.

This application note describes the volume verification process with an MVS-incompatible microtiter plate or small liquid container where:

- (1) a target volume of MVS Sample Solution is dispensed into an MVS-incompatible microtiter plate, tube, or vial;

- (2) a partial addition of Diluent is added to the sample volume in the plate or liquid container, which is also referred to as a *wash* step;
- (3) the reagents are *mixed*;
- (4) the mixed solution is *transferred* to an MVS-compatible 96-well or 384-well test plate;
- (5) steps 2-4 above are repeated (Diluent wash, mix, transfer) until the working volume of the MVS-compatible test plate is obtained;
- (6) the MVS-compatible test plate is mixed on an orbital shaker before measuring the absorbance values in each well; and
- (7) the volumes determined well-by-well in the test plate are indicative of the target volume transferred in the original small liquid container.

The schematic in **Figure 1** shows the overall process for a target volume dispensed into an MVS-incompatible microtiter plate type followed by transfer to an MVS-compatible test plate. This application note shows proof-of-concept work using gravimetrically-calibrated syringes, which have been shown internally to be reliable volume

transfer standards. The syringes enable the direct comparison of the target volumes measured in the MVS-compatible test plate after they were transferred via multiple wash steps from the original liquid container.

Requirements

- (1) MVS with Data Manager software 2.0 or higher
- (2) Training on MVS operation
- (3) MVS Sample and Diluent Solutions
- (4) MVS Calibrator Plate
- (5) MVS-incompatible microtiter plate or small liquid container
- (6) MVS-compatible microtiter plate(s)
- (7) Pipettor or liquid handler

Materials & Methods.

Materials for testing included an MVS, 96-well Artel Verification Plates (VP), 384-well Corning 3711 plates, MVS Calibrator Plate, Diluent, Baseline Solution, Stock 1 Solution, and Range C Sample Solution. A 7.97- μ L gravimetrically-calibrated syringe (Artel SN# 01487) was employed to dispense the target volume into the various microtiter plates for proof-of-concept and validation testing. An 8-channel 20-200 μ L Rainin

Table 1. Examples of MVS-compatible microplates and MVS-incompatible microplates and liquid containers*

MVS-compatible microtiter plates	MVS-incompatible plates and liquid containers
Optically-clear, flat-bottom microtiter plates	Microplates characterized by:
<ul style="list-style-type: none"> ▶ Artel Verification Plates ▶ 96-well standard profile ▶ 384-well standard profile ▶ 384-well low-profile plates ▶ 384-well low-volume plates 	<ul style="list-style-type: none"> ▶ a total number of wells not equal to 96 or 384 ▶ wells that are not flat-bottom or optically-clear
	Deep-well or custom or unique microtiter plates
	Tubes
	Vials

* Volumes dispensed into MVS-compatible microplates may be directly used with the MVS for volume verification. Target volumes dispensed into MVS-incompatible microplates and liquid containers cannot be directly measured with the MVS. In these situations, the target volume must be transferred to an MVS-compatible microplate before determining the target volume.

electronic handheld pipette was used to dispense Diluent for the wash, mix, and transfer steps. All target volumes dispensed with the calibrated syringe consisted of eight replicates and were dispensed into empty wells (dry-dispense; no solution in the wells). For the 96-well plates, the replicate dispenses completely filled one column within the plate. For a 384-well plate, the target volume was dispensed into every other well within one column so that the 8-channel pipette was properly aligned for simultaneously adding and mixing Diluent in all wells at once. Between replicate dispenses, a plate cover was used to minimize evaporation and protect the solution from dust and ambient light. The Diluent is used to reach the working volume in each test plate, but it is important to note that the Diluent volume

should be close to the desired amount, but does not have to be exact¹. The total working volume per well for each of the MVS-compatible microtiter plate types are shown in **Table 2**.

Proof-of-concept testing. A 96-well Artel VP and three MVS-incompatible microtiter plate types were employed: (1) a clear, Corning 3790 96-well cell culture cluster plate with round-bottom wells; (2) a white Matrical 384-well plate with inverted pyramidal-shaped wells (MP101-2-PP); and (3) an opaque Applied Biosystems 384-well plate with conical tube-shaped wells (A30075TH). After the target volume of Range C was dispensed with the syringe into the three MVS-incompatible plates, Diluent was added to the target volume and transferred to the 96-well Artel VP with multiple

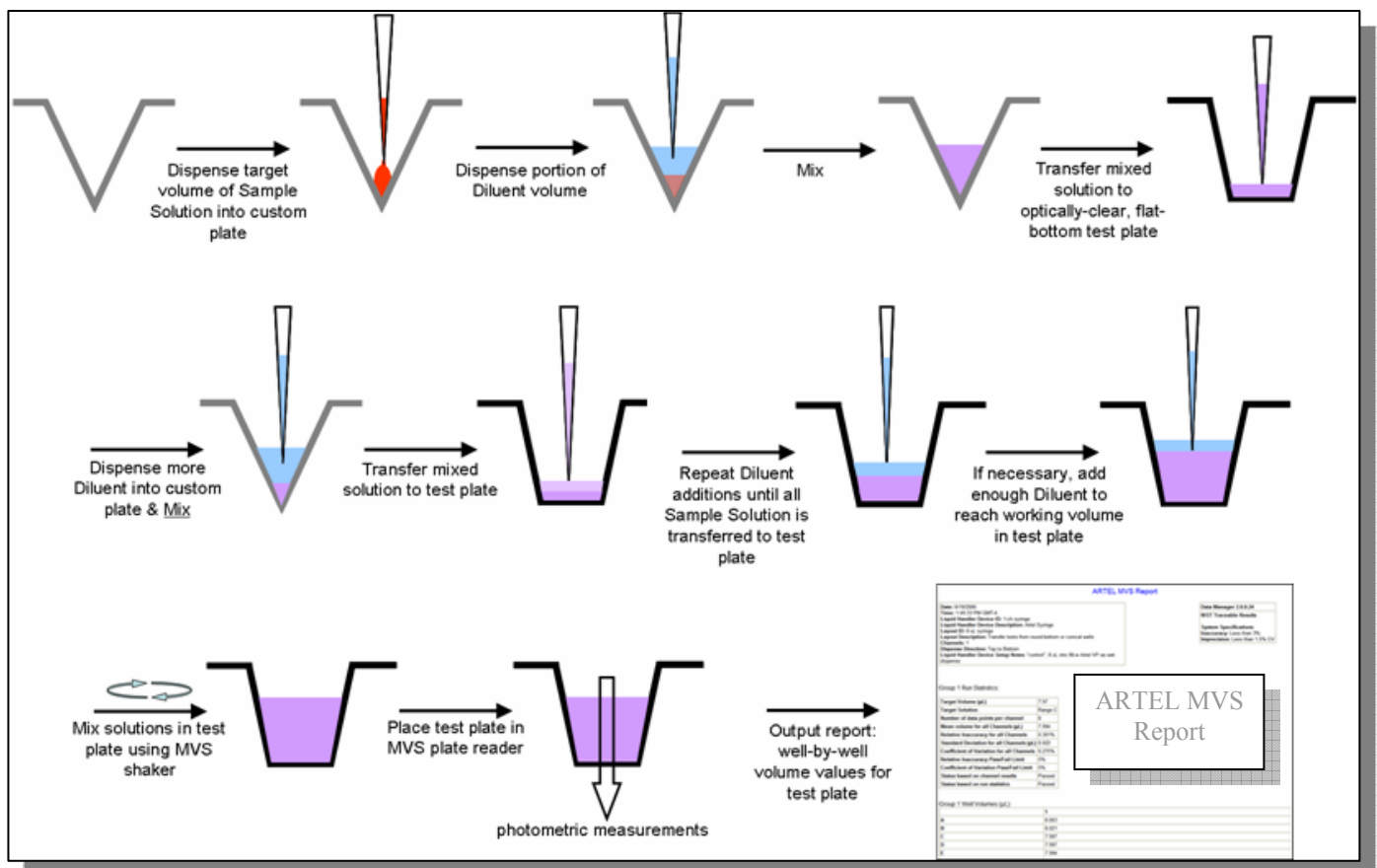


Figure 1. Schematic flow diagram for testing target volumes in MVS-incompatible plates or small liquid containers.

Table 2. Measureable volume range and desired working volume for each MVS-compatible microtiter plate type

Microtiter Plate Type	Desired Working Volume Per Well (μL)	Volume Range for MVS Sample Solutions (μL)	Alternative Test Solutions - Approximate Volume Range (μL)
96-well Artel Verification Plate (VP)	200	0.1 – 200	0.4 – 49.9
96-well standard profile	200	0.1 – 200	0.4 – 49.9
384-well standard profile	55	0.03 – 55	0.1 – 9.9
384-well (round-well) low-volume	28	0.019 – 28	0.05 – 3.99
384-well low-profile	20	0.01 – 20	0.04 – 2.49

steps. The amount of Diluent used in each step can be varied, and it may depend on the target volume plus plate type, i.e. trial and error experimentation may be required. After all of the transfer steps to the 96-well Artel VP (detailed below and in **Table 3**), the Artel VP was shaken on an orbital shaker (60 s at 1300 rpm) before performing volume measurements. Two control experiments were performed in a 96-well Artel VP. In one control, the syringe was used to dry-dispense Range C into eight wells, and in the other control, the syringe was used to dispense Range C into eight wells previously filled with 192 μL Diluent (wet-dispense). In both controls, the target was not transferred to another plate, i.e., the same Verification Plate was used for the target dispense and volume measurement (see below).

The five proof-of-concept (poc) tests are detailed herein and the summary of each experiment is included in **Table 3**. **Poc-1:** The 7.97- μL syringe was used to wet-dispense Range C Sample Solution into 192 μL of Diluent in a 96-well Artel VP. **Poc-2:** The 7.97- μL syringe was used to dry-dispense Range C into a (dry) 96-well Artel VP followed by addition of 192 μL of Diluent to create a working volume of nearly 200 μL . **Poc-3:** The 7.97- μL syringe was used to dispense target volume into a 96-well Corning round-bottom plate. Following the eight replicate, dry-dispenses of target volume, multiple Diluent mix and transfer steps were employed to transfer the

theoretical 7.97- μL target volume from each well into separate wells of a 96-well Artel VP. In each step, 48 μL of Diluent was added to the target volume, the solution was mixed with a pipette using three aspirate/dispense cycles (50- μL cycles) before being transferred to an Artel VP. In all, there were four Diluent addition steps of 48 μL to equal nearly 200 μL working volume (48 $\mu\text{L} \times 4 + 7.97 \mu\text{L}$ target volume) in each well of the test plate. **Poc-4:** The syringe was used to dispense target volume into a 384-well Matrical plate characterized by inverted pyramidal-shaped wells. Diluent was added to the target volume in seven 20- μL steps. After each Diluent addition, the solutions were mixed with three pipette mix cycles (22- μL cycles), which was followed by an aspirate and transfer to the 96-well Artel VP. After the seven Diluent transfer steps, 50 μL of Diluent was added to the Artel VP to reach the working volume of 200 μL . **Poc-5:** The same syringe was then used to dispense the target volume into a 384-well Applied Biosystems plate characterized by conical tube-shaped wells. For this plate, after each of the five, 20- μL Diluent transfers, the solutions were mixed with a pipette using three mix cycles (22- μL cycles) before transferring to the 96-well Artel VP. After the Diluent wash, mix and transfer steps, 90 μL of Diluent was added to the Artel VP to approximately achieve the 200 μL working volume per well before volume verification with the MVS.

Field testing with a 384-channel Beckman Coulter Biomek FX. A target volume of 1 μL DMSO-based alternative test solution was dispensed into three 384-well v-bottom plates, then transferred into three MVS-compatible 384-well Corning 3711 test plates for volume determination. Per the MVS Alternative Solution Library calculations, the DMSO-based alternative test solution was prepared to incorporate 75.3% DMSO (vol/vol) and 24.7% MVS Stock 1 Solution (vol/vol)². More information for preparing and testing DMSO solutions with the MVS is published elsewhere³. The following method was employed with the Biomek FX using pre-wetted tips for each of the three plates: (a) 1 μL DMSO test solution dispensed into 384-well v-bottom plate; (b) tips were washed; (c) 19 μL Diluent was added to the 1- μL target in the v-bottom plate; (d) the solution was mixed in each well with three aspirate/dispense mix cycles; (e) the mixed solution was aspirated and transferred to an MVS-compatible 384-well Corning 3711 plate; (f) the tips were washed; (g) 20 μL Diluent was dispensed into the v-bottom plates; (h) three aspirate/dispense mix cycles; (i) the solution was aspirated and transferred to the same MVS-compatible 384-well test plate; (j) the tips were washed; (k) 15 μL Diluent was dispensed into the v-bottom plate, mixed and transferred to the test plate. The entire method with 6 plates (three v-bottom plates with volume transfer to three test plates), required about 7 minutes to perform with the liquid handler. Each of the three test plates was centrifuged at 2000 rpm for 1 min. Each test plate was individually placed on an orbital mixer for 2 minutes at 2750 rpm before collecting volume measurements with the MVS.

Results and Discussion.

The data for the proof-of-concept experiments are shown in **Table 3** and are summarized in **Figure**

2, where the relative inaccuracy and CV data for each of the five experiments can be directly compared. The measured performance of the control experiments nearly mirrors the results for the three custom plates. In each of the five experiments, the measured CV values were all approximately 0.30%, indicating that there was no substantial variability in the measured well-to-well target volume for each of the different test plates. Additionally, all relative inaccuracy values for the five experiments were below 0.8% for the syringe's target volume of 7.97 μL . The use of the gravimetrically-calibrated syringes helped prove that a specific volume dispensed into a custom plate can be efficiently transferred to a test plate.

For the field test data using the 384-channel Biomek FX, the overall MVS measurement for three test plates for a 1- μL target volume of DMSO-based test solution showed relative inaccuracy and CV values of 2.4% and 3.9%, respectively. As a note, there were only four tips with relative inaccuracy values close to 10% and there were seven tips with CVs close to 10% (data not shown; the tips did not overlap between these two categories). The overall mean volume and

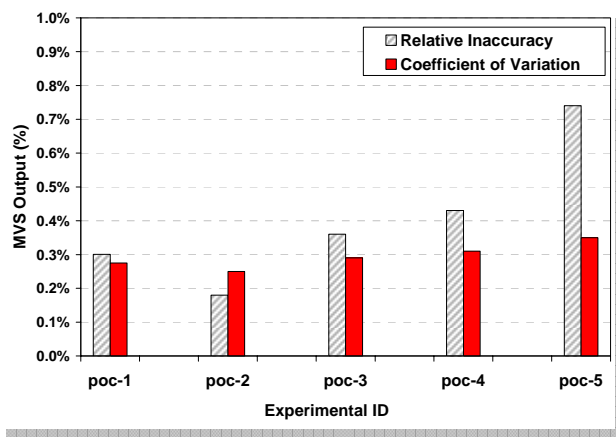


Figure 2. Graphic representation of the relative inaccuracy and CV data for the five proof-of-concept (poc) test plates. See **Table 3** and the text for testing details.

Table 3. Proof-of-concept (poc) testing. An 8- μ L target volume dispensed into an MVS-incompatible microtiter plate followed by transfer to an MVS-compatible test plate for volume verification

Experimental ID ¹	poc-1	poc-2	poc-3	poc-4	poc-5
Target volume of gravimetrically-calibrated syringe (μ L)	7.97	7.97	7.97	7.97	7.97
Number of replicate dispenses	8	8	8	8	8
Microtiter plate type for original target volume dispense	96-well Artel VP (control)	96-well Artel VP (control)	96-well, round-bottom	384-well, "inverted" pyramid wells	384-well, conical tube-like wells
Description of target volume dispense	wet dispense into 192 μ L Diluent	dry dispense into empty wells	dry dispense into empty wells	dry dispense into empty wells	dry dispense into empty wells
Number of Diluent additions; Volume of Diluent; number of pipette aspirate/dispense mix cycles before each transfer to the MVS-compatible test plate	—	1 x 192 μ L; no mix cycles	4 x 48 μ L; 3 mix cycles	7 x 20 μ L; 3 mix cycles	5 x 20 μ L; 3 mix cycles
Plate type (test plate) used to receive transferred target volume	same 96-well Artel VP (no transfer)	same 96-well Artel VP (no transfer)	96-well Artel VP	96-well Artel VP	96-well Artel VP
Amount of Diluent added to test plate before shaking and volume verification (μ L)	—	—	—	50 μ L	90 μ L
Test plate mean volume (μ L)	7.994	7.984	7.999	8.004	8.029
Test plate relative inaccuracy	0.30%	0.18%	0.36%	0.43%	0.74%
Test plate standard deviation (μ L)	0.022	0.02	0.023	0.025	0.028
Test plate coefficient of variation (CV)	0.28%	0.25%	0.29%	0.31%	0.35%

standard deviation for the three test plates were 1.024 μ L and 0.04 μ L, respectively. **Figure 3** shows a surface plot for the overall mean volume measured for each of the 384-tips.

The designed experiments prove that a target volume can be dispensed into an MVS-incompatible microtiter plate or small liquid container and then successfully transferred to an MVS-compatible microtiter plate for volume measurements. The measurements in the test plate are indicative of the original target volume dispense. The advantages of this approach include a way to determine the amount of target volume delivered for assay-specific volumes in assay-specific microtiter plates or liquid containers. For instance, this process allows the volume dispensing device, such as a robotic liquid handler, to be checked for dispensing performance exactly as it is employed for an assay (tip touches, aspirate/dispense heights, etc.). Users of automated equipment sometimes prefer to

measure the volume in the same microtiter plate type as used in their assays. In the field test application of this process, the users of the Biomek insisted that the target volume was to be dispensed into the v-bottom plate (assay plate). They did not want to dispense the target volume directly into the MVS-compatible plate because *“.it was not the same geometry and the automated methods could act differently...the tip depth and tip touches might not perform the same in the different plate and then I won't know if the source of error is the plate or my method or both.”* Following the results, the users of the Biomek were more than satisfied with the measured inaccuracy and precision of the transfer for the 1 μ L DMSO solution into the specific v-bottom plate.

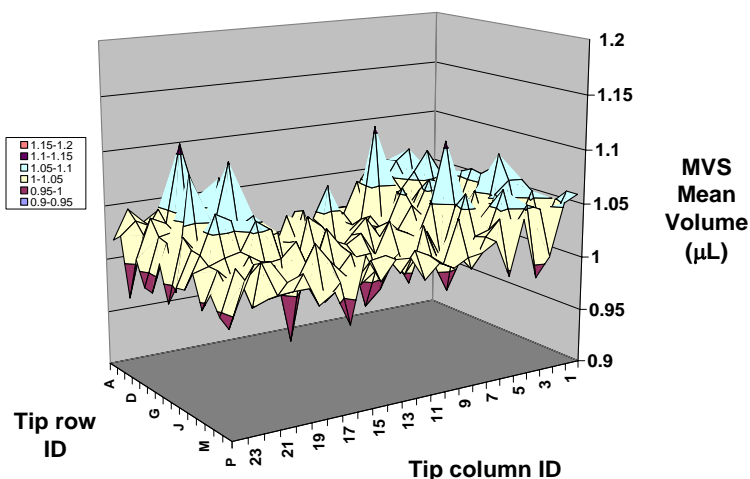


Figure 3. A surface plot for the measured mean volume (μL) for each of the 384 independent dispensing tips on the Biomek FX. These data were measured after the $1 \mu\text{L}$ DMSO alternative test solution was dispensed into three 384-well v-bottom plates and then transferred to three MVS-compatible 384-well test plates. The mean volume and standard deviation for the three test plates were $1.024 \mu\text{L}$ and $0.04 \mu\text{L}$, respectively.

Considerations.

There may be some limitations and considerations to this transfer process. If the original dispense requires a wet-dispense into an MVS-incompatible microtiter plate, the user has to make sure that Diluent is added to the plate before the target volume of MVS Sample Solution. In this case, it should be understood that there may be fewer allowed Diluent additions to transfer the target volume to the test plate *if* the total volume in the test plate is close to the working volume. Refer to **Table 2** for listed working volume values per microtiter plate type. In any case, the absolute goal is to transfer all of the Sample Solution from the custom plate to the test plate. If any of the Sample Solution hangs on the well sides and is not recovered during the Diluent additions, the plate should be centrifuged before or directly after Diluent addition. If any Diluent is left behind in the custom plate, there should not be any concern as long as the multiple wash steps have sufficiently removed all Sample Solution. If performed correctly, this procedure will work very

well for small target volumes. As the target volume increases, the amount of Diluent added and the total number of allowed Diluent transfers may decrease. In this case, the transfer of higher target volumes is possible, but it may require more attention due to the smaller amount of Diluent allowed. For large target volumes, it is recommended to first transfer the target volume to the MVS-compatible plate type that holds the largest working volume. For Data Manager Software versions 2.0 – 2.2, this plate type is a 96-well flat-bottom, optically-clear plate and holds $200 \mu\text{L}$ of working volume. The remaining target volume can be subsequently transferred with Diluent steps. If a 384-well plate will be used as the MVS-compatible test plate, it should be noted that efficient mixing in 384-well plates is more challenging compared to mixing reagents in 96-well plates. For more information, Artel has published and presented information on evaluating the efficiency of mixing methods (and microtiter plate shakers) at numerous conferences⁴. Efficient mixing steps are *extremely* important for properly measuring the target volume on a well-by-well basis.

References

- (1) Bradshaw, J.T.; Knaide, T.; Rogers, A.; Curtis, R.H. Multichannel Verification System (MVS): A Dual-Dye Ratiometric Photometry System for Performance Verification of Multichannel Liquid Delivery Devices. *J. Assoc. Lab. Autom.*, **2005**, *10*, 35-42.
- (2) Protocols and information for determining the proper amount of MVS Stock Solution to add to the corresponding amount of Solvent (or starter) Solution are detailed in multiple places, including the MVS User Guide, the MVS Help menu (MVS Data Manager software 2.0 and higher), as well as the Alternative Solution Helper software program found as part of MVS Data Manager 2.2 and higher. Additionally, Artel Technical Support can be contacted with any questions between 8 am – 5 pm

Eastern Time (888.406.3463 x109) or via e-mail at technical.support@artel-usa.com.

- (3) Albert, K.J.; Bradshaw, J.T.; Knaide, T.R.; Rogers, A.L. Verifying Liquid Handler Performance for Complex or Non-Aqueous Reagents: A New Approach. *J. Assoc. Lab. Autom.*, **2006**, *11*, 172-180.
- (4) B. W. Spaulding, J. T. Bradshaw, A. Rogers. *A Method to Evaluate Mixing Efficiency in 384 Well Plates*, Poster presented at SBS 2006 (Seattle, WA); B. W. Spaulding, L. Borrmann, J. T. Bradshaw, W. Wentz. *Photometric Measurement of Mixing Efficiency Using the Eppendorf MixMate Mixer*, Poster presented at SBS 2007 (Montreal, Canada); B. W. Spaulding, J. T. Bradshaw, P. Chang, I. Feygin. *Optimization of the TechElan TEOS Orbital Shaker Using a Dual-Dye Photometric Protocol*, Poster presented at Lab Automation, **2007** (Palm Springs, CA).

