Using the MVS to Measure Residual Volumes Remaining in a Plate After Sample Aspiration: General Approach

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Abstract

The purpose of this application note is to demonstrate an alternative use for the Artel MVS® Multichannel Verification System. As opposed to measuring the accuracy and precision for a volume transfer of sample into a microtiter plate, the MVS is instead employed to measure the volume of sample left behind after attempting to aspirate off the entire sample from each well of a microplate, i.e., residual volume of sample. *Measuring the residual volume after sample aspiration is a direct way for assessing the performance of the sample removal step.* The experiments discussed herein show how residual volumes can be measured using the MVS.

Introduction

In most scenarios, the MVS is employed to measure the accuracy and precision of a dispensed, or transferred, sample volume within a microplate. This study demonstrates a related, but unique, application: using the MVS to measure the amount of residual volume left behind within a well of a plate after some or all of the sample volume is *aspirated out*. The experiments discussed herein illustrate the use of the MVS to measure small, residual volumes following one or more removal steps of aqueous sample solutions. Aspirating off reagents and contaminants in wells of a microplate can be an important step in some assays, such as with the removal of ethanol during some DNA purification procedures.

Requirements

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

Materials and Methods

Various experiments were conducted to demonstrate how the residual volume remaining after sample aspiration could be measured. All sample solutions were aqueous and all MVS measurements were performed in 96-well MVS Verification Plates (VP). For each volume transfer, aspiration, mix and dispense steps, a Rainin 8-tip 20-200 µL LTS manual pipette was employed. All gravimetric measurements were performed with a Sartorius Research R160D balance and laboratory environmental factors (temperature, humidity, and pressure) were *not* employed in the calculation when converting weight to volume or vice versa. The balance measurements were mainly used as a comparison check before and

after sample volumes were aspirated out of a plate.

For each sample aspiration (removal) step, the pipette was simply dialed to the desired volume value before inserting the tips into the sample within the plate. Each aqueous test solution (MVS Range A, B, and C) was employed at least once to measure theoretical residual volumes of 60 and 140 µL (Range A), 15 µL (Range B), and 2 µL (Range C). Within the MVS system software, plate layouts were created to coordinate with the above mentioned target volumes for an 8-tip dispenser for 12 replicates. The different MVS Sample Solutions and associated (theoretical) residual volume values were coordinated in order to stay within the working range of what was anticipated to be the amount of residual volume left in each well of the 96-well VP following sample removal. For instance, in the second experiment below, 185 µL is aspirated out of a 96-well VP containing approximately 200 µL of test solution per well, thus leaving a theoretical residual volume of 15 µL per well. Range B, which covers a test volume range of 10 – 49.9 µL in a 96-well plate, was employed for this test because its volume range is coordinated with the assumed residual volume value of 15 µL.

Experiment 1. In the first experiment, the following procedure was conducted in a 96-well VP:

1. An empty 96-well VP ("plate 1a") was placed on the balance and then tared. A second new 96-well VP ("plate 1b") was also weighed in comparison to the tared balance for *plate 1a* (the net difference in grams was applied to the experiments for *plate 1b* measurements).
2. 200 µL of Range A was dispensed into all 96 wells of *plate 1a*. The plate was then weighed and the well-by-well volumes were measured using the MVS.
3. The pipette was dialed down and 140 µL was aspirated out of each well in *plate 1a*. After each aspiration step, each 140-µL aliquot was transferred to *plate 1b*, whereby the aspirated volume from column 1 in *plate 1a* was transferred to column 1 in *plate 1b*, and this procedure was repeated for all 12 columns.
4. Both *plates 1a* and *1b* were re-weighed.
5. To *plate 1a*, 140 µL Diluent was added to each well, the plate was weighed again, and the MVS was used to measure the residual volume (theoretical amount of 60 µL).
6. To *plate 1b*, 60 µL Diluent was added to each well, the plate was weighed again, and the MVS was used to measure the well-by-well volumes that were transferred from *plate 1a* (theoretical amount of 140 µL).

Experiment 2. In the second trial, the following procedure was conducted:

1. An empty 96-well VP ("plate 2") was placed on the balance and then tared; 200 µL of Range B was dispensed into all 96 wells and the plate was weighed.
2. 185 µL of Range B was then aspirated out of each well and the plate was re-weighed. During sample aspiration, the plate was tilted on the benchtop, *i.e.*, the right side of *plate 2* was propped up on two stacked 96-w VPs and the left side of the *plate 2* remained on the bench. During sample aspiration, the pipette was essentially placed at the junction of the well wall and the bottom of the well (as much as possible for all 8-tips simultaneously).
3. To *plate 2*, 185 µL Diluent was added to each well, the plate was re-weighed, and then the MVS was used to measure a theoretical residual volume value of 15 µL. Refer to **Figure 1**.

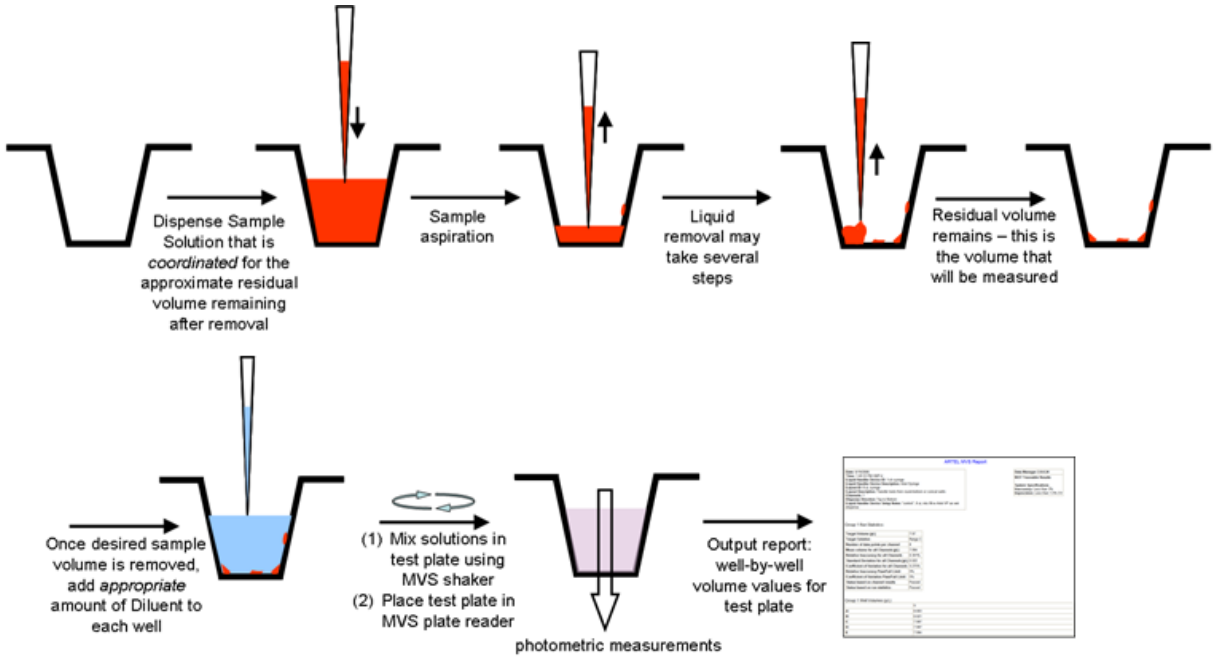

Figure 1. Overview of the approach employed to use the MVS for measuring residual volumes in a microtiter plate following sample aspiration.

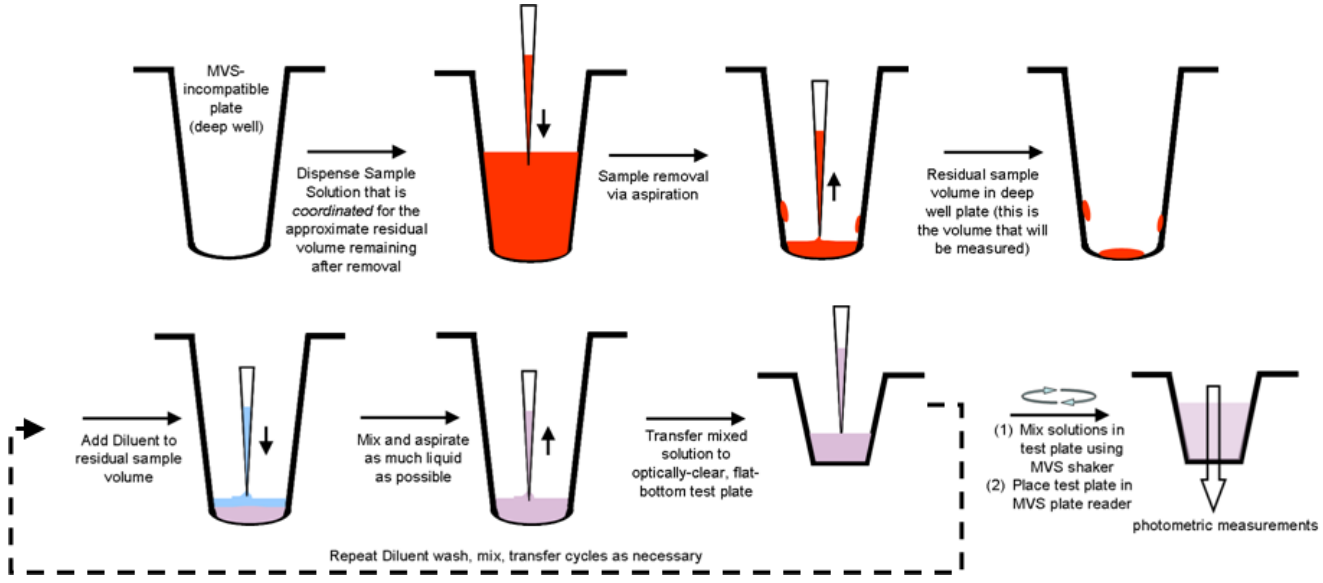

Figure 2. Overview of the approach employed to use the MVS for measuring residual volumes in an MVS-incompatible plate, such as a deep well plate, where the residual volume is transferred to an MVS-compatible plate for volume measurement (see Methods and reference 1 for more information).

Experiment 3. In the third experiment, the following procedure was conducted:

1. An empty NUNC deep 96-well plate (U96 PP 2 mL; 278752) was placed on the balance and tared. The well bottoms in this plate are round.
2. 200 µL of Range C was dispensed into all 96 wells and the deep plate was weighed.
3. 198 µL Range C was then aspirated out of each well and the deep well plate was re-weighed. The deep well plate was then centrifuged for 1 min at 1100 rpm.
4. Using the approach outlined in **Figure 2** and the methods described in reference 1, the theoretical residual volume of 2 µL was transferred to a 96-well VP ("plate 3") using multiple Diluent wash, mix and transfer steps. In all, 4 cycles of Diluent additions were used (50 µL for each wash, mix, and transfer step; totaling 200 µL of Diluent).
5. After all Diluent wash, mix, and transfer cycles, the theoretical residual volume (2 µL) in each well of *plate 3* was measured with the MVS. The volume measured in *plate 3* is the actual residual volume value not aspirated out of the original deep well plate during the initial transfer step.

Results and Discussion

The goal was to demonstrate that the performance of a sample aspiration (removal) step within a microplate could be assessed by quantifying the amount of volume remaining in the plate. In some cases, the sample aspiration step may be required to remove as much sample as possible. In other cases, only a specific amount of sample is aspirated out. The three experiments described herein, use the later approach.

The overall approach for experiments 1 and 2 (*plates 1a, 1b,* and *2*) employed the approach shown in **Figure 1** and demonstrate that residual volumes could be measured in an MVS-compatible plate. After 200 µL of Range A was transferred into *plate 1a*, it was weighed with the balance and the volumes were also measured on a well-by-well basis with the MVS. The starting (initial) volume was measured with the MVS in order to know how much volume was in each well before 140 µL was aspirated out and transferred to a new plate (*plate 1b*). Range A was employed because it covers the volume range for measuring the theoretical remaining amount of liquid in each well in *plate 1a* (60 µL) as well as the transferred amount in *plate 1b* (140 µL). Both the balance and the MVS were used to measure the residual volume in *plate 1a* as well as the 140 µL that was removed from *plate 1a* and transferred to *plate 1b*. In all MVS measurements for *plates 1a* and *1b*, the relative inaccuracy and CV values were <1.2% and <0.66%, respectively (see **Table 1**, first three columns). The percent differences in total liquid weight between the MVS and measurements made with the analytical balance, are all within 1.54% (see **Table 2**, first three columns). Moreover, the percent difference (for total liquid volume) between the initial MVS measurement of 200 µL, as compared to the summed portions, *i.e.*, 60 µL residual from *plate 1a* and 140 µL transferred into *plate 1b*, was only 0.21% (**Table 2**). The accuracy and precision in these measurements show that the 140-µL sample aspiration step was very effective and this example demonstrates that residual volumes can be accurately measured with the MVS.

Table 1. MVS measurement results for the three experiments detailed in the Methods section

	Plate 1a -- 200 µL initial volume transfer	Plate 1a -- 60 µL residual volume after 140 µL transferred to plate 1b	Plate 1b -- 140 µL (transferred from plate 1a)	Plate 2 -- 15 µL residual volume after 185 µL aspirated out	Plate 3 -- 2 µL residual volume in deep well plate after 198 µL aspirated out
MVS Target Volume (µL)	200	60	140	15	2
MVS Mean Volume (µL)	202.3	60.23	141.6	14.85	2.435
MVS Overall Relative Inaccuracy %	1.20%	0.38%	1.10%	-1.00%	21.75%
MVS Overall Standard Deviation (µL)	1	0.4	0.7	0.38	2.86
MVS Overall CV %	0.50%	0.66%	0.50%	2.56%	117.45%

Table 2. Summing all 96 volume measurements per plate (from MVS Output Reports) and converting to a *total liquid weight* before comparing to the gravimetric values for total liquid weight

	Plate 1a -- 200 µL initial volume transfer	Plate 1a -- 60 µL residual volume after 140 µL transferred to plate 1b	Plate 1b -- 140 µL (transferred from plate 1a)	Plate 2 -- 15 µL residual volume after 185 µL aspirated out	Plate 3 -- 2 µL residual volume in deep well plate after 198 µL aspirated out
MVS, Total Plate Volume (Summing All 96 Wells, mL)	19.418	5.782	13.595	1.426	0.234
MVS, Total Plate Volume Converted to grams	19.519	5.812	13.666	1.433	0.235
Analytical Balance, Net Liquid Weight (g)	19.276	5.723	13.482	1.418	0.229
% Difference: Total Liquid Weight in Grams (MVS vs. Gravimetry) *	1.25	1.54	1.35	1.04	2.46
MVS, % Difference Between Initial 200 µL Volume Transfer and Summed Portions (60 µL in Plate 1a; 140 µL in Plate 1b) **	0.21	—	—	—	—

Initial starting volume in all plates is 200 µL. * Using total plate volume values in g; Percent difference = (MVS - Balance) / [(MVS + Balance)/2]*100. ** Using total plate volume values in mL; Percent difference = [MVS200µL - (MVS60µL+140µL)] / [(MVS200µL + (MVS60µL+140µL))/2]*100

As a second example of measuring residual volumes, 200 µL of Range B was dispensed into a 96-well VP before 185 µL was aspirated out. Range B was employed because it covers the volume range for measuring the theoretical remaining amount of liquid in each well (15 µL) after the 185-µL aliquot was aspirated out. The residual volume of 15 µL was measured with the MVS to have an accuracy and CV of -1% and 2.56%, respectively (**Table 1**). The CV shows that the 185-µL sample aspiration step was not as uniform (repeatable) on a well-by-well basis as the aspiration step employed in *plate 1a*.

Regardless, the percent difference between the measurements for MVS and the balance (total weight for the residual liquid in *plate 2*) was only 1.04% (**Table 2**).

The third demonstration of measuring residual volumes employed an approach detailed in a different Artel Application Note[1]. In experiment 3 (*plate 3*), the approach for testing is shown in **Figure 2** and the experiment demonstrates that residual volumes can be measured in an MVS-incompatible plate after the residual volume is transferred to an MVS-compatible plate[1].

The experiment started by dispensing 200 µL of Range C into a deep 96-well plate. Range C was employed because it covers the volume range for measuring the theoretical remaining amount of liquid (2 µL) after 198 µL of sample was aspirated out of each deep well. Following the sample aspiration step, the residual volume was transferred to a 96-well VP for MVS measurements using multiple Diluent wash, mix and transfer cycles[1]. The residual volume of 2 µL was measured with the MVS to have an accuracy and CV of ~22% and 117%, respectively (**Table 1**). The percent difference between the MVS and balance measurements for total liquid residual weight was 2.46% (**Table 2**). The small relative percent difference for such a low residual volume in combination with the high overall CV from the MVS measurements, *indicates that the sample removal step from the deep well plate was not as efficient or repeatable* on a well-by-well basis as in previous experiments with 96-well VPs. Most of the error comes during the sample aspiration step and not during the transfer to the 96-well VP. The removal of 198 µL was not repeatable from well-to-well, as indicated by the cross-section image shown in **Figure 3**. During a few of the sample aspiration steps from individual columns of the deep well plate, it was noticed that some, but not all, pipette tips were occasionally pinned on the bottom of the plate. This pinning, along with the difficulty in fully aspirating out nearly-all of the sample liquid, resulted in many wells having more liquid than others. This observation was made visually as well as from the measurements on the MVS. For instance, the wells with *visually* more (red) liquid remaining in the wells after sample aspiration matched the wells that showed higher volume values with the MVS, data not shown). The very high CV value indicates that the amount of residual volume varied quite a bit from well-to-well within the deep well plate.

Conclusions

Measuring the residual volume after sample aspiration is a direct way for assessing the performance of a sample removal step. For instance, depending on the assay, very small (nL to low µL) amounts of residual volume remaining in the wells may correspond to a very good sample aspiration step. It may be up to the user to determine how much residual volume a particular assay can withstand through trial and error experiments.

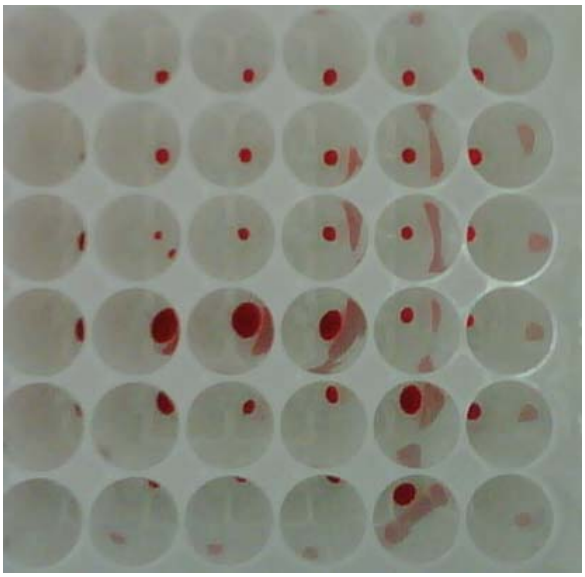

Figure 3. A photo of a 6 x 6 well cross-section of the deep well plate after the sample removal step. This image, taken from the top of the plate, shows that some wells have more liquid remaining than others.

If the goal is to aspirate all of the volume out of each well, the MVS is well suited for such low measurements. The concept and/or process of measuring residual volumes can be performed with the MVS for residual aqueous solutions for volumes as low as 0.1 µL (standard 96-well plate) or 0.03 µL (standard 384-well plate) or even as low as 0.01 µL (384-well low profile plate).

References

(1) Artel application note: “MVS Volume Verification Using Any Microtiter Plate or Small Volume Container, Such as a Tube or Vial”, Artel controlled document number: 12A5553A; http://www.artel-usa.com/resources/applications.aspx (accessed Jan 2011)

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