Artel MVS as a Tool For Measuring Liquid Mixing Efficacy in Microplates

Tanya Knaide, Rachel Parshley, Matt Wilkinson PhD MBA

Artel

ABSTRACT:
You wouldn't bake a cake without mixing the ingredients first, yet when important assays are being conducted the liquids dispensed into a microplate are often assumed to have mixed through simple diffusion or after only a cursory agitation of the plate.

For assays results to be truly trustworthy, every part of the methodology employed needs to be assessed for efficacy and repeatability. This application note describes how the ARTEL MVS® Multichannel Verification System can be used as a tool for measuring the efficacy of liquid mixing protocols.

In addition, the data presented herein highlights the importance of ensuring that effective mixing of the solutions dispensed into a microplate has occurred during every step of the assay procedure. This is particularly poignant as experiments conducted within the Artel laboratory show that diffusion-based mixing can take as long as 24 hours, and even a plate shaker set at 2200 RPM can take close to 10 minutes to achieve complete mixing if the shaker protocol has not been optimized.

INTRODUCTION:
Homogeneity of solutions in microplates plays a crucial role in assay effectiveness, yet is an area of assay method development and verification that is often overlooked. Without ensuring that solutions have been effectively mixed, it is possible for non-homogenous samples to be used for subsequent assay steps leading to aspiration of very concentrated or very dilute aliquots.[1]

In this study, the MVS was used to measure the absorbance of dye solutions in microplate wells at multiple time points. The methods discussed herein may be applied to any mixing method including diffusion, aspirate and dispense steps with liquid handling instruments or other mixing methods.

Complete mixing of two solutions following a wet dispense protocol is particularly difficult to achieve. In order to mimic this challenging mixing scenario, studies involving the wet dispensing of a small volume of sample into a much larger volume of Diluent were performed.

For each measurement set, the average, standard deviation and %CV were calculated for the absorbance values for the control and the test data sets. The control solution was pre-mixed and therefore should have displayed no change in absorbance, regardless of the number of mix cycles performed. Therefore, the %CV values for the control samples should remain constant. Conversely, the test samples typically exhibited large initial absorbance readings due to a concentrated area of sample solution in the well resulting from the wet dispense. The %CV is also high due to lack of homogeneity of the samples in the wells prior to mixing. As the mixing action achieves homogeneity of the samples and diluent in the wells, the %CV is reduced, ultimately becoming constant from measurement to measurement.

MATERIALS:

- Artel MVS Plate Reader
- MVS Sample Solutions: Range C and Range E
- MVS Diluent Solution

12A6128A

- 96 and 384-well microplates
- Electronic syringe
- Multichannel pipette and tips
- Automated plate shaker

PROCEDURE:
A pre-determined number of wells within a microplate were filled with a pre-mixed control solution comprised of Sample and Diluent Solutions. The absorbance of the control solution needed to fall within the readable range of the plate reader, but did not need to match the absorbance of the dispensed samples. A schematic of the pre-mixed control solution added to the wells is shown in Figure 1[2].

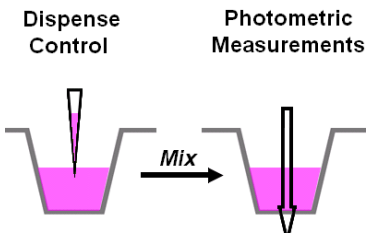

Figure 1. 55 µL of pre-mixed control solution was added to a portion of the microplates during testing.

For the diffusion studies, which will serve as a baseline comparison for the evaluation of the mixing procedure, a pre-mixed control solution of Sample and Diluent was dispensed into 48 wells of the 384-well microplates and 24 wells of the 96-well microplates studied. Next, 55 µL of Diluent was dispensed into the remaining wells before 0.2 µL of Range E sample solution was added.

Absorbance measurements were then taken every 5 minutes using the MVS and the coefficient of variation (%CV) calculated.

In the 2 µL test cases (both for non-optimized and optimized studies), 48 wells in the microplate were filled with the pre-mixed control solution of Diluent and Sample. The remaining wells in the plate were filled with 53 µL of Diluent using a handheld 8-tip 20-200 µL multichannel pipette. A 2-20 µL multichannel pipette was used to dispense Range C Sample Solution into the wells that contained Diluent. The absorbance of the plate was measured immediately to determine the initial, unmixed absorbance in each well of the plate.

In the non-optimized studies, a Big Bear plate shaker was used to agitate the plate at 2200 RPM for 1 minute. In the optimized studies, a Q. Instruments Bioshake 3000 shaker was used to agitate the plate at 2600 RPM.

Upon completion of the mixing cycle, the plate was immediately measured again to obtain the second set of absorbance values for each well. These steps were repeated until the %CV values remained unchanged.

The procedure for the optimized study was repeated for a sample volume of 0.2 µL using Range E Sample Solution. The sample was dispensed into 55 µL of Diluent solution using a 5 µL electronic syringe. While all of the remaining wells not containing the control solution did not additionally contain dispensed sample in this case, the plate was adequately filled to simulate the weight of a filled plate during the mixing process.

RESULTS & CONCLUSION:
The relative differences from measurement to measurement were used to determine the extent of mixing following each time period or mixing cycle. When the %CV values are unchanging for multiple mix-read cycles, mixing was deemed complete, regardless of the magnitude of the %CV value. High %CV values were attributed to dispense inconsistencies as a result of using the dispensers at their lowest settings.

The 96-well plate diffusion study data shown in Figures 2 and 3 indicate that it can take as long as 2 hours before complete mixing is observed if plates are exposed to minimal agitation. This time scale has been mirrored in studies using 384-well microplates. However, it should be noted that the movement of the microplate both to and from, and within the plate reader causes some liquid agitation. One-hit studies on individual plates have shown

that it can take as long as 24 hours before complete mixing occurs by diffusion alone.

As can be seen from Figures 4 and 5, when solutions are mixed with an automated plate shaker that has not been optimized for the plate format in question, it can take over 8 minutes of shaking before the solutions are homogenously mixed.

Figures 6-9 illustrate that effective mixing occurs in just 1 minute at 2600 RPM when the automated plate shaker program has been optimized.

If efforts to reduce assay result uncertainty are to be truly comprehensive, then it is clear that the microplate mixing protocol needs to be included as one of the key variables to be optimized during method development.

The procedure described for measuring mixing efficacy may be applied to virtually any method used for mixing solutions in microplates allowing for greater confidence in assay results.

Figures 2-3: Diffusion Studies

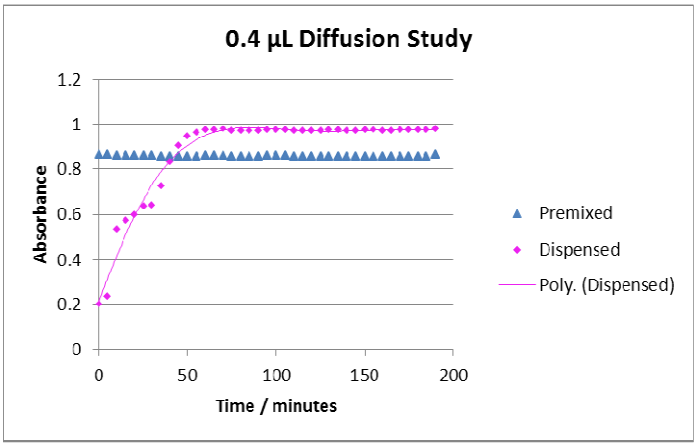

Figure 2. Diffusional mixing of 0.4 µL Range E solution into 200 µL of Diluent in a 96-well microplate takes more than 90 minutes before a homogenous mixture is achieved.

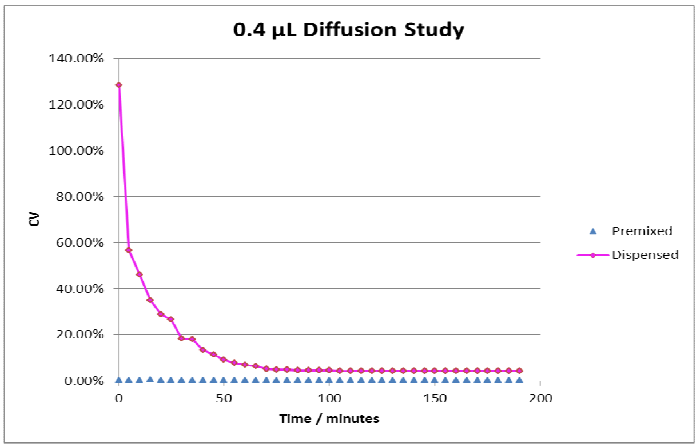

Figure 3. Diffusional mixing of 0.4 µL Range E solution into 200 µL of Diluent.

Figures 4-5: Unoptimized Mixing Tests

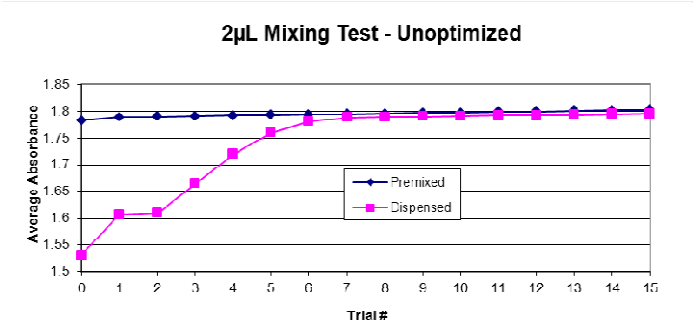

Figure 4. The unoptimized mixing protocol for a 384 well microplate at 2200 RPM takes around 8 minutes before consistent absorbance values are achieved.

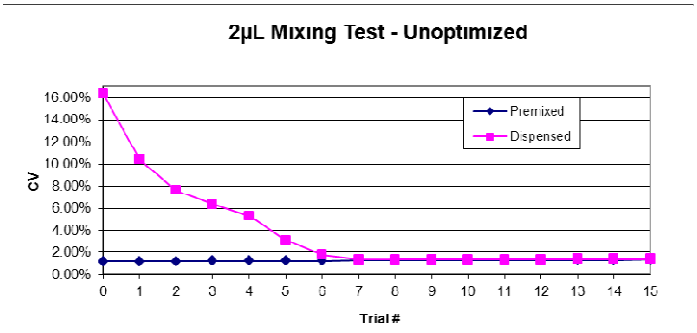

Figure **5**. The unoptimized mixing protocol at 2200 RPM takes around 8 minutes before consistent %CV values are reached.

Figures 6-9: Optimized Mixing Tests

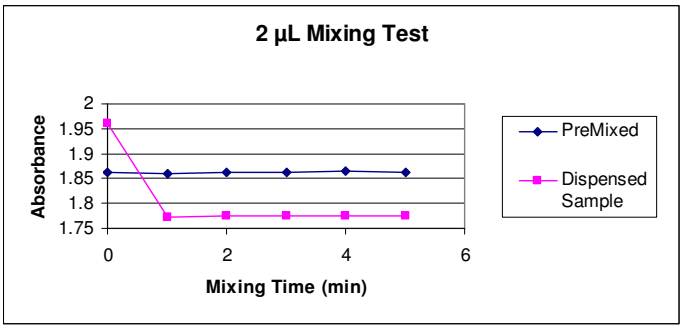

Figure 6. Mixing of the 2 µL sample in 53 µL of Diluent was achieved in 1 minute as illustrated by the consistent %CV values recorded after the initial, unmixed absorbance measurement. The mixing cycle duration was 60 seconds for each trial and speed was 2600 RPM. Control wells show consistent absorbance values for all measurements, as expected.

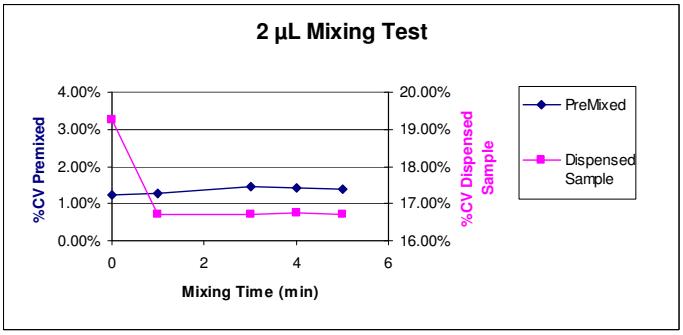

Figure 7. Control wells show consistent %CV values for all measurements, corresponding to the left y-axis. Test wells show consistent %CV values after 1 minute of mixing at 2600 RPM corresponding to the right y-axis.

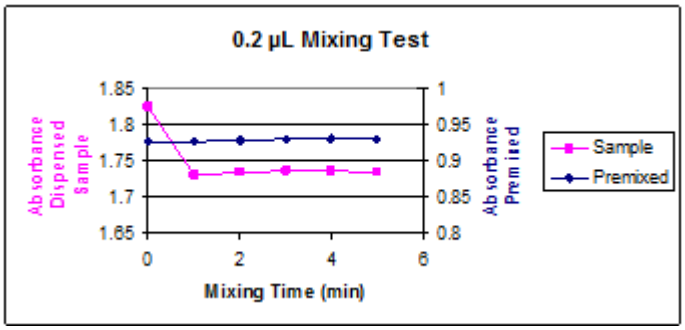

Figure 8. Mixing efficiency of the 0.2 µL sample is indicated after 1 minute of mixing at 2600 RPM by the consistent absorbance measurements after the initial measurement.

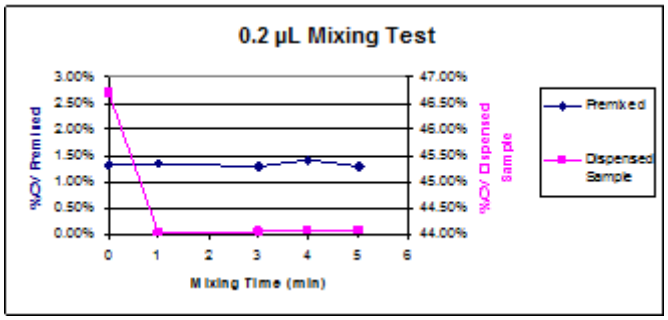

Figure 9. Control wells show consistent %CV values for all measurements, corresponding to the left y-axis. Test wells show consistent %CV values after 1 minute of mixing at 2600 RPM corresponding to the right y-axis.

REFERENCES:

1. Albert, K.J. and J. T. Bradshaw, "Importance of Integrating a Volume Verification Method for Liquid Handlers: Applications in Learning Performance Behavior," *J. Assoc. Lab. Autom.*, 2007, *12*, 172-180; the electronic file is located under the same title at: "Labautopedia." <http://www.labautopedia.com/mw/index.php/Importance_of_Integrating_a_Volume_Verification_Method_for_Liquid_Handlers>.
2. Spaulding, B.; Bradshaw, J.T. (Artel); Wente, W.; Borrmann, L. (Eppendorf), "Photometric Measurement of Mixing Efficiency Using the Eppendorf MixMate Mixer," in Artel's on-line Resource Library (assessed 30 April 2009). http://www.artel-usa.com/resources/default.aspx#Measuring_Mixing_Efficiency

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