Mixing Efficiency of Q-Instruments Bioshake 3000® Plate Shaker

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ABSTRACT:
The goals of the study were to assess the amount of time required for complete mixing in 384-well microplates with the Q-Instruments Bioshake 3000® plate shaker and to determine the feasibility of including this shaker with the Artel MVS® Multichannel Verification System. Mixing efficiency was measured using the MVS where absorbance and coefficient of variation (%CV) values were monitored for two dye solutions over multiple mixing steps. Mixing was deemed complete when the absorbance and %CV values leveled off, indicating a homogenous (mixed) solution in each well, as discussed in reference 2. A pre-mixed dye solution was used as a control in this analysis. The shaker time and speed required for proper mixing are 1 minute and 2600 RPM, respectively.

INTRODUCTION:
Mixing of solutions in 384-well microplates is a challenging, yet highly important task for many laboratories and assays. Effective mixing is of particular importance for volume verification using the MVS where two dyes must be completely mixed before photometric measurements are made. The absorbance values of the mixed solutions are subsequently used to calculate the sample volume dispensed from each tip of a liquid handler under test on a well-by-well basis in a microplate. For the photometric-based volume verification method used by the MVS, proper sample mixing is paramount and without it, the verification method may not be useful in monitoring a liquid handler's performance. For instance, without proper mixing the performance for the liquid handler under test could be misinterpreted, especially when dispensing into 384-well or higher density plate formats, where the mixing step becomes even more critical and challenging.[1] Therefore, an effective mixer must be used with the MVS to ensure that the two dyes are completely mixed in every well of the microplate before any absorbance measurements are collected and volume calculations are performed. The Q-Instruments Bioshake 3000 was tested for mixing effectiveness using the following procedure to determine feasibility for inclusion with the MVS.

MATERIALS:
- MVS Sample Solutions: Range C (2-µL testing) and Range E (0.1-µL testing)
- MVS Diluent Solution
- 384-well microplates (black with clear bottom – Corning 3711)
- Hamilton electronic syringe
- Multichannel pipette and tips
- Plate reader
- Bioshake 3000 with control software (Q-Instruments, Jena, Germany)

PROCEDURE:
The plate reader was used to collect absorbance readings for all filled wells at 520 and 730 nm. A pre-determined number of wells were filled with a pre-mixed control solution made up of the sample and diluent solutions. A schematic of the pre-mixed control solution being added to the wells is shown in **Figure 1**.[2] For the 2-µL testing, the premixed control was added to 48 wells and for the 0.1-µL testing, the pre-mixed control was dispensed into 20 wells of a 384-well plate.

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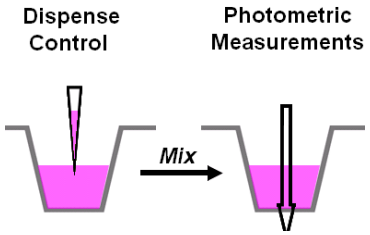

Figure 1. 55 µL of pre-mixed control solution was added to a portion of the 384-well plate during testing.

The remainder of the wells in the plate were filled with 53 µL of the MVS dye-based aqueous buffer solution (diluent) using a handheld 8-tip multichannel pipette. A different 8-tip multichannel pipette was used to dispense 2 µL of MVS sample solution into the wells containing diluent. The plate was read immediately to determine the initial, unmixed absorbance values for each well.

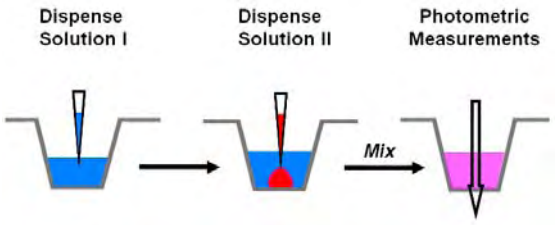

Figure 2. (left) 53 µL of diluent was added to wells of a 384-well plate; (middle) 2 µL of dye solution was added to the diluent before mixing and photometric measurements were collected.

After the initial reading, the plate was mixed with the Bioshake 3000 at 2600 RPM for 1 minute and immediately measured for absorbance values with the plate reader, as indicated by **Figure 2**. These steps were repeated a total of five times, which included five 1-minute mixing steps and six total plate reader measurements (not included is the time required for the plate reader to read the plate, which was approximately 1 minute per read). The entire procedure was repeated for a sample volume of 0.1 µL, which was dispensed into 55 µL diluent in a 384-well plate using a 5-µL barrel electronic syringe. Due to the "difficulty" in achieving complete mixing after a wet dispense, the most difficult mixing case scenario was simulated by using small dispensed volumes in the experiment.

For each absorbance measurement set, the average, standard deviation and %CV were calculated for the control data set as well as the test data set. Since the control solution is pre-mixed, neither the absorbance nor %CV should change from read-to-read. In other words, a properly mixed solution in each well should show absorbance and %CV values leveled off and not changing significantly with added time or more mixing steps. In contrast, the test wells typically display large absorbance values for the initial read due to a concentrated area of sample solution in the well, i.e., pockets of unmixed dye solution are present. The %CV of the test wells is also commonly high due to lack of homogeneity of the sample and diluent solutions. As the mixing action achieves the desired effect, the absorbance and %CV will level off and approach relative consistency in values similar to the control solution. However, it is important to note that the absorbance and %CV values for the two solutions (pre-mixed control vs. test solution) do not have to be identical to prove mixing efficiency as stated in reference 2.

RESULTS & CONCLUSION:

The **relative differences** from read-to-read are used to determine effectiveness of mixing. The degree of mixing is determined by the absorbance values *within* each well from read-to-read. When the value is unchanging over multiple trials, the solution is mixed. Therefore, regardless of the magnitude of %CV, the leveling off and consistency of the results are the points of interest. Consideration: *It should be pointed out that the high %CV's are due to volume transfer inconsistencies and not mixing inconsistencies.* In the use of the handheld pipette and syringe, they were both used at their lowest volume settings and consistent volume transfer was difficult to achieve (so some wells received more dye and others received less). To reiterate, the overall %CV's are quite high due to the varying amounts of dye solution being dispensed across the plate.

Figures 3 - 6 illustrate that the Bioshake 3000 effectively mixes two aqueous dyes in 1 minute.

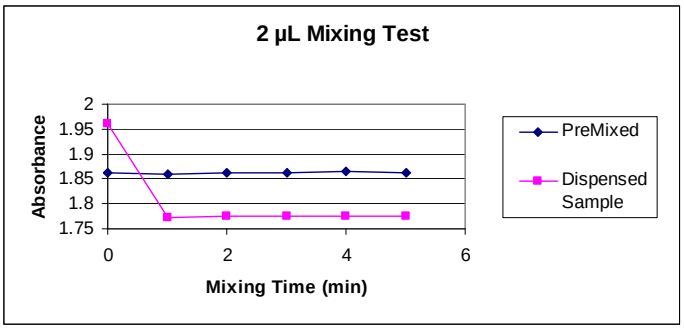

Figure 3. The 2-µL sample that was wet-dispensed into 53 µL diluent shows mixing efficiency as indicated by the consistent raw absorbance values after 1 minute of mixing. Mixing occurred within the first mixing cycle, where each mixing cycle was 60 seconds at 2600 RPM.

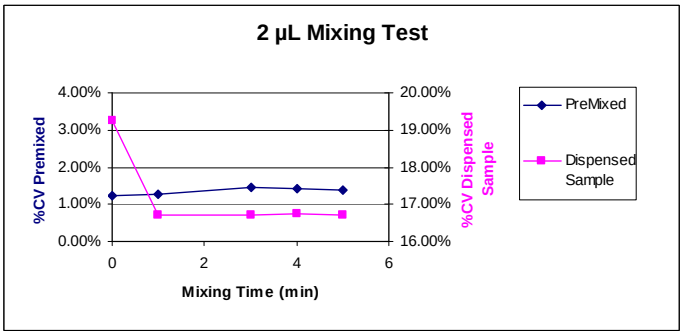

Figure 4. %CV of absorbance readings in all control wells and all test wells filled with the 2-µL sample solution.

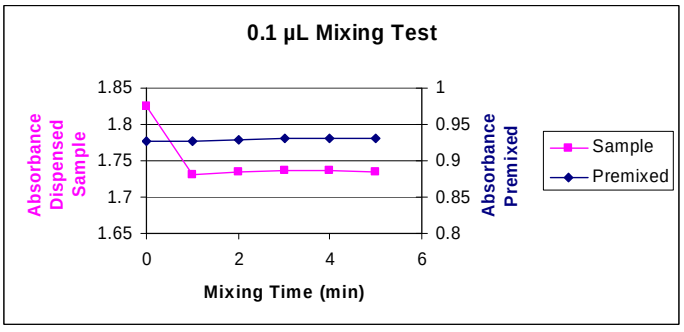

Figure 5. The 0.1-µL sample that was wet-dispensed into 55 µL diluent shows mixing efficiency as indicated by the consistent raw absorbance values after 1 minute of mixing. Mixing occurred in the first mixing cycle, where each mixing cycle was 60 seconds at 2600 RPM.

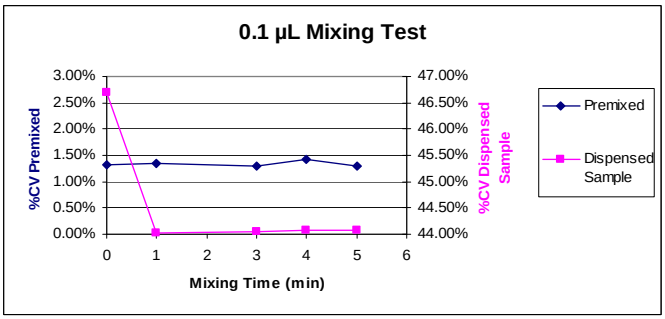

Figure 6. %CV of absorbance readings in all control wells and all test wells filled with the 0.1-µL sample solution.

The Bioshake 3000 plate shaker effectively mixes aqueous solutions in standard volume 384-well plates in 1 minute at 2600 RPM, has a small footprint and is software (rather than manually) controlled, meeting all requirements for use with the Artel MVS.

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

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