A METHOD TO EVALUATE MIXING EFFICIENCY IN 384-WELL MICROTITER PLATES

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OVERVIEW

Achieving optimal mixing results, especially in the 384-well format, can be an elusive goal. Mixing efficiency is frequently determined through visual observations. Our research indicates that these observations for effective mixing are too qualitative and subjective, which can lead to acceptance of non-optimally mixed sample solutions and false conclusions. Therefore, we have developed a quantitative method that allows a user to test a specific mixing device or protocol by using dual-dye photometry.

To test for optimal mixing, two solutions are dispensed into a microtiter plate, which is then subjected to a predetermined mixing program. A premixed control solution of the two solutions is used to account for potential non-mixing related factors. The average absorbance, standard deviation, and coefficient of variation (CV) of each solution, computed over multiple mixing trials, provide data regarding the efficiency of the protocol and mixer. We describe here this new measurement method in detail and present data that shows the characteristics of non-mixed, and optimally mixed solutions in 384-well microtiter plate formats.

PURPOSE

- To demonstrate that visual observations may be insufficient to judge mixing efficiency in microtiter plates.
- Inform personnel about a dual-dye photometry method that can be used to quantitatively measure mixing efficiency.
- Present the method and experimental results in a manner that would allow an operator to test a mixing device or protocol for efficiency in their laboratory environment.

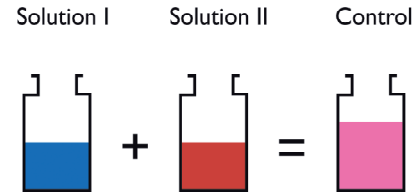
MATERIALS

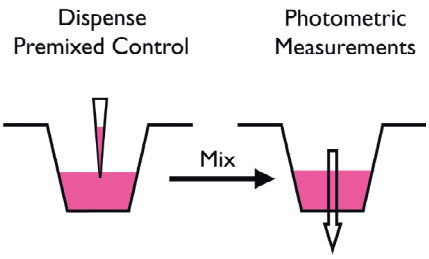
- Solution I (MVS® Diluent, ARTEL) contains a fixed, known concentration of blue dye.
- Solution II (MVS® Range C, ARTEL) contains the same concentration of blue dye as solution I, but also contains a known concentration of red dye.
- Plate reader to measure absorbance at 520 nm & 730 nm (Bio-Tek, ELx800nb).
- 384-well, flat bottom, non-treated black polystyrene assay plates (Corning, #3711).

METHOD

Control

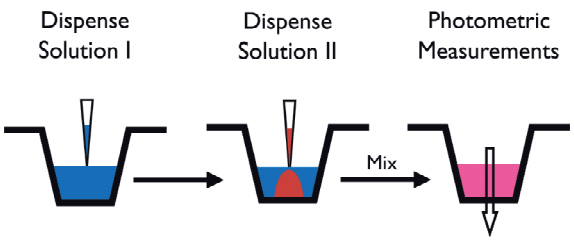
A set number of columns (or wells) are filled with 55 µL of a premixed control solution. This control solution, which is mixed before being dispensed into the test plate, consists of the two dyes in the same concentration as the test solutions. Since the blue dye is at the same concentration in both solutions, it is used as an internal standard.





Sample

- Wells under test are filled with 53 µL of solution I.
- Using a multichannel pipette, 2 µL of solution II is added to the wells containing 53 µL of solution I.



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- Remaining empty wells are filled with 55µL of solution I. This replicates the normal weight of the plate during the mixing process.

- The plate is read for initial/premixing absorbance values. This pre-mix read is referred to as Trial 0 in the Figures.

- After the initial reading, the plate is subjected to a predetermined mixing program performed by the mixing device under test. Once the program is complete, the plate is re-read. The process of mixing/reading is continued for a set number of trials.

- For each trial at both wavelengths, the average absorbance, standard deviation, and coefficient of variation (CV) for each test well and control well are computed. This analysis includes the internal standard, which acts as a control in both solutions. Thus the data provides information regarding the efficiency of the mixer and mixing program.

- Due to the fact that the control solution is already mixed before it is dispensed into the microtiter plate, the average absorbance and CV should start and remain approximately the same over the multiple mixing trials. The data generated from the control solution allows for the removal of the effects of any potential delivery artifacts (e.g. solution evaporation and splashing). Finally, the reader should note that by design the absorbance of the control and sample do not need to converge.

- The initial average absorbance and CV data for test wells filled with solution II will often produce inflated values (although it is possible to observe lower values) compared to post mixing data. These values are primarily due to an area of concentrated red dye of solution II in the center of the wells. After mixing, the measured parameters will shift to a new value. As the red dye is mixed and is evenly distributed, the absorbance changes and the CV decreases since CV is a measure of relative dispersion. If the two solutions are optimally mixed, further mixing trials will not produce absorbance values with significant variation beyond a certain point.

- In some cases, mixing parameters are not sufficient to optimally mix the solutions with a single trial. To the eye, the solutions may appear to be completely mixed. However, further mixing trials may provide quantitative evidence that this conclusion is false. As the additional mixing trials are completed, measured parameters will shift or trend towards a relatively stable plateau. Therefore, once the values are no longer changing (i.e. the plateau), optimal mixing has been achieved.

EXAMPLES

Incomplete Mixing

- Using the described method, a mixing device was tested for optimal mixing.

- The method was executed with an arbitrary protocol of 60 seconds at 3100 RPM.

FIGURE 1A

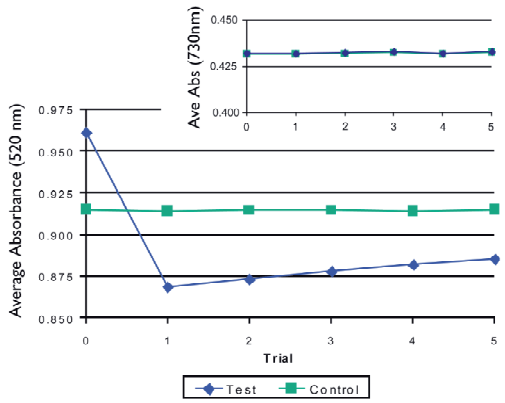
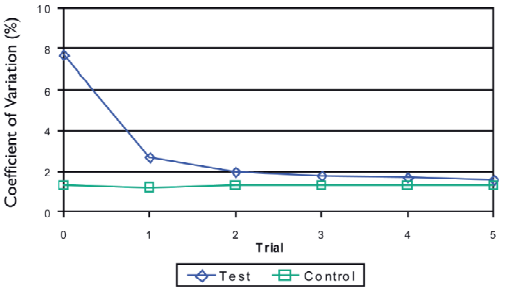


FIGURE 1B



- In Fig. 1A, the average absorbance for the test wells in Trials 1-5 increases after each mixing step. The values fail to reach a plateau. A different pattern is observed in Fig. 1B for the CV data. The CV continues to decrease from trial-to-trial. Although the two solutions are approaching an optimally mixed state, a plateau value was not reached during the five trials. This indicates that the solutions never reached a completely mixed state.

- The 730 nm data, shown as the inset in Fig. 1A, exhibits the expected pattern for both sample and control solutions (both data profiles are overlapped). The flat appearance of the average absorbance line indicates a successful delivery of the solutions to the microtiter plate. In addition, this pattern also indicates that no mixing artifacts were introduced into the test solutions (such as splashing, significant meniscus change, non-uniform removal of bubbles, etc.).

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Complete Mixing

- After reviewing the results for the first test, a second test was performed for the same mixing device. For this test, the mixing speed was increased to 3570 RPM.

FIGURE 2A

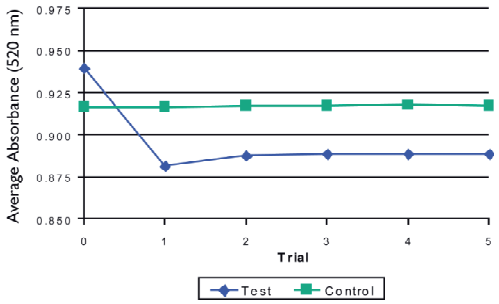
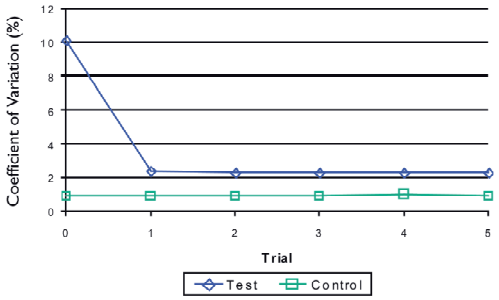


FIGURE 2B



- In Fig. 2A, the average absorbance reaches a relatively constant value after the second mixing trial. The control solution value starts and remains constant over the multiple trials. The plateau pattern is observed in Fig. 2B, for the CV. These patterns indicate that after Trial 2, the two solutions have been optimally mixed by the device/ mixing program under test.

- The corresponding 730 nm data (not shown) for both the sample and control produced a nonchanging response, as expected. This indicates that no delivery artifacts occurred during the test

MIXING EFFICIENCY

Eppendorf MixMate Vortex Shaker

- Testing the standard factory preset “384” soft key mixing protocol, which entails a 15 second mix at 2000 RPM.

- Control solution wells n = 48, test wells n = 64. Average plate weight ~ 88 g.

FIGURE 3A

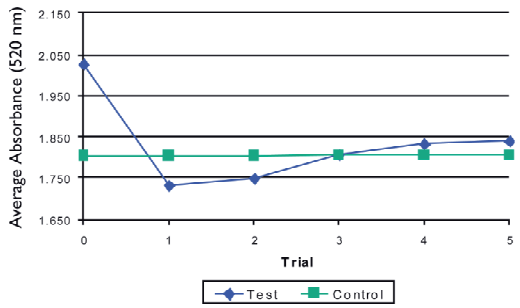
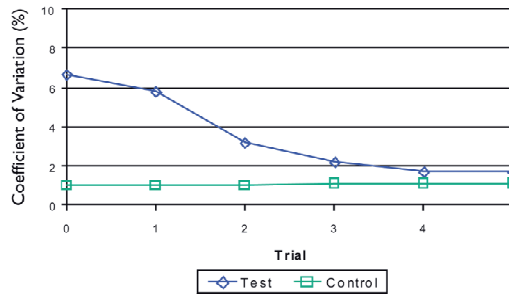


FIGURE 3B



- The Eppendorf MixMate Vortex Shaker does produce the plateau pattern when tested under the specified conditions (Fig. 3A, 3B).

- The MixMate visually appeared to produce optimally mixed wells after three trials. However, the photometric measurement tests concluded that a total of four mixing trials were needed to produce an optimally mixed solution.

- Therefore, tested under these specific conditions, the MixMate generated an optimally mixed solution after four mixing trials (60 seconds). Thus, the operator may now adjust the mixing parameters to 60 seconds at 2000 RPM to ensure optimal mixing during future protocols.

CONCLUSIONS

The method discussed in this poster provides a quantitative test to measure and evaluate mixing protocols. This method is an improvement over many commonly used methods that rely upon visual observations and/or high speed video images for making judgments about mixing efficiency. A control is implemented in this method to ensure that any non-mixing artifacts are accounted for during testing. Additionally, an internal standard (blue dye) is used in both the sample and control solutions to further account for issues that would affect the measurements. Examples include a change in pathlength from bubble removal, loss of liquid through splashing, and drastic meniscus changes. While not employed herein, the internal standard could potentially be used to correct for and remove these observed mixing artifacts from the analysis.

Although this approach was applied to 384-well plates, it should be equally applicable to both 96- and 1536-well microtiter plates. Furthermore, evaluation of onboard mixing protocols with automated liquid handlers and mixing efficiency for serial dilution based assays are testable with this method. In summary, without proper and effective mixing protocols, an assay or device could be falsely interpreted based on the experimental results. This method provides a quantitative test for these particular instances to help ensure the quality and integrity of the results.