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Poster Presentation

Performance Verification of BioTek's Precision 2000™ using Artel's Multichannel Volume Verification (MVV™) System

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Abstract

Biomolecular screening often entails repetitive pipetting of fluids into and out of 96- and 384-well microplates. The standardized matrix and spacing of the wells in microplates, 8 x 12 and 16 x 24, for 96- and 384-well microplates, respectively, has allowed the use automated systems to pick up and dispense liquids when these microplates are employed. The use of automation has allowed tremendous numbers of samples to be screened using microplates as the reaction vessel. Towards that end, BioTek® Instruments (Winooski, VT) has developed the Precision 2000™ Automated Pipetting System to eliminate the need for manual pipetting into these plate formats. Due to the absence of human intervention, the increased reliance on automation to perform repetitive pipetting tasks on large numbers of samples makes the need for pipetting accuracy even more important than it a;ready was. Artel has developed a system that photometrically measures liquid handler performance from 2-200 µl. This Multichannel Volume Verification (MVV™) System uses dimensionally characterized microplates and accuracy controlled dual-dye sample solutions to measure both the accuracy and precision of liquid dispensed into 96-well plates from multichannel liquid handler heads. Because of the small volumes associated with the wells of plates, both 96- and 384-well, the ability to dispense small volumes accurately and precisely is necessary to perform many assays. While the performance of the Precision 2000™ Automated Pipetting System has been specified at relatively large volumes such as 100 µl, its typical performance at much lower volumes is quite remarkable. Here we describe the process of using the MVV system to validate the Precision 2000 in a matter of minutes, as well as demonstrate the performance of the Precision 2000 Automated Pipetting System.

Introduction

The use of automation has allowed tremendous numbers of samples to be screened using microplates as the reaction vessel. Towards that end, BioTek Instruments has developed the Precision 2000™ Automated Pipetting System. The Precision 2000 has a completely configurable multi-station platform to hold the required pipette tips, reagent troughs, and microplates (96- and 384-well) for fluid transfer (Figure 1). The platform is removable, allowing for multi-user friendliness, easy cleaning, and setup of the instrument. The 8- or 12-channel pipette arm moves up and down as well as side to side, while the platform moves front to back to provide complete access to all locations on the work platform and complete configurability. The pipette arm uses a proprietary technology to reliably pick up and seal any standard tip with

individual, free-floating barrels that compensate for tips out of position (Figure 2). An optional bulk-dispense 8- or 12-channel manifold, which uses a precise bidirectional syringe pump to accurately and rapidly dispense fluids from a large unpressurized reservoir, is also available.

Precision Power™ PC software accompanies the Precision 2000 Automated Pipettor to provide complete programming flexibility. In addition to the external PC software, the Precision 2000 has a built-in microprocessor that can control all movements, which allows for program downloading. Once downloaded, the PC can be disconnected and the pipetting programs can be run via a keypad. The flexible onboard software, which provides complete programming for the most complex fluid transfers, can store up to 80 programmed assays. For more complete automation robotics, interfaces can be developed using ActiveX® software commands. The Precision 2000's small size, with a 15 x 21-inch footprint and a height of 16 inches, allows it to be used almost anywhere including most biological safety cabinets or chemical fume hoods. Also available is an optional aerosol cover for use on the laboratory bench.

Building upon their expertise in liquid delivery verification, Artel has developed a system (MVV™) that photometrically measures liquid handler performance from 2-200 µl. The MVV system uses dimensionally characterized microplates and accurately controlled concentrations of dual-dye sample solutions to measure both the accuracy and precision of liquid dispensed into 96-well plates from multichannel liquid handler heads. The MVV system uses two distinctly different dye solutions, which are very stable over time and traceable to national standards. The blue dye, with an absorbance peak at 730 nm, is used at the same concentration for all volumes to be tested. The red dye, with an absorbance peak of 520 nm, is used at different concentrations depending on the dispense volume to be tested. The microplates have truncated conical wells that are uniform and have been well characterized with regard to their geometry. Using the device to be tested, the "test" sample is pipetted into the microplate. Note that the sample contains both blue and red dyes. However, with very small test-volumes, additional diluent will be required in order to have a sufficient volume to adequately cover the bottom of the well. Because the diluent contains the same concentration of blue dye as is present in the test sample, the precise amount of diluent is not critical.

Once the test sample and any diluent necessary have been pipetted, the microplate's absorbance at 520 nm and 730 nm is measured. The first calculation performed determines the liquid depth or pathlength of each well. This calculation is based on the ratio of the absorbance of each well at 730 nm to an absorbance per unit pathlength constant that has been determined by Artel (Figure 3A). Once the depth of the liquid has been calculated, the total volume of liquid can be calculated for each well using the volume formula for a truncated cone (Figure 3B). The test sample volume that has been pipetted into the well is then calculated using the calculated total volume, the ratio of absorbance at 520 nm to 730 nm, as well as the ratio of the absorbance per unit of pathlength constants of both dyes (Figure 3C).

In order to calibrate microplate reader response (i.e., the absorbance values) to the test dyes of the MVV system, a calibrator plate that contains the same solutions as the sample and diluent solutions in sealed precision cuvettes is placed into the microplate reader and the absorbance at 520 nm and 730 nm determined. Each calibrator plate is bar-coded to specifically identify the factory-determined absorbance properties of the calibrator plate to the MVV software. This establishes a direct correlation between the factory reference spectrophotometer and the microplate reader. With the reader calibrated and the test plate pipetted, the absorbance of the test plate is measured and the performance of the instrument (e.g., Precision 2000) determined in a matter of minutes.

Materials and Methods

Using the Multichannel Volume Verification (MVV™) system from Artel, the dispense accuracy and precision of the Precision 2000™ Automated Pipetting System was tested. For each indicated volume, microplate wells were filled with the indicated volume of dye using a Precision 2000 Automated pipettor. The absorbance at 520 and 730 nm was determined using an ELx800NB Microplate Absorbance Reader (BioTek® Instruments, Winooski, Vermont) according to the MVV instructions. Dispense volumes and precisions were calculated using MVV software (Artel® Westbrook, Maine). The calculated accuracy and precision data were then exported to Microsoft Excel for the purposes of graphing. For single dye experiments, a concentrated blue dye solution was dispensed into clean dry plates and colorless TRIS buffer diluent was added using the Precision 2000 (BioTek Instruments, Inc.). The resulting absorbance was then read at 595 nm using a microplate absorbance reader. Volume data were then exported to Microsoft® Excel for analysis.

Results

As demonstrated in Figure 4, the Precision 2000 is very accurate over the range of volumes tested using the MVV system from Artel. The specification for accuracy with the Precision 2000 is an error of less than 2.0% at 100 µl. In these experiments the percent error at all volumes tested was well below specification, and at volumes greater than 50 µl the error was less than 0.5%. In addition to being accurate, the Precision 2000 was found to be precise. Despite having 8 or 12 independent pipette channels that work in unison, there was very little channel-to-channel variability. Figure 5 demonstrates the dispense-precision of the Precision 2000. As an entire plate, the coefficient of variance (%CV) was found to be less than 2.0% at volumes down to 5 µl. When a direct comparison between calculated volume and expected volume is plotted, the resultant linear regression shows a very high degree of correlation, with a correlation coefficient (r^2) of 0.9999 (Figure 6). In addition, these data correlate well with precision data generated using a one-dye test (Figure 7).

Conclusions

Reliance on automation to perform repetitive pipetting tasks on large numbers of samples makes pipetting accuracy and precision even more important than they already were. These data indicate that the Precision 2000 can be used to dispense aqueous solutions precisely and accurately. Using either a one-dye test procedure, which tests precision, or the two-dye MVV system, which tests both accuracy and precision, the Precision 2000 performed admirably. Several factors influence the ability to dispense small volumes with the Precision 2000. The positive displacement syringes depend on the displacement of air to push the fluid out of the barrel of the tip. Unfortunately, gaseous materials are prone to compression when the gas volume is large relative to the fluid volume to be dispensed (e.g., 100 µl). This often precludes the ability to be accurate at distinctly smaller volumes. Because air is also prone to expansion and contraction as a result of temperature changes, having samples and reagents equilibrated to ambient temperature will result in more accurate and precise dispensing as well. For more accurate and precise dispenses, it is advisable to include an initial pre-pickup of a small volume. This will provide some compensation for air gas compression, as well as provide a humid environment inside the pipette tip. Additional steps can be taken to ensure accurate dispensing as well. If multiple dispenses of small volumes are needed, it is faster and more accurate to aspirate a large volume and perform multiple dispenses from the same pipette tip. However, in many instances the reuse of tips is precluded by cross-contamination concerns. In the case of dispensing into dry microplates, it is advisable to perform a bottom touch-off, which will assist in transferring the remaining droplet caused by the surface tension of the liquid from the pipette tip to the well. Contacting the liquid to the well bottom will caused the droplet to adhere to the well rather than remain with the tip.

Summary:

- The Precision 2000™ is an accurate and precise automated multichannel pipettor.
- The MVV™ System is a fast and reliable method to test the calibration of multichannel pipetting devices.
- The MVV™ System can test both accuracy and precision at the same time in a matter of minutes.

Figure 1. Precision™ 2000 Automated Pipettor with Rapid-Dispense Manifold and Aerosol Cover.

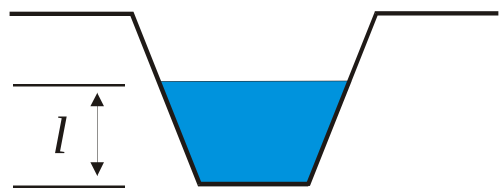
Figure 2. Closeup of Pipette head of an 8-channel Precision™ 2000 Automated Pipettor.

Calculate depth of liquid in each well

Based on the absorbance at 730 nm

Independent of the ratio of sample to diluent

a_b = absorbance per unit pathlength of blue dye in both solutions

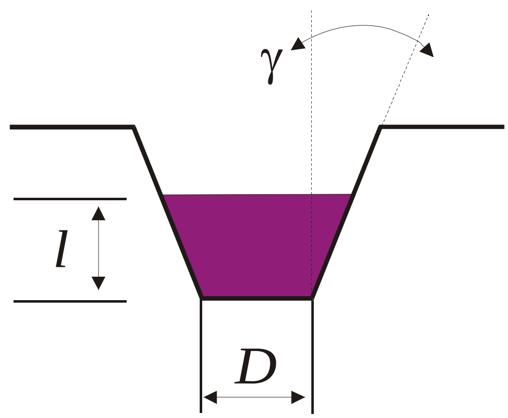


$$l = \frac{A_{730}}{a_b}$$

Figure 3A. Liquid Depth Calculation.

Volume calculation is based on a truncated cone

Total volume calculated from liquid depth and known dimensions



$$V_T = \pi l \frac{D^2}{4} + \pi D l^2 \frac{\tan(\gamma)}{2} + \pi l^3 \frac{\tan(\gamma)}{3}$$

Figure 3B. Liquid Volume Calculation.

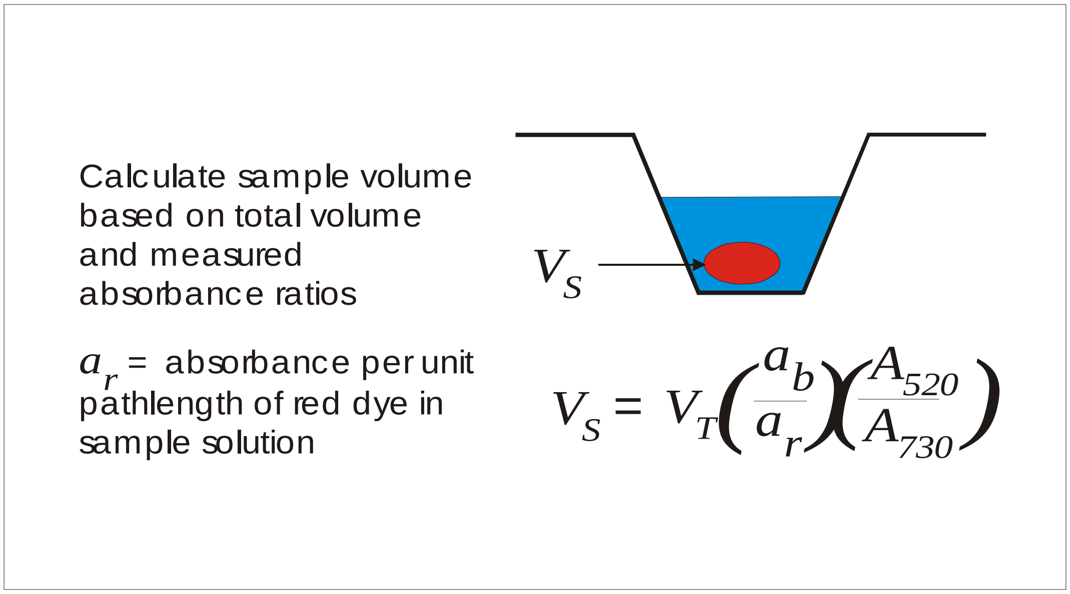


Figure 3C. Sample Dispense Volume Calculation.

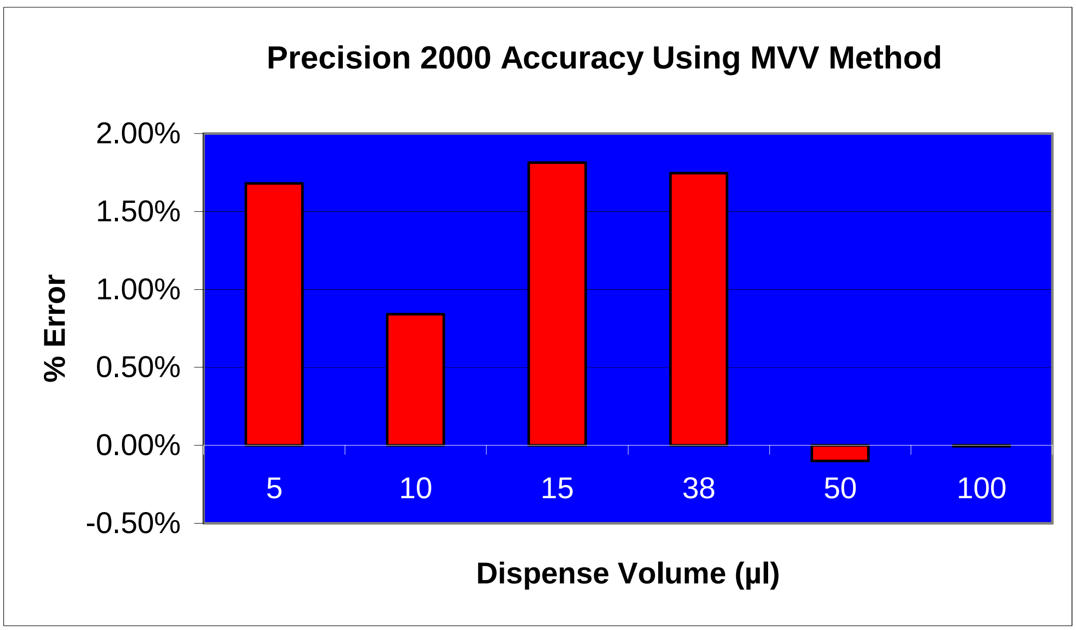


Figure 4. Accuracy of the Precision 2000™ Automated Pipettor. Using the MVV™ System, the volume of liquid dispensed by the Precision 2000 was determined for several different volumes. The percent error of the calculated mean at each volume was then plotted.

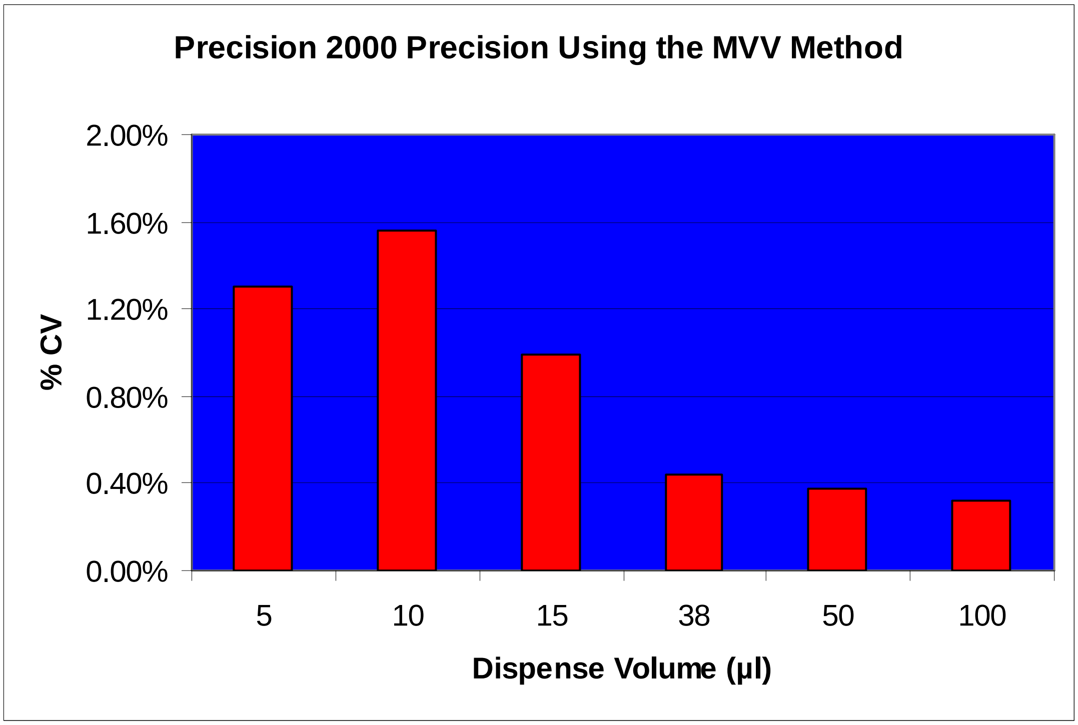


Figure 5. Dispense precision of the Precision 2000™ Automated Pipettor. Using the data depicted in Figure 4, the coefficient of variance (%CV) of the data at each volume was calculated and plotted.

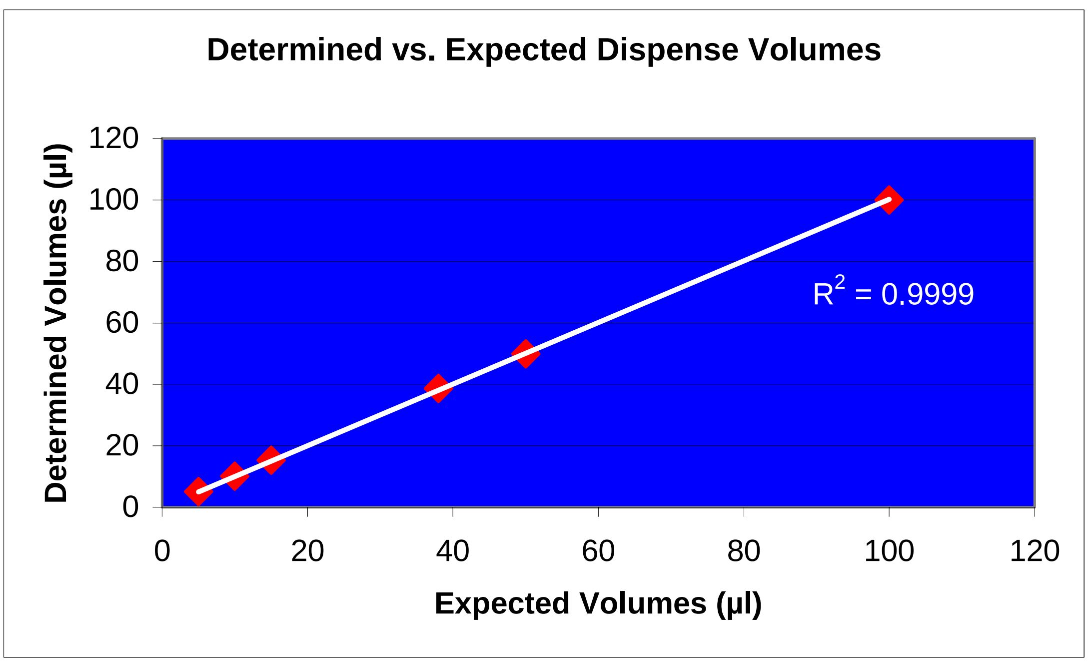


Figure 6. Comparison between expected and determined dispense-volumes. Using the MVV™ System (Artel®, Westbrook, ME) the volume of liquid dispensed by the Precision 2000™ (BioTek® Instruments, Winooski, VT) was determined for several different programmed volumes. The calculated volume was then plotted against the expected volume and a linear regression analysis performed. The resultant correlation coefficient of the regression is indicated.

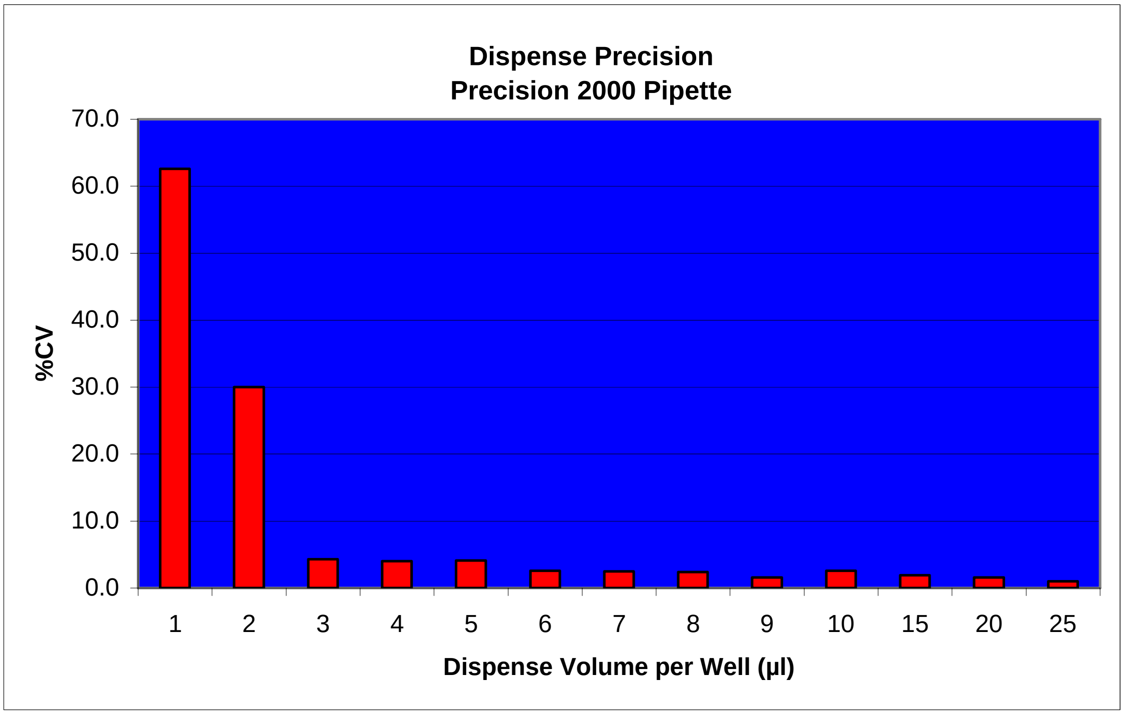


Figure 7. Dispense precision into dry 96-well plates using the Precision 2000™ pipette at various dispense volumes. The indicated volume of TRIS buffer containing blue food coloring was dispensed to all the wells of a 96-well plate using the pipette. After dispensing the colored dye, 100 µl of TRIS buffer was dispensed into each well using the dispense manifold at a speed setting of 5. The absorbance at 595 nm for each well of every plate was determined using a microplate absorbance reader. Note that each data bar represents the %CV of an entire plate.

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