# Pipetting Characteristics of Serum and Serum-like Solutions versus Water

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## Introduction

Many types of chemical and biological analyses, which are core to bio-pharmaceutical laboratories, are based upon analytical techniques involving accurate delivery of liquid components. A common tool developed to deliver these liquid components is the handheld micropipette, as well as automated pipettors which range in complexity from single-tip to upwards of 384-tips. These pipetting tools have become commonplace especially in many biological and pharmaceutical laboratories. Modern liquid delivery tools have been engineered to deliver liquid volumes with an increasing degree of accuracy and precision, which is required to attain the analytical results of the tests with which they are tasked. While these tools are familiar to many, their operational differences when pipetting different types of solutions are often over-looked aspects that relate directly to the accuracy and reproducibility of pipetting performance. For example, it is commonly assumed that water pipettes differently than serum when using air-displacement pipetting systems. While this is somewhat understood to be true, the pipetting performance of liquid delivery devices is often checked using only water as a reference solution. Presumably the idea is that if a pipetting device is calibrated for water performance, and checked periodically using water, that at least it can be shown that the pipetting device was behaving properly with a known solution. What this type of approach does not show is what type of bias might exist for real test solutions compared to the water test standard.

This presentation will address the difference in performance of a handheld micropipette when dispensing water versus various types of animal and human serum, as well as a serum-like dye solution. These differences are quantifiable and can be accounted for through careful experimentation and attention to physical pipetting details. Specifics on achieving ideal performance when pipetting serum versus water are discussed, along with the validity of water-only calibration methods.

## Experimental

### Pipetting Protocol:

When serum solutions are dispensed with a pipetting device, the amount delivered is dependent on various details of the aspiration and dispense protocol. The purpose of this testing was to identify the differences in behavior between water and various types of sera. Hence it was critical to establish a fixed aspirate/dispense protocol to ensure that observed differences were attributed to real differences in rheological properties, and not due to differences in technique.

Atmospheric conditions, especially temperature play a critical role in pipetting. Temperature control was achieved by performing all pipetting experiments in a temperature and humidity controlled laboratory. All equipment and reagents were allowed to thermally equilibrate for a minimum of 2 hours.

A new tip was placed on the pipette and pre-wet ted by immersing the tip into the sample solution, aspirating, removing the tip completely from solution and dispensing back into the reservoir, repeated three times. Completely removing the tip from the solution during the dispense step is critical to relieve pressure inside the tip. If not, the first dispense from the tip will be lower than subsequent dispenses. Aspiration occurred by carefully inserting the tip into the sample solution to a depth of 2-3 mm, followed by a rapid, even movement of the plunger. After aspiration, the tip was left in solution for a 1 second pause. This pause is critical for full aspiration. The aspirated solution was then dispensed into a glass vial sitting on a 6-place balance. Dispense occurred by gently touching the sidewall of a receiving glass vial at a 10-20˚ angle, approximately 5-10 mm above the vial bottom, or the liquid level in the vial. Delivery occurred using a rapid, even movement of the plunger to the first stop. Following this dispense, the pipette was moved away from the side-wall and held in place for five (5) seconds to allow remaining solution to pool at the tip bottom. After the 5 second pause, the plunger was moved to the second stop, delivering the remaining sample and blow-out volume (using the same tip-touch procedure). After the full volume transfer, the tip was removed and the delivered volume was measured by the balance. This process was repeated for a total of ten (10) deliveries using the same tip, at which point the tip was discarded.

## Results

Figure 1: Target Volume = 200 µL

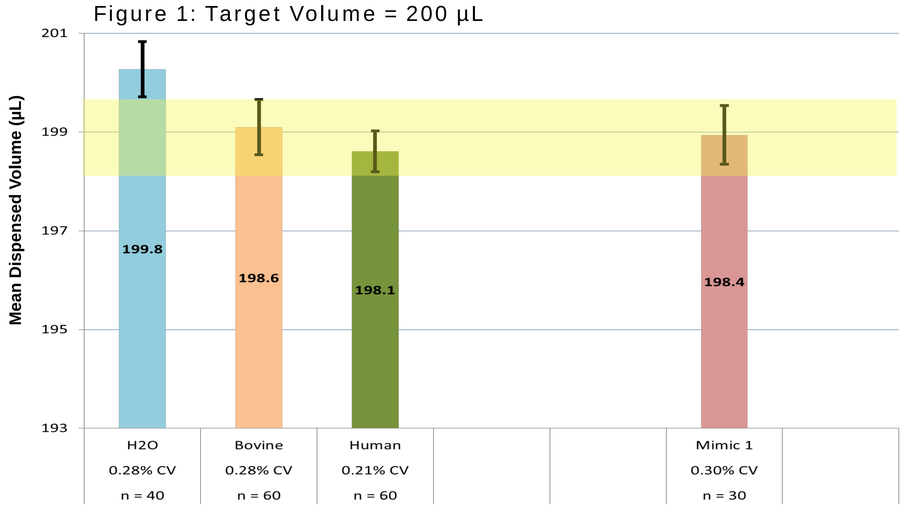



Figure 2: Target Volume = 50 µL

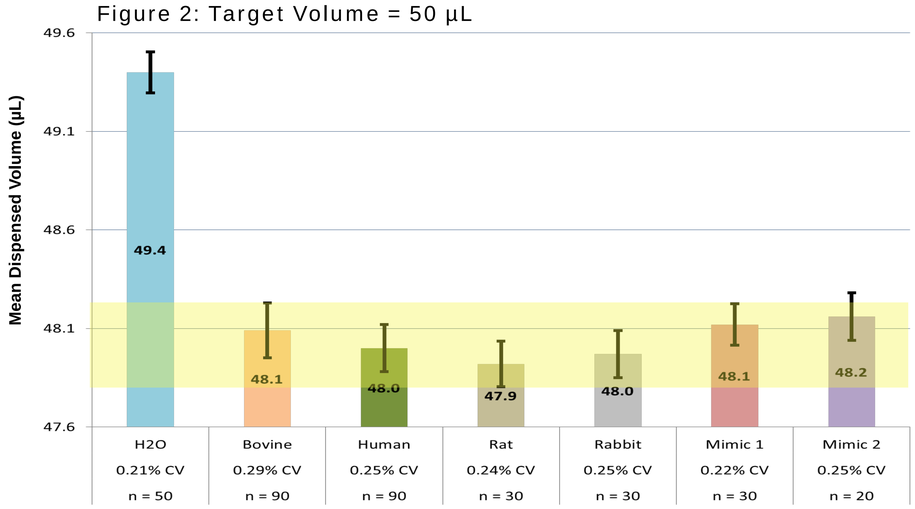



Figure 3: Target Volume = 10 µL

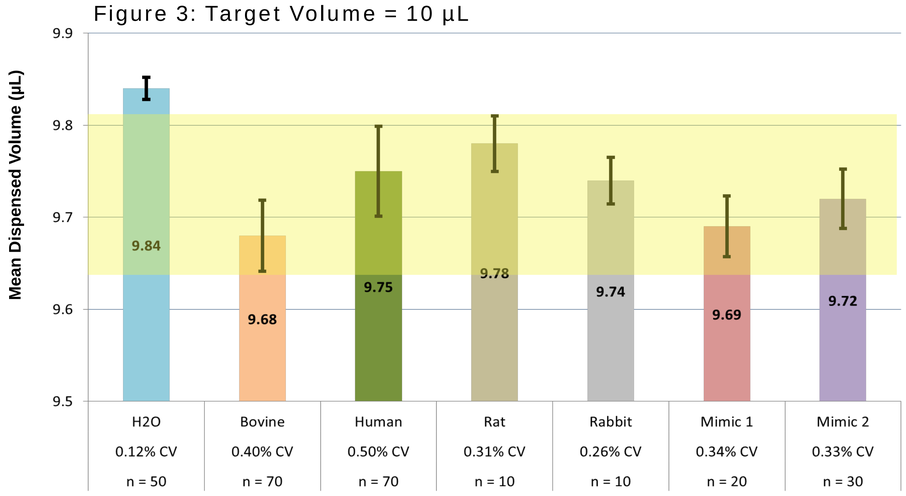
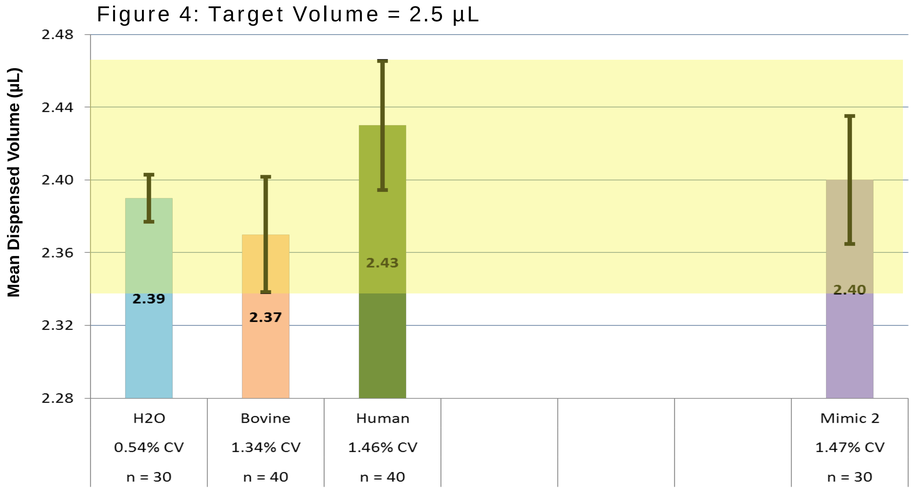



Figure 4: Target Volume = 2.5 µL




### Materials:

1. Deionized water (> 10 MΩ·cm) was generated by an in-house reverse osmosis system. This water meets ISO 3696 (Grade 1) and ASTM D1193-91 (Type II) standards.
2. Fetal Bovine Serum (FBS) from two different suppliers was used in these pipetting studies: 1) Catalog number SFBU30-2524 from Equitech-Bio (Kerrville, TX), and 2) Catalog number F1051 from Sigma-Aldrich (Allentown, PA).
3. Human Serum also came from two different suppliers: 1) Catalog number SHS33-266 from Equitech-Bio, and 2) Catalog number S7023 from Sigma-Aldrich.
4. Rat Serum (R9759) and Rabbit Serum (R9133) were both purchased from Sigma-Aldrich.
5. Mimic 1 and Mimic 2 were manufactured from various aqueous components, and included red and blue dyes in similar concentrations as found in Artel MVS Range A and Range B Sample solutions. The goal of testing the mimics was to determine if they behave sufficiently like serum when pipetted using the protocols defined above.

### Equipment:

1. 6-place microbalance (MT5, Mettler-Toledo, Columbus, OH), using a custom evaporation trap.
2. Programmable single channel electronic pipette (0.5 - 10 µL Research Pro, Eppendorf, Hauppague, NY).
3. Programmable single channel electronic pipette (5 - 100 µL Research Pro, Eppendorf).
4. Programmable single channel electronic pipette (10 - 300 µL eLine 300, Biohit, Bohemia, NY).
5. Polypropylene pipette tips, specified by the manufacturer for each pipette.

## Discussion

Figures 1-4 above demonstrate the pipetting performance of the various types of sera, DI water, and serum mimics previously described. The pipetting protocol used for this study was the culmination of many different iterations of a base protocol. Early testing with a manual handheld pipette resulted in too much variability caused by the inherent variability of the manual device. We found it necessary to use electrically driven pipettes that allowed for programming the plunger motion, and surprisingly found the fastest settings to give the best results.

Other key points to the test protocol were the delays during the aspiration and dispense steps. The pause during aspiration was needed to completely dissipate the applied force and allow for equilibration of the internal and external pressures. Removing the pipette before the 1 second pause results in a variable under aspiration. A surprising find was that a longer pause during aspiration was not necessary. The pause during the dispense routine was needed to allow the “viscous” serum to run to the bottom of the tip. Early experiments applied a slow plunger dispense. However, best results were achieved using a fast, even plunger force to the first stop, followed by a delay. This delay allowed the solution coating the inside pipette wall enough time to pool at the tip bottom. Not using this long delay resulted in variability due to an uncontrolled under-delivery.

The pipetting protocol developed herein resulted in nicely controlled deliveries at volumes of 10, 50 and 200 µL. The maximum coefficient of variation (CV = StDev/Mean) for all samples tested at these three volumes was 0.50%. By way of comparison, the test results at 2.5 µL show significantly more variability, with water showing a CV of 0.54%. While larger CVs are to be expected at this lower test volume, the magnitude of the difference, especially for the sera would indicate that this pipetting protocol is not sufficient for this volume range.

The overlying yellow band on each graph indicates the span between the largest positive error bar and the largest negative error bar of all the serum samples. This yellow region provides a visual indication of the difference in dispensed volume of serum versus DI water using a precisely controlled dispense protocol. With exception to the 2.5 µL results, the data above demonstrate a clear difference between water, the various sera samples, and the serum mimics. The delivered volume of DI water was larger than for any of the serum samples, which demonstrates the different rheological properties of serum versus water. Interestingly, the 50 and 200 µL test volumes resulted in a 1.39 µL offset between the average water delivery, and the average serum delivery. This trend did not hold for the 10 µL test point.

Another important observation at all 4 test volumes is that the mimic samples behave considerably like the various sera. While the 2.5 µL data raises questions about the propriety of the pipetting protocol, it still seems to indicate that Mimic 2 continues to behave more serum-like than water-like.

## Conclusions

Unsurprisingly, DI water pipettes differently than serum samples. However, various types of sera seem to fall within a definable range of performance. This may indicate that establishing one set of aspirate/dispense parameters for a general “serum liquid class” will result in reliable delivery of multiple types of serum.

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