Improving Automated Liquid Handling Performance for Serum Based Assays

Michael Grimaldi
Rachel Parshley
Heather Macken
Nathaniel Hentz, PhD.
John Thomas Bradshaw, PhD.

Introduction

Serum is a complex fluid component extracted from blood and is used in a variety of clinical diagnostic tests as well as biomedical research applications. The rheological properties of serum differ from ideal aqueous solutions, yet there is a need for accurate and precise assay results that doctors and scientists depend on to make critical decisions. Liquid handling precision and accuracy both directly impact assay results for both large-, and small-scale operations. Automated liquid handlers (ALH) often employ liquid classes to differentiate different fluid types. Determining the optimal liquid class (LC) for this complex solution can be an arduous process made only more difficult by the fluid handling properties of the solutions themselves. In many cases, optimizing liquid class settings with the actual assay solutions are problematic due to prohibitive cost, rarity, or complexity. For these types of high value assay solutions, users often create an analog solution that hopefully has the same liquid handling characteristics as the sample it is supposed to mimic.

Artel is developing a serum mimic solution with liquid handling properties that are analogous to several commonly used sera. This mimic will provide a serum-like reference solution that can be used to assist in liquid class optimization, volume verification, and calibration of automated liquid handlers. This solution will be the latest in the Artel MVS product line and will be specifically engineered to reduce cost and time requirements in addition to generating the most accurate and reliable results for assays that contain serum. In this study we show the similarities of serum mimic solution compared to various commercially available sera and the importance of choosing the appropriate liquid classes.

Methods & Materials

Equivalency Tests & Liquid Class set up

Experimental Set-up:

Normal sera from several species were selected and purchased from listed suppliers (Table 1).

Liquid class settings were established for an Eppendorf epMotion 5073 ALH using Artel's MVS serum mimic solution prior to data collection. Three pipetting tools were used with appropriate filtered pipet tips (Table 2).

Data were collected with solutions at both room temperature (RT) and chilled on-ice (Cold). Cold data not shown.

Table 1. Sera used for equivalency testing.

Serum	Vendor	Cat#
Horse Serum	Gibco	16050
Rabbit Serum	Gibco	16120
Human Serum	Innovative Research	IPLA-SER-AHBS
Rat Serum	Millipore Sigma	S24-100mL

Table 2. epMotion tools used for testing.

Tool	Volume Range	Experimental volumes
TM300	20 – 300 µL	200 µL, 100 µL
TM50	1 – 50 µL	50 µL, 25 µL, 10 µL
TM10	0.2 – 10 µL	5 µL, 2.5 µL

Protocol – Liquid Class optimization:

1. Collect initial measurements using default serum LC with MVS.
2. Repeat process making two or less adjustments until desired CV (%) and relative inaccuracy (%) are achieved.

Protocol – Equivalency Testing:

1. Use predetermined liquid class settings for serum A and serum B.
2. Place empty 96-well Verification Plate on microanalytical balance.
3. Tare balance with empty plate.
4. Move empty plate to deck of ALH and run liquid transfer protocol. (1 dispense = 8 wells filled).
5. Move filled plate back to balance and record weight.
6. Repeat steps 1-5 with each solution at each target volume.

Note: all results are averages n=3 with error bars representing 95%CI

Gravimetric and MVS Method Comparison

Experimental Set-up:

Serum B was measured at three different volumes (10, 25 and 49.9 µL) with the serum B LC using both the gravimetric method, and the MVS method.

All data was collected in environmentally controlled laboratory in accordance with ISO17025.

Protocol – Method Comparison:

1. Tare empty verification plate on balance.
2. Move empty plate to deck of ALH and run liquid transfer protocol.
3. Return filled plate back to the balance and record weight.
4. Remove plate from balance and fill remaining volume left with Diluent.
5. Read on MVS.

Note: all results are averages n=3

Viscosity Measurement

The rheological properties of serum mimic solutions and other sera samples(human and horse) were tested offsite using Brookfield DV- III Ultra Sample Volume Adapter at a temperature of 5°C (all results are averages n=3). Test temperature was chosen because viscosity increases at lower temperatures.

Results

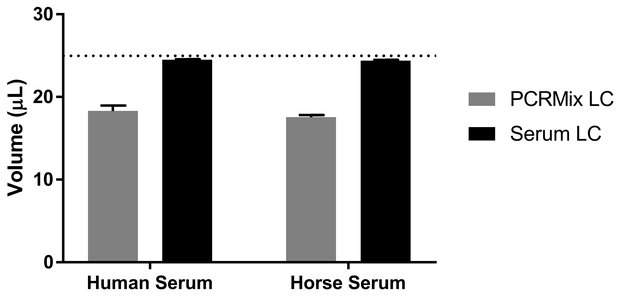

***Figure 1**. Mean volume (µL) measurements of serum B, human serum, horse serum with two different liquid classes. 25 µL target volume represented by dotted line. PCRMix LC are represented by grey bars and serum LC are represented by black bars. Tests were completed at room temperature. (n=3 for each measurement).*

Results-cont.

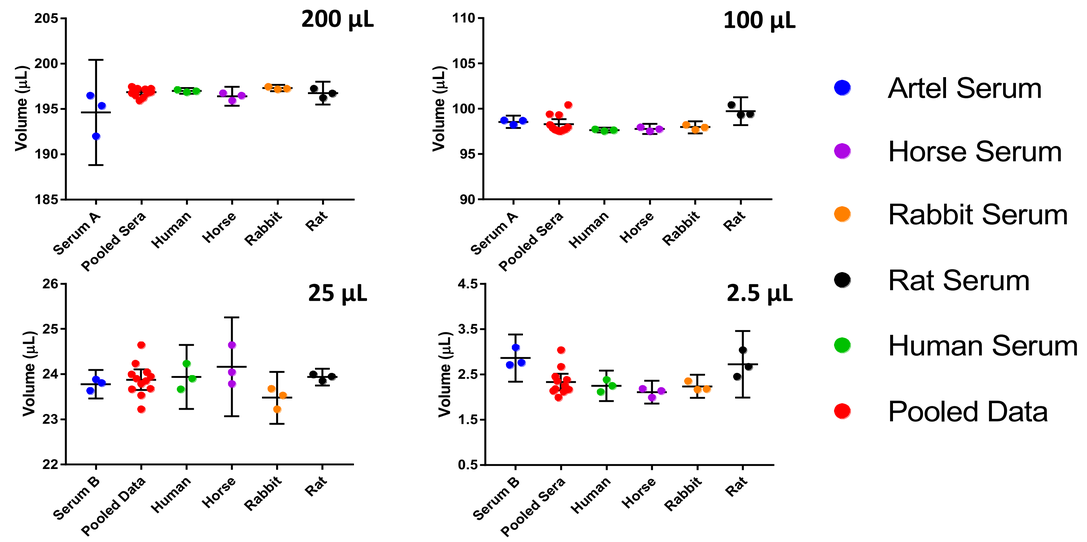

***Figure 2**. Measured mean volume (µL) for each sera test solution; human, horse, rabbit, and rat compared to Artel serum A/B at four target volumes; 2.5 µL, 25 µL, 100 µL and 200 µL collected at room temperature (RT). 200 µL and 100 µL data were collected using the TM300, 25 µL data were collected with the TM50 and the 2.5 µL data were collected with TM10. Pooled data displays data points from all four sera solutions. Error bars represent 95%CI (n=3 for each measurement).*

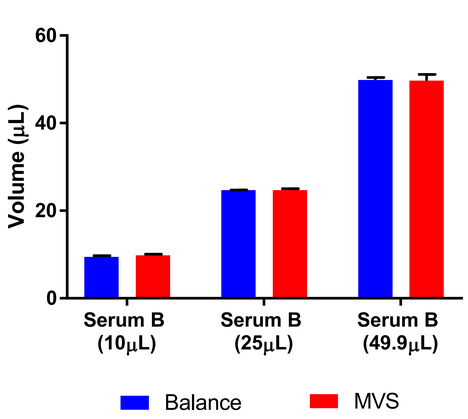

***Figure 3**. Mean volume (µL) comparison at 10, 25 and 49.9 µL between MVS (red bar) and gravimetry (blue bar). The same plate was used for both MVS and gravimetric measurements. Tests were completed at room temperature. (n=3 for each measurement).*

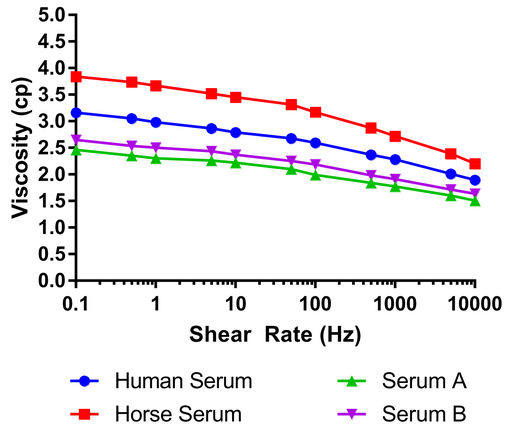

***Figure 4**. Viscosity as a function of shear rate. Human serum, horse serum, serum A and serum B viscosity comparison. All solutions were tested at 5°C (n=3 for each measurement).*

Discussion

To show the importance of liquid class selection we tested human serum, and horse serum using two different liquid classes (Figure 1). We reveal that using a liquid class similar to serum but not optimized for serum shows an inaccuracy (>25%) and with less precision when compared to a liquid class optimized uniquely for serum. Both sera pipetted more accurately (≤3%) and precisely (≤3%) with a liquid class specifically tailored for their unique liquid handling properties.

Equivalency testing was performed gravimetrically to show the similar pipetting behaviors between serum mimic and four commercially available normal serum products (Figure 2). From the data above we show at volumes ranged from 2.5 to 200 µL that the serum mimic consistently pipets analogous to a variety of sera. We see similar trends at other volumes within that 2.5 to 200 µL volume range (all data not shown). In addition, we repeated these gravimetric tests with cold samples and found results with similar accuracy (≤5%) and precision (≤5%).

Comparing MVS results to our gravimetric results show nearly identical outcomes across 10, 25 and 49.9 µL test volumes (Figure 3). The standard deviation is slightly larger for the MVS results due to the well-to-well detail we gain with the MVS, but we lose when using the gravimetric method. The MVS also reduces time for optimization and consumables used.

We appreciate the fact that all sera are not created equal, and to make sure we are developing a solution that is truly comparable to sera we compared viscosity of the serum mimic solutions to human and horse sera (Figure 4). We show that both serum mimic solution viscosities track very close to human and horse sera at lower temperatures where solution viscosity increases.

Conclusions

1. Identifying and adjusting liquid class settings with like-solutions is essential for accurate and precise liquid handling results.
2. Artel serum mimic solutions display comparable rheological properties to commercially available sera and should be used for volume verification, calibration and optimization in place of alternatives.
3. The MVS provides similar results as the gravimetric method but offers better detail, faster results, reduces consumables cost and does not require specialized environmental conditions for operation.

25 Bradley Drive, Westbrook, Maine 04092
888-406-3463 | info@artel.co

artel.co