Predicted Dispense Volume vs. Gravimetric Measurement for the Microlab® 600

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HAMILTON®

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Abstract

The Hamilton Microlab® 600 syringe pump is compatible with syringes from 10 µL to 50 mL. Dispense accuracy across the various syringe sizes is dictated by two factors. The first factor is the inner diameter (ID) of the syringe barrel; the second is the positional accuracy of the syringe drive. The ID of a Hamilton syringe is held to the tightest possible specifications but some tolerance is expected and allowed. Since the customer installs the syringes after the instrument has been purchased it is critical that the Microlab 600 provide sufficient positional accuracy to compensate for the allowable variation in syringe ID. The following experiment will show that the positional accuracy of the Microlab 600 is such that a syringe on the large side and the small side of allowable tolerance will provide an accurate dispense. The result is that direct measurement of the drive stem displacement is validated to predict dispense volume and provides a suitable means for calibrating the instrument.

Introduction

We have the capability to measure the linear syringe drive stem displacement with an accuracy of ± 0.0025 mm (2.5 µL) traceable to N.I.S.T measures. The linear motion delivered by the syringe pump is one of two components that account for the dispense volume. The inner diameter, and thus the cross-sectional area, of the installed syringe makes up the second component. By calculating the dispense volumes that would result with a syringe manufactured at the minimum diameter tolerance per the manufacturing print, and the maximum diameter tolerance per the manufacturing print, the range of possible dispense volumes can be predicted. If the predicted dispense volume falls within the accuracy specifications for the Microlab 600, then the pump is accurate for any syringe that meets manufacturing tolerance specifications. The accuracy specifications for a Microlab 600 configured with a 1 mL syringe are listed below.

Table 1: Microlab 600 accuracy specifications for 1%, 5% and 30% stroke of a 1 mL syringe

	Microlab 600 Accuracy Specification	Allowable Volume Range for a 1 mL Syringe (µL)
1% Syringe Stroke	± 3.0%	9.7–10.3
5% Syringe Stroke	± 1.2%	49.4 – 50.6
30% Syringe Stroke	± 1.0%	297 – 303

All Hamilton syringes are manufactured to specifications with a defined minimum **(d1)** and maximum **(d2)** allowable tolerance for the inner diameter. For example, the diameter of a 1 mL (1000 µL) syringe must fall between **d1** = 4.600 mm and **d2** = 4.620 mm.

Each syringe is designed to dispense its nominal volume over a 60 mm stroke length. Assuming a theoretically perfect 60 mm stroke, the dispense volume for a syringe built with the minimum and maximum allowed inner diameter would be calculated using the following equations.

Dispense Volume = Stroke Length (mm) x Area (mm^2)
Area = π x $(Diameter/2)^2$

Minimum Dispense Volume	Maximum Dispense Volume
Stroke Length x π x $(d1/2)^2$	Stroke Length x π x $(d2/2)^2$
60 mm x π x $((4.600)/2)^2$	60 mm x π x $((4.620)/2)^2$
997.1 µL	1005.8 µL

The theoretical extremes above fall within 990 µL and 1010 µL which corresponds to the Microlab 600's ± 1% accuracy specification for a 1 mL dispense from a 1 mL syringe. The next step is to incorporate the actual measured stroke of a Microlab 600 into the calculations above to show that the accuracy of the entire system meets specification with extreme scenario syringes. In practice, actual syringes will almost never be at exactly the extreme edges of tolerance, but will fall on average somewhere on a bell curve between the extremes.

Methods & Results

1. Ten 1 mL syringes (p/n 59000-35) pulled randomly from stock were labeled and the barrel inner diameters were measured using the appropriate air gauge. The diameter was measured at the top, middle and bottom of the barrel and these measurements were averaged to obtain the Measured Inside Diameter. See Table 2 for details.

   Table 2: Cross sectional areas of ten 1 mL syringes pulled from stock

Syringe #	Measured Inside Diameter (mm)	Cross-Sectional Area (mm^2)
1	4.61391	16.71969
2	4.60866	16.68166
3	4.60917	16.68534
4	4.61264	16.71048
5	4.60705	16.67002
6	4.60908	16.68473
7	4.61086	16.69760
8	4.61035	16.69392
9	4.60917	16.68534
10	4.61383	16.71907

2. Using a calibrated Mitutoyo Digimatic Digital Indicator, the linear displacement of the Microlab 600 was measured 10 times at 1% stroke (0.6 mm), 10 times at 5% stroke (3 mm) and 10 times at 30% stroke (18 mm), ending each stroke at the zero position. The digital interface was used to enter the pump movement commands. The average of 10 measurements for each range was calculated and recorded in Table 3.

Table 3: Average measured stroke for Microlab 600 Serial Number ML600AG1026

Stroke To Zero Position	Average Measured Stroke (mm)
1% (0.6 mm)	0.6022
5% (3.0 mm)	3.0072
30% (18 mm)	18.0171

3. The average measured stroke and measured syringe diameters were used to calculate the expected dispense volume using the equations below. Predicted gravimetric results for each syringe are shown in Table 4.

Dispense Volume = Stroke Length (mm) x Area (mm^2)

Table 4: Predicted dispense volume from measured linear displacement and measured syringe diameters

	Predicted Volume		
Syringe #	**1% Stroke (µL)**	**5% Stroke (µL)**	**30% Stroke (µL)**
1	10.068	50.279	301.240
2	10.046	50.165	300.555
3	10.048	50.176	300.621
4	10.063	50.252	301.074
5	10.039	50.130	300.345
6	10.048	50.174	300.610
7	10.055	50.213	300.842
8	10.053	50.202	300.776
9	10.048	50.176	300.621
10	10.068	50.278	301.229

4. Using the same pump and the same commands as in step 2, each syringe was tested gravimetrically at 1% (0.6 mm), 5% (3.0 mm) and 30% (18.0 mm), using deionized water. The test apparatus was calibrated to N.I.S.T traceable weights and the procedure followed is outlined in Appendix A. As per normal calibration procedures, each volume measurement was repeated ten times and the average is reported in Table 5.

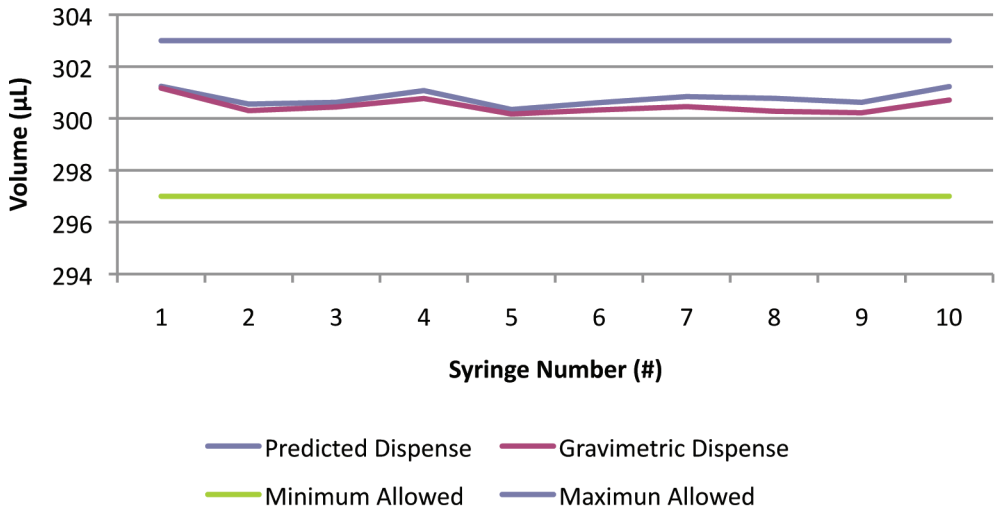
Table 5: Gravimetrically measured dispense volumes

	Average Measured Volume		
Syringe #	**1% Stroke (µL)**	**5% Stroke (µL)**	**30% Stroke (µL)**
1	9.954	50.031	301.170
2	9.888	49.906	300.302
3	9.971	50.031	300.443
4	9.919	50.012	300.770
5	9.901	49.930	300.172
6	9.913	49.974	300.329
7	9.898	49.986	300.456
8	9.956	50.007	300.278
9	9.893	50.012	300.218
10	9.804	49.944	300.710

Data Analysis

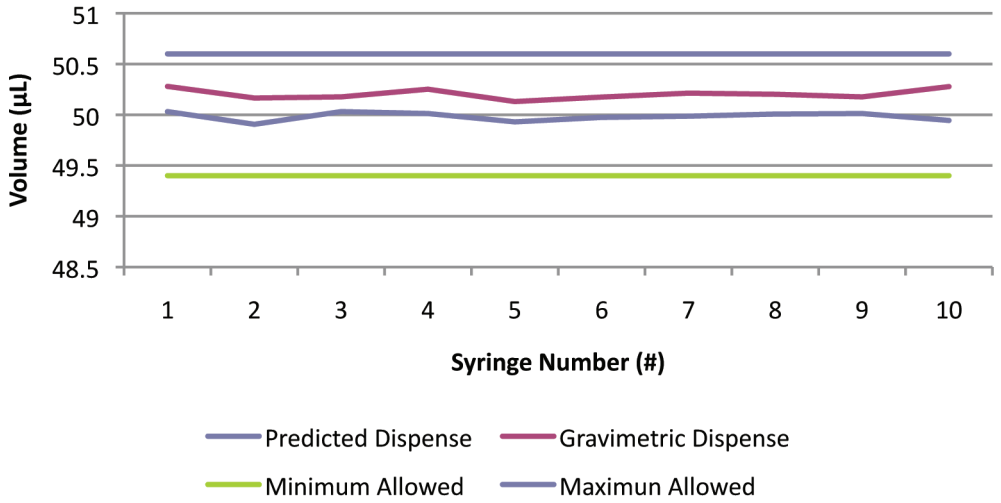
The data collected for Predicted and Gravimetric dispense volumes are displayed in Figures 1, 2 and 3 below. The graphs show the correlation between the predicted dispenses and the gravimetric dispenses. They also show the error bands which indicate the allowed dispense volumes at each stroke level.

Figure 1: Comparing the Predicted vs. Gravimetrical Volume for a 300 µL dispense



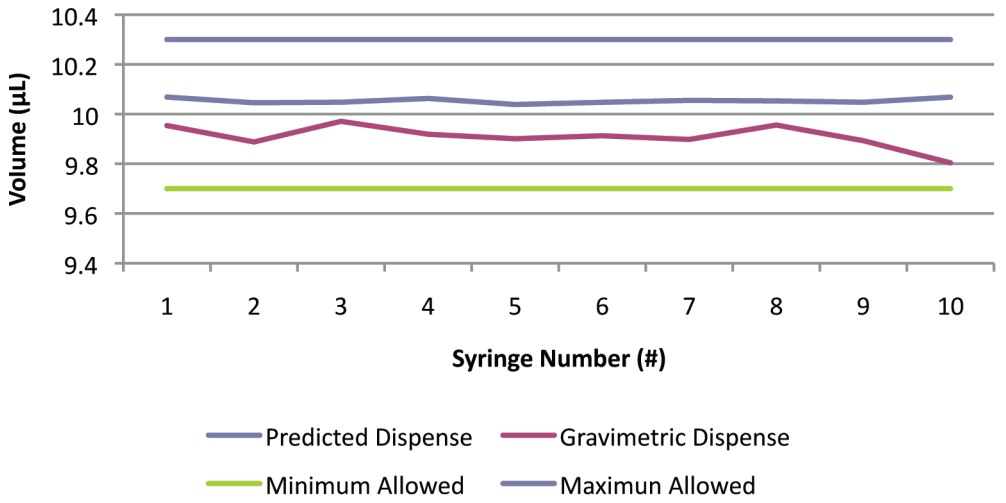
The Predicted vs. Gravimetric graph for the 300 µL dispense shows an almost perfect correlation. Syringes that were predicted to dispense slightly above the mean did dispense above the mean. Syringes that were predicted to dispense below the mean did dispense below the mean. Additionally all dispenses fell well within the minimum and maximum allowable dispenses according to the Microlab 600 specifications.

Figure 2: Comparing the Predicted vs. Gravimetrical Volume for a 50 µL dispense



The Predicted vs. Gravimetric graph for the 50 µL dispense shows a correlation with slightly more deviation than the 300 µL graph. The Gravimetric line is consistently less than the Predicted line. This is related to Z factors like evaporation, humidity, density of water and compressibility of the system. What is critical to note is that all dispenses continued to fall within the minimum and maximum allowable dispense specifications.

Figure 3. Comparing the Predicted vs. Gravimetrical Volume for a 10 µL dispense



The Predicted vs. Gravimetric graph for the 10 µL dispense shows that all dispenses fall within the minimum and maximum allowable dispense specifications. The peaks and valleys of the graph do not exactly correlate. This is expected because the cross sectional area used to calculate the predicted volume in Table 4 was an average across the entire length of the syringe barrel. A 10 µL dispense only uses 1% of the barrel, so the predicted dispense could be improved by measuring the exact barrel ID over this short distance. In reviewing Figure 3, it is useful to note that from the aspect of the pump, the maximum and minimum allowable volumes shown represent a linear displacement of ± 0.0178 mm. This distance is smaller than the diameter of the average human hair.

Conclusion

The Microlab 600 is designed to accurately dispense a variety of different liquids over a wide range of volumes. Since it is not practical to test all possible combinations of syringe size, dispense volume and liquid type, a test protocol was established to measure the linear accuracy of the syringe drive. This testing was experimentally shown to be predictive of the actual dispense volumes achieved using the same syringes with deionized water. As the data shows, under test conditions, the Microlab 600 system is easily capable of achieving the published specification. The specification is intentionally broad in an attempt to take into account unexpected factors that can influence dispense results. Potential factors include compressibility of the solvent, atmospheric pressure, humidity, etc. For these reasons it is good practice to test the accuracy and precision of the Microlab 600 under true laboratory conditions using the actual solvents to be dispensed.

Appendix A

Gravimetric Procedure for Calibrating a Microlab 600

Summary

This general procedure is based on determining the weighing result of water samples delivered by the syringe. True volume is calculated based on the density of water at specific temperatures.

Limitations

This method is not recommended for volumes below 2 µL. There is no upper volume limit.

Equipment, Materials, Environment

1. Laboratory balances required for the test method should meet or exceed the following performance specifications. They must be regularly maintained and calibrated with the appropriate N.I.S.T. traceable weights.

Test Volume, µL	Balance Sensitivity, mg
1–10 µL	0.001 mg
10–100 µL	0.01 mg
100–1000 µL	0.1 mg

2. Use a balance table, or suitable equivalent to minimize vibration. Cover the working surface directly in front of the balance with a dark, smooth, non-glare material. Keep the balance area reasonably free of draft currents and the ambient area free of excessive dust.
3. Use a weighing vessel that has a total volume 12 to 40 times the test volume, or 500 µL, whichever is larger (this is for evaporation control). If possible, use a cover that fits over the outside of the vessel top (do NOT allow the cover to come into contact with the test liquid). The vessel should be plastic, glass, metal or some other non-porous material. The cross-sectional area of the opening should be as small as possible to further control evaporation.
4. Handle the vessel with forceps or tweezers.
5. Use deionized water that has equilibrated to room temperature.
6. Use a calibrated thermometer to measure the temperature of the water.

Test Procedure

1. Turn on all equipment and allow all test materials to equilibrate to room temperature.
2. Place a small amount of water in the weighing vessel (between 2 and 30 test volumes).
3. Prime the Microlab 600 to eliminate all air bubbles from the fluid path.
4. Run the method to be validated.
5. Open the door of the balance chamber, place the weighing vessel on the balance pan and close the door of the balance chamber.
6. Tare the balance. Retrieve the weighing vessel from the balance chamber, deliver the sample and return the vessel to the balance pan, closing the door to the chamber. Observe and record balance readout.
7. Deliver a total of *n* samples (*n*=10 is recommended) into the weighing vessel, and weigh each sample after delivery. Replicate all motions and time intervals in each sampling cycle as precisely as possible. Keep the distance between the balance and the diluter/dispenser to a minimum.
8. Measure and record the water temperature.

Calculations

1. Calculate the volume of each dispense (Vi) by dividing each mass value by the density of water at the measured temperature. Refer to the table below for density values.

Density of Water at Various Temperatures

°C	g/mL	°C	g/mL
17	0.998774	24	0.997296
18	0.998595	25	0.997044
19	0.998405	26	0.996783
20	0.998203	27	0.996512
21	0.997992	28	0.995646
22	0.997770	29	0.995944
23	0.997538	30	0.995646

Taken from CRC Handbook of Chemistry and Physics, 50th edition, 1969, page F-4

2. Calculate the average dispensed volume from the individual dispensed volumes, Vi (where *i* is 1 to 10): V avg = (V1 + V2 + V3 + … + V10) / 10
3. Calculate the syringe accuracy: Accuracy (%) = (Vavg – Vo) / Vo x 100
   Note: Vo is equal to the expected dispense volume
4. Calculate the standard deviation (STDEV) of the calculated volumes, then determine the coefficient of variation: CV (%) = STDEV / V avg x 100

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HAMILTON®

Web: www.hamiltoncompany.com
USA: 800-648-5950
Europe: +40-356-635-055

Hamilton Americas & Pacific Rim
Hamilton Company, Inc.
4970 Energy Way
Reno, Nevada 89502 USA
Tel: +1-775-858-3000
Fax: +1-775-856-7259
sales@hamiltoncompany.com

Hamilton Europe, Asia & Africa
Hamilton Central Europe S.R.L.
str. Hamilton no. 2-4
307210 Giarmata, Romania
Tel: +40-356-635-055
contact.lab.ro@hamilton-ce.com

To find a representative in your area, please visit hamiltoncompany.com/contacts.