Calibration of a motorized syringe (single stroke dispenser without valve) - Comparison between the procedure of ISO 8655-6 and the new working document ISO 8655-8

Final Report V2

EURAMET Project no. 1486

IPQ – Coordinator of the comparison

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1. Introduction

In order to validate and evaluate the accuracy and precision of the photometric reference method for volume measurements of piston operated apparatus, described in the draft ISO/CD 8655-8, Artel, the project leader of this document, requested IPQ to conduct a comparison study regarding the calibration of a motorized syringe at different volumes. IPQ used the gravimetric method as described in ISO 8655-6:2002. The measurements were performed in September and October of 2019. Calibrations at IPQ and Artel were conducted in a "blind" manner - neither Artel nor IPQ knew each other's results prior to the conclusion of the study. After the final calibration at IPQ was finished, Artel submitted its results to IPQ for comparison and the generation of this report.

Table 1 – Participants

Country	Laboratory	Periods	Responsible	Contact
USA	Artel	September 2019	George Rodrigues	Email: grodrigues@artel-usa.com Tel: 207-591-6326
Portugal	IPQ	September/October 2019	Elsa Batista	Email: ebatista@mail.ipq.pt Tel: +351212948167

2. The instrument

For this comparison study, Artel supplied the measurement instrument, an eVol® motorized syringe made by SGE Analytical Science. This instrument was chosen because it combines fairly robust traveling standard capabilities, with an ease of use that is similar to a hand-held air displacement pipette.
The syringe set used in this comparison consists of a motorized control handle, exchangeable syringes with five different nominal volumes (5, 50, 100, 500 and 1000 µl), charger, stand, user instructions, packing materials, and a cushioned traveling case. The instrument and removable syringes are described in Table 2 and shown in Figure 1 and Figure 2.

Table 2 – Syringe used in the comparison

Manufacturer	Model	Glass removable syringes	Serial number
SGE	eVol XR	handle 5 µL 50 µL 100 µL 500 µL 1000 µL	5041455 AS54J AR41J AS82D AR33O AS78N

Figure 1 – Motor eVol XR and removable glass syringes

Figure 2– Syringe assembly

3. Experimental tests

The syringe set was calibrated at 0,5 µL, 5 µL, 50 µL, 100 µL, 500 µL and 1000 µL. Point 0,5 µL and 5 µL were calibrated with the 5 µL removable syringe. The set was programmed with settings such as plunger speed, pause times, etc. These programs are saved inside of the electronic handle and were used by all participants in this study. The set is used in a "non-adjusted" as-found state as provided by the manufacturer. These syringe systems have a function, which allows adjustment for each syringe; however this adjustment was not performed by any participants. Each test was performed with 10 replicates.

4. Calibration method

IPQ used the gravimetric method to determine the volume of the syringes and applied the equation described in ISO 4787 [2]. The experimental procedure is based on the standard ISO 8655-6:2002.

Artel used the photometric method described in ISO/CD 8655-8. All test solutions and calibrator mixtures were specially prepared for this study according to the procedure in ISO/CD 8655-8. The PCS-500 photometer was used to obtain the absorbance values and liquid temperature of the solution in the cuvette. These values were then applied in the formulas described in ISO/CD 8655-8 in order to determine the delivered volume of the syringes (using Microsoft Excel).

5. Equipment used

Table 3 – Equipment characteristics

Balance	**Type**	**Range**	**Resolution**
IPQ	Electronic, AX26 with evaporation trap	(0-22) g	0,001 mg
Spectrophotometer	**Type**	**Range**	**Resolution**
Artel	PCS-500	0-1,65 AU	0,001 mAU
Liquid thermometer	**Type**	**Range**	**Resolution**
IPQ	PT100	(-30 to 150) ºC	0,01 ºC
Artel	PCS-Internal Fluke/Hart 1521	(15 to 30) ºC (0 to 100) ºC	0,01 ºC 0,001 ºC
Air Thermometer	**Type**	**Range**	**Resolution**
IPQ	Digital	(0 to 50) ºC	0,1 ºC
Artel	PTU-301	(-40 to 60) ºC	0,01 ºC
Barometer	**Type**	**Range**	**Resolution**
IPQ	Digital	(800 - 1150) hPa	0,01 hPa
Artel	PTU-301	(500 - 1100) hPa	0,01 hPa
Hygrometer	**Type**	**Range**	**Resolution**
IPQ	Digital	(0-100) %	0,1%
Artel	PTU-301	(0-100) %	0,01%

6. Ambient conditions

The ambient conditions of both laboratories were the following:

Table 4 - Ambient conditions

Laboratory	**Air Temperature (°C)**	**Pressure (hPa)**	**Relative Humidity (%)**	**Air Density (g/ml)**
IPQ	20,3 – 22,6	1008,27 – 1009,60	60,5 – 73,4	0,0012
Artel	20,09 – 21,17	1012,47 – 1018,15	49,83 – 60,69	0,0012

7. Measurement results

7.1. Determination of the reference value and stability

In order to determine the reference value and access the stability of the instrument two measurements were performed by IPQ - one at the beginning and other at the end of the comparison study.

Table 5 – Stability of the transfer standards

	IPQ1		IPQ2		
Syringe	**Volume (μl)**	**Uncertainty (μl)**	**Volume (μl)**	**Uncertainty (μl)**	**ΔV (μl)**
0,5	0,482	0,022	0,491	0,019	-0,009
5	5,010	0,029	5,011	0,023	-0,001
50	49,969	0,072	50,026	0,066	-0,057
100	100,32	0,12	100,31	0,12	0,013
500	499,86	0,58	499,82	0,58	0,046
1000	996,9	1,2	996,3	1,2	0,59

The result variation of IPQ is smaller than the declared uncertainty and therefore it is assumed that the syringe was stable during the comparison.

The mean value of IPQ was considered the reference value, along with the largest value of uncertainty.

Table 6 – Reference value

Syringe	Volume (μl)	Uncertainty (μl)
0,5	0,487	0,022
5	5,011	0,029
50	49,998	0,072
100	100,31	0,12
500	499,84	0,58
1000	996,6	1,2

The results for all the syringes are presented is the following tables and figures.

7.2. Results analysis

The results analysis was performed according to the E_n number [3, 4]:

$$E_n = \frac{V_{lab} - V_{ref}}{\sqrt{U_{lab}{}^2 + U_{ref}{}^2}}$$

where V_{lab} and U_{lab} are the volume and the expanded uncertainty obtained by the laboratory and V_{ref} and U_{ref} the volume and the expanded uncertainty obtained by the reference laboratory (IPQ).

Absolute values of $E_n \leq 1$ represent a satisfactory performance by the laboratory.

7.3. Calibration results

7.3.1. Syringe calibration at 0,5 μL

Table 7 – Volume measurement results – syringe at 0,5 μl

Laboratory	Volume (μl)	Uncertainty (μl)	En value
IPQ - 1	0,482	0,022	
Artel	0,4863	0,0051	-0,01
IPQ - 2	0,491	0,019	
Vref	0,487	0,022	

7.3.2. Syringe calibration at 5 μl

Table 8 – Volume measurement results – syringe at 5 μl

Laboratory	Volume (μl)	Uncertainty (μl)	En value
IPQ - 1	5,010	0,029	
Artel	5,014	0,012	0,11
IPQ - 2	5,011	0,023	
Vref	5,011	0,029	

7.3.3. Syringe calibration at 50 μl

Table 9 – Volume measurement results – syringe at 50 μl

Laboratory	Volume (μl)	Uncertainty (μl)	En value
IPQ - 1	49,969	0,072	
Artel	50,094	0,071	0,95
IPQ - 2	50,026	0,066	
Vref	49,998	0,072	

7.3.4. Syringe calibration at 100 µl

Table 10 – Volume measurement results – syringe at 100 µl

Laboratory	Volume (µl)	Uncertainty (µl)	En value
IPQ - 1	100,321	0,12	
Artel	100,22	0,14	-0,50
IPQ - 2	100,308	0,12	
Vref	100,31	0,12	

7.3.5. Syringe calibration at 500 µl

Table 11 – Volume measurement results – syringe at 500 µl

Laboratory	Volume (µl)	Uncertainty (µl)	En value
IPQ - 1	499,86	0,58	
Artel	499,83	0,96	-0,01
IPQ - 2	499,82	0,58	
Vref	499,84	0,58	

7.3.6. Syringe calibration at 1000 µl

Table 12 – Volume measurement results – syringe at 1000 µl

Laboratory	Volume (µl)	Uncertainty (µl)	En value
IPQ - 1	996,7	1,2	
Artel	996,3	1,6	-0,14
IPQ - 2	996,3	1,2	
Vref	996,6	1,2	

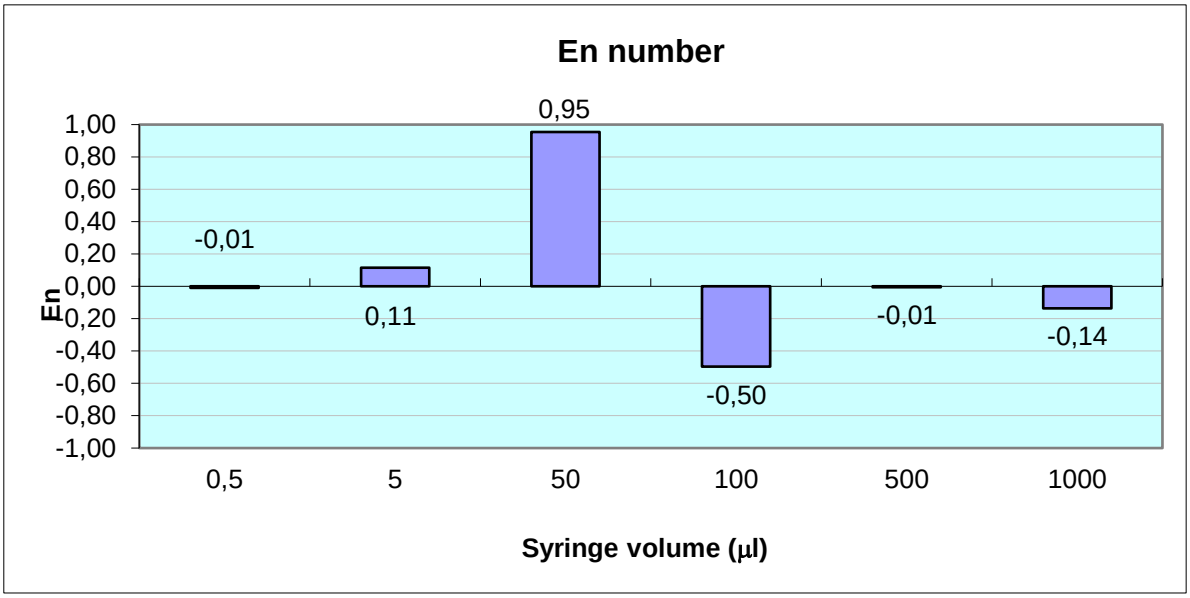

Figure 3 – E_n number

As can be seen from tables 7 to 12 and figure 3, all of the results using the photometric method are satisfactory.

8. Uncertainty calculation

Artel estimated uncertainties of the mean volume measured by the photometric method of ISO 8655-8 as described in the revised draft of ISO/TR 16153 (ISO/TC 48/WG04 N0311). The standard uncertainty of the method (k=1) is provided as Method uncertainty in Table 13. The eVol syringe also contributed uncertainty due to repeatability within the n=10 replicates of a single calibration, and also due to reproducibility of calibrations between days and operators.

Repeatability in Table 13 is the standard deviation of the ten replicates in the single calibration, divided by the square root of 10. This is an estimated repeatability of the mean based on the statistics of the single calibration.

Reproducibility is based on historical calibrations of eVol motorized syringes including different operators and different days. Artel has some information on reproducibility for the 50 µL device, but very limited information for the other volumes. The reproducibility values in Table 13 were based on the data from 600 calibrations, over all volumes, that were supplied by Webers GmbH. They represent one standard deviation of the mean volume based on different calibrations.

The three contributions are combined as a root-sum-square, then multiplied by k=2 to estimate the expanded uncertainty. There is a potential for double counting since reproducibility includes the effect of repeatability. However, for the six calibrations in Table 13, including repeatability causes no significant increase to the combined uncertainty. (note: A similar behaviour is seen with other Piston operating apparatus, where reproducibility between different calibrations is larger than the estimated repeatability within a single calibration).

There are some differences in the expanded uncertainty presented by Artel compared with those of the reference laboratory. For the two largest volumes (1000 µL and 500 µL) Artel presented higher uncertainty values than IPQ. For the two mid volumes (100 µL and 50 µL) the values are similar. For the two smallest volumes (5 µL and 0,5 µL) the Artel photometric uncertainties are lower. This is expected based on the technical differences between the methods.

Table 13 - Uncertainties components considered by Artel. All values in μL

Test Volume	Method	Repeatability	Reproducibility	Expanded uncertainty
0,5	0,000 24	0,000 54	0,002 5	0,005 1
5	0,002 2	0,002 6	0,005	0,012
50	0,025	0,005 5	0,025	0,071
100	0,051	0,016	0,05	0,14
500	0,40	0,065	0,25	0,96
1000	0,62	0,17	0,5	1,6

Notes:

a) Method, Repeatability and Reproducibility values are not expanded, and equivalent to one standard deviation.
b) Expanded uncertainty is the combination of all three contributions, then multiplied by k=2.

9. Conclusions

Under the work of ISO TC48/WG4, Artel requested a comparison study regarding the calibration of a motorized syringe at different volumes in order to validate and evaluate the accuracy and precision of the photometric reference method for volume measurements of piston operated apparatus, described in the draft ISO/CD 8655-8.

The results presented for all volumes were satisfactory.

For the two largest volumes (1000 μL and 500 μL) Artel presented higher uncertainty values than IPQ. For the two mid volumes (100 μL and 50 μL) the values are similar. For the two smallest volumes (5 μL and 0,5 μL) the Artel photometric uncertainties are much lower.

10. References

1. ISO 4787: 1984 – Laboratory Glassware – Volumetric Glassware – Methods for use and Testing of Capacity
2. ISO 8655-6:2002 – Piston-operated volumetric apparatus - Gravimetric methods for the determination of measurement error
3. ISO 13528:2005 - Statistical methods for used in proficiency testing by interlaboratory comparisons
4. ISO/IEC 17043:2010 - Conformity assessment — General requirements for proficiency testing
5. JCGM 100:2008 - Guide to the expression of uncertainty in measurement (GUM)