



Instituto Português da Qualidade  
PORTUGUESE INSTITUTE FOR QUALITY



## Final Report

### **Volume comparison on Calibration of micropipettes – Gravimetric and photometric methods**

**EURAMET Project no. 1353**

IPQ – Coordinator of the comparison

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## 1. Introduction

There are two different methods generally used for calibration of micropipettes. The gravimetric method described in ISO 8655-6:2002 and the photometric method described in ISO 8655-7. In order to verify the degree of agreement between the two methods and different operators in each laboratory, a bilateral comparison between IPQ (pilot laboratory) and Artel was proposed in February 2015 to EURAMET Technical Committee for Flow.

**Table 1 - Participants in the EURAMET project 1353**

Country	Laboratory	Periods	Responsible	Contact
Portugal	IPQ	May 2015	Elsa Batista	Tel: +351212948167 Email: ebatista@ipq.pt
USA	Artel	March 2015	George Rodrigues	Tel : 207-591-6326 Email: grodrigues@artel-usa.com

## 2. Instrument

There are several types of micropipettes, single channel or multichannel. The type suggested for this comparison was the single-channel piston pipette, which is the most commonly used in laboratories and easy to handle. The micropipette needs to have attached a removable plastic tip in order to aspirate the liquid. The tips were supplied by Artel.

Micropipettes may be factory-preset to deliver a given volume, or have selectable volumes within a useful volume range [1].

In the following figures and table it is presented 4 micropipettes used for this comparison made essentially of plastic with a coefficient of thermal expansion of  $2,4 \times 10^{-4} \text{ } ^\circ\text{C}^{-1}$  [2].

**Table 2 – Micropipettes characteristics**

Volume range (µL)	Calibrated Volume (µL)	Type	Number	Brand
100 - 1000	1000	Variable	BB50509	Gilson
10 - 100	100	Variable	KC26141	Gilson
1 - 10	10	Variable	KB30293	Gilson
0,1 – 2	1, 0,2 and 0,1	Variable	A0724524A	Rainin



Figure 1- variable micropipette of 1000 µl



Figure 2- variable micropipette of 100 µl



Figure 3- variable micropipette of 10 µl



Figure 4- variable micropipette of 2 µl

### 3. Calibration methods

#### 3.1 Gravimetric method

The gravimetric method is the standard method used by National Metrology Institutes (NMI's) and by accredited laboratories to calibrate volume instruments. This method consists on measuring the delivered volume of the micropipette into a beaker placed on a balance [1]. The liquid used is generally pure water (distilled, bi-distilled, or deionized) with a conductivity lower than 5 µS/cm and was chosen to suit the level of accuracy required relative to the amount of water used. A conversion is then performed from mass to volume at a reference temperature of  $t_0$  (normally 20 °C). The recommended equation is described in ISO 4787 standard [3] and given below (1).

$$V_{20} = (I_L - I_E) \times \frac{1}{\rho_W - \rho_A} \times \left(1 - \frac{\rho_A}{\rho_B}\right) \times [1 - \gamma(t - 20)] \quad (1)$$

#### 3.2 Photometric method

The photometric method uses a high-resolution photometer and colorimetric solutions to determine the volume delivered by a micropipette. The basic principle behind photometric measurement is the conservation of mass. Two additional assumptions are also made to allow the photometric method to be used easily for volume measurements: conservation of volume and the Lambert-Beer Law [4].

In the dual-dye radiometric photometry two colorimetric solutions are used. Each solution (one red, one blue) has an absorbance peak at a specific analytical wavelength. The basis of this technique is the following: an unknown volume of red dye is delivered into a vial containing a known volume and concentration of blue dye. After mixing, the change in absorbance of the resulting volume can be calculated as a ratio. The equation that describes this measurement principle is as following:

$$V_S = V_B \left( \frac{\frac{A_S}{A_B}}{K - \frac{A_S}{A_B}} \right) \quad (2)$$

Where,

$A_S/A_B$  is the absorbance ratio measured in the Photometer

$K$  is the calibration factor for the dyes

$V_B$  is the volume of the blank solution

$V_S$  is the volume delivery to be determined



Figure 5- Artel Photometer - PCS3

#### 4. General conditions for calibration

The transfer package consists of a set of 4 variable micropipettes; all artefacts have been calibrated in different volumes; the gravimetric results of IPQ were expressed at a reference temperature of 20 °C and 10 measurements were performed for each micropipette in each selected point.

The micropipettes were handled with care, i.e., only by qualified metrology personnel.

Each participating laboratory used its own instruments and procedures.

The gravimetric method and the photometric method were used by each laboratory. Artel presented results for 5 different operators. IPQ used only one operator.

Two different runs were made by each operator in each point in order to obtain the reproducibility of each operator. Except for point 0,1 µl, where only one run was performed.

To reach temperature uniformity, the tips and the water used in these tests were placed in the measurement laboratory at least 24 hours before any measurement was performed, at a temperature near 20 °C.

The Humidity was higher than 50 %.

The ambient temperature was between 17 °C and 23 °C.

Each laboratory described the equipment used in the calibration.

**Table 3 – Equipment characteristics**

<b>Balance</b>	<b>Type</b>	<b>Range</b>	<b>Resolution</b>
IPQ	Electronic	(0 - 22) g	0,001 mg
Artel	Electronic	(0 - 5,1) g	0,001 mg
Artel	Electronic	(0 - 220) g	0,01 mg
<b>Water thermometer</b>	<b>Type</b>	<b>Range</b>	<b>Resolution</b>
IPQ	Digital	(-30 to 150) °C	0,01 °C
Artel	Digital	(-50 to 150) °C	0,001 °C
<b>Air Thermometer</b>	<b>Type</b>	<b>Range</b>	<b>Resolution</b>
IPQ	Digital	(0 to 50) °C	0,1 °C
Artel	Digital	(-40 to 60) °C	0,01 °C
<b>Barometer</b>	<b>Type</b>	<b>Range</b>	<b>Resolution</b>
IPQ	Digital	(800 - 1150) hPa	0,01 hPa
Artel	Digital	(500 - 1100) hPa	0,01 hPa
<b>Hygrometer</b>	<b>Type</b>	<b>Range</b>	<b>Resolution</b>
IPQ	Digital	(0 - 100) %	0,1 %

Artel	Digital	(0 - 100) %	0,01 %
<b>Photometer</b>	<b>Type</b>	<b>Reagents</b>	
IPQ	PCS3	Lot Code 43253	
Artel	PCS3	Lot Code 6802	

## 5. Evaluation of the measurement results

### 5.1 Reference value

The comparison reference value and the uncertainty of the reference value are based on the results presented by IPQ.

### 5.2 Consistency determination

To verify if the results are consistent it is used the well-known  $E_n$ . This value is defined as:

$$E_{n_{lab-i}} = \frac{\varepsilon_{lab-i} - \varepsilon_{RV}}{\sqrt{U^2(\varepsilon_{lab-i}) + U^2(\varepsilon_{RV})}}$$

where  $\varepsilon_{lab-i}$  is the error of lab-i for a certain point,  $\varepsilon_{RV}$  is the comparison reference value (RV) for the error and  $U(\varepsilon_{lab-i})$  and  $U(\varepsilon_{RV})$  and the expanded uncertainties ( $k=2$ ) of those values.

With the value of  $E_n$  one can conclude that:

- The results of the laboratory for a certain point are consistent (passed) if  $E_n \leq 1$
- The results of the laboratory for a certain point are inconsistent (failed) if  $E_n > 1$

## 6. Results

### 6.1 Volume obtained by gravimetric method

#### 6.1.1 - 1000 $\mu$ L variable micropipette

The volume measurements obtained by IPQ and by Artel operators are presented in the following figures:

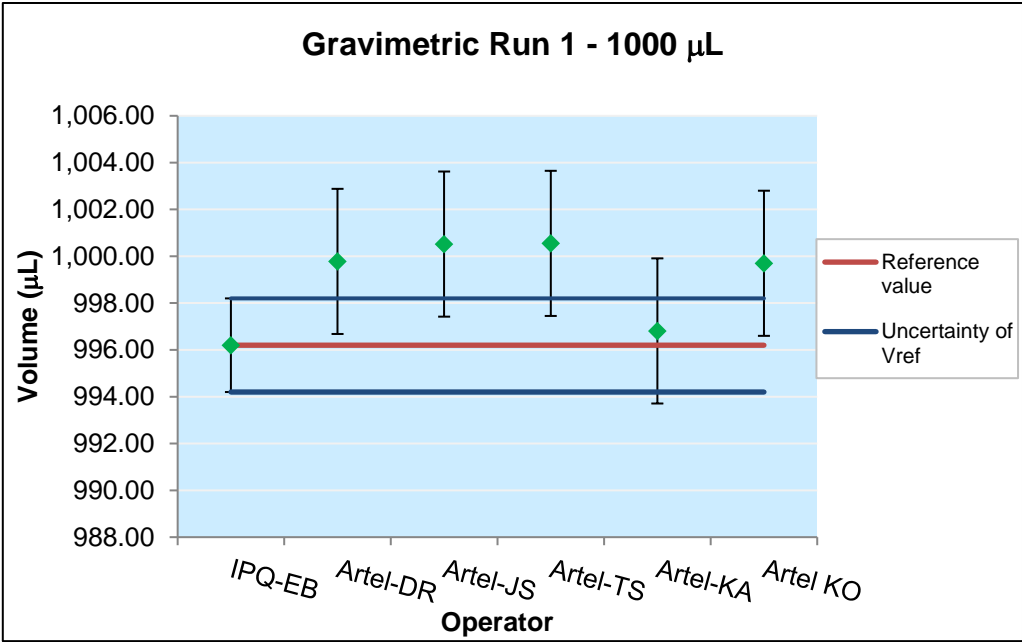


Figure 6- Results 1000 µL, run 1

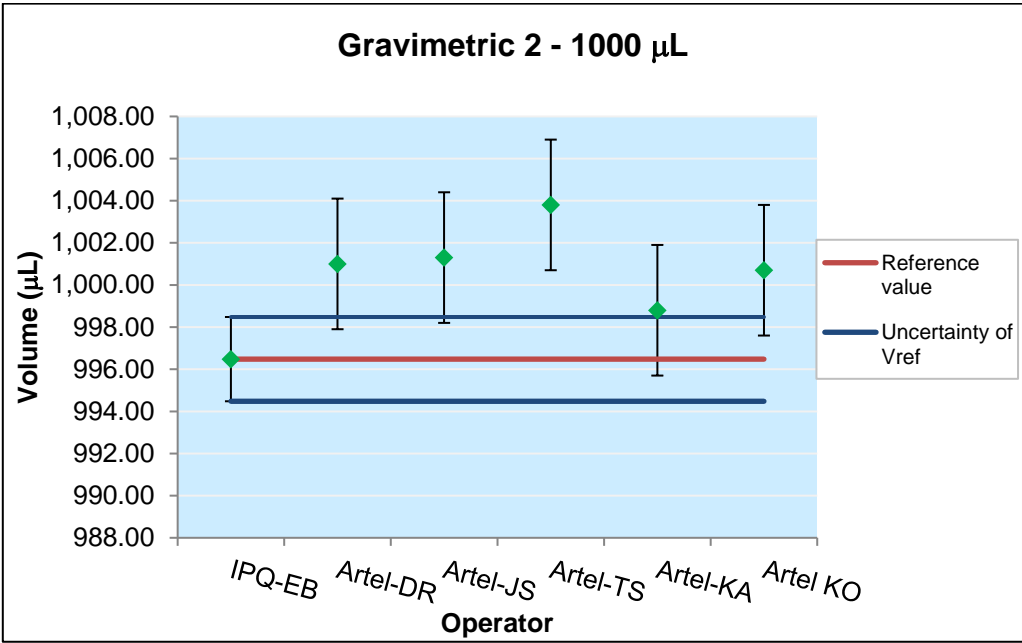


Figure 7- Results 1000 µL, run 2



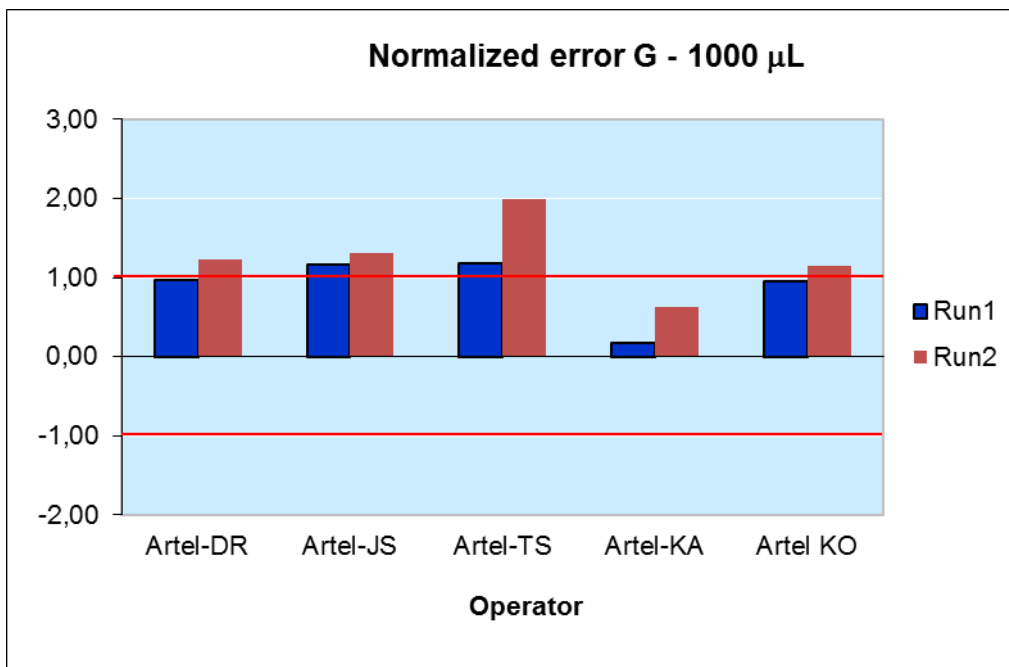


Figure 8-  $E_n$  Results 1000 µL Gravimetric

It can be verified from the figures that four results from Artel are consistent with IPQ.

6.1.2 - 100 µL variable micropipette

The volume measurements obtained by IPQ and by Artel operators are presented in the following figures:

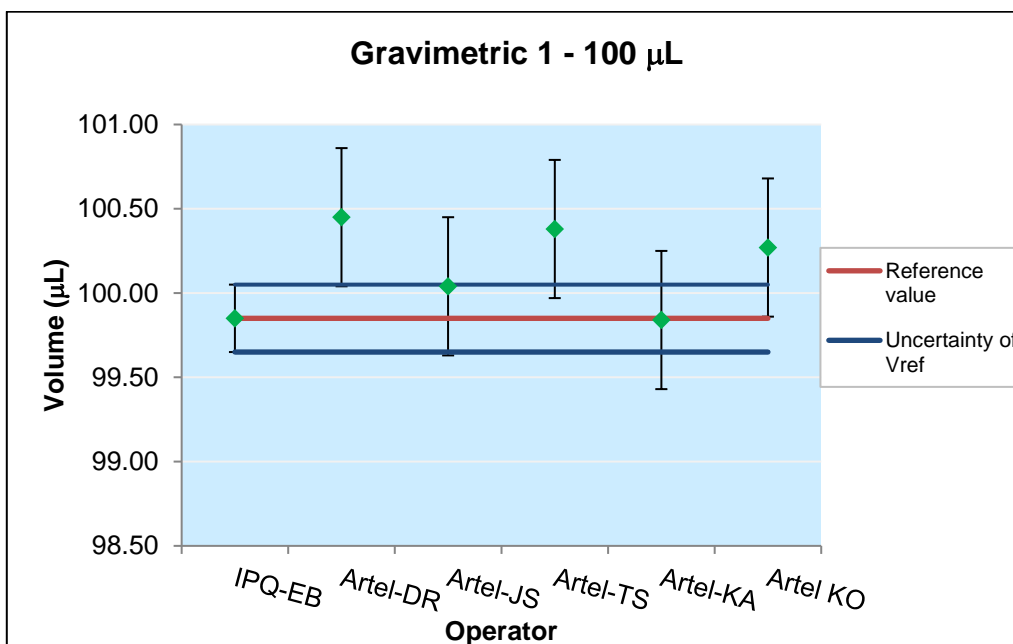


Figure 9- Results 100 µL, run 1

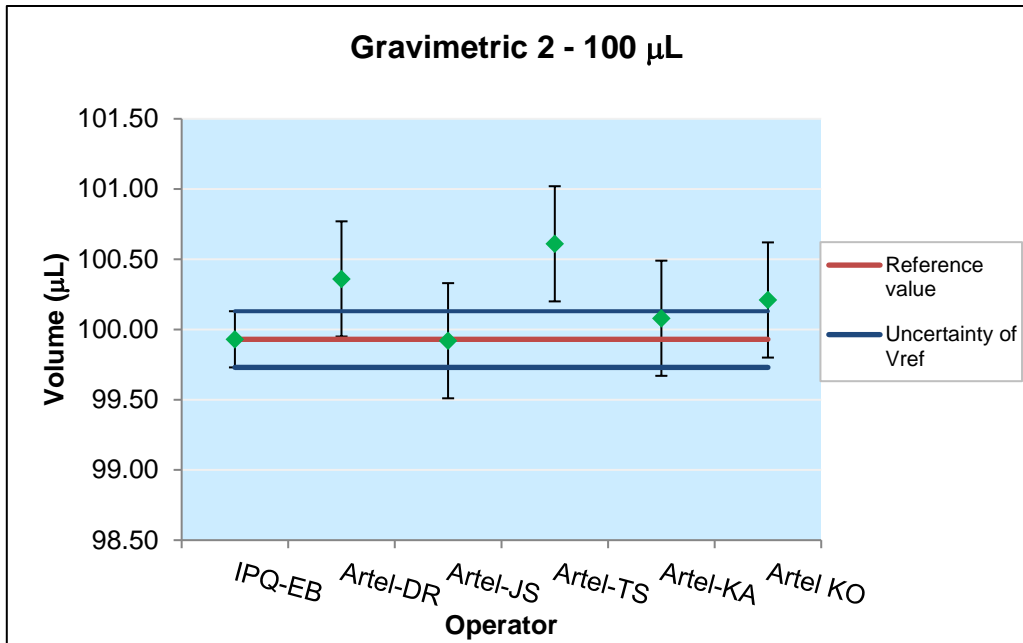


Figure 10- Results 100 µL, run 2

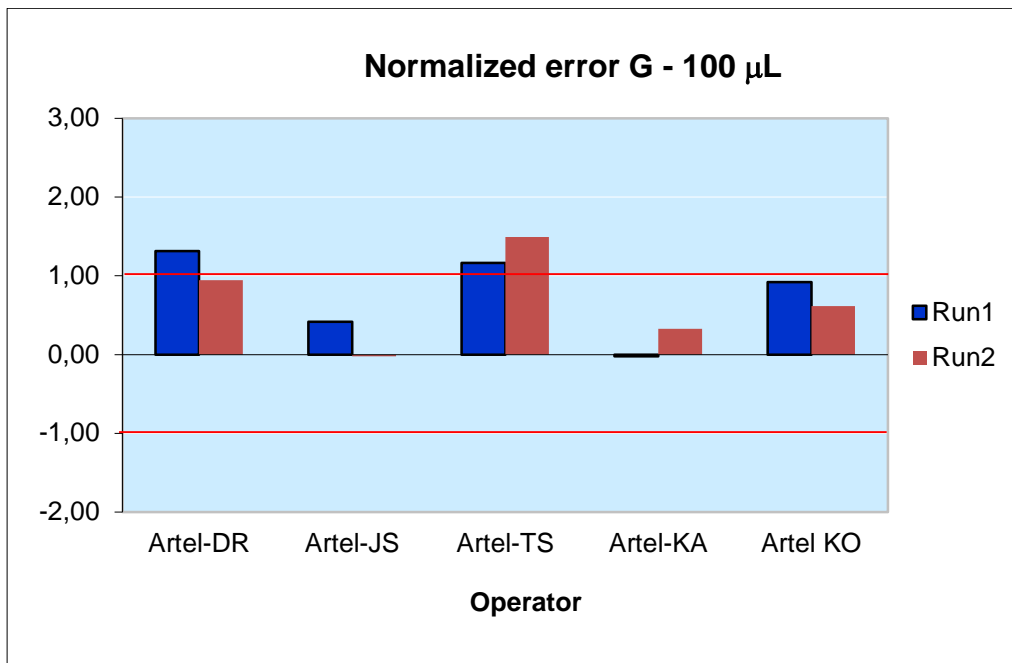


Figure 11-  $E_n$  Results 100 µL Garvimetric

For this micropipette only operator TS is inconsistent in both runs. Operator DR is inconsistent in one run.

### 6.1.3 - 10 µL variable micropipette

The volume measurements obtained by IPQ and by Artel operators are presented in the following figures:

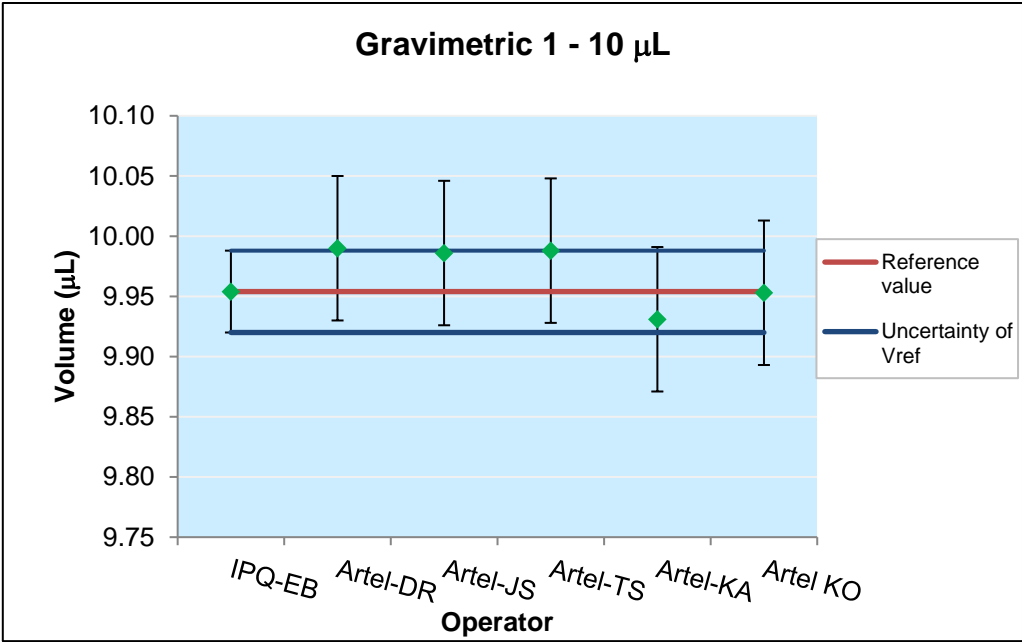


Figure 12- Results 10 µL, run 1

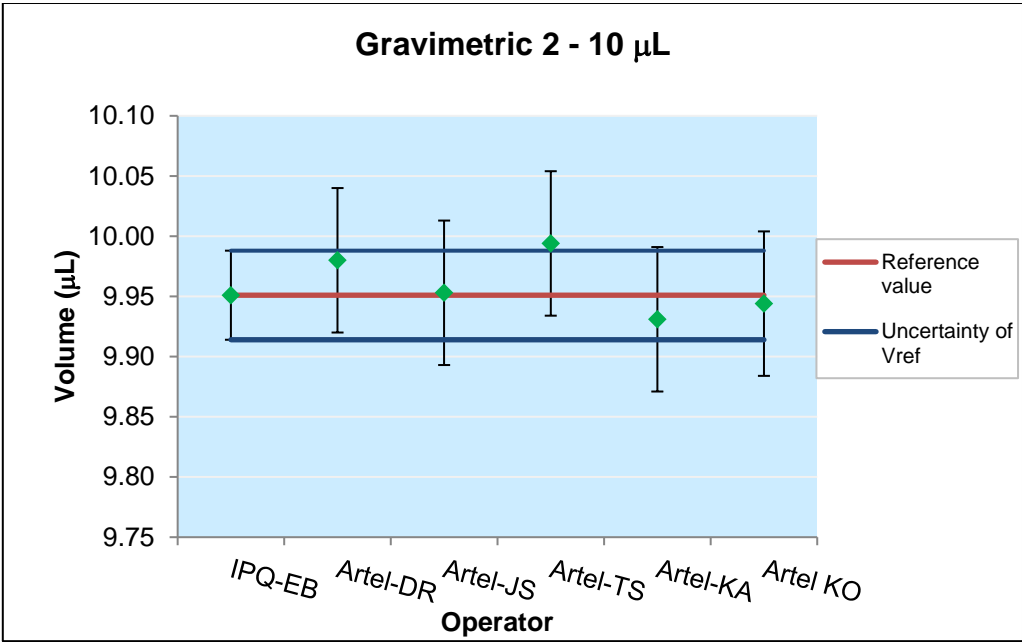


Figure 13- Results 10 µL, run 2

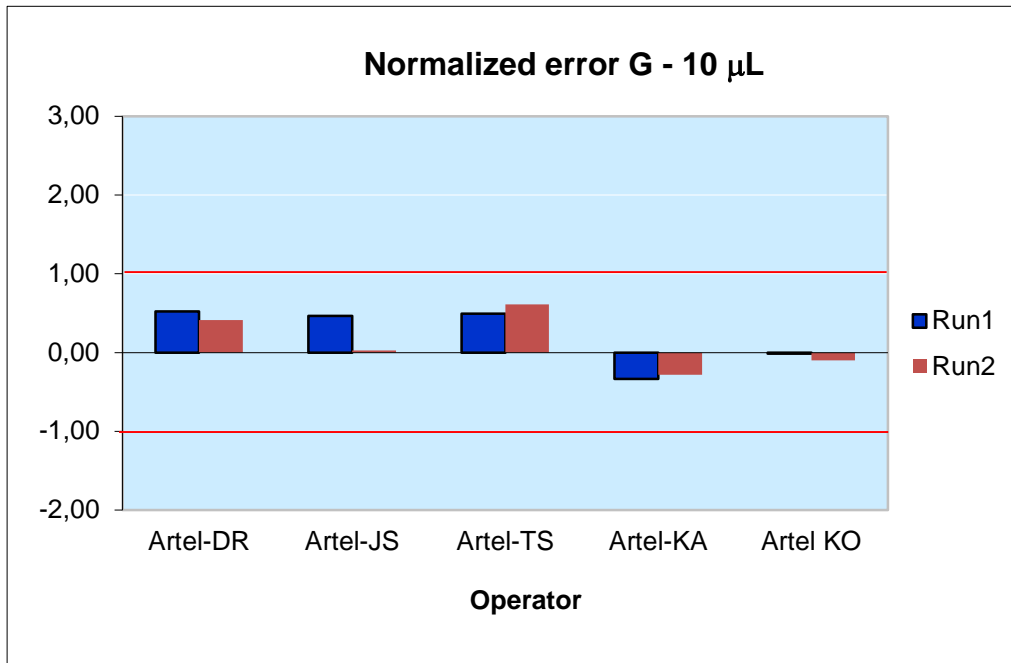


Figure 14-  $E_n$  Results 10 µL Gravimetric

From the pictures above it can be verified that all Artel operators are consistent with IPQ.

6.1.4 - 2 µL variable micropipette

In this micropipette three different volumes were measured 1 µL, 0,2 µL and 0,1 µL.

1 µL volume measurements

The volume measurements obtained by IPQ and by Artel operators are presented in the following figures:

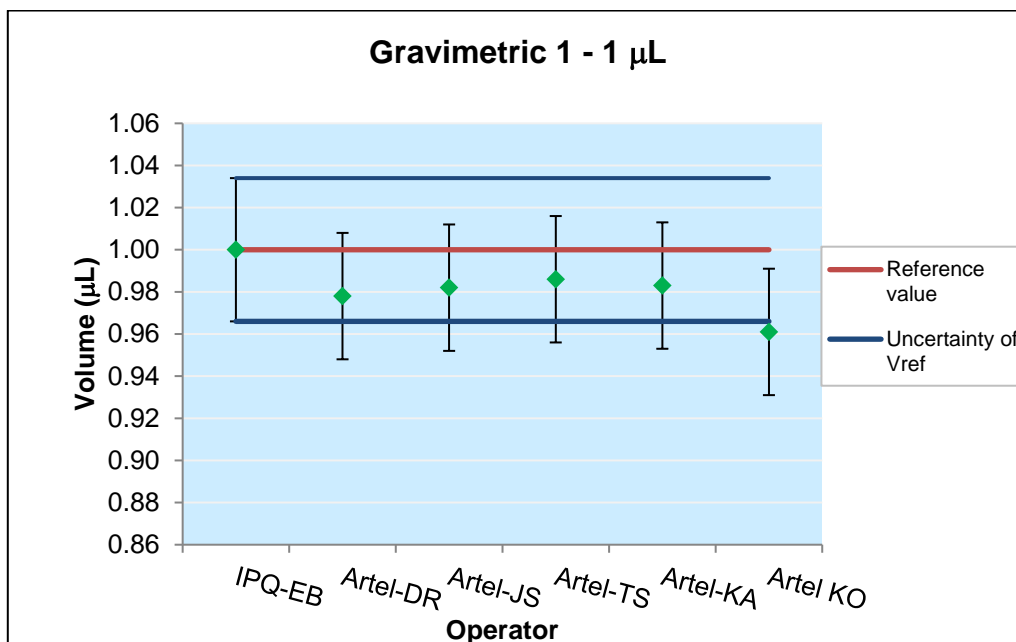


Figure 15- Results 1 µL, run 1

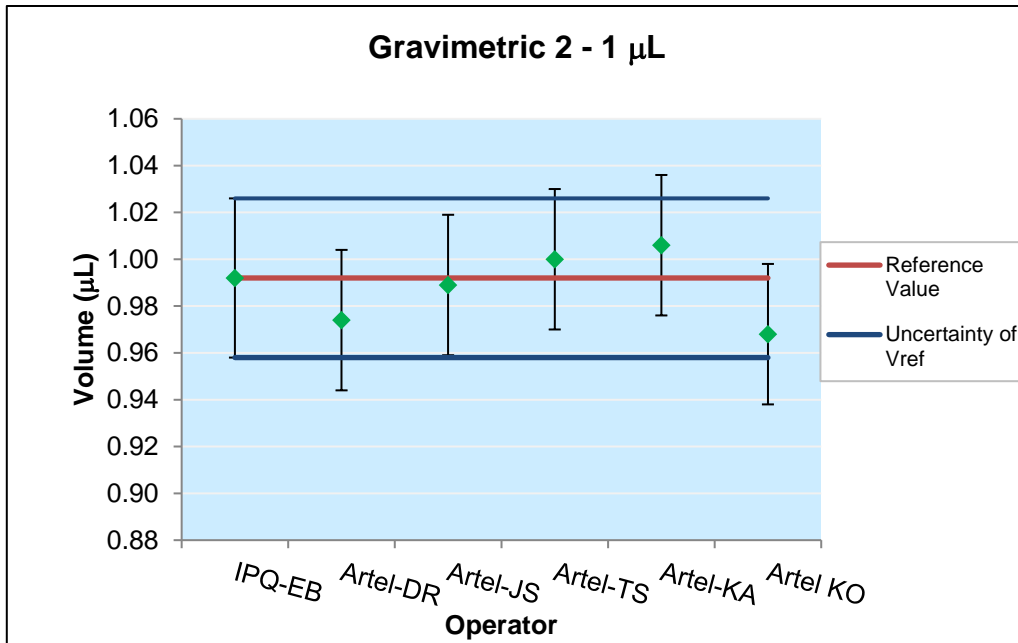


Figure 16- Results 1 µL, run 2

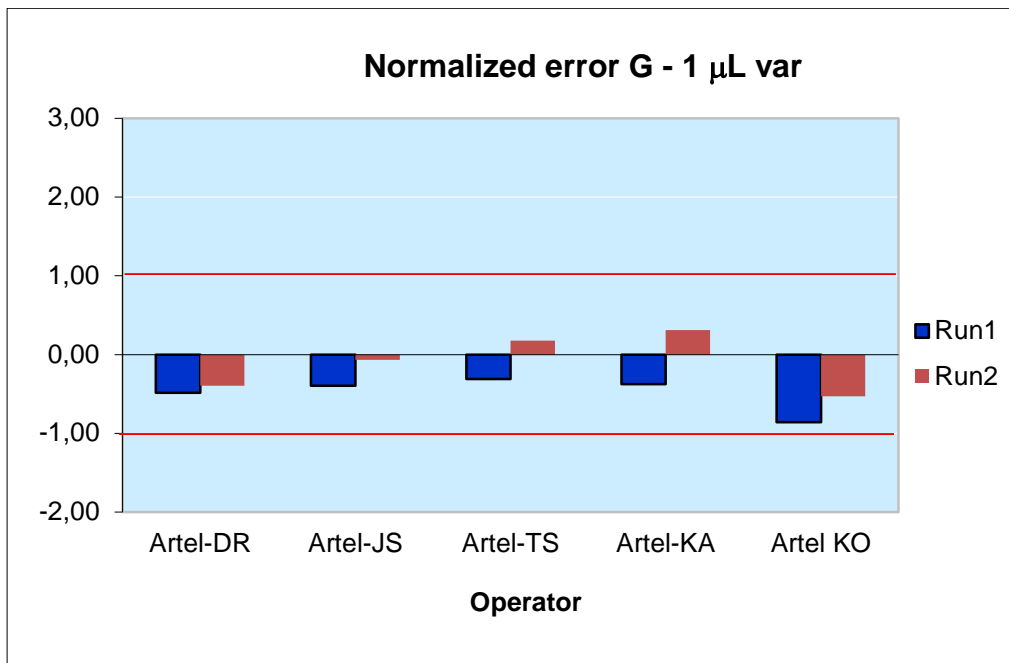


Figure 17-  $E_n$  Results 1 µL Gravimetric

From the pictures above it can be verified that all Artel operators are consistent with IPQ.

*0,2 µL volume measurements*

The volume measurements obtained by IPQ and by Artel operators are presented in the following figures:

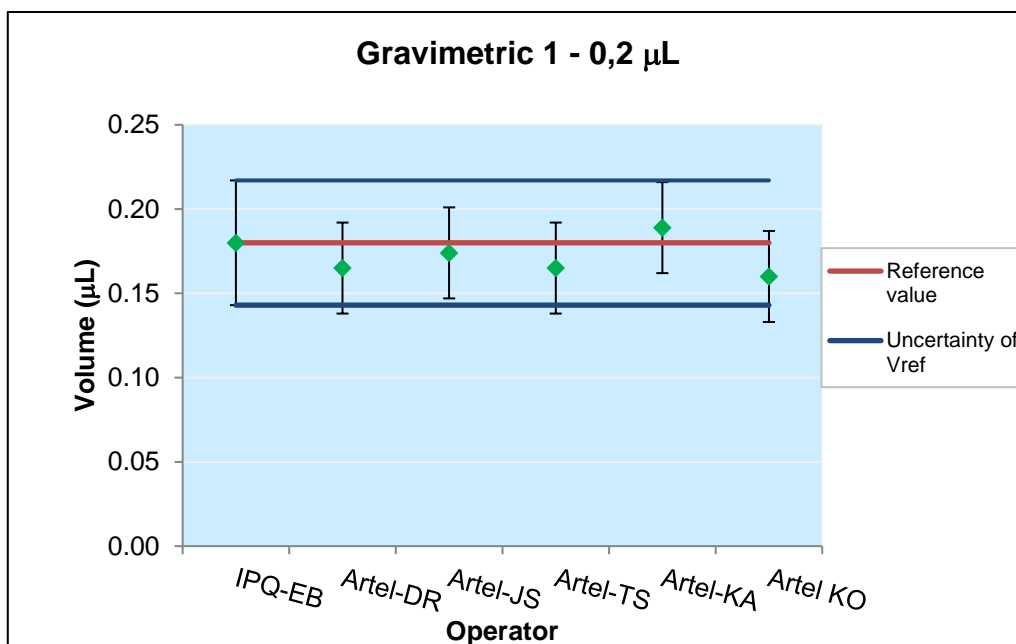


Figure 18- Results 0,2 µL, run 1

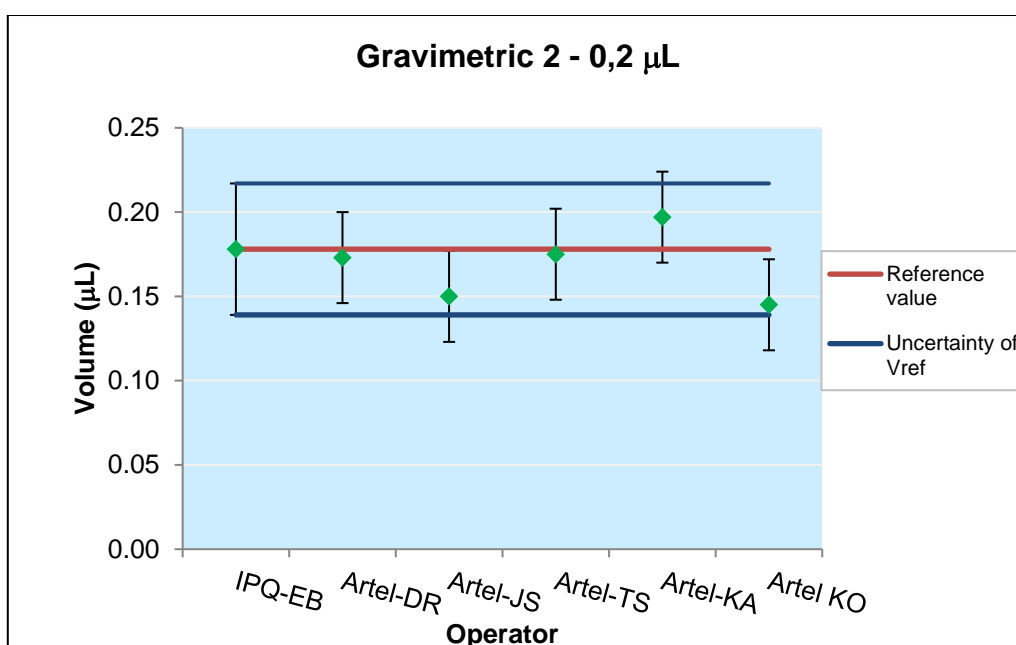


Figure 19- Results 0,2 µL, run 2

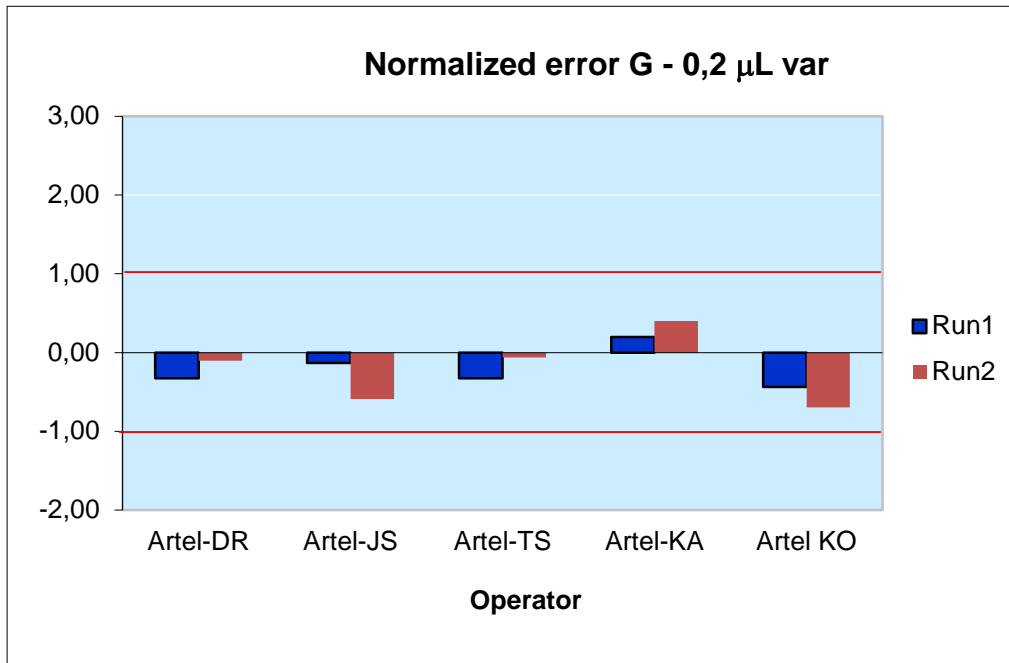


Figure 20-  $E_n$  Results 0,2 µL Gravimetric

From the pictures above it can be verified that all Artel operators are consistent with IPQ.

*0,1 µL volume measurements*

The volume measurements obtained by IPQ and by Artel operators are presented in the following figures, only the results from one run are presented:

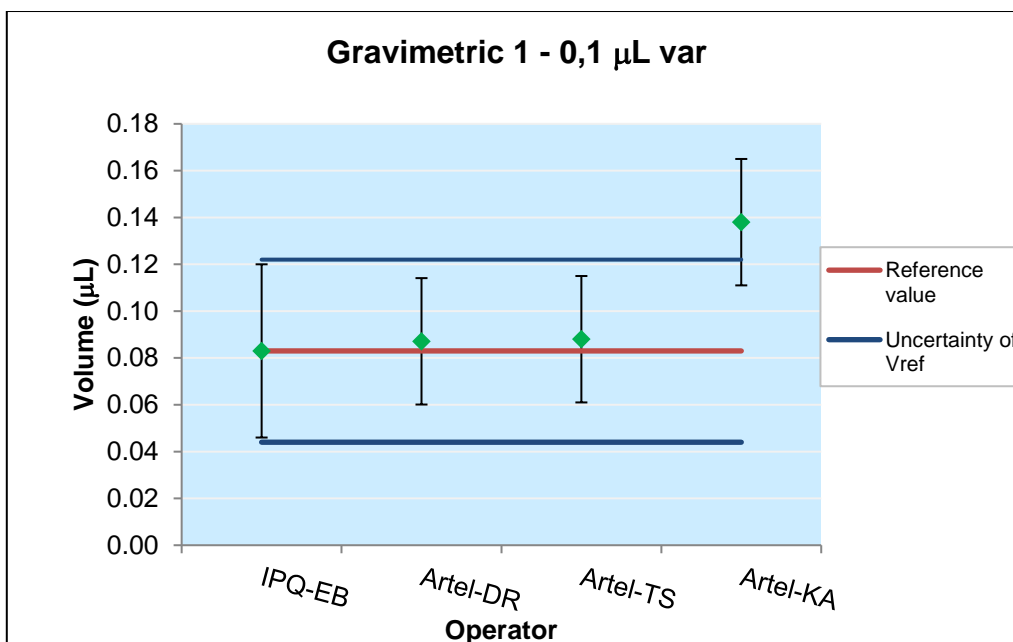


Figure 21- Results 0,1 µL

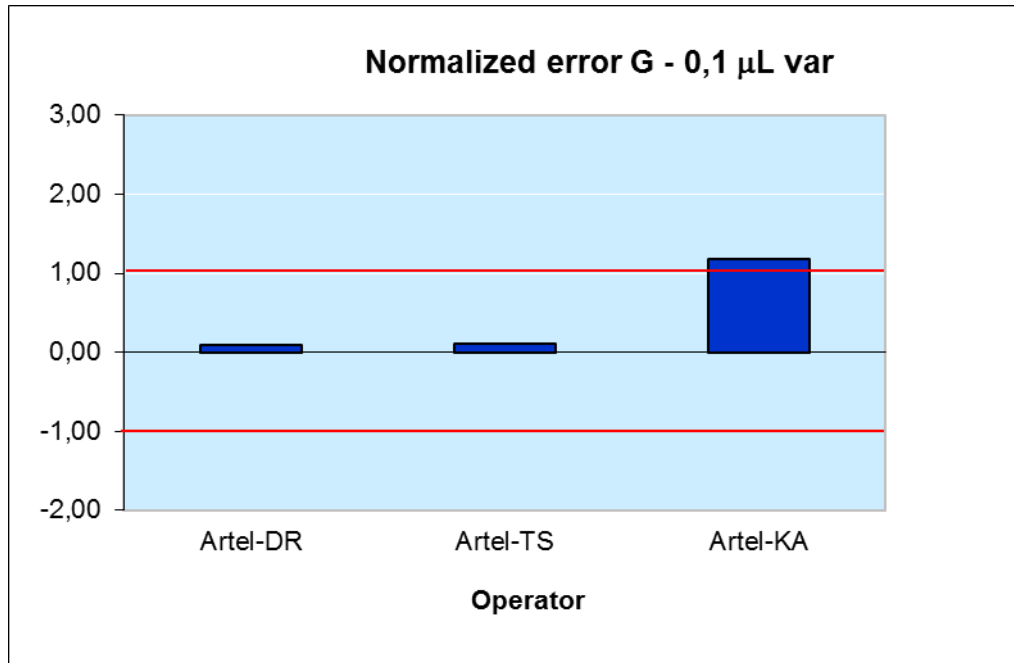


Figure 22-  $E_n$  Results 0,1 µL Gravimetric

From the pictures above it can be verified that only one Artel operator is inconsistent with IPQ.

## 6.2 Volume measurements photometric

### 6.2.1 - 1000 µL variable micropipette

The volume measurements obtained by IPQ and by Artel operators are presented in the following figures:

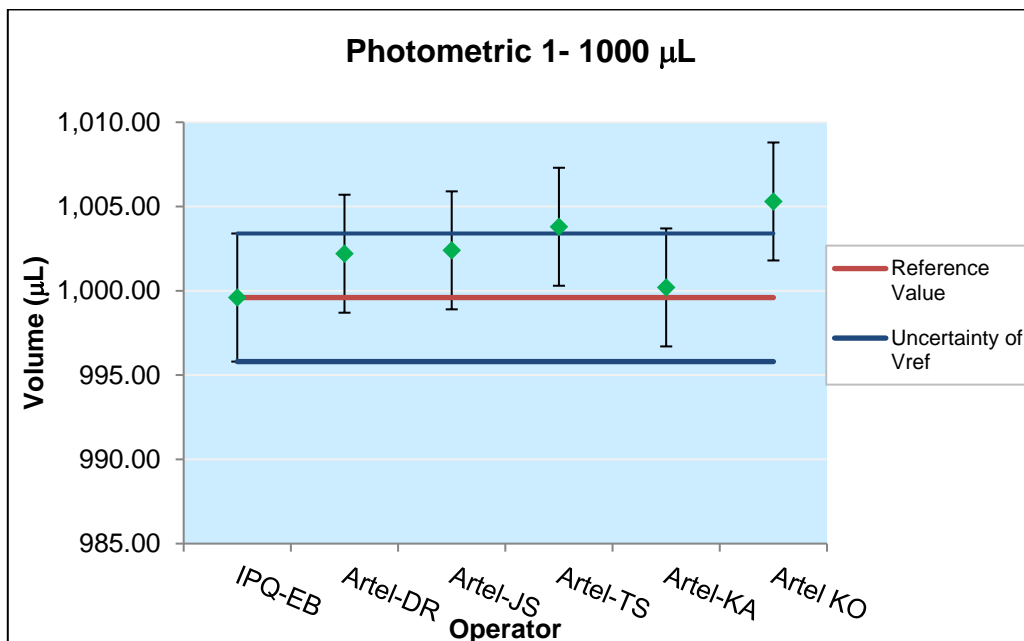


Figure 23- Results 1000 µL, run 1



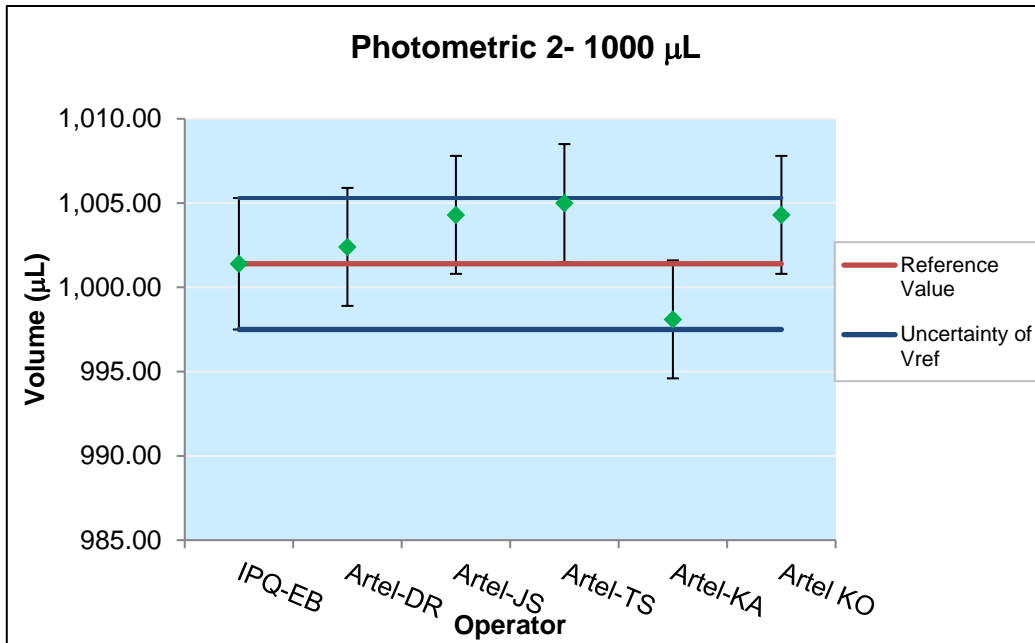


Figure 24- Results 1000 µL, run 2

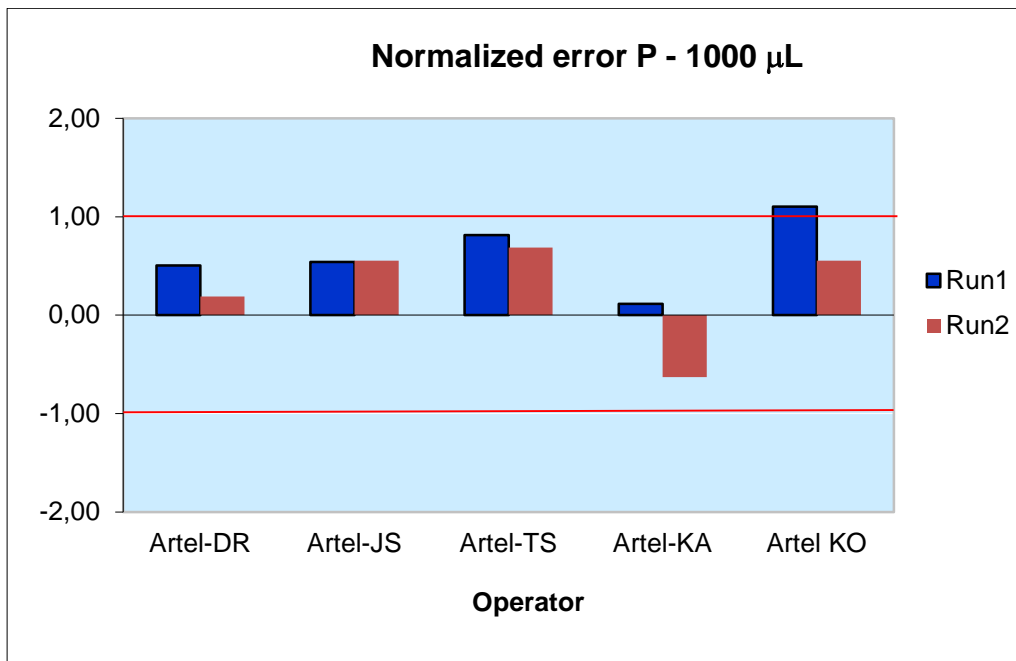


Figure 25-  $E_n$  Results 1000 µL Photometric

It can be verified from the figures that the operator KO from Artel is inconsistent with IPQ only in run 1.

### 6.2.2 - 100 µL variable micropipette

The volume measurements obtained by IPQ and by Artel operators are presented in the following figures:

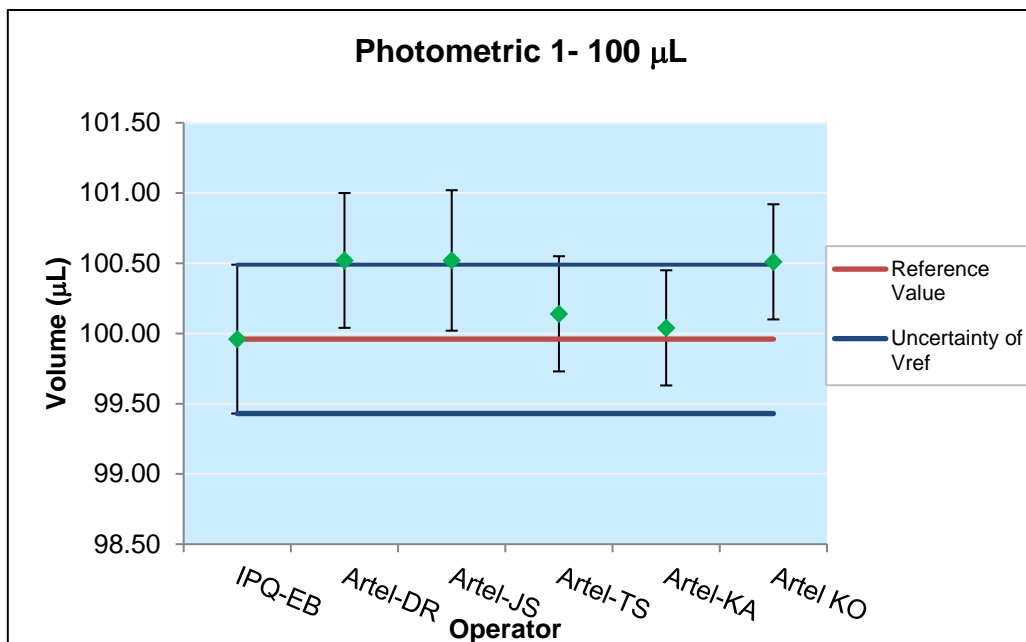


Figure 26- Results 100 µL, run 1

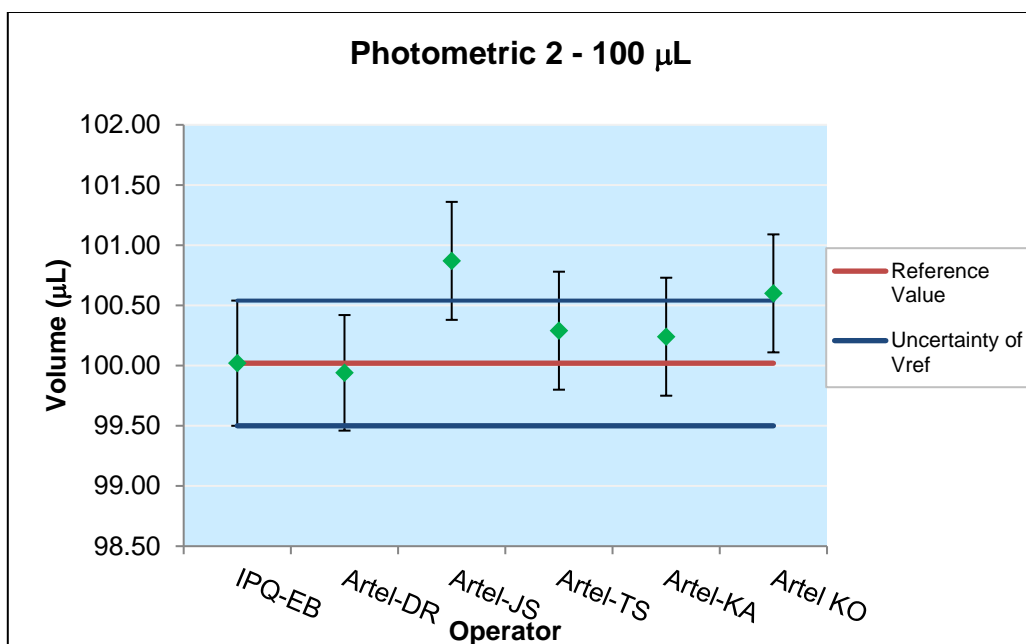


Figure 27- Results 100 µL, run 2

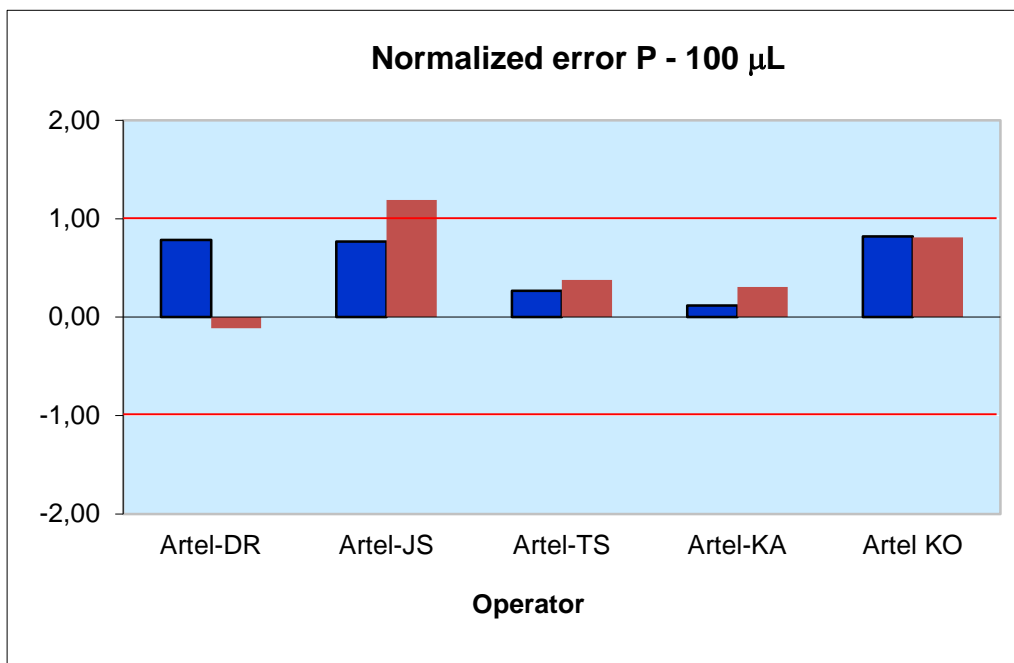


Figure 28-  $E_n$  Results 100 µL Photometric

For this micropipette operator JS is inconsistent only in run 2.

### 6.2.3 - 10 µL variable micropipette

The volume measurements obtained by IPQ and by Artel operators are presented in the following figures:

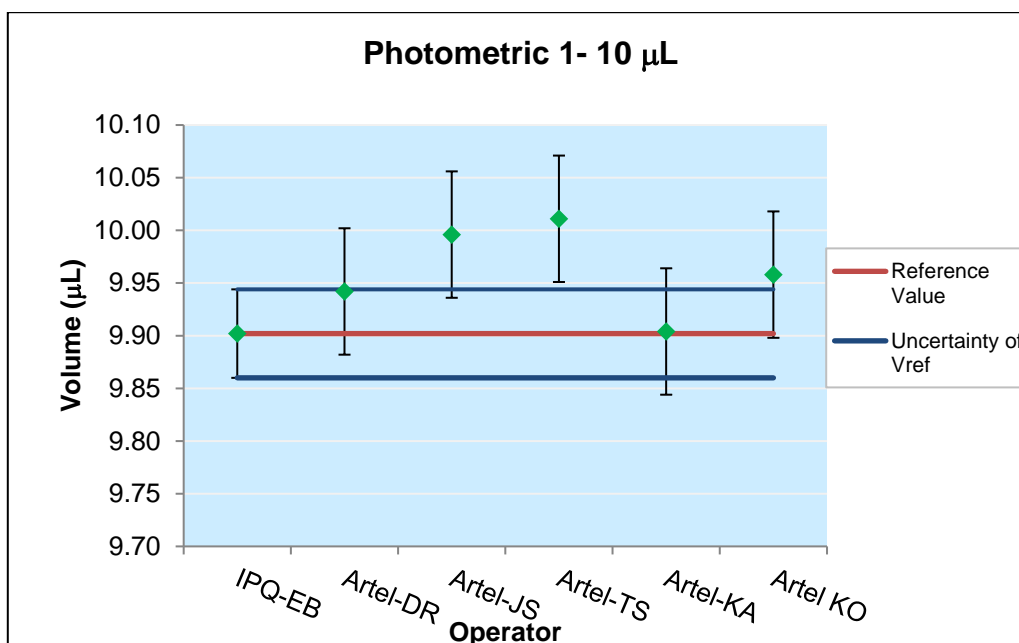


Figure 29- Results 10 µL, run 1

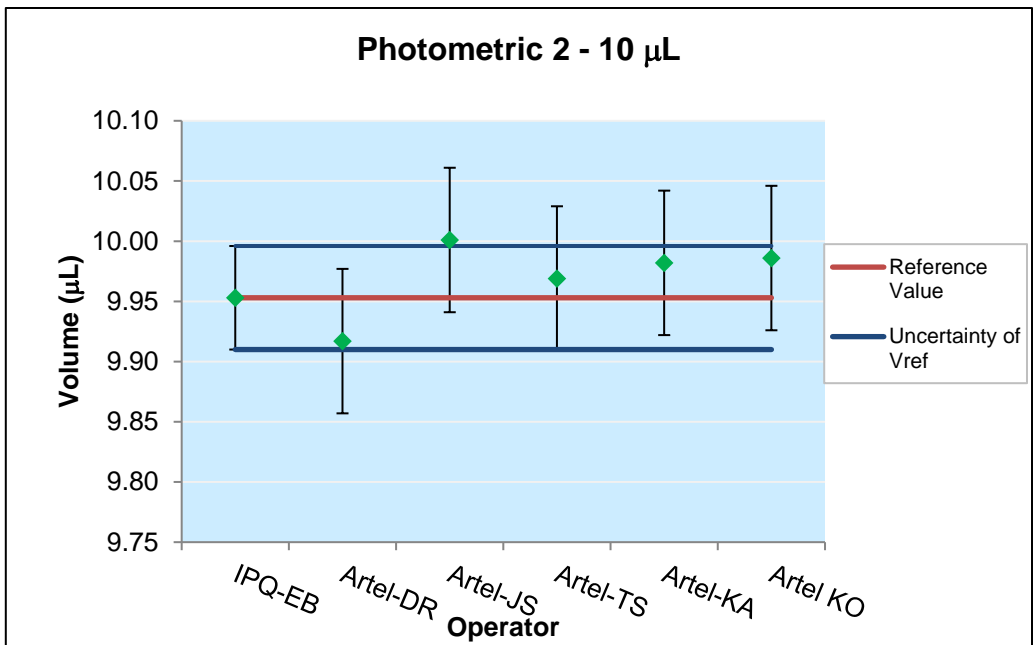


Figure 30 - Results 10 µL, run 2

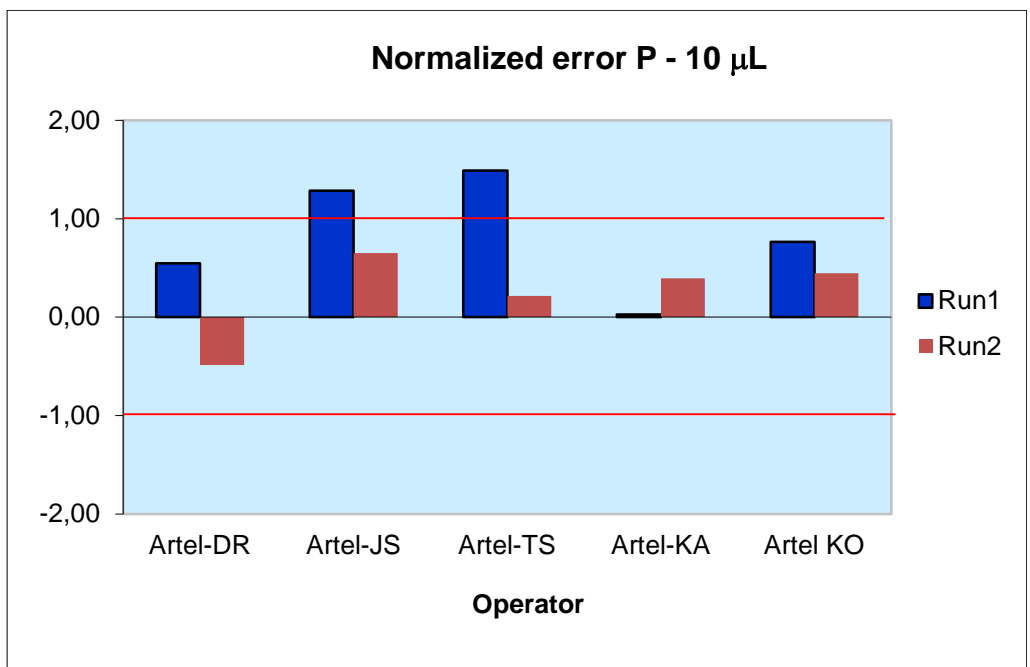


Figure 31-  $E_n$  Results 10 µL Photometric

From the pictures above it can be verify that operators JS and TS are both inconsistent in run 1.

6.2.4 - 2 µL variable micropipette

In this micropipette three different volumes were measured 1 µL, 0,2 µL and 0,1 µL.

*1 µL volume measurements*

The volume measurements obtained by IPOQ and by Artel operators are presented in the following figures:

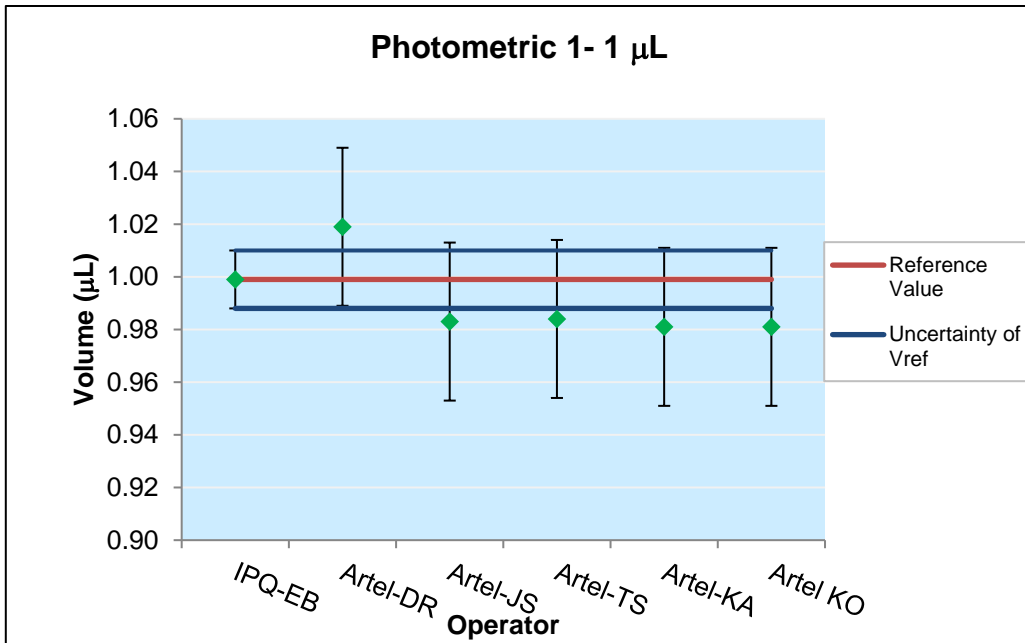


Figure 32 - Results 1 µL, run 1

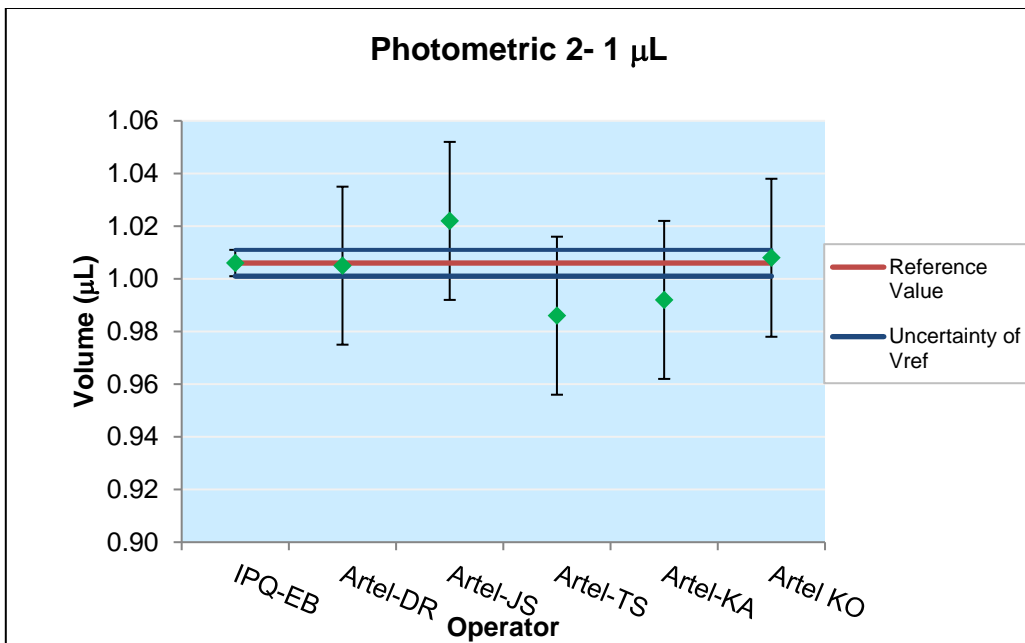


Figure 33 - Results 1 µL, run 2

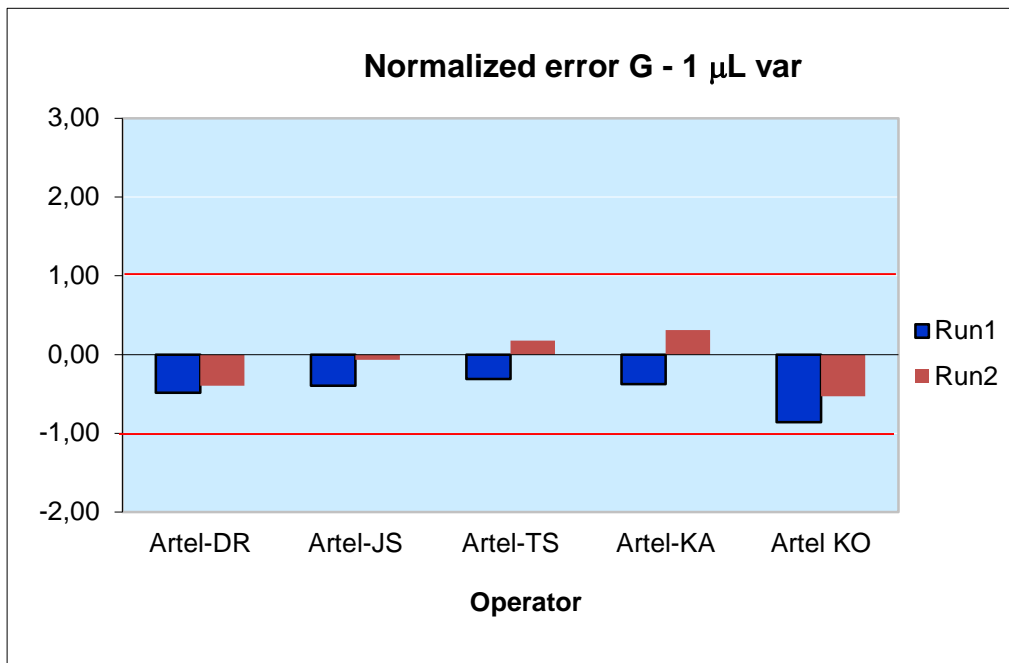


Figure 34-  $E_n$  Results 1 µL Photometric

From the pictures above it can be verified that all operators are consistent with IPQ.

*0,2 µL volume measurements*

The volume measurements obtained by IPQ and by Artel operators are presented in the following figures:

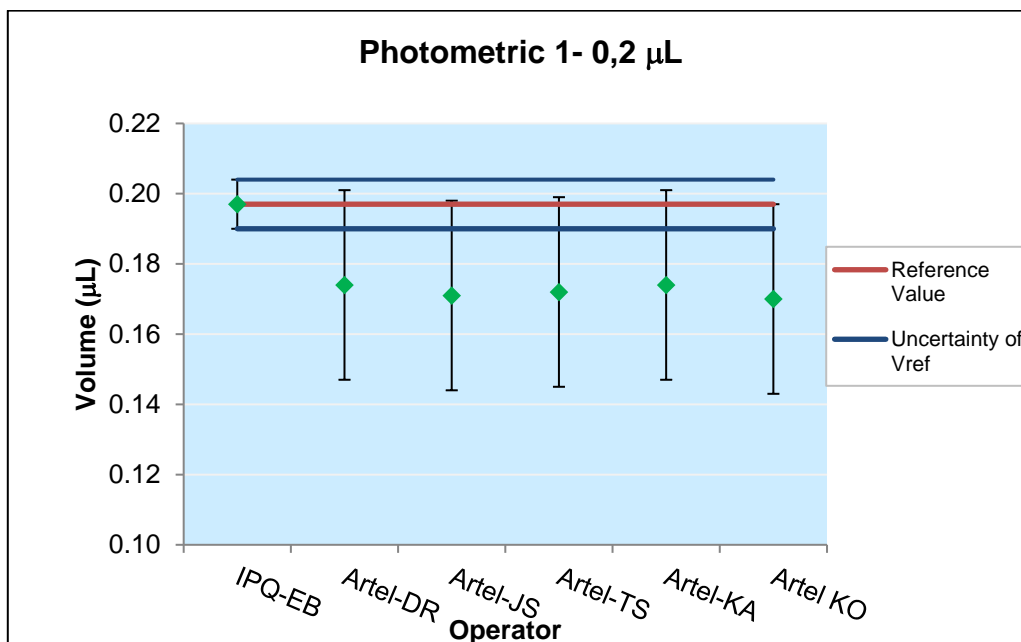


Figure 35- Results 0,2 µL, run 1

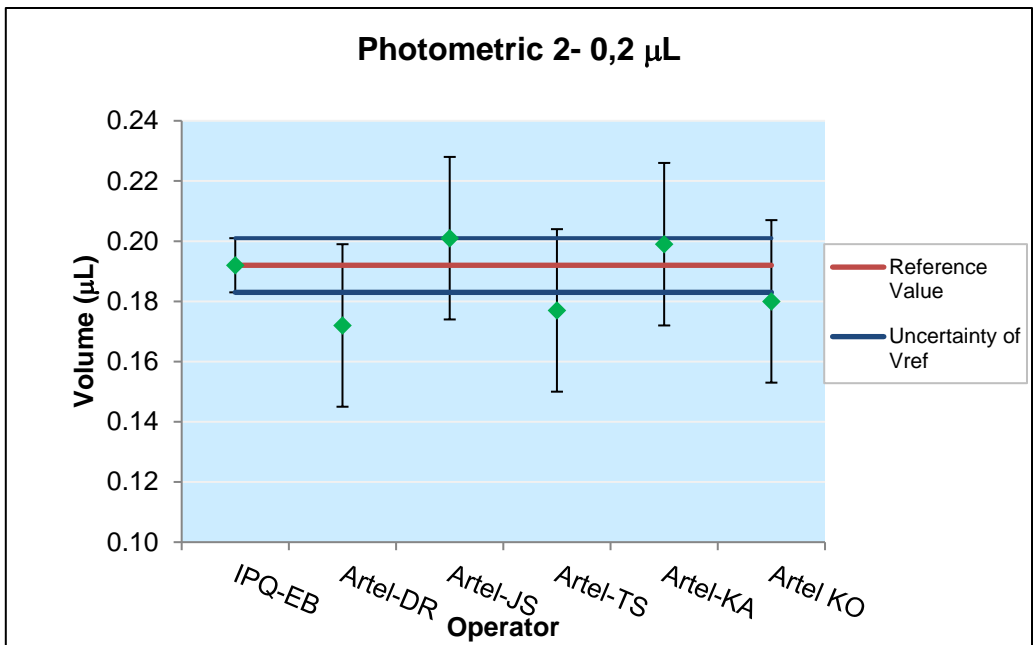


Figure 36- Results 0,2 µL, run 2

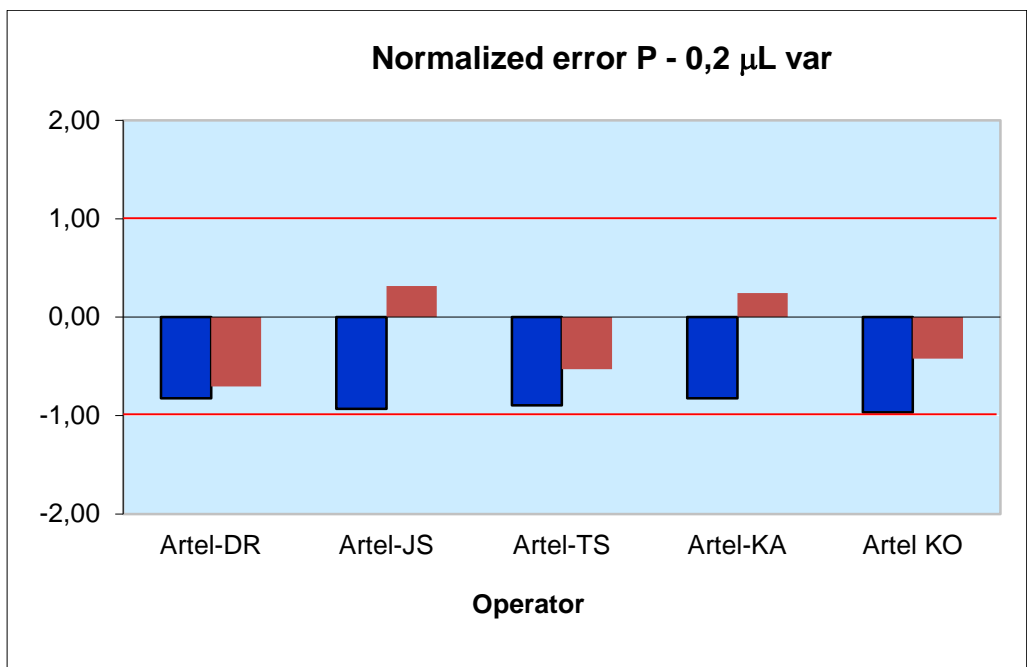


Figure 37-  $E_n$  Results 0,2 µL Photometric

From the pictures above it can be verified that all Artel operators are consistent with IPQ.

*0,1 µL volume measurements*

The volume measurements obtained by IPQ and by Artel operators are presented in the following figures, only one run was performed:

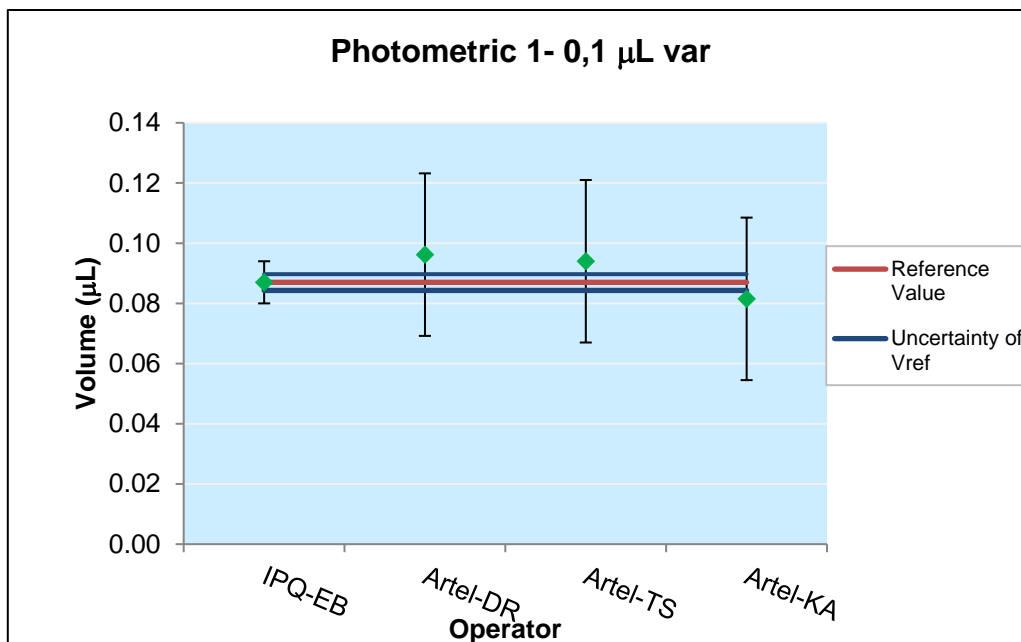


Figure 38- Results 0,1 µL

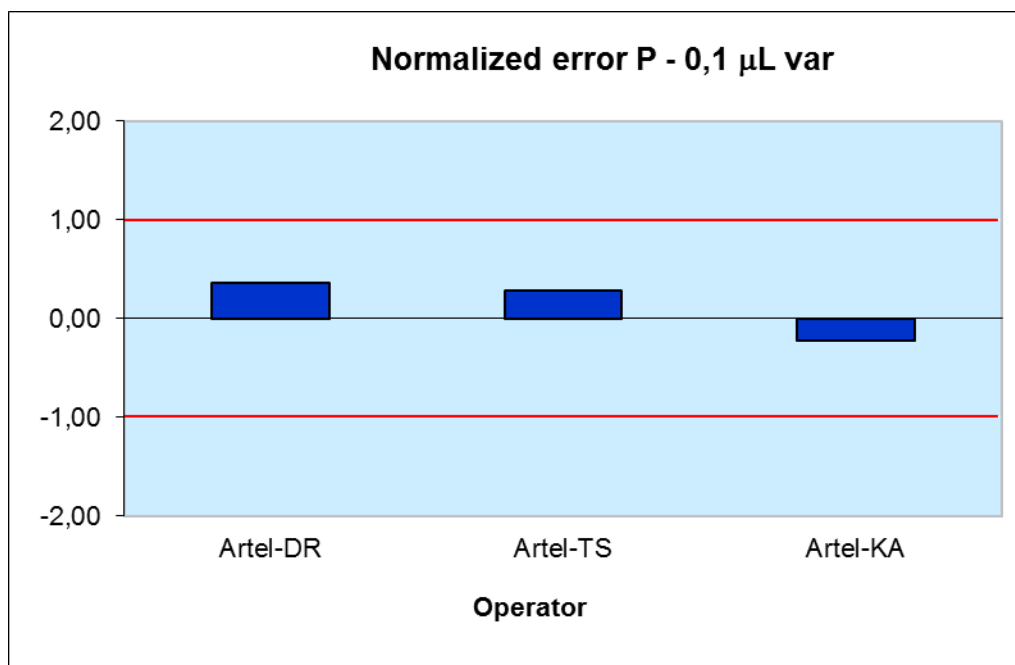


Figure 39-  $E_n$  Results 0,1 µL Photometric

From the pictures above it can be verified that all Artel operators are consistent with IPQ.

## 7. Agreement of results

All the results from Artel were analyzed and compiled in figure 40, which describes the percentage of consistent results for each method at each volume measured of the total runs and operators.



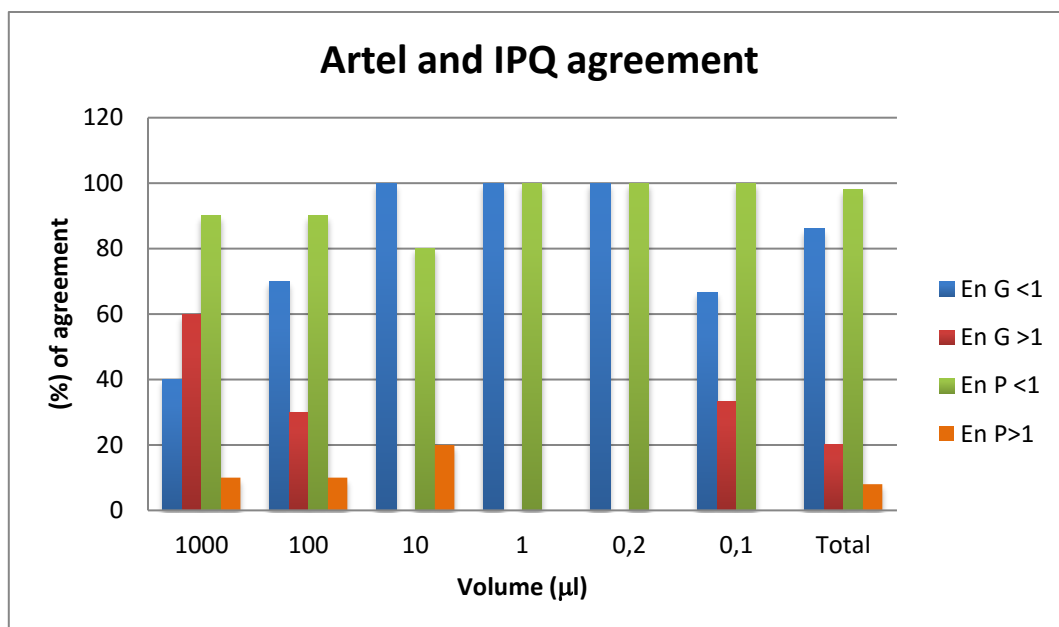


Figure 40- Agreement between laboratories

It can be verified from the figure that the photometric method yields 98 % consistency in the results. For the gravimetric method the value is 86 % which means that the majority of the results are consistent, except for the 1000 μL micropipette, where the worst agreement can be found, 40 %.

Also, it was observed that each one of the operators is consistently higher or lower. For example operator KA is consistently lower than the rest of Artel team and very consistent with IPQ-EB operator. One reason for that might be that the method of delivery and other bias methodology for liquid delivery are similar for both operators, mainly the strength necessary for descending the piston of the micropipette.

The variability of results for large volumes found among the operators reflects the need to include an operator-to-operator standard deviation in the calibration uncertainty and calibration measurement capability.

## 8. Method comparison

The average results on the two runs from IPQ and Artel using both gravimetric and photometric method were compared for each volume based on the  $E_n$  determination, considering the gravimetric method of each laboratory results as reference. For IPQ the results are represented in figure 41, for Artel the results are represented at figure 42.

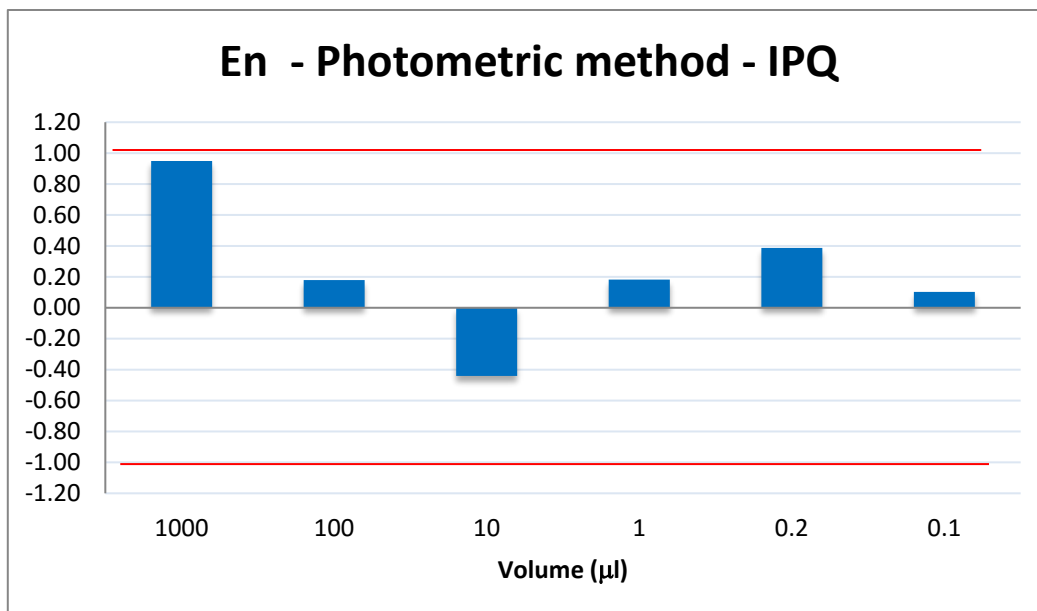


Figure 41- Agreement between methods, IPQ

From the figure above it can be verified that all the results obtain by IPQ using the photometric method are consistent with the ones obtained with the gravimetric method.

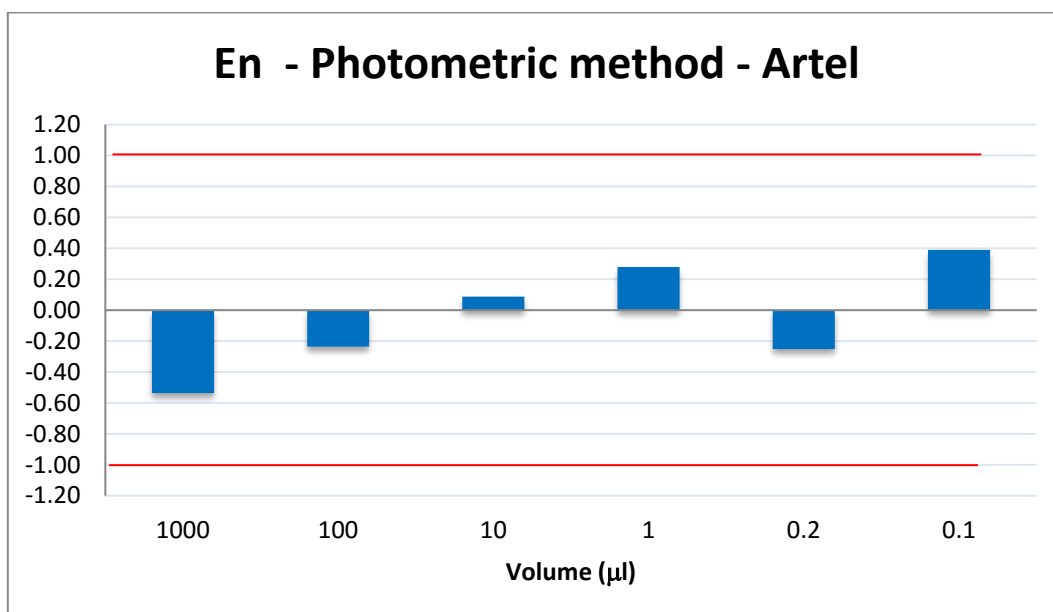


Figure 42- Agreement between methods, Artel

From the figure above it can be verified that all the results obtain by Artel using the photometric method are consistent with the ones obtained by the gravimetric method.

## 9. Uncertainty determination

Both laboratories determined the expanded uncertainty for each method according to the GUM – guide to the expression of measurement uncertainty [5].

## 9.1 Gravimetric method

### 9.1.1 - IPQ

The sources of uncertainty used by IPQ regarding the gravimetric method are [6]:

- Water temperature
- Water density
- Air density
- Mass pieces density
- Cubic thermal expansion coefficient of the material of the instrument under calibration
- Reading of the meniscus
- Evaporation
- Mass
- Measurement repeatability

Depending on the determined volume there are two main sources of uncertainty: the repeatability for 1000  $\mu\text{L}$  to 100  $\mu\text{L}$ , and the mass for values lower than 100  $\mu\text{L}$ .

The CMC declared by IPQ for this method from 20 mL to 1  $\mu\text{L}$  is an uncertainty of 0,1 % to 0,3 %.

### 9.1.2 - Artel

The sources of uncertainty used by Artel regarding the gravimetric method are:

- Calibrated mass weights
- Balance repeatability and reproducibility
- Balance resolution
- Evaporation
- Z factor
- UUT imprecision
- Measurement repeatability and reproducibility

## 9.2 Photometric method

### 9.2.1 - IPQ

Regarding the photometric method only two uncertainty sources were considered. One obtained from the manufacturer specification of the PCS3 (reagents, resolution, instrument), and another from the repeatability of the measurements.

Depending on volume to be calibrated, different samples solutions can be used and this fact will be reflected on the standard uncertainty of the instrument.

Range 1 - 200  $\mu\text{L}$  to 5000  $\mu\text{L}$  with 0,19 % standard uncertainty

Range 2 - 50  $\mu\text{L}$  to 199  $\mu\text{L}$  with 0,26 % standard uncertainty

Range 3 - 10  $\mu\text{L}$  to 49  $\mu\text{L}$  with 0,21 % standard uncertainty  
Range 4 - 2  $\mu\text{L}$  to 9  $\mu\text{L}$  with 0,21 % standard uncertainty  
Range 5 - 0,5  $\mu\text{L}$  to 1,9  $\mu\text{L}$  with 0,21 % standard uncertainty  
Range 6 - 0,1  $\mu\text{L}$  to 0,49  $\mu\text{L}$  with 0,23 % standard uncertainty

For the photometric method the largest source of uncertainty depends also on the range used. For small volumes repeatability will be the largest contribution but for large volumes, bigger than 100  $\mu\text{L}$  the instrument will be the most significant source of uncertainty. This is the opposite of what happens with the gravimetric method.

### 9.2.2 - Artel

#### Instrument Uncertainty

- wavelength uncertainty at 520 nm and 730 nm
- air zero uncertainty at 520 nm and 730 nm
- glass uncertainty
- imprecision of measurement at 520 nm and 730 nm
- system linearity
- temperature
- mixing
- instrument resolution

#### Reagent Uncertainty

- blue & red dye absorbance
- stability
- blank volume
- glass

#### UUT Imprecision

Depending on the volume to be calibrated, different samples solutions can be used and this will reflect on the standard uncertainty of the instrument.

Range 1 - 200  $\mu\text{L}$  to 5000  $\mu\text{L}$  with 0,19 % standard uncertainty  
Range 2 - 50  $\mu\text{L}$  to 199  $\mu\text{L}$  with 0,26 % standard uncertainty  
Range 3 - 10  $\mu\text{L}$  to 49  $\mu\text{L}$  with 0,21 % standard uncertainty  
Range 4 - 2  $\mu\text{L}$  to 9  $\mu\text{L}$  with 0,21 % standard uncertainty  
Range 5 - 0,5  $\mu\text{L}$  to 1,9  $\mu\text{L}$  with 0,21 % standard uncertainty  
Range 6 - 0,1  $\mu\text{L}$  to 0,49  $\mu\text{L}$  with 0,23 % standard uncertainty

## **10. Conclusions**

This bilateral comparison between IPQ and Artel comprised the calibration of four different micropipettes in five volume points, IPQ acting as the pilot laboratory determined the reference value.

The volume results obtained by Artel are 86 % consistent with the reference value for the gravimetric method and 98 % consistent for the photometric method.

The values obtained for higher volumes had the most percentage of inconsistent results, this may be due to a larger operator effect or the balance characteristics.

The value obtained for the expanded uncertainty for the 1000  $\mu\text{L}$ , 100  $\mu\text{L}$  and 10  $\mu\text{L}$  volumes is quite similar in both laboratories however for the smaller volumes the uncertainty of the reference value in both methods is smaller than Artel claims.

The uncertainty component that has a major contribution to the final uncertainty depends on the volume determined. In the photometric method for small volumes the repeatability will be the largest uncertainty component but for large volumes, bigger than 100  $\mu\text{L}$ , the instrument will be the most significant source of uncertainty. This is the opposite of the results obtained by the gravimetric method. Conclusion the best method to be used for volumes smaller than 100  $\mu\text{L}$  is the photometric method.

The variability found between the operators for large volumes reflects the need to include the operator-to-operator standard deviation in the calibration uncertainty and calibration measurement capability.

In this report it was also determined the equivalence of results for both laboratories between the gravimetric method and the photometric method. The results obtained for all micropipettes for both laboratories had an  $E_n$  value lower than 1.

One of the goals of this comparison was to validate a new set of CMCs submitted by IPQ in the future regarding the photometric method from the range of 1000  $\mu\text{L}$  to 0,1  $\mu\text{L}$ , with an uncertainty range from 0,4 % to 2,7 % and based on the results obtained and described in this report this goal was achieved.

## 11. References

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