Instituto Português da Qualidade

In house verification of piston pipettes

In House Verification of Piston Pipettes

A handy Metrological Approach in the COVID-19 Crisis

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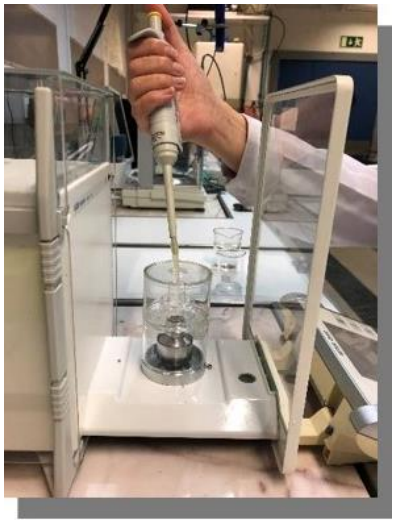
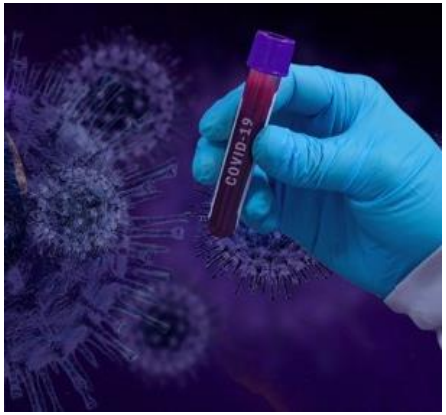


Introduction

The unprecedented conditions public health institutions experience due to COVID-19 pandemic crisis have forced hospital laboratories to ***perform on a daily basis a vast amount of diagnostic tests using pipettes for the handling of the samples***.



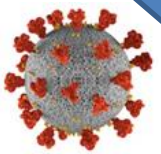
COVID-19 situation

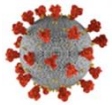
Correct measurement of sample volume depends on the calibration status of the pipette

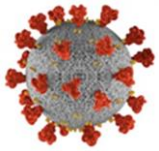
In pandemic situations the analytical laboratories may not have the time to send the pipettes to a calibration laboratory

COVID-19 situation

Operational status of the pipette non verified by Calibration ?







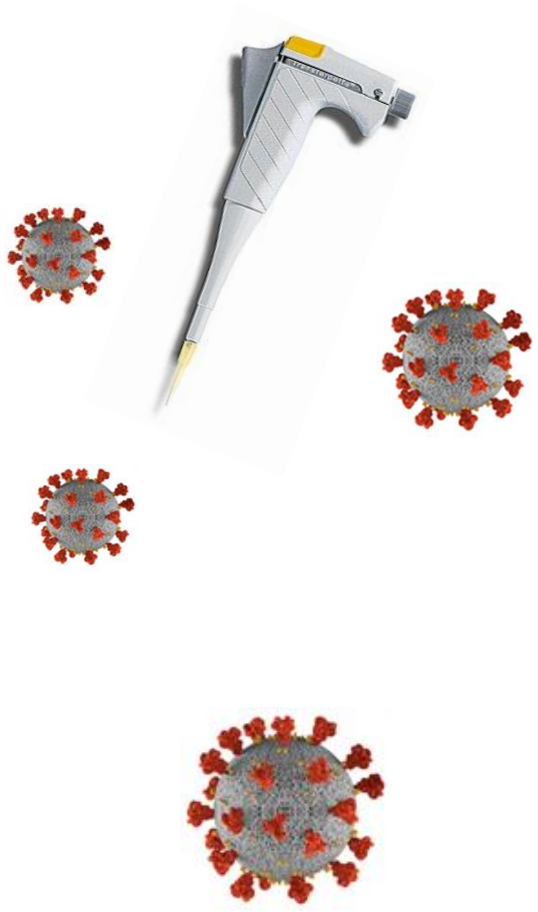
Diagnostic test result may not be reliable

TRACING THE INFECTION

PROBABILITY OF FALSE POSITIVE
VS
FALSE NEGATIVE RESULTS

COVID-19 situation

This document intends to provide hospital laboratories with a brief, handy "*Rescue Manual*" for performing a quick *in-house verification* of the measuring status of pipettes in order to identify the ones which do not perform safely within specifications



Calibration[1] of piston operated pipettes

Routine calibration at 3 points:

- *nominal volume*
- *50 % of the nominal volume*
- *10 % of the nominal volume*

In house verification (COVID-19 emergency)

- One point (nominal volume)

Method: Gravimetric

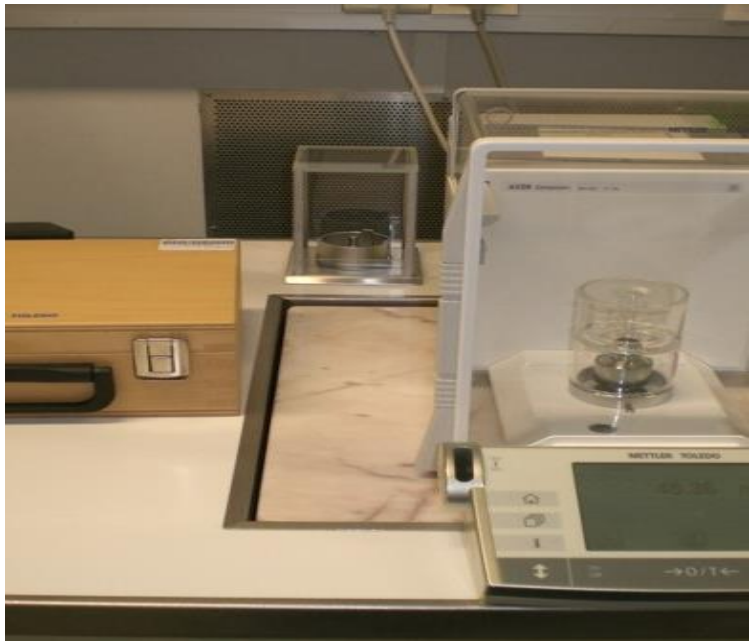
- A good analytical balance is needed

[1]*ISO 8655 - 1 to 7:2002 – Piston-operated volumetric apparatus*

Principle of Gravimetric Method

A COVID-19 EMERGENCY APPROACH

"The Mass of water delivered by the pipette is measured"



Equipment

Recommended Balance characteristics *

Nominal Delivered Volume	Resolution (mg)	Repeatibility (mg)	Standard uncertainty (mg)
1 µL < V < 10 µL	0,001	0,002	0,002
10 µL < V ≤ 100 µL	0,01	0,02	0,02
100 µL < V ≤ 10 mL	0,1	0,2	0,2
10 mL< V ≤ 200 mL	1	2	2

* *To be considered necessary*

Equipment

Thermometer 1
Measurement of the liquid temperature in order to determine its density.

Thermometer 2
Measurement of the air temperature

Barometer *
Measurement of the atmospheric pressure

Weighing vessel
For the weighing of the water mass preferably with a lid to minimize evaporation

* *If not available, ask for the current local weather report*

General Techniques

Calibration Liquid

The calibration liquid is normally distilled or de-ionized water

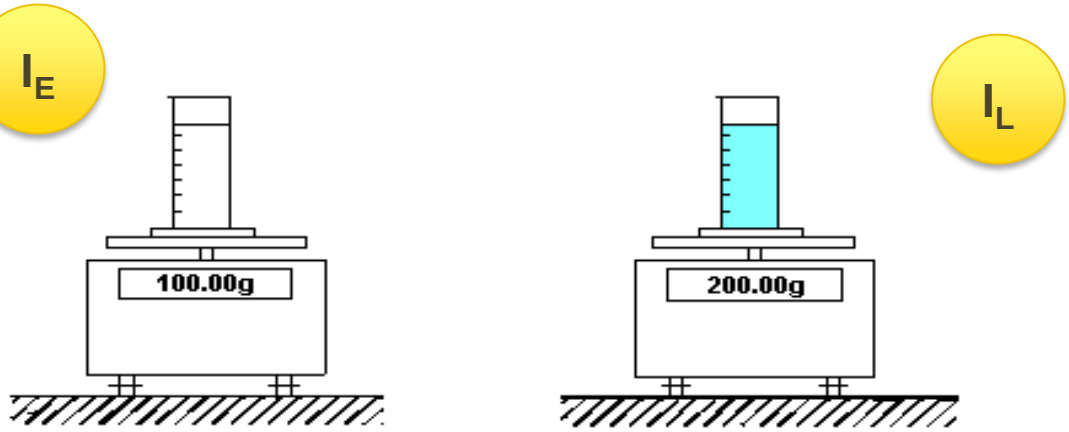
Temperature stabilisation

It is recommended that the piston apparatus and the liquid used in calibration are placed near the balance for 2 hours before calibration

Ambient conditions

It is recommended to control the ambient conditions mainly be as close as possible to 20 ºC and have a humidity larger than 50%.

General Procedure



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

Volume Calculation [1]

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

Where:

V_{20} = Volume, at reference temperature equal to 20 º C, in ml
I_L = Balance reading of vessel with water, in g
I_E = Balance reading of empty vessel, in g
ρ_W = Density of water at calibration temperature t in g / ml
ρ_A = Density of air in g / ml
ρ_B = Density of balance weights (8,0 g / ml)
γ = Coefficient of cubic thermal expansion of the material of which the piston operating apparatus is made, 0,00024 /ºC
t =Temperature of water used for calibration in ºC

[1] ISO 4787: 2010 (E)

Volume Calculation – Short version

$$V_{20} = \left(I_L - I_E\right) \times Z \qquad \text{(Eq. 1)}$$

$$Z = \left(\rho_W - \rho_A\right)^{-1} \times \left(1 - \frac{\rho_A}{\rho_B}\right) \times \left[1 - \gamma\left(t - 20\right)\right]$$

To facilitate an easy calculation of the pipette's volume at reference temperature, V_{20} , using Eq. 1, **the factor Z** has been calculated for polypropylene at common air pressure versus temperature (**Table 1**)

Volume Calculation – Z factor

Table 1.

Factor Z for Polypropylene ($\gamma = 240 \times 10^{-6}$)													
T [°C]	Water density	Air Density (for relative humidity 50%)											
		Air Pressure [mbar]											
	[kg/m3]	930	940	950	960	970	980	990	1000	1010	1020	1030	1040
15	999,100	1,00309	1,00310	1,00311	1,00312	1,00313	1,00314	1,00315	1,00316	1,00317	1,00318	1,00319	1,00320
15,5	999,023	1,00304	1,00305	1,00306	1,00307	1,00308	1,00310	1,00311	1,00312	1,00313	1,00314	1,00315	1,00316
16	998,943	1,00300	1,00301	1,00302	1,00303	1,00304	1,00305	1,00306	1,00307	1,00309	1,00310	1,00311	1,00312
16,5	998,860	1,00296	1,00297	1,00298	1,00299	1,00300	1,00301	1,00303	1,00304	1,00305	1,00306	1,00307	1,00308
17	998,775	1,00293	1,00294	1,00295	1,00296	1,00297	1,00298	1,00299	1,00300	1,00301	1,00302	1,00303	1,00304
17,5	998,686	1,00289	1,00290	1,00291	1,00292	1,00293	1,00294	1,00296	1,00297	1,00298	1,00299	1,00300	1,00301
18	998,595	1,00286	1,00287	1,00288	1,00289	1,00290	1,00291	1,00292	1,00294	1,00295	1,00296	1,00297	1,00298
18,5	998,502	1,00283	1,00284	1,00285	1,00287	1,00288	1,00289	1,00290	1,00291	1,00292	1,00293	1,00294	1,00295
19	998,405	1,00281	1,00282	1,00283	1,00284	1,00285	1,00286	1,00287	1,00288	1,00289	1,00290	1,00291	1,00292
19,5	998,306	1,00279	1,00280	1,00281	1,00282	1,00283	1,00284	1,00285	1,00286	1,00287	1,00288	1,00289	1,00290
20	998,204	1,00277	1,00278	1,00279	1,00280	1,00281	1,00282	1,00283	1,00284	1,00285	1,00286	1,00287	1,00288
20,5	998,099	1,00275	1,00276	1,00277	1,00278	1,00279	1,00280	1,00281	1,00282	1,00283	1,00284	1,00285	1,00286
21	997,992	1,00274	1,00275	1,00276	1,00277	1,00278	1,00279	1,00280	1,00281	1,00282	1,00283	1,00284	1,00285
21,5	997,882	1,00272	1,00273	1,00274	1,00276	1,00277	1,00278	1,00279	1,00280	1,00281	1,00282	1,00283	1,00284
22	997,770	1,00271	1,00273	1,00274	1,00275	1,00276	1,00277	1,00278	1,00279	1,00280	1,00281	1,00282	1,00283
22,5	997,655	1,00271	1,00272	1,00273	1,00274	1,00275	1,00276	1,00277	1,00278	1,00279	1,00280	1,00281	1,00282
23	997,538	1,00270	1,00271	1,00272	1,00274	1,00275	1,00276	1,00277	1,00278	1,00279	1,00280	1,00281	1,00282
23,5	997,418	1,00270	1,00271	1,00272	1,00273	1,00274	1,00275	1,00276	1,00277	1,00279	1,00280	1,00281	1,00282
24	997,296	1,00270	1,00271	1,00272	1,00273	1,00274	1,00275	1,00277	1,00278	1,00279	1,00280	1,00281	1,00282
24,5	997,171	1,00271	1,00272	1,00273	1,00274	1,00275	1,00276	1,00277	1,00278	1,00279	1,00280	1,00281	1,00282
25	997,044	1,00271	1,00272	1,00273	1,00274	1,00275	1,00276	1,00277	1,00278	1,00279	1,00280	1,00282	1,00283

In house verification of micropipettes in 9 simple steps *

1. *Operate the pipette as usually*
2. *Set the pipette volume to be verified*
3. *Measure the water temperature in the water reservoir*
4. *Put the water receiving vessel on the balance and zero the balance*
5. *Aspirate the set volume of water*
6. *Transfer the water to the receiving vessel*
7. *Record balance indication*
8. *Record environmental conditions (Barometric pressure)*
9. ***Use Equation 1 and Table 1 to calculate the volume***

* *But keep in mind Good Laboratory Practices (see Flyer in last slide)*

Analysis of results

The obtained error can be compared with the systematic error of the pipette described in ISO 8655-2 or with the previous error described in the calibration certificate.

Calculate the systematic error e_S of the piston pipette using the formula:

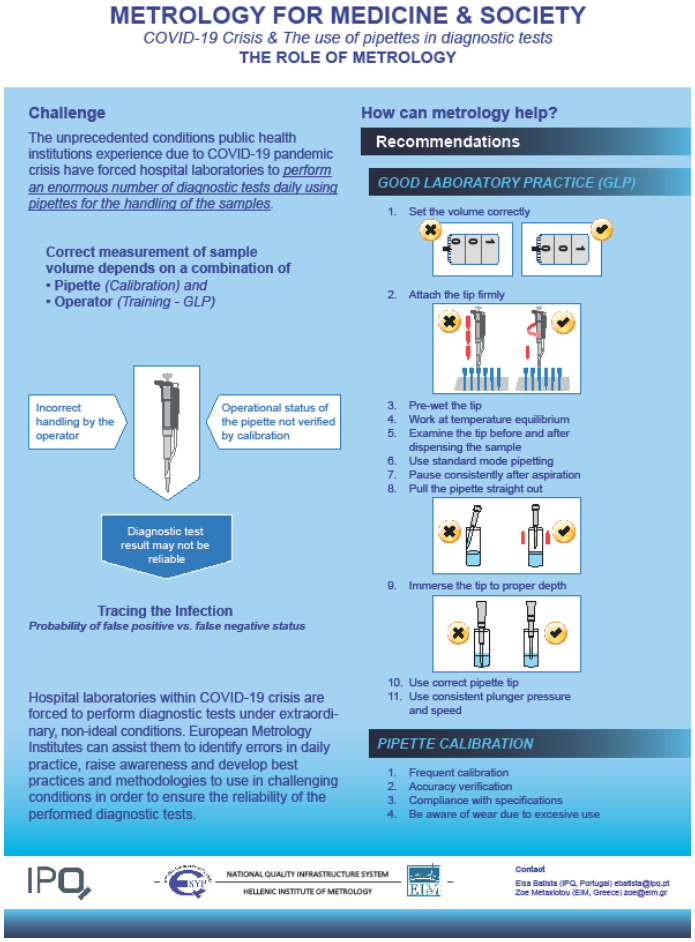
$$e_S = V - \bar{V}$$

Where

e_S is the absolute systematic error, expressed in units of volume;
V_S is the selected test volume at the piston pipette under test;
$\bar{V}$ is the average result of the volume measurements.

The **European National Metrology Institutes** can assist hospital laboratories to identify errors in daily practices, raise awareness and develop best practices and methodologies to use in challenging conditions in order to ensure the reliability of the performed diagnostic tests.

[Watch the video 'Internal verification of piston pipettes']



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