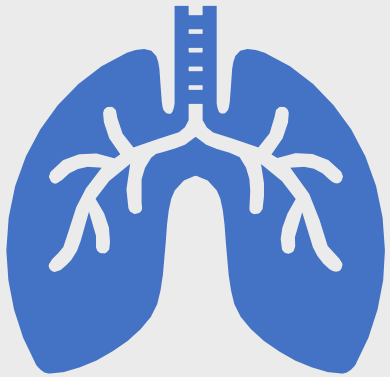


"DEVELOPMENT OF BASIC METROLOGY INFRASTRUCTURE TO SUPPORT MEDICAL TESTING EQUIPMENT (VENTILATORS)"

Calibration of Piston-operated pipettes with air cushion as part of a quality assurance program

USE OF PISTON PIPETTES IN SAR SARS-CoV-2 (COVID-19) DETECTION

1. Quality control of virus detection kits SARS-COVID-19
2. In the different SARS- COVOD 19 virus detection tests:
   a) Molecular (PCR), Active infection
   b) Antigen, active infection
   c) Antibody, Past infection

3. Quality control of drugs for the treatment of COVID-19

Piston pipettes of (0.5-10) µL, (2-20) µL, (20 -200) µL, and (100 µL -1000) µL

Filter tips

They are used in taking samples for direct use or preparation of sample solutions and dilutions

Aim of the calibration

Guideline DKD-R 8-1 (2011

The calibration of piston-operated pipettes with air cushion serves to define the deviation of the dispensed volume from the selected testing volume

The measurement trueness and the measurement uncertainty analysis of the determined measuring results are decisive for the implementation of quality-related metrological specifications in fields such as medicine and pharmacology

Thereby, metrological traceability to national or international standards must be ensured.

Pipette quality control

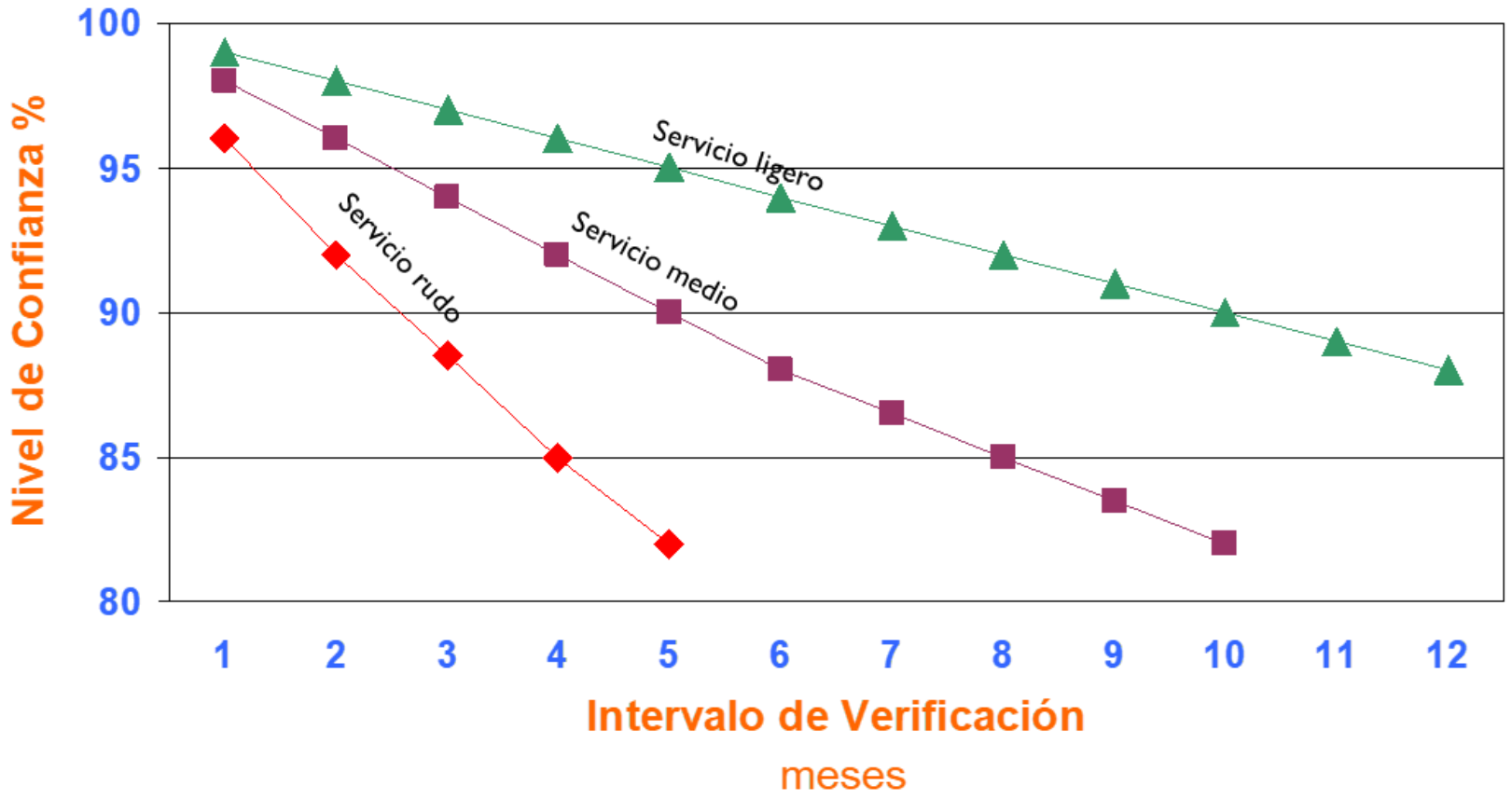
1. Piston pipettes

+

2. Operator:

- Preventive maintenance
- Calibration
- Performance Verification
- Training
- Procedure

Verification frequency for piston pipettes



General requirements for the calibration capability of piston-operated pipettes with air cushion

The general requirements can be divided into three categories:

1. Requirements of standard EN ISO 8655

apparatus, ambient conditions, procedure, etc

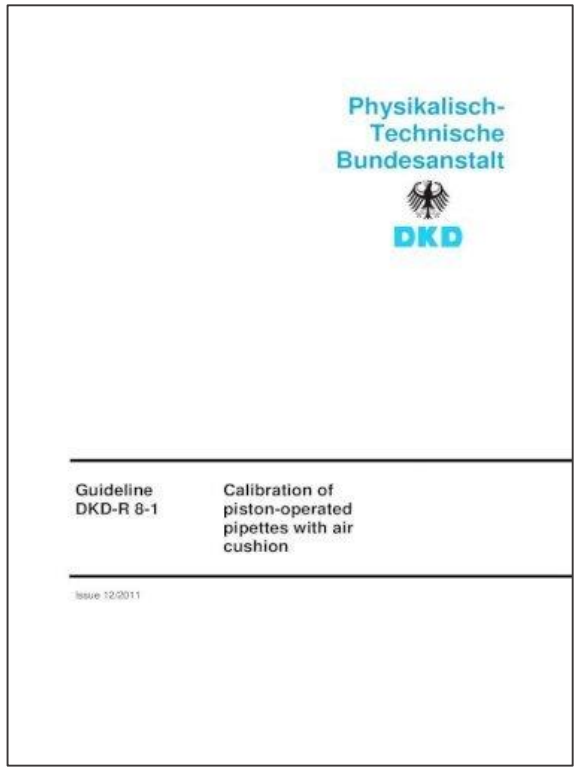
2. Requirements contained in the product information of the manufacturers

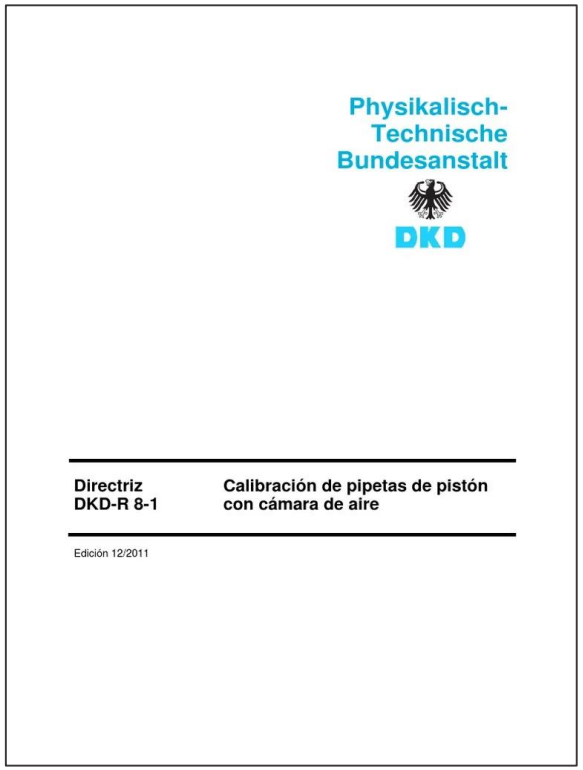
maintenance, cleaning and servicing, Indication of the approved pipette tips to be used, among others.

3. Additional requirements of standard practice

ninternal or external damage, use of original tips, tight and secure sealing, etc.

Additional notes for the calibration process (as a supplement to EN ISO 8655-6)





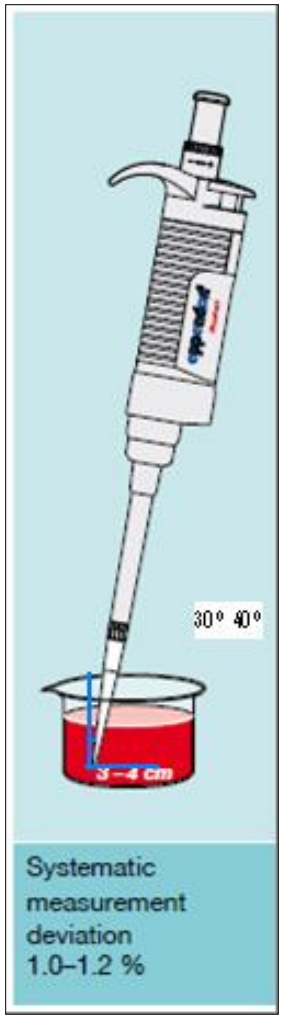
Process-related handling practices

- Pipette's angle of inclination
- Waiting period after the aspiration
- Pipetting frequency
- Immersion depth
- Operating force *
- Reproducibility of the piston stroke
- Hysteresis (variable* pipettes)
- Hand warmth

* not in the case of electronic pipettes

Angle of inclination

hydrostatic pressure of a liquid column is proportional to the cosine of the angle of inclination



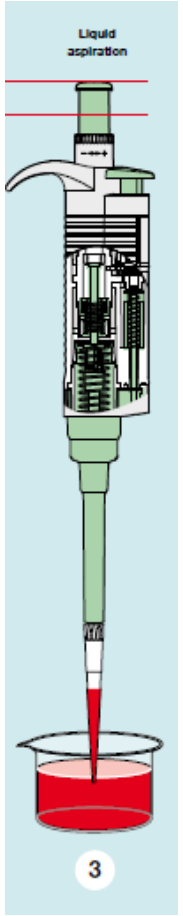
If a pipette is not handled vertically during aspiration, a higher volume is pipetted.

The maximum modification off vertical line should be 10°.

Pipeta 100 µL :
60° inclinacion → incremento 0.53% Vol

Pipeta 1000 µL :
45° inclinacion → incremento 0.29% Vol

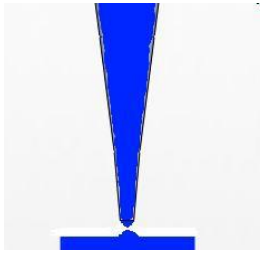
Waiting Period



ISO 8655 defines a waiting period of 1-2 seconds

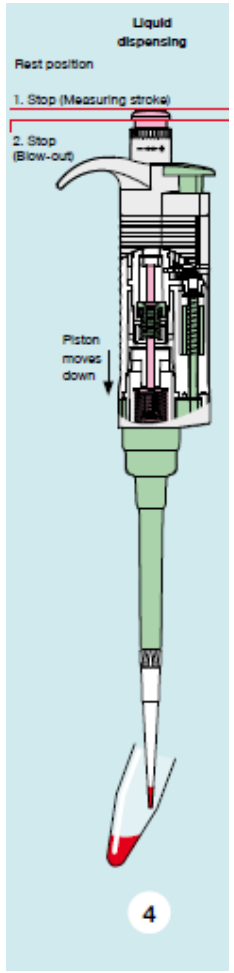
A period of 1 second is recommended for small piston pipettes

Pipettes with a nominal volume greater than 1 000 µL need 3 seconds to make sure that aspiration is completed



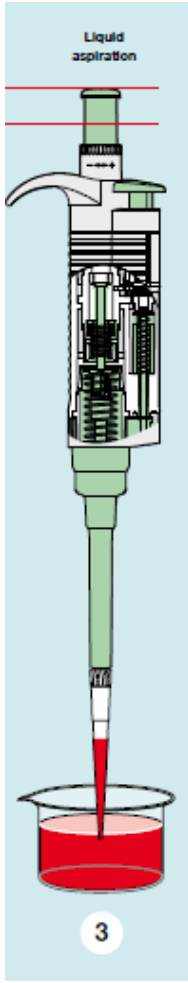
evaporation into the air cushion causes volume decrease

Pipetting Frequency



The time delay between delivery and the next aspiration of the liquid decreases the internal humidity of the pipette

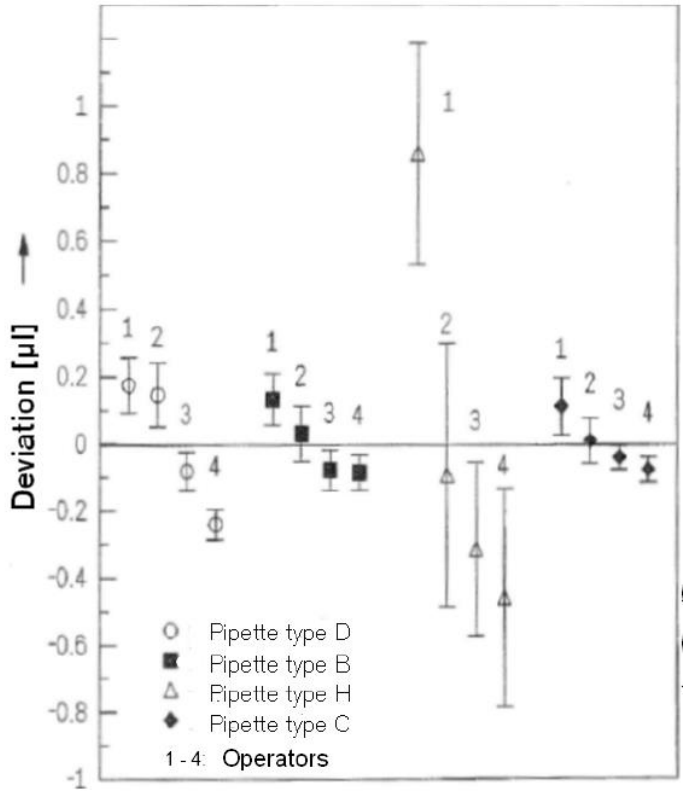
Decreased internal humidity leads to volume decrease in the subsequent pipetting cycle.



Immersion Depth and waiting time

Volumen (µl)	Profundidad inmersión (mm)	Tiempo de espera (s)
0.1-1	1	1
>1-100	2	1
>100-1000	3	1
>1000	4	3

Operational Force



It has been experienced that well-trained employees reach more even results (lower standard deviation of random error) than untrained employees

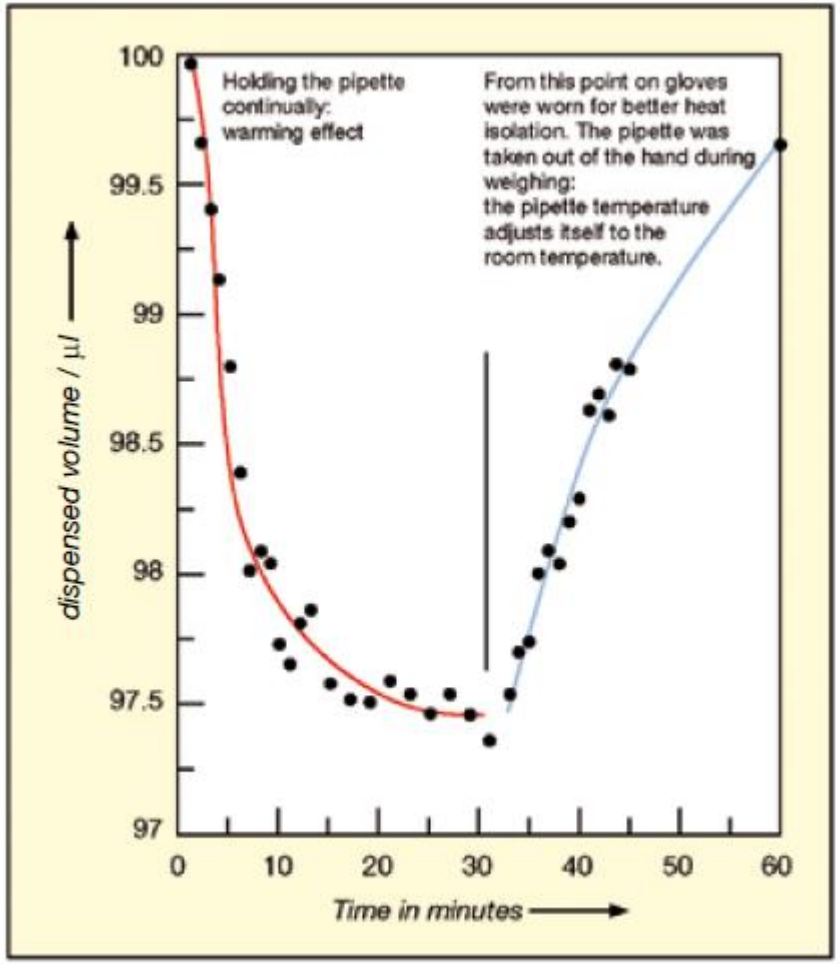
Results of 4 operators using 4 pipette types.
Volume deviation caused by the variation of operational force from person to person.

Counter Hysteresis

The piston stroke depends on from which direction a value is set on the counter at variable mechanical pipettes. Effect is eliminated by routinely setting value from only one direction

Heat Transfer of Hand Warming

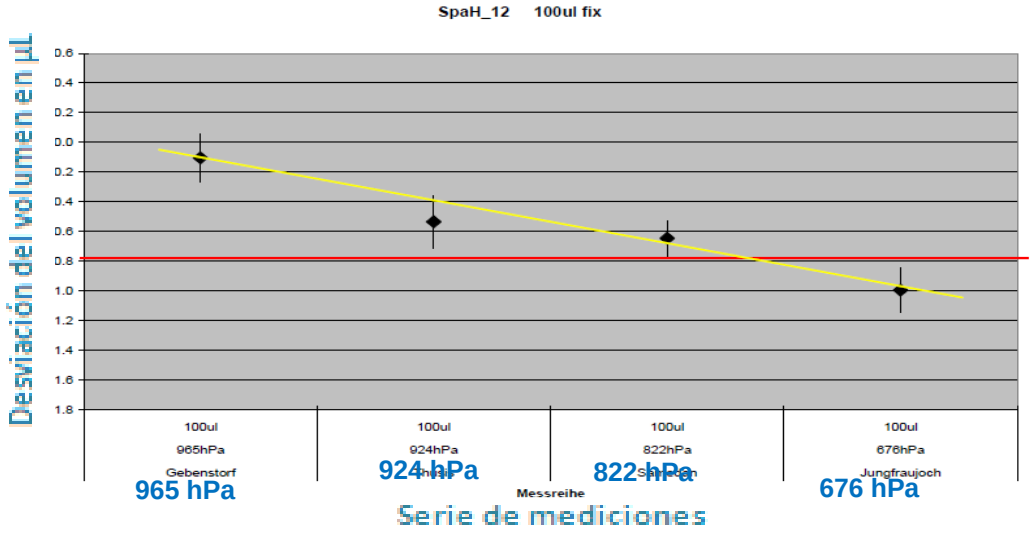
Red line:
Warming effect by holding the pipette continually

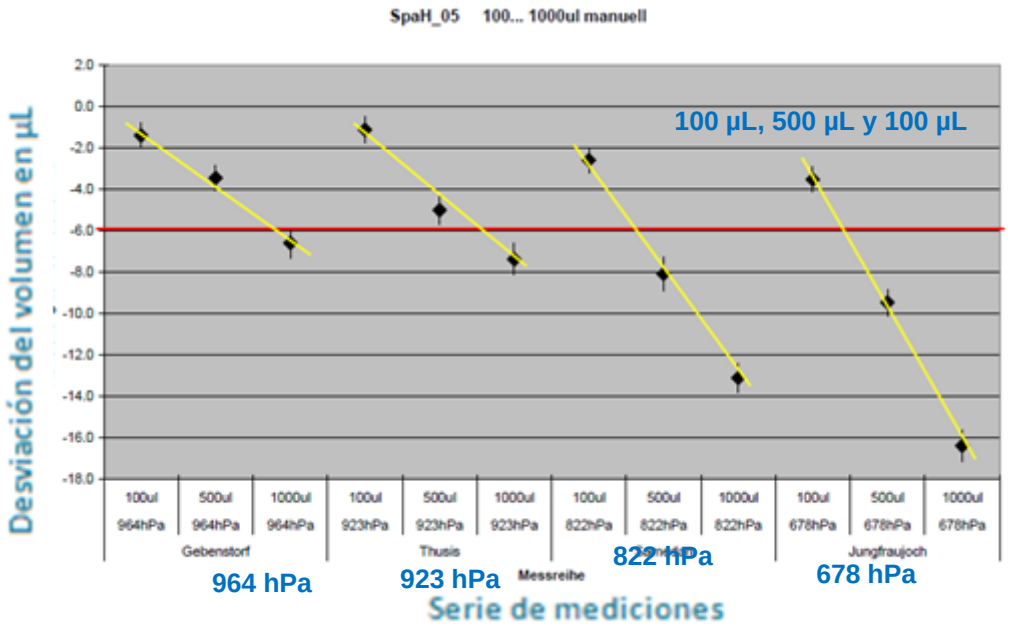


Blue line:
Globes were worn for better heat isolation.

Influence of hand temperature on pipetted volumen with a 100 µL pipette.

Atmospheric pressure - Altitude



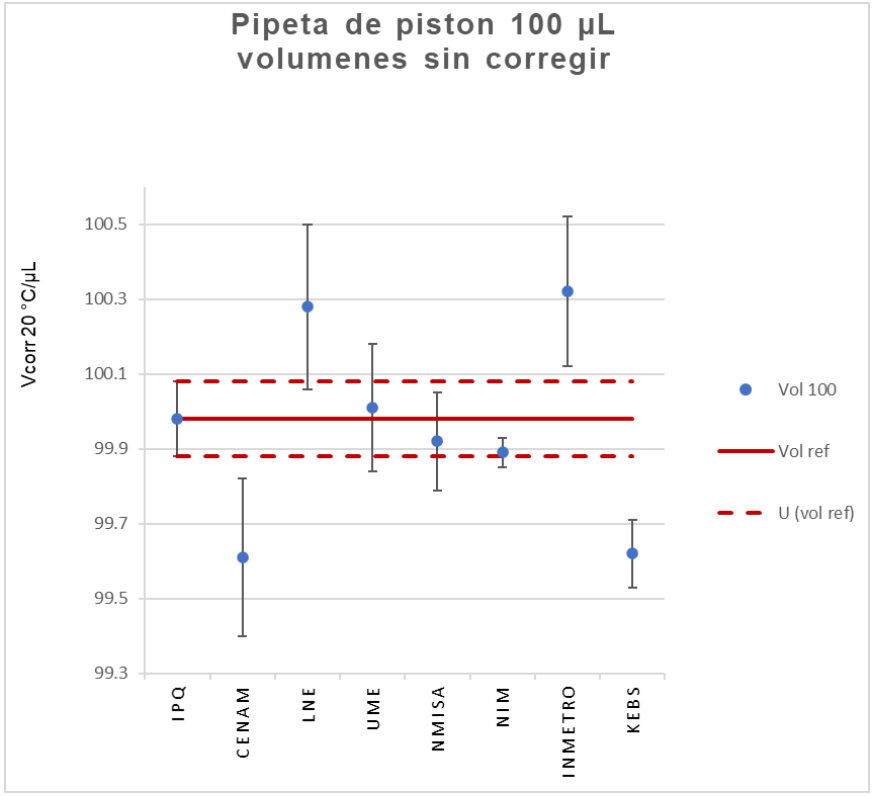


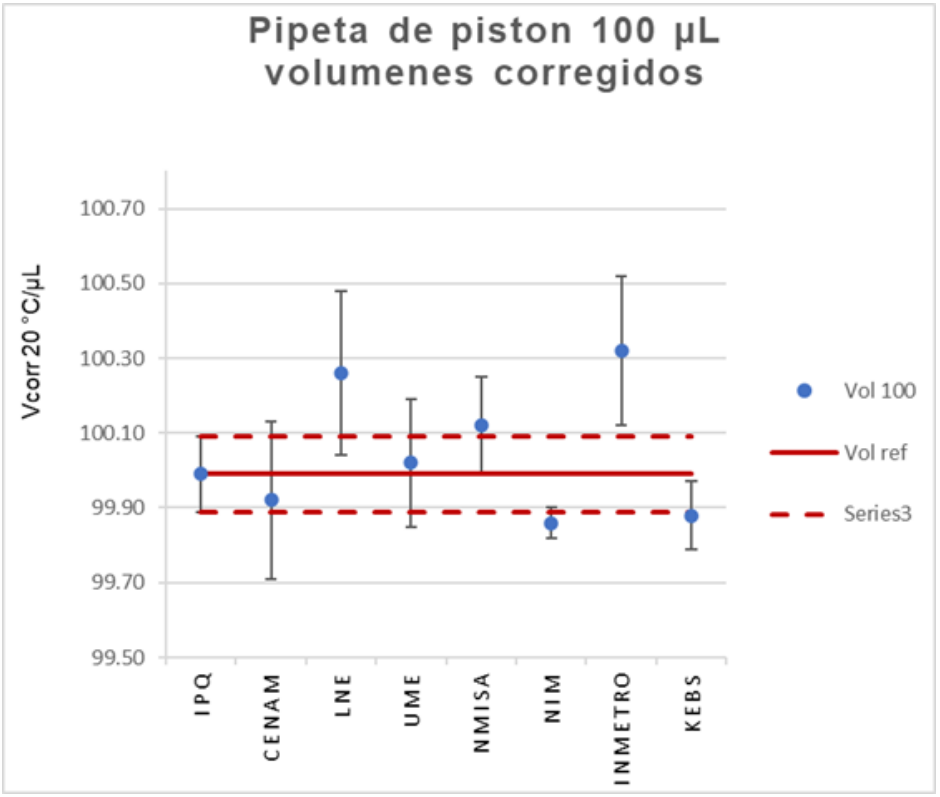
Atmospheric pressure - Altitude

Table 8 – Volume change determination for micropipette 35428Z

NMI	ρ_w/(kg/m^3)	p_{LX2}/Pa	ΔV/µL	V/µL	V_{corr}/µL
IPQ	997,76	100 667	-0,008	99,98	99,99
CENAM	998,664	81 413	-0,312	99,61	99,92
LNE	998,13	102 911	0,020	100,28	100,26
UME	998,31	100 214	-0,014	100,01	100,02
NMISA	998,1955	87 289	-0,205	99,92	100,12
NIM	998,038	104 400	0,037	99,89	99,86
INMETRO	998,204	101 100	-0,003	100,32	100,32
KEBS	997,607	83 950	-0,263	99,62	99,88

Example of results published in the final report of the comparison CCM.FF-K4.2.2011 of piston pipettes where the altitude correction is applied to calculate the volume delivered by the pipette


Pipeta de piston 100 µL
volumenes sin corregir
Vcorr 20 °C/µL
100.5
100.3
100.1
99.9
99.7
99.5
99.3
IPQ
CENAM
LNE
UME
NMISA
NIM
INMETRO
KEBS
Vol 100
Vol ref
U (vol ref)


Pipeta de piston 100 µL
volumenes corregidos
Vcorr 20 °C/µL
100.70
100.50
100.30
100.10
99.90
99.70
99.50
IPQ
CENAM
LNE
UME
NMISA
NIM
INMETRO
KEBS
Vol 100
Vol ref
Series3

Bibliografia

- ISO 8655-2:2002, Piston-operated volumetric apparatus – Parte 2: Piston pipettes.
- ISO 8655-6: 2001 Piston-operated volumetric apparatus – Parte 6: Gravimetric test methods.
- Richard Curtis, Controlling Pipette Performance in Real World, Artel, USA (1999)
- Guideline DKD-R 8-1 Calibration of piston –operated pipettes with air cushion

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