European Directorate for the Quality of Medicines & HealthCare | Direction européenne de la qualité du médicament & soins de santé

Funded by the European Union and the Council of Europe

EUROPEAN UNION

COUNCIL OF EUROPE

CONSEIL DE L'EUROPE

Implemented by the Council of Europe

General European OMCL Network (GEON)
QUALITY MANAGEMENT DOCUMENT

PA/PH/OMCL (09) 64 R5

QUALIFICATION OF EQUIPMENT

ANNEX 6: QUALIFICATION OF PISTON PIPETTES

Full document title and reference	Qualification of Equipment Annex 6: Qualification of piston pipettes *PA/PH/OMCL (09) 64 R5*
Document type	Guideline
Legislative basis	Council Directive 2001/83/EC and 2001/82/EC, as amended
Date of first adoption	May 2010
Date of original entry into force	July 2010
Date of entry into force of revised document	September 2018
Previous titles/other references / last valid version	*PA/PH/OMCL (09) 64 2R*
Custodian Organisation	The present document was elaborated by the OMCL Network / EDQM of the Council of Europe
Concerned Network	GEON

ANNEX 6 OF THE OMCL NETWORK GUIDELINE
"QUALIFICATION OF EQUIPMENT"

QUALIFICATION OF PISTON PIPETTES

Note: Mandatory requirements in this guideline and its annexes are defined using the terms «shall» or «must». The use of «should» indicates a recommendation. For these parts of the text other appropriately justified approaches are acceptable. The term «can» indicates a possibility or an example with non-binding character.

INTRODUCTION

The present document is the 6th Annex of the core document "Qualification of Equipment", and it should be used in combination with it when planning, performing and documenting the qualification process of piston pipettes.

The core document "Qualification of Equipment" contains the Introduction and general forms for Level I and II of qualification, which are common to all type of instruments.

Level III and IV testing must be carried out being an ISO 17025 requirement.

This Guideline has been elaborated according to the norm ISO 8655 (Parts-1,-2 and -6). Requirements and (if applicable) corresponding typical acceptance criteria given in bold should be applied, however other appropriately justified deviations are acceptable provided they are traceable.

Exemplary procedure described in Annex I, refers to the method given in ISO 8655-6 ("Gravimetric method for the determination of the measurement error") which is the most commonly used. Alternative procedures (e.g. "Non-gravimetric method for the estimation of the performance of the equipment" given in ISO 8655-7) can be applied.

AIM AND SCOPE OF THE GUIDELINE

This guideline describes the requirements for piston pipettes used in chemical and biological tests irrespective of the mode of operation (manual or electronical).

Requirements and test procedures are based on the EN ISO 8655.

The following types of pipettes have been considered in this guideline:

1. Fixed volume monochannel pipettes (air-displacement (type A) and positive displacement or direct displacement pipettes (type D))

2. Variable volume monochannel pipettes (air-displacement (type A) and positive displacement or direct displacement pipettes (type D))

3. Fixed volume multichannel pipettes (air-displacement (type A))

4. Variable volume multichannel pipettes (air-displacement (type A))

NOTE: Repetitive piston pipettes are also covered in the guideline as "variable volume monochannel pipettes (positive displacement or direct displacement.

CONSIDERATIONS FOR LEVEL I AND II OF EQUIPMENT QUALIFICATION

It is recommended to:

1) Level I - select a manufacturer of pipettes that can certify its compliance with the requirements of ISO 8655 norm.

2) Level II - check if all requirements set up during the selection are met and all necessary requirements are covered and fulfilled in the provided certificate.

GLOSSARY

Nominal volume (as ISO 8655-1): (piston-operated volumetric apparatus) volume specified by the manufacturer and used for identification and for indication of the measuring range.

NOTE: for a variable-volume piston-operated volumetric apparatus, the nominal volume corresponds to the maximum volume that can be set by the user and that is specified by the manufacturer.

For the other terms refer to JCGM 200:2012 International vocabulary of metrology – Basic and general concepts and associated terms (VIM)

GENERAL CONSIDERATIONS FOR LEVEL III VERIFICATION

The periodic calibration of pipettes should follow a pre-established calibration plan, in the frame of the Quality Management System of the laboratory.

It is recommended to calibrate the pipettes:

- at reception (unless already calibrated by the supplier).
- once a year during the use of the pipette.
- after any maintenance, repair or adjustment.

As described in ISO 8655-1, the laboratory can consider to perform more frequent and regular calibrations depending on:

- workload of the pipette and number of operators using the pipette
- type of liquids used
- maximum permissible errors selected, depending on the type of use and the accuracy required.

If calibration is carried out by a service provider, the laboratory should verify and preferably choose a provider that is accredited in accordance with ISO/IEC 17025 to perform calibrations in accordance with the specifications of ISO 8655.

If the calibration is performed by internal laboratory operators, the appropriate procedure for calibration should be selected. Using the gravimetric procedure, the laboratory should be equipped by suitable balances in relation to the volume of pipettes to be calibrated and calibration should be carried out under controlled environmental conditions (as described in ISO 8655-6). In case the above cannot be fulfilled, the laboratory should consider sub-contracting calibration to an accredited service provider.

Calibration shall be carried out reflecting the routine operations (e.g. operational range of use; e.g. tips used for the calibration of pipettes preferably same type used in daily work; e.g. using same pipetting technique i.e. direct mode or reverse mode).

If the laboratory is working with infectious materials, decontamination (e.g. disassembling) should be done before calibration, according to the instructions provided by the manufacturer.

Environmental Conditions

Ambient conditions (i.e. air temperature, air relative humidity, atmospheric pressure and water temperature) may influence weighing of the dispensed volume and water density (Z factor), therefore the calibration results can be impacted. As a result, calibration must be performed under controlled environmental conditions (i.e. temperature, humidity and pressure) to minimise the evaporation of water, which is critical for pipettes with dispensing volumes lower than 50 µL. Recommendations related environmental conditions are given in Annex I (Settings).

Preliminary checks before calibration

Correct functionality of pipettes shall be checked before calibration, as follows:

1) Function of piston: smooth and positive.
2) Tip holder: no marks or distortions, no liquid residues.
3) Leakage: no drop is formed at the tip after 10 seconds from withdrawing the nominal volume.
4) No internal or external contaminations.

TABLE I

Level III. Periodic and motivated instrument verification

Requirements and related typical acceptance criteria are indicated in bold.

1. FIXED AND VARIABLE VOLUME MONOCHANNEL PIPETTES

1a. Air-displacement pipettes (type A)

Parameter to be checked	***Typical criteria***
maximum permissible systematic error	see Annex I
maximum permissible random error	see Annex I

1b. Positive displacement or direct displacement pipettes (type D)

Parameter to be checked	***Typical tolerance limits***
maximum permissible systematic error	see Annex I
maximum permissible random error	see Annex I

2. FIXED VOLUME AND VARIABLE VOLUME MULTICHANNEL PIPETTES

Air-displacement pipettes (type A)

Parameter to be checked	***Typical tolerance limits***
maximum permissible systematic error	see Annex I
maximum permissible random error	see Annex I

Level IV. Before-use checks

Before routine use, traceability and labelling (e.g. unique identification code, valid calibration date) of the pipettes should be checked.

ANNEX I

Level III. Periodic and motivated instrument checks

In accordance to ISO 8655-6, calibration and verification requirements are fulfilled if the calculated experimental systematic and random errors are within the limits defined in the ISO norm as given below in Tables 4-6. This approach is considered to provide a good metrological estimation of the conformity of pipettes, providing that the conditions given below under "settings" are fulfilled.

GRAVIMETRIC TEST

The principle of the gravimetric method is described in ISO 8655-6. In case of selected volume is lower than 50 µL, the effect of the evaporation of the water used is significant. Therefore the loss of mass should be taken into account in the calculation, by following the instructions given in ISO 8655-6 points 7.2.7. and 7.2.8..

Settings:

- The laboratory where the calibration is performed should be draught free, with a relative humidity ≥ 50 %. In case the volumes selected are lower than 50 µL, an evaporation trap or an open vessel containing water shall be placed inside the balance chamber.
- In addition, the temperature of the laboratory should be between 15° and 30°C and stable within (± 0.5 °C) during calibration.
- All the material needed for the test (e.g. glassware, water, etc.) must be equilibrated for at least 2 hours in the room where the test is performed.

Materials:

- Analytical balance of appropriate resolution should be used depending on the selected volume of the pipette under test (according to ISO 8655-6), as given below in Table 2

Table 2

Selected volume of the pipette	**Resolution**
1 µl to 10 µl	0.001 mg
> 10 µl to 100 µl	0.01 mg
>100 µl	0.1 mg

- Thermometer with a maximum standard uncertainty of 0.2 °C or better.
- Barometer with a maximum standard uncertainty of 0.5 kPa.
- Alternatively, seek information at the local meteorological station or website of the region. Note: it is important to take into account that the air pressure in an air-conditioned laboratory might be significantly different *versus* the external, so barometric reading from a meteorological station may be misleading.
- Hygrometer with a maximum standard uncertainty of 10%.
- Weighing vessel of suitable size filled (height / diameter ratio of at least 3:1), with purified water to a depth of at least 3 mm.

- A reservoir vessel with purified water (minimum quality level 3, see norm ISO 3696) to an excess that allows performing all tests e.g. greater than 10 times the dispensed volume.
- Tips appropriate for the pipette to be calibrated.

Method:

1) Selection of volumes:

- Variable volume pipettes are tested at 3 different volumes, each with 10 replicates:
  - the nominal volume
  - 50 % of the nominal volume
  - the lower limit of the useful volume range or 10 % of the nominal volume (whichever is greater)

- Each channel of multi-channel pipettes is checked separately with 3 different volumes, each with 10 replicates:
  - the nominal volume
  - 50 % of the nominal volume
  - 10 % of the nominal volume

- For repetitive piston pipettes the nominal volume is depending on the tips used. Thus, these pipettes shall be calibrated with the tips routinely used. If other tips are used, accuracy and precision may be assured by the use of tips with a manufacturer's certificate confirming compliance with ISO 8655 requirements, or the calibration must be repeated with all types of tips used. The calibration shall be carried out by selecting the lowest, intermediate and the higher dispensed volume of the aliquots. For each selected volume 10 replicates should be made, for a total of 12 replicates considering that the first and the last ones should be discarded.
  The laboratory should document if different volumes than the above specified are chosen for calibration (e.g. in case of specific operational needs).

2) Measure the temperature of the test liquid and atmospheric pressure at beginning of the calibration.
3) Place the appropriate tip on the pipette.
4) For variable volume pipettes select the volume to be tested.
5) Dip the pipette into the test liquid
6) For air-displacement pipettes, fill the pipette at least 5 times with the test liquid and discard the fillings (pre-wetting).
7) Hold the pipette vertically.
8) Before withdrawing the liquid, dip the tip below the surface to be immersed for 2-3 mm in the test liquid.
9) During liquid withdrawing, push the plunger smoothly until coming to rest with a light and consistent force at the first stop and then release the plunger at a constant rate.
10) Dispense the volume slowly and evenly.
11) Pull the pipette tip slowly out of the liquid, wiping it on the vessel wall.
12) Determine the tare mass (reset balance).
13) Deliver the test liquid slowly to the weighing vessel.
14) When delivering the test liquid into the weighing vessel, touch with the tip against the recipient wall near the liquid surface, holding the pipette at an angle of 30-45°, sliding up the tip in the wall over 8-10 mm before releasing the plunger. After withdrawing and before removing the tip from the liquid, pause for 1 s.

15) Record the weighted mass.
16) Repeat withdrawing and dispensing of the test liquid 9 times.
17) Measure the temperature of the test liquid at the end of calibration.

NOTES:

1. When performing the calibration tests, the pipette tips can be changed as frequently as considered necessary. It should be kept in mind to pre-wet every new tip.
   This procedure can be adapted depending on manufacturer's instructions and technical specifications for different types of pipettes and tips.

1. To minimize handling of the pipette and tip, hold the pipette loosely. The heat transfer from hands and pipettes and/or tips may affect the volumes delivered.

Calculations:

- Calculate the mean temperature *t* of the test liquid (rounded to the nearest 0.5 °C)

$$t = \frac{t_1 + t_2}{2}$$

t mean temperature
t_1 temperature before the first weighing
t_2 temperature after the last weighing

- Use the barometric pressure B and mean temperature *t* to find the corresponding Z-factor from the Table 3 below

- Calculate the volume V_i in µl from the individual masses m_i.

$$V_i = Z \times m_i$$

V_i individual volume (calculated)
m_i weight of the individual volume

- Calculate the mean volume $\overline{V}$ from the series of volumes.

$$\overline{V} = \frac{\sum V_i}{n}$$

$\overline{V}$ mean of the individual volumes from the series
V_i individual volume (calculated)
n number of weighings in the series

- Calculate the systematic error (accuracy) *e*, which is the difference between the mean volume of actual measurements and the true value as specified by the selected volume V_s. Accuracy is expressed in µL.

$$e = \overline{V} - V_s$$

e accuracy
$\overline{V}$ mean of the individual volumes from the series
V_s selected volume

Systematic error can be also expressed as percentage %

$$e_s = 100(\overline{V} - V_s)/V_s$$

- Calculate the random error (repeatability) *s*, which quantifies the scattering of individual weighing of dispensed volumes as absolute value (µL). Random error can be also expressed as percentage % by the variation coefficient (CV). Formulas are provided below:

$$s = \sqrt{\frac{\sum (V_i - \overline{V})^2}{n-1}}$$

s repeatability
Vi individual volume (calculated)
$\overline{V}$ mean of the individual volumes from the series
n number of weighings in the series

$$CV = \frac{100 \times s}{\overline{V}}$$

CV variation coefficient

Limits:

The limits for the maximum permissible systematic error and the maximum permissible random error are given in Tables 4-6 for the different types of pipettes. They are applicable to pipettes used for quantitative determinations.
The laboratory may specify limits notwithstanding the limits given in Tables 4-6 in order to meet the laboratory's requirements or manufacturer's specifications. For the definition of these differing limits the uncertainty of measurement should be taken into consideration. In this case, the limits defined must be justified and must take into account the use of each pipette

Table 3

t (°C)	*B* (kPa)	80	85	90	95	100	101.3	105
					Z (µL/mg)			
15.0		1.0017	1.0018	1.0019	1.0019	1.0020	1.0020	1.0020
15.5		1.0018	1.0019	1.0019	1.0020	1.0020	1.0021	1.0021
16.0		1.0019	1.0020	1.0020	1.0021	1.0021	1.0021	1.0022
16.5		1.0020	1.0020	1.0021	1.0021	1.0022	1.0022	1.0022
17.0		1.0021	1.0021	1.0022	1.0022	1.0023	1.0023	1.0023
17.5		1.0022	1.0022	1.0023	1.0023	1.0024	1.0024	1.0024
18.0		1.0022	1.0023	1.0023	1.0024	1.0025	1.0025	1.0025
18.5		1.0023	1.0024	1.0024	1.0025	1.0025	1.0026	1.0026
19.0		1.0024	1.0025	1.0025	1.0026	1.0026	1.0027	1.0027
19.5		1.0025	1.0026	1.0026	1.0027	1.0027	1.0028	1.0028
20.0		1.0026	1.0027	1.0027	1.0028	1.0028	1.0029	1.0029
20.5		1.0027	1.0028	1.0028	1.0029	1.0029	1.0030	1.0030
21.0		1.0028	1.0029	1.0029	1.0030	1.0031	1.0031	1.0031
21.5		1.0030	1.0030	1.0031	1.0031	1.0032	1.0032	1.0032
22.0		1.0031	1.0031	1.0032	1.0032	1.0033	1.0033	1.0033
22.5		1.0032	1.0032	1.0033	1.0033	1.0034	1.0034	1.0034
23.0		1.0033	1.0033	1.0034	1.0034	1.0035	1.0035	1.0036
23.5		1.0034	1.0035	1.0035	1.0036	1.0036	1.0036	1.0037
24.0		1.0035	1.0036	1.0036	1.0037	1.0037	1.0038	1.0038
24.5		1.0037	1.0037	1.0038	1.0038	1.0039	1.0039	1.0039
25.0		1.0038	1.0038	1.0039	1.0039	1.0040	1.0040	1.0040
25.5		1.0039	1.0040	1.0040	1.0041	1.0041	1.0041	1.0042
26.0		1.0040	1.0041	1.0041	1.0042	1.0042	1.0043	1.0043
26.5		1.0042	1.0042	1.0043	1.0043	1.0044	1.0044	1.0044
27.0		1.0043	1.0044	1.0044	1.0045	1.0045	1.0045	1.0046
27.5		1.0045	1.0045	1.0046	1.0046	1.0047	1.0047	1.0047
28.0		1.0046	1.0046	1.0047	1.0047	1.0048	1.0048	1.0048
28.5		1.0047	1.0048	1.0048	1.0049	1.0049	1.0050	1.0050
29.0		1.0049	1.0049	1.0050	1.0050	1.0051	1.0051	1.0051
29.5		1.0050	1.0051	1.0051	1.0052	1.0052	1.0052	1.0053
30.0		1.0052	1.0052	1.0053	1.0053	1.0054	1.0054	1.0054

Table 4

Type A: Fixed and variable volume monochannel pipettes (air-displacement)

nominal volume	**maximum permissible systematic error**		**maximum permissible random error**	
[µl]	[%]	[µl]	[%]	[µl]
1	± 5.0	± 0.05	± 5.0	± 0.05
2	± 4.0	± 0.08	± 2.0	± 0.04
5	± 2.5	± 0.125	± 1.5	± 0.075
10	± 1.2	± 0.12	± 0.8	± 0.08
20	± 1.0	± 0.2	± 0.5	± 0.1
50	± 1.0	± 0.5	± 0.4	± 0.2
100	± 0.8	± 0.8	± 0.3	± 0.3
200	± 0.8	± 1.6	± 0.3	± 0.6
500	± 0.8	± 4.0	± 0.3	± 1.5
1000	± 0.8	± 8.0	± 0.3	± 3.0
2000	± 0.8	± 16.0	± 0.3	± 6.0
5000	± 0.8	± 40.0	± 0.3	± 15.0
10000	± 0.6	± 60.0	± 0.3	± 30.0

NOTE 1:
For variable volume piston pipettes, the limits for permitted systematic error and permitted random errors (expressed as absolute values i.e. µL) are set up at the higher volume of the range. E.g. variable volume piston pipette working in a range from 10 µl to 100 µl, the maximum permissible systematic/random errors are respectively ± 0.8 µL and ± 0.3 µL for every volume within the range 10-100 µL.

NOTE 2:
For pipettes having a nominal volume not mentioned in the table above the limits of the nearest higher volume are valid.

Table 5

Type D: Fixed and variable volume monochannel pipettes (positive displacement or direct displacement)

nominal volume	**maximum permissible systematic error**		**maximum permissible random error**	
[µl]	[%]	[µl]	[%]	[µl]
5	± 2.5	± 0.13	± 1.5	± 0.08
10	± 2.0	± 0.2	± 1.0	± 0.1
20	± 2.0	± 0.4	± 0.8	± 0.6
50	± 1.4	± 0.7	± 0.6	± 0.3
100	± 1.5	± 1.5	± 0.6	± 0.6
200	± 1.5	± 3.0	± 0.4	± 0.8
500	± 1.2	± 6.0	± 0.4	± 2.0
1000	± 1.2	± 12.0	± 0.4	± 4.0

NOTE 1:
For variable volume piston pipettes (i.e. working in a nominal range of volumes), the limits for permitted systematic error and permitted random errors (expressed as absolute values i.e. µl) are set up at the higher volume of the range. E.g. variable volume piston pipette with a volume range from 10 µL to 100 µL the maximum permissible systematic errors and maximum permissible random errors are respectively ± 1.5 µL ± 0.6 µL for every volume within the range 10-100 µL

NOTE 2:
For pipettes having a nominal volume not mentioned in the table above the limits of the nearest higher volume are valid.

Table 6

Type A: Fixed and variable volume multichannel pipettes (air-displacement)

nominal volume	**maximum permissible systematic error**		**maximum permissible random error**	
[µl]	[%]	[µl]	[%]	[µl]
1	± 10	± 0.10	± 10.0	± 0.1
2	± 8	± 0.16	± 4.0	± 0.08
5	± 5	± 0.25	± 3.0	± 0.150
10	± 2.4	± 0.24	± 1.6	± 0.16
20	± 2	± 0.40	± 1.0	± 0.2
50	± 2	± 1.0	± 0.8	± 0.4
100	± 1.6	± 1.6	± 0.6	± 0.6
200	± 1.6	± 3.2	± 0.6	± 1.2
500	± 1.6	± 8.0	± 0.6	± 3.0
1000	± 1.6	± 16.0	± 0.6	± 6.0
2000	± 1.6	± 32.0	± 0.6	± 12.0
5000	± 1.6	± 80.0	± 0.6	± 30.0
10000	± 1.2	± 120.0	± 0.6	± 60.0

NOTE 1:
For variable volume piston pipettes (i.e. working in a nominal range of volumes), the limits for permitted systematic error and permitted random errors (expressed as absolute values i.e. µl) are set up at the higher volume of the range. E.g. variable volume piston pipette with a volume range from 10 µl to 100 µl the maximum permissible systematic/random absolute errors are respectively ± 1.6 µL ± 0.6 µL for every measure volume within the above range.

NOTE 2:
For Pipettes having a nominal volume not mentioned in the table above the limits of the nearest higher volume are valid.

NON-CONFORMITY OF PIPETTES

In case a calibration gives no-compliant results, the pipettes should be placed out of service until adjustment and successful calibration occurred.

INTERNAL CALIBRATION REPORT/CERTIFICATE

After the calibration, the laboratory should appropriately document the obtained results.
If an internal calibration report or calibration certificate is issued, the following minimum information should be included:

- Title of the report/certificate.
- Identification of the report/certificate.
- Entity having performed the calibration.
- Page numbering.
- Date of calibration.
- Reference to the gravimetric method or alternative procedure used.
- Conditions of measurement (temperature, pressure).
- Z-factor.
- Unique identification of the material used to perform the calibration (balance, hygrometer, thermometer, etc) unless specified in another quality document.
- Unique identification of the pipette.
- Nominal volume of the pipette.
- Volumes selected for the calibration.
- Calibration results (where applicable before and after maintenance).
- Uncertainty of measurement (if appropriate).
- Calculation of systematic error (accuracy) and random error (repeatability).
- Acceptance criteria (if appropriate).
- Conclusion (e.g. PASS/NOT PASS).
- Name and signature of the operator who performed the calibration.
- Name and signature of the supervisor responsible for the release for use of the pipette (preferably a different person from the operator who performed the calibration).

Note: If the laboratory requests calibration to an external company, it should be ensured that this minimal information is contained in the external calibration report/certificate. The responsible person in the laboratory should evaluate and approve this report/certificate as a release for use of the pipette.

REFERENCES

(For all references, if the version is not mentioned, the latest version applies)

1) JCGM 200:2012 International vocabulary of metrology – Basic and general concepts and associated terms (VIM).
2) EN ISO 8655 part 1: Piston-operated volumetric apparatus.
3) EN ISO 8655 part 2: Piston pipettes
4) EN ISO 8655 part 6: Gravimetric methods for determination of measurement error
5) EN ISO 8655 part 7: Non-gravimetric methods for the estimation of the equipment performance
6) FX07-011: Métrologie - Essais - Métrologie dans l'entreprise - Constat de vérification des moyens de mesure.
7) Measurement Good Practice Guide No. 69. The Calibration and Use of Piston Pipettes. ISSN 1368-6550. July 2004. National Physical Laboratory, United Kingdom. www.npl.co.uk.
8) Annex 1 of the OMCL Guideline on Validation of Computerized Systems: "Validation of computerised calculation systems. Example of validation of in-house software document" (PA/PH/OMCL (08) 87).
9) WHO Good Practices for Pharmaceutical Control Laboratories. WHO Expert Committee on Specifications for Pharmaceutical Preparations. Forty-fourth Report. Geneva, World Health Organization, 2010 (WHO Technical Report Series, No. 957), Annex 1. http://www.who.int/medicines/publications/44threport/en/
10) Guide Technique d'Accréditation Étalonnage des instruments volumétriques à piston. Cofrac (LAB GTA 90).
11) ISO 3696: Water for analytical laboratory use -- Specification and test methods.