VITLAB® micropipette

Standard Operating Procedure (SOP)

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1. Introduction

The standard DIN EN ISO 8655 describes both the design and the testing of piston operated pipettes such as the VITLAB® micropipette. The following testing instructions describe how to apply the ISO standard in practice.

We recommend a testing of the piston operated pipette every 3 - 12 months. This interval may be adjusted to individual requirements. For example, when working very frequently or when using aggressive media, the instrument should be tested more frequently.

These instructions may be used as a basis for the supervision of testing devices according to DIN EN ISO 9001, DIN EN ISO 10012 and DIN EN ISO / IEC 17025.

Owing to its 8 respectively 12 channels, the VITLAB® micropipette -8 / -12 can carry out 8 resp. 12 pipettings in one action. However, this advantage results in an increased effort in testing because acc. to ISO 8655, each channel has to be tested seperately. Therefore, 240 weighings are necessary for the VITLAB® micropipette -8 and even 360 weighings have to be carried out for the 12-channel pipette. This results in a high expenditure of time for the testing of multichannel pipettes.

For the regular examinations required by DIN EN ISO 9001, DIN EN ISO 10012 and DIN EN ISO / IEC 17025 and the GLP guidelines, VITLAB additionally provides an optimised calibration service acc. to ISO 8655. For more detailed information, please contact us or your labware supplier.

2. Preparation of the pipette for testing

2.1 Instrument identification

Read instrument type and nominal capacity	► Enter in test record
Read serial number (located behind tip ejection button)	► Enter number in test record
Read customers identification, if present	► Enter identification in test record

2.2 Minimum configuration for VITLAB® micropipette

Have the operating manual ready	
VITLAB® micropipette	► Use only manufacturerer's original parts
Pipette tips	► Use only appropriate tips. Optimum results can only be obtained with VITLAB® pipette tips.

2.3 Cleaning

Clean pipette shaft	► Wipe off with a soft cloth, so that no media residues remain
Clean the exterior sufficiently	► Use a damp cloth (Water or diluted soap solution)
Any liquid residues inside the device?	► Disassemble and clean the instrument (see operating manual)
For multichannel pipettes: V- resp. O-rings damaged?	► No media residues! V- and O-rings can be replaced (see operating manual)

2.4 Visual examination for damage or leakage

Housing and tip ejector	▶ General damage?
Piston	▶ Scratches or soilings on the surface?
Seal	▶ Scratches or soilings on the surface?
For multichannel pipettes: V- resp. O-rings damaged?	▶ Scratches on the surface? Deformation? Visual damage?
	▶ Enter results in the test record

Possible errors and the resulting measures
(For other measures and more troubleshooting advice, please check the operating manual)

Error	Possible cause	Measures
Pipette tip is no longer tight	Scratches on the pipette shaft tip	▶ Obtain spare parts (see operating manual)
Device is stiff and / or leaking	Seal / Piston soiled or damaged	▶ Obtain spare parts (see operating manual)
V- resp. O-rings damaged	Mechanical damage Wear	▶ Replace V- resp. O-rings (see operating manual)

3. Test instruments and environment

Test room

The calibration should be performed in a draught-free room with constant temperature and humidity.

Temperature

The test pipette and the test liquid must be at equilibrium with the room temperature. For this, leave the pipette (without the packaging) and the test liquid for at least 2 hours in the test room. Try to avoid temperature changes (e.g.from sun radiation). Then carry out a comparison of the temperature from pipette, liquid and room.

Test liquid

Distilled or deionised water of a minimum quality 3 according to ISO 3969. The maximum difference of room and water temperature should be 0.5 °C.

Balance

Recommended specifications, see table:

Selected volume* of the device to be tested V	Resolution of the Balance display mg	Repeatability and linearity mg	Standard uncertainty µl
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 ≤ 1000 µl	0.1	0.2	0.2
1 ml < V ≤ 10 ml	0.1	0.2	0.2

*For practical purposes, the nominal volume may be used to choose the balance.

Thermometer

Use only thermometers with a maximum measurement error of 0.2 °C.

Hygrometer

Considering the measuring tolerance of the hygrometer a relative atmospheric humidity of 40 - 60% should be reached.

Recipient vessel

Vessel (e.g. Erlenmeyer flask) filled with test liquid. Prevent a cooling of the water in the recipient vessel through evaporation.

Weighing vessel

Vessel (e.g. Erlenmeyer flask) filled with water to a level that the bottom is completely covered. Provide an evaporation protection for testing volumes < 100 µl.

4. Functional testing

Mount new pipette tip and set the nominal volume

Take up the test liquid	▶ Aspiration of the liquid not possible or very slow: See notes in the table below
Leak test: Hold the filled pipette vertically for approx. 10 seconds and observe if a drop forms at the tip	▶ If a drop forms: See notes in the table below
Release the test liquid. Hold pipette tip against wall of the vessel and wipe off against the wall	▶ The pipetting button must move smoothly and jerk-free
Eject the tip	▶ Enter results in the test record

Possible errors and the resulting measures
(For other measures and more troubleshooting advice, please check the operating manual)

Error	Possible cause	Measures
Aspiration not possible or very slow	Pipette shaft or pipette shaft tip blocked	▶ Clean the instrument (see operating manual)
Drop forming at the pipette tip	Pipette tip not properly attached	▶ Attach new pipette tip
	Seal or piston damaged	▶ Clean or replace seal and / or piston (see operating manual)
	V- resp. O-ring damaged	▶ Replace V- resp. O-ring (see operating manual)

Pipettes with nominal volume ≤ 50 µl

With pipettes of a nominal volume ≤ 50 µl, the error limits are usually smaller than 0.5 µl. Due to this small error limit, the evaporation of water during the test procedure has a relatively large influence on the result. Therefore, the testing of pipettes of this size requires a test procedure which largely prevents evaporation. To provide a sufficient evaporation protection, special pipette calibration balances including an evaporation trap can be used.

Traceability of test results to national standards

Through the use of calibrated testing devices (balance and thermometer), the requirement of DIN EN ISO 9001, DIN EN ISO 10012 and DIN EN ISO / IEC 17025 to refer the test to national standards is fulfilled. The calibration of the balance e.g. can be carried out either by DAkkS calibration or official certification of the balance, or by calibrating the balance with appropriate weights that are traced to the national standard. The calibration of the thermometer, hygrometer and barometer can also be carried out by DAkkS calibration or official certification, or by a comparison with thermometers that are traced to the national standard (under defined conditions).

5. Calibration - Checking the volume

Note: For multichannel pipettes, each channel has to be tested seperately!

1. Set the nominal volume at 10% or 20%.

2. Determine temperature of the test liquid.
   ► Enter temperature into test record.

3. Place the weighing vessel filled with test liquid on the balance and tare balance.

4. Attach new pipette tip. For multichannel pipettes, attach a new pipette tip only to one channel. Perform conditioning of the pipette. Therefore, aspirate and release test liquid 5 times.
   ► The conditioning increases the accuracy of the test.

5. Aspirate test liquid from the recipient vessel. Push the pipetting button to the first stop. Immerse the pipette tip vertically 2 - 4 mm into the test liquid. For 5 ml and 10 ml pipettes, immerse the tip 3 - 6 mm. Then release the pipetting button steadily and smoothly.
   ► Observe waiting time: Remain approx. 1 second in the test liquid. For 5 ml and 10 ml pipettes, wait approx. 10 seconds.

6. Release the test liquid into the weighing vessel. Lean pipette tip in an angle of 30° - 45° against the wall of vessel. Push the pipetting button to the first stop and hold. Push to the second stop to empty the pipette tip completely. Wipe off pipette tip against the wall of the vessel over a distance of approx. 10 mm. Then release the pipetting button smoothly.

7. Place weighing vessel on the balance and record weighing value.
   ▶ Enter value into test record.

8. Tare balance again.

9. Repeat steps 5 - 8 ten times (for multichannel pipettes ten times per channel).
   ▶ Enter weighing values into test record.This results in a minimum of 24 (8-channel) resp. 36 (12-channel) weighing values.

10. Afterwards, repeat the same procedure for 50% and 100% of the nominal volume. Always start with step 4.
    ▶ Enter the weighing values into the test record. This results in 30 values for the singlechannel pipette, in 72 values for the 8-channel pipette and 108 values for the 12-channel pipette.

6. Evaluation

The obtained weighing values from the gravimetric test are only the mass values of the pipetted volume without the correction of the air buoyancy. To obtain the actual volume, a correction calculation taking into account the water density and the air buoyancy has to be carried out.

For multichannel pipettes, the following calculations have to be carried out for each channel seperately.

Mean weighing value (of one channel for multichannel pipettes)
Example for 10 weighing values:

$$\bar{x} = \frac{x_1 + x_2 + x_3 \ldots + x_{10}}{10}$$

Mean volume (of one channel)

$$\bar{V} = \bar{x} \cdot Z$$

▶ Factor Z; see table
▶ Enter value into the test record

Standard deviation volume (of one channel)

$$s = Z \cdot \sqrt{\frac{(x_1 - \bar{x})^2 + (x_2 - \bar{x})^2 + (x_3 - \bar{x})^2 + \ldots + (x_{10} - \bar{x})^2}{9}}$$

▶ Factor Z; see table
▶ Enter value into the test record

Accuracy (of one channel)

$$A\,[\%] = \frac{\overline{V} - V_0}{V_0} \cdot 100$$

▶ Enter value into the test record

Coefficient of variation (for one channel)

$$CV\,[\%] = \frac{100\ s}{\overline{V}}$$

▶ Enter value into the test record

Comparison actual values - nominal values (per channel)
Error limits: see tables 7.2.1 and 7.2.2 (for multichannel pipettes see tables 7.3.1 and 7.3.2) or definition of individual error limits.

Result

If the calculated values for A [%] and CV [%] are (for multichannel pipettes per channel) smaller than or equal to the error limits, the pipette is in good working order.

If the calculated values are **larger** than the error limits:
▶ Verify, if all above instructions have been carefully followed step by step
▶ Observe notes from the „Troubleshooting" section in the operating manual
▶ Recalibrate the VITLAB® micropipette according to the steps in the operating manual

If these measures are not successful, we recommend you to make use of the VITLAB calibration service.

Possible volume errors and the resulting measures:

Error	Possible causes	Measures
Volume too small	Pipette tip not properly attached	▶ Use a new pipette tip and attach it tightly
	Seal or piston damaged	▶ Clean or replace seal resp. piston (see operating manual)
Multichannel pipettes:	V- / O-rings damaged	▶ Replace V- / O-rings
Volume too large	Pushed pipetting button too far	▶ Be very vigilant to not press beyond the first stop
Other influences	Pipette is not correctly calibrated	▶ Recalibrate pipette
	Temperature adjustment of the device, room and water temperature was not completed	▶ Carry out temperature adjustment

7. Tables

7.1 Factor z - Excerpt from DIN EN ISO 8655, Part 6.
Table refers to 1013 hPa. Valid from 950 hPa to 1040 hPa.

Temperature °C	Factor z ml / g	Temperature °C	Factor z ml / g
15	1.0020	23	1.0035
15.5	1.0020	23.5	1.0036
16	1.0021	24	1.0038
16.5	1.0022	24.5	1.0039
17	1.0023	25	1.0040
17.5	1.0024	25.5	1.0041
18	1.0025	26	1.0043
18.5	1.0026	26.5	1.0044
19	1.0027	27	1.0045
19.5	1.0028	27.5	1.0047
20	1.0029	28	1.0048
20.5	1.0030	28.5	1.0050
21	1.0031	29	1.0051
21.5	1.0032	29.5	1.0052
22	1.0033	30	1.0054
22.5	1.0034		

7.2 Volume error limits for piston operated pipettes

The stated volume error limits for the VITLAB® micropipette (Table 7.2.2) are final test values relative to the nominal capacity. These error limits refer to new instruments under optimised test conditions (qualified operators and standardised ambience conditions). Typically these error limits are two times better under ideal testing conditions (experience of the manufacturer). For partial volumes the absolute value (µl) corresponding to the nominal volume is applied.

Table 7.2.1: Excerpt from DIN EN ISO 8655, Part 2

Nominal volume µl	A ≤ ± %	CV* ≤ %
10	1.2	0.8
20	1.0	0.5
100	0.8	0.3
200	0.8	0.3
1000	0.8	0.3
5000	0.8	0.3
10000	0.6	0.3

Table 7.2.2: Values from operating manual VITLAB® micropipette

Nominal volume µl	A ≤ ± %	CV* ≤ %
10	1	0,5
5	1.6	1
1	7	4
20	0.8	0.4
10	1.2	0.7
2	5	2
100	0.6	0.2
50	0.8	0.4
10	3	1
200	0.6	0.2
100	0.8	0.3
20	3	0.6
1000	0.6	0.2
500	0.8	0.3
100	3	0.6
5000	0.6	0.2
2500	0.8	0.3
500	3	0.6
10000	0.6	0.2
5000	0.8	0.3
1000	3	0.6

For calibration, the error limits to be observed by the operator must individually defined by the user. For this purpose, the following methods can be applied:

- If required by the application and if the optimised conditions for measuring are present, the stated error limits can also be expected in the case of used volumetric instruments are in good working order.
- In correspondence to the German regulations for official testing, it is also admissible to apply limits which are typical for practice. These practice limits correspond to double the limits for official testing. In this case, the values in table 7.2.2. should be doubled!
- The user may also define his own individual tolerance limits corresponding to his particular application, and apply these error limits for the calibration of the instrument.

The above procedures fulfil the requirements of DIN EN ISO 9001, DIN EN ISO 10012 and DIN EN ISO / IEC 17025.

7.3 Volume error limits for multichannel piston operated pipettes

The stated volume error limits for the VITLAB® micropipette -8/-12 (Table 7.3.2) are final test values relative to the nominal capacity. These error limits refer to new instruments under optimised test conditions (qualified operators and standardised ambience conditions). Typically these error limits are two times better under ideal testing conditions (experience of the manufacturer). For partial volumes the absolute value (µl) corresponding to the nominal volume is applied.

Table 7.3.1: Excerpt from DIN EN ISO 8655, Part 2

Nominal volume µl	A ≤ ± %	CV* ≤ %
10	2.4	1.6
50	2.0	0.8
100	1.6	0.6
200	1.6	0.6
300	1.6	0.6

Table 7.3.2: Values from operating manual VITLAB® micropipette -8/-12

Nominal volume µl	A ≤ ± %	CV* ≤ %
10	1.6	1.0
5	2.0	2.0
1	8.0	6.0
50	0.8	0.4
25	1.4	0.8
5	6.0	3.0
100	0.8	0.3
50	1.4	0.6
10	4.0	2.0
200	0.8	0.3
100	1.4	0.6
20	4.0	1.5
300	0.6	0.3
150	1.2	0.6
30	3.0	1.5

For calibration, the error limits to be observed by the operator must individually defined by the user. For this purpose, the following methods can be applied:

- If required by the application and if the optimised conditions for measuring are present, the stated error limits can also be expected in the case of used volumetric instruments are in good working order.
- In correspondence to the German regulations for official testing, it is also admissible to apply limits which are typical for practice. These practice limits correspond to double the limits for official testing. In this case, the values in table 7.3.2. should be doubled!
- The user may also define his own individual tolerance limits corresponding to his particular application, and apply these error limits for the calibration of the instrument.

The above procedures fulfil the requirements of DIN EN ISO 9001, DIN EN ISO 10012 and DIN EN ISO / IEC 17025.

Test Record for Volumetric Instruments (EX)

1. Instrument:

❑ VITLAB® continuous E / RS
❑ VITLAB® genius
❑ VITLAB® simplex
❑ VITLAB® TA
❑ VITLAB® micropipette
❑ _____

Nominal volume: _____
Serial number: _____
Customers identification: _____

2. Damage:
❑ None
❑ Type of damage
.....
.....
.....
❑ Damage repaired

3. Functional defects:
❑ None
❑ Type of functional defect:
.....
.....
.....
❑ Functional defect repaired

4. Water temperature:.....°C
Balance:.....
Thermometer:.....

Air pressure:.....
Relative humidity (at least 35%):.....
Correction factor z:.....

5. Weighing values and results of the gravimetric test:

Weighing value No.	10 % (resp. 20 %)	50 %	Nominal volume
x_1			
x_2			
x_3			
x_4			
x_5			
x_6			
x_7			
x_8			
x_9			
x_{10}			

6. Evaluation of the gravimetric test:

Calculated values		10 % (resp. 20 %)	50 %	Nominal volume
a	$\overline{V}$			
b	s			
c	A [%] Found			
d	CV [%] Found			
e	A [%] Nominal			
f	CV [%] Nominal			
g	Result			

The testing was carried out according to DIN EN ISO 8655.

Date _____ Signature _____

VITLAB®

Test Record for Volumetric Instruments (EX)

1. Instrument:
❑ VITLAB® micropipette -8/-12
❑ _____

Type:
❑ 8-channel
❑ 12-channel
Nominal volume: _____
Serial number: _____
Customers identification: _____

2. Damage: ❑ None
❑ Type of damage:
.....
.....
.....
❑ Damage repaired

3. Functional defect: ❑ None
❑ Type of functional defect:
.....
.....
.....
❑ Functional defect repaired

4. Water temperature:.....°C
Balance:.....
Thermometer:.....
Air pressure:.....
Relative Humidity (at least 35%):.....
Correction factor Z:.....

5. Weighing values and results of the gravimetric test:

Testing volume: 10 %:.....µl
Nominal volume: (mg) A(%) (nominal) CV(%) (nominal)

Channel No. / Weighing value	1	2	3	4	5	6	7	8	9	10	11	12
X_1 (mg)												
X_2 (mg)												
X_3 (mg)												
X_4 (mg)												
X_5 (mg)												
X_6 (mg)												
X_7 (mg)												
X_8 (mg)												
X_9 (mg)												
X_{10} (mg)												
X mean (mg)												
V mean (µl)												
A(%) found												
VK(%) found												
A(%) nominal												
CV(%)nominal												
Result A												
Result CV												

Testing volume: 50 %:.....µl

Nominal volume: (mg) A(%) (nominal) CV(%) (nominal)

Channel No. / Weighing values	1	2	3	4	5	6	7	8	9	10	11	12
X_1 (mg)												
X_2 (mg)												
X_3 (mg)												
X_4 (mg)												
X_5 (mg)												
X_6 (mg)												
X_7 (mg)												
X_8 (mg)												
X_9 (mg)												
X_{10} (mg)												
X mean (mg)												
V mean (µl)												
A(%) found												
CV(%) found												
A(%) nominal												
CV(%)nominal												
Result A												
Result CV												

Test volume: Nominal volume: .....µl

Nominal volume: (mg) A(%) (nominal) CV(%) (nominal)

Channel No. / Weighing values	1	2	3	4	5	6	7	8	9	10	11	12
X_1 (mg)												
X_2 (mg)												
X_3 (mg)												
X_4 (mg)												
X_5 (mg)												
X_6 (mg)												
X_7 (mg)												
X_8 (mg)												
X_9 (mg)												
X_{10} (mg)												
X mean (mg)												
V mean (µl)												
A(%) found												
CV(%) found												
A(%) nominal												
CV(%)nominal												
Result A												
Result CV												

The testing was carried out according to DIN EN ISO 8655.

VITLAB®

Date Signature

Declaration on the Abscence of Health Hazards

To be sent together with the instrument or via mail (if urgent with fax **in advance**).

VITLAB GmbH

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Germany

Fax: +49 (0) 6026 9 77 99 - 30

We intend to give our staff a maximum of protection from health hazards caused by contaminated instruments. We therefore ask for your understanding that we cannot carry out any calibration or repair unless this declaration is submitted completed and signed.

For consignment dd. ..... **/ for delivery note No.** ..... **:**

The undersigned hereby declares:

- That the instruments have been carefully cleaned and decontaminated before shipment.
- That the instruments pose no danger through bacteriological, chemical, radiological or viral contamination
- That she / he is aware that shipment of contaminated instruments is a violation of the law, and she / he personally and the Institution represented may be held liable for any damages caused contaminated instruments.
- For calibration service only: minor repairs of a value up to € 50,- + VAT shall be carried out and invoiced without further queries. (Cross out if not applicable).

Company / Laboratory (Stamp)

Tel./Fax/E-Mail:

.....
Name

.....
Position

.....
Date, Signature

- In case of return for repair, please provide us with the following supplementary information:

  Detected defect:.....

  Media which the instrument has been used with: .....