Determination of laboratory glassware precision and pipetting accuracy

Introduction:

Laboratory glassware precision can be determined by measuring the mass of a test liquid (usually distilled or deionised water). The volume measured is then calculated from the mass value using the density, which is dependent on temperature.

$$V = m / \rho$$

V ... volume

m ... mass

ρ... density (see **Table 1**)

Material:

- Balances with appropriate precision
- Distilled or deionised water
- Glass thermometer
- Beakers, Erlenmayer flasks, graduated cylinders, volumetric flasks, glass pipettes
- Automatic piston pipettes with fixed or adjustable volume, various ranges

Procedure

A. Verification of laboratory glassware precision

Distilled water temperature is measured using a glass thermometer, the temperature value is noted. The water of known temperature will be subsequently used for precision verification.

The desired volume of distilled water is measured using various measuring glassware (beakers, Erlenmayer flasks, graduated cylinders, volumetric flasks, glass pipettes). Measuring glassware can be calibrated either for *inclusion* (IN) or *exclusion* (EX).

The vessels designated for *inclusion* should be weighed and the balance set to zero (tare) before distilled water addition. After water addition, they are weighed again, the value is let to stabilise and the mass is noted.

Distilled water, whose volume is measured using exclusion glassware, is weighed in a beaker, the mass of which is in advance set to zero. The mass value is let to stabilise and noted.

All measurements are repeated at least two more times.

The volume is calculated for all measurements with each piece of glassware and then the mean value and standard deviation are calculated. The precision of various laboratory glassware types is evaluated.

B. Determination of automatic piston pipette precision and pipetting accuracy

Correct pipetting technique:

There are three possible positions of the automatic pipette button: the highest is the detachment position, then the calibration stop and subsequently to the lowest stop. Two ways of pipetting are used:

a) The piston is pressed to the calibration stop, the tip is submerged ca 1 cm vertically in the liquid that is to be taken in and the tip is filled by a **slow** release of the button to the detachment position. The sample can then be removed from the tip by pressing the button to the lowest stop. Pressing the button several times is recommended. This procedure is suitable for greater volumes.
b) The button is pressed to the lowest stop, the liquid is taken as in a). The calibrated volume is then pipetted by pressing the button only to the calibration stop, surplus liquid remains in the tip. This procedure is more precise for smaller volumes (below 20 µL).

Attention: *Pressing and releasing the button must be done slowly and smoothly (to avoid risk of taking in air bubbles and to avoid entry of liquid into the pipette mechanism). When the pipette is filled, it must NEVER be in a horizontal position to prevent the liquid from getting into the pipette.*

The pipettes, tips and the test liquid are placed in the room, where the measurement will take place, at least one hour in advance.

1. Measurement with pipettes for **continuously adjustable** (by a micrometric knob) **volume** is performed using **i)** the highest volume of the range allowed and then **ii)** 10 % of the highest volume or the lowest volume of the range (the higher of these). The range of volumes is always marked on the pipette in µL and THE VOLUME SET MUST NEVER EXCEED THIS RANGE (below or above – risk of pipette damage!).

2. Using pipettes with **fixed volume**, naturally only this volume is measured.

The test liquid must be taken into the tip in a vertical position with the tip submerged at 1 cm for the highest pipetting precision. A new tip is first 3-5× moistened with the test liquid. Aspirate the test volume slowly and uniformly to the first stop. Remove the pipette tip from the test liquid slowly and uniformly. Remove any remaining liquid by placing the pipette tip against the inside of the vessel. Dispense the test volume into the weighing vessel (beaker) as follows: Touch the filled tip against the inside of the weighing vessel at an angle of approx. 30 ° to 45 °. Dispense the test volume slowly and uniformly up to the first stop (measuring stroke). Press the control button to the lowest stop (blow-out) and dispense any liquid

remaining in the tip. Hold down the control button and pull the tip up along the inside of the weighing vessel. Let the control button slide up again and determine the mass.

Repeat the procedure with the same pipette four more times.

The volume is again calculated using water density from Table 1.

The mean volume and standard deviation (SD) are calculated using the values from five consecutive measurements. Then the values mean + SD and mean – SD are compared with **Table 2**, which contains the range of permissible volumes.

Note:

The values in **Table 2** are taken as real values used in our accredited testing Laboratory of Cell Cultures of the Faculty of Medicine and Dentistry, Palacký University in Olomouc, which acts in the field of cytotoxicity testing on cellular models *in vitro*. The values depend, besides the quality and calibration of the pipette, on the worker who performs the measurement, on its practise, experiences and accuracy. The results obtained by students can thus be out of the range in **Table 2**. A different mean value means rather an incorrect pipette, while a large SD is usually caused by incorrect pipetting technique.

Table 1. Distilled water density depending on temperature.

Temperature [°C]	**Density ρ** [g/ml]
15	0.9980
15.5	0.9980
16	0.9979
16.5	0.9978
17	0.9977
17.5	0.9976
18	0.9975
18.5	0.9974
19	0.9973
19.5	0.9972
20	0.9971
20.5	0.9970
21	0.9969
21.5	0.9968
22	0.9967
22.5	0.9966
23	0.9965
23.5	0.9964
24	0.9962
24.5	0.9961
25	0.9960

Temperature [°C]	**Density ρ** [g/ml]
25.5	0.9959
26	0.9956
26.5	0.9956
27	0.9955
27.5	0.9953
28	0.9952
28.5	0.9950
29	0.9949
29.5	0.9948
30	0.9946

Table 2. The range of permissible volumes for pipettes.

Measured volumes (µl)	**Lower limit (µl)**	**Upper limit (µl)**
20	19.0	21.0
40	38.0	42.0
100	97.0	103.0
200	196.0	204.0
500	490.0	510.0
1 000	980.0	1 020.0
2 000	1 960.0	2 040.0
5 000	4 900.0	5 100.0
10 000	9 800.0	10 200.0