

Chapter 16

Pipettes

GMDN Code	15166
ECRI Code	15-166
Denomination	Pipettes

Pipettes are devices used for measuring or transferring small volumes of liquid from one container to another with great precision. There are many pipette models. Initially, they were made of glass; at present, there is a wide range of options. Fixed volume and variable volume pipettes with mechanical controls are highlighted herein. Recently, pipettes with electronic controls have been introduced into the market. This chapter deals with aspects referring to the maintenance and calibration¹ of mechanical pipettes.

¹ Calibration must be done exclusively by trained personnel according to current international standards as BS ES ISO 8655-6:2002 or updated ones. Reference work instruments must be suitably calibrated by national or international institutions, responsible for verifying the compliance with international measurement standards.

PHOTOGRAPHS OF PIPETTES

Single channel pipette



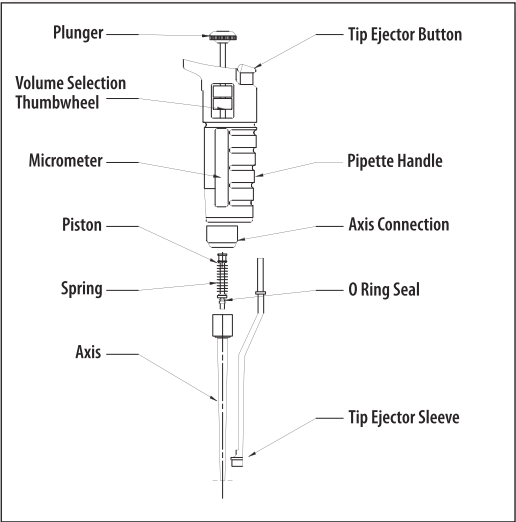
Photo courtesy of Gilson S.A.S.

Multichannel pipette



Photo courtesy of Eppendorf AG

Figure 50. Diagram of a pipette



PURPOSE OF THE PIPETTES

Pipettes are devices widely used in clinical and research laboratories to supply very exact quantities of fluids.

OPERATION PRINCIPLES OF THE PIPETTE

The mechanical or piston pipette generally functions by manually transmitting force exercised on a plunger. The plunger is an axis connected to a piston which moves along a fixed length cylinder, forcing a predetermined volume of liquid outside or inside the pipette.

There are two types of piston pipettes: the fixed volume type with a predetermined liquid volume known as *nominal volume* [Nv] and the variable volume type, which allows adjusting of the volume dispensed within a determined range depending on the pipette’s specifications. Volume adjustment is achieved by modifying the range of the piston’s movement inside the plunger. In variable volume pipettes, the nominal volume is the maximum volume the pipette can hold according to the manufacturer’s specifications.

Fixed volume and variable volume pipettes can be subdivided into two types: A and B. Pipettes of the type A are named *air displacement pipettes* due to the fact that there is a volume of air between the head of the piston and the liquid in the cylinder (see pipette No. 1, Figure 51). Type B pipettes are called *positive displacement pipettes* or direct displacement pipettes as the piston is in direct contact with the liquid (see pipette No. 2). Figure 44 shows differences between these types of pipettes.

Air displacement pipettes have the advantage of presenting less risks of contamination when heavily used. However, they are not as precise as positive displacement pipettes when working with very small volumes of liquid due to the

compressibility of air. All piston pipettes have disposable tips for minimizing risks of contamination. It is recommended to exclusively use tips provided by the manufacturer or compatible with the specific pipette to guarantee their correct adjustment to the pipette’s body as well as volumes dispensed. In order to facilitate identifying these volumes, some manufacturers have adopted a colour code which simplifies the identification of the volumes to be dispensed. The following table demonstrates this colour convention.

Table of pipette colour coding

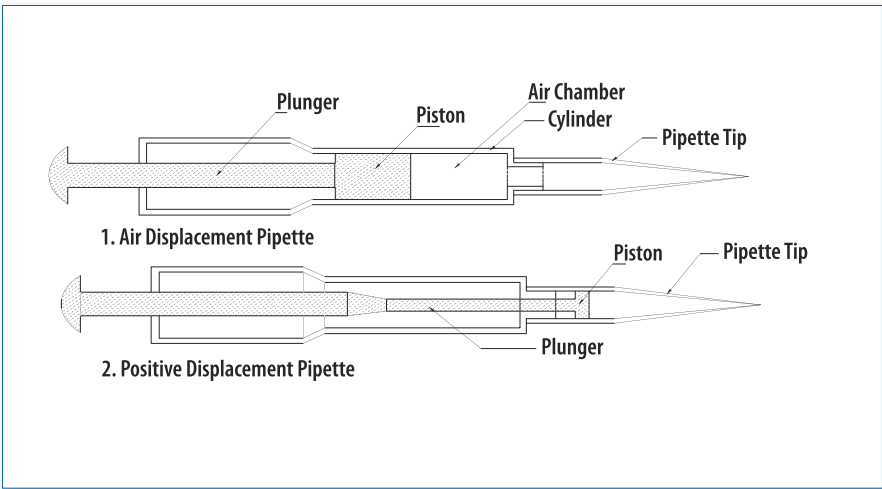
Volume range in microlitres (µl)	Colour
0.1–2.5 µl	Black
0.5–10 µl	Grey
2.0–20 µl	Grey/Yellow
10–100 µl	Yellow
50–200 µl	Yellow
100–1000 µl	Blue
500–2500 µl	Red

REQUIREMENTS FOR USE

To use a pipette, the laboratory must be suitably clean and well lit. The general conditions are the following:

- 1. Verify that room temperature is stable with an optimum temperature of 20 °C with a variation range of ± 5 °C (between 15 °C and 30 °C).
- 2. Confirm that the relative humidity is higher than 50 %.
The pipettes and samples or liquid materials must be stabilized to the conditions of the laboratory. Typically it is recommended to equilibrate these in the laboratory two to three hours before the work is performed.
- 3. Avoid working with pipettes under direct sunlight.
- 4. Use the appropriate protective elements if working with toxic materials or those carrying a biological risk.

Figure 51. Types of pipettes



USING THE PIPETTE

In order to obtain precise, exact and reliable results, it is necessary for pipette operators to know in detail correct pipetting procedures. This is achieved by training and detailed follow-up regarding the use of pipettes. The general outlines for the appropriate use of pipettes are as follows:

Warning: Before using a pipette, verify that it is correctly calibrated and suitable for the transfer of liquid volume to be performed.

General recommendations

1. Verify that the pipette is in a vertical position to aspirate a liquid. The vertical position guarantees that there is no uncertainty due to minimal variation at the surface of the liquid.
2. Use the recommendation outlined by the manufacturer for the minimum immersion depth of the pipette's tip to aspirate liquids. The depths vary according to the pipette type and capacity. A general guide is shown in the following table¹:

Table of tip immersion depth according to the pipette volume range

Volume range of the pipette (µl)	Depth of the immersion (mm)
1–100	2–3
100–1000	2–4
1 000–5000	2–5

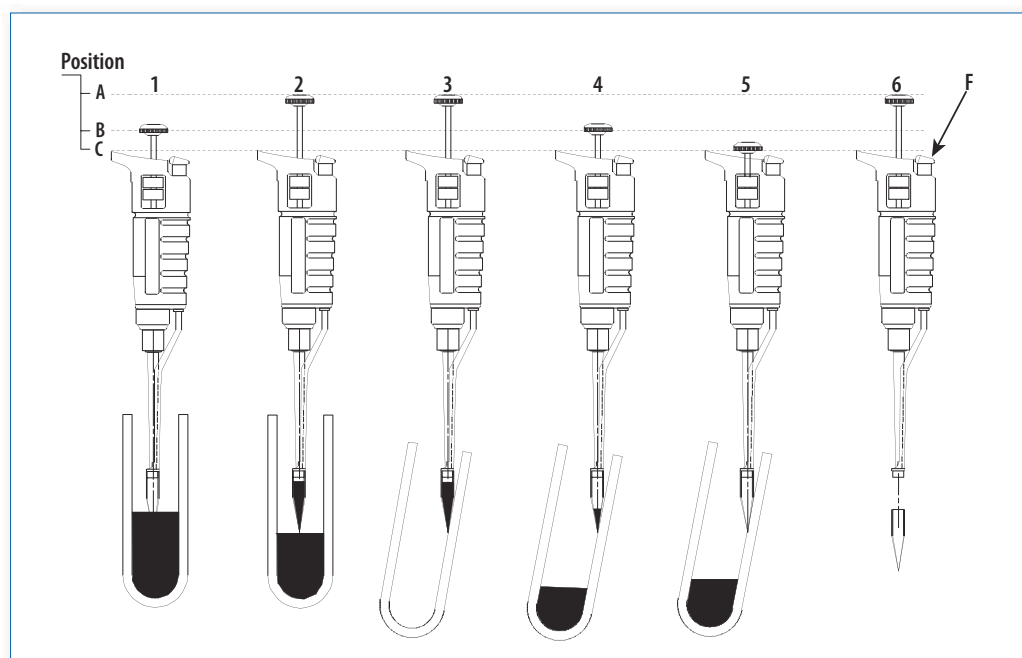
¹ Blues, J., Bayliss, D., Buckley, M., *The calibration and use of piston pipette*, UK, National Physical Laboratory, Teddington, Middlesex, 2004, page. 6. (www.npl.co.uk)

3. Humidify tips of air displacement pipettes for better pipetting accuracy. To humidify the tip, draw working solution several times, dispensing its contents into the waste container. This reduces the possibility of air bubbles being aspirated when dense or hydrophobic liquids are aspirated. The process mentioned allows humidity to be homogeneous in the pipette's air chamber (area between the piston's head and the liquid's surface). Pre-humidifying is not necessary in pipette dispensing volumes lower than or equal to 10 µl. Neither is humidifying necessary for positive displacement pipettes.
4. After filling the pipette tip, remove any drop on the tip by gently sliding the pipette tip against the wall of the original tube. Absorbent material may be required to avoid touching the pipette's tip and taking necessary precautions in case the material shows any sign of contamination.
5. Dispense the liquid drawn by letting the tip touch the wall of the receiving tube. The pipette's tip must form an angle ranging between 30 and 45° with the tube at 8 to 10 mm above the surface of liquid.

Correct pipetting technique

The following is a description of the general steps required when using a mechanical air displacement pipette. The operator must take into account specific recommendations of the manufacturer. This observation must also be respected when using electronically-controlled pipettes. The diagram in Figure 52 shows the description of the process.

Figure 52. Phases of pipette use



1. Place a new tip according to the pipette specifications on the pipette tip holder. Avoid contaminating the tip with other substances. Verify that it remains well adjusted.
2. Press the plunger gently until it reaches the first limit. Until this point, the tip of the pipette must not touch the liquid.
3. Put the extremity of the tip in the liquid. Verify the recommended depth included in table 2 or use the recommendation provided by the manufacturer. Confirm that the pipette is in a vertical position. This process corresponds to the position 1B (first to the left) in the figure.
4. Release the plunger gently for the pipette to aspirate the liquid (position 2A). Verify that the plunger is completely released. Wait at least two seconds before removing the pipette's tip from the liquid.
5. Place the pipette's tip against the wall of the receiving tube. Verify that the angle formed between the pipette's tip and its wall is between 30 and 45°. If the receiving tube already contains liquid, avoid the pipette's tip from being submerged (position 3A).
6. Dispense the contents of the pipette by pressing the plunger gently but firmly, until reaching the first limit (position 4B). At all times, maintain contact between the pipette's tip and the wall of the receiving container. Gently slide the tip against the inside wall at 8 to 10 mm from the tube edge to ensure that there are no drops of liquid left on the pipette tip.
7. Press the plunger gently until it reaches the second limit on the piston's path (position 5C). This expels any fraction of liquid still in the pipette's tip, by forcing out the air in the chamber through the opening of the tip. Keep the plunger pressed at the second limit while the pipette is removed from the receiving tube. Once the pipette is removed, gently release the plunger to the higher limit position.
8. Discard the pipette's tip. To do this, press the expulsion mechanism's button (position 6).

Note: If a variable volume pipette is used, the volume to be dispensed must first be selected. To do this, instructions indicated by the manufacturer must be followed. Normally the volume controls are found in the upper part of the pipette. It is necessary that the operator understands and learns to differentiate the scales.

ROUTINE MAINTENANCE

General outlines of the required routine maintenance for mechanical pipettes are featured next. Specific maintenance must be carried out on the different models according to the instructions manuals provided by the manufacturers.

Inspection:

Frequency: Daily

Pipettes require frequent inspection in order to detect abnormal wear and tear or damage and/or to verify that they are in good working condition. Inspection must cover the following aspects:

1. Verify the integrity and adjustment of the mechanisms. These must move smoothly. The piston must move smoothly.
2. Confirm that the tip holder is not displaying distortions or signs of being worn out, as it is essential for the exactitude of measurements. Verify the adjustment of the tips.
3. Put on a tip and fill it with distilled water. The pipette must not show any leak.

Cleaning and decontamination

1. Every day, verify that the pipette is clean. If dirt is detected, it must be cleaned using a suitable solvent or a mild detergent solution. Check the manufacturer's recommendation regarding the compatibility of the pipette with solvents to select the appropriate one.
2. Sterilize the pipette according to the manufacturer's indications. Some pipettes can be sterilized in an autoclave using a cycle of 121 °C for approximately 20 minutes. Some will need to be disassembled for the vapour to come into contact with their internal components¹. Disassembly consists of liberating and unscrewing the central body of the pipette according to the procedures indicated by the manufacturer. To disassemble or assemble some pipettes, a set of tools (keys) provided by the manufacturers with the pipette at the time of sale must be used. After the sterilization cycle, the pipette must only be reassembled once at room temperature. Prior to assembly, it should be verified that the components are dry. Some manufacturers recommend sterilizing the pipette using a 60 % isopropanol solution and washing the components with distilled water, drying and assembling.
3. If a pipette has been used with harmful substances, it is the responsibility of the user to ensure that it is completely decontaminated before it is used in other procedures or removed from the laboratory. It is advisable to expeditiously prepare a report indicating its brand, model, serial number, contaminating substances and substances or procedures with which it was treated or cleaned.

¹ Pipettes which can be sterilized with vapour have a mark with such identification; the manufacturer supplies the requirements for disassembly.

Maintenance

Frequency: Bi-annually

A pipette used daily must be submitted to the following procedures for guaranteeing its correct functioning:

1. Disassemble the pipette. Follow the procedure described by the manufacturer in the user manual (the procedure varies depending on the brand and model). Normally, the main body of the pipette is disassembled from the tip ejector system unscrewing the body of the pipette from the cylinder.
2. Clean the O rings, the plunger and the inside of the cylinder before lubricating. If the internal components were contaminated accidentally, all the surfaces should be cleaned with a mild detergent and then with distilled water. If the O rings or gaskets need to be changed, replacement parts with the same characteristics as the original should be used. The type of ring or gasket varies depending on the pipette brand, type and model.

3. Lubricate the plunger and piston with silicone grease¹ specially developed for pipettes. Always use the lubricant recommended by the manufacturer. Remove any excessive lubricant with absorbent paper.
4. Assemble following the reverse process to that of disassembly.
5. Calibrate the pipette before use.

Concepts of pipette calibration

Calibration of pipettes is done using standardized procedures.

The calibration method depends mainly on the volume the pipette handles. The smaller the volume range of the pipette, the more demanding and costly the calibration process is. A brief description of the gravimetric process used with pipettes dispensing volumes between 20 µl (microlitres) and 1 ml (millilitre) is explained in this chapter.

Required materials and equipments²

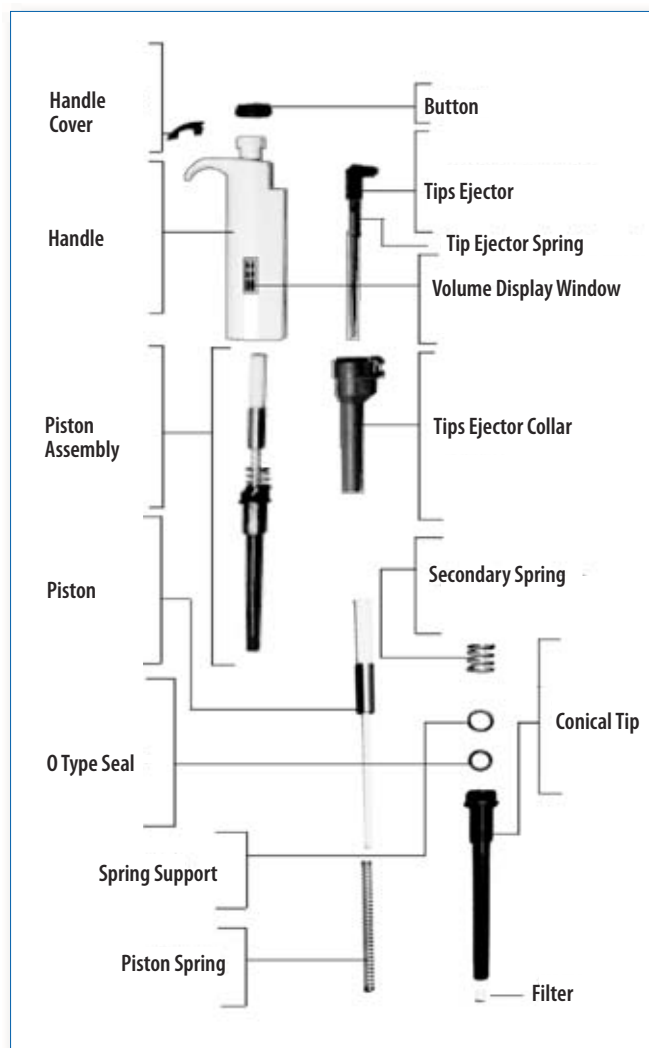
1. Analytical balance.
2. Electronic thermometer with a 0.1 °C or greater resolution, of suitable temperature range with a submersible probe
3. Hygrometer with a standard uncertainty of 10 % or less.
4. Barometer with a standard uncertainty of 0.5 kPa or less.
5. Timer.
6. Micropipettes of various volumes.
7. Disposable tips of various volumes.
8. Flat bottom vials.
9. Bi- or tri-distilled degassed water.
10. Trained operator.

Recommended Pipette Calibration Frequency (Quarterly)

Principle

The procedure is based on measuring the volume of a water sample from the mass of water dispensed by the pipette and dividing that mass by the water density. In practice, a group of measurements is done, to which corrections are applied to compensate for any variation due to non standard temperature and atmospheric pressure and to any significant evaporation during test.

Figure 53. Disassembly of a pipette



¹ There are different specifications for silicone grease; therefore the grease recommended by the pipettes manufacturer must be used.

² The equipment used in pipette calibration must be certified by an accredited calibration laboratory.

This type of test allows the following:

1. To compare different types of pipettes to each other to detect if there are differences among them.
2. To check the precision and exactitude of a pipette.
3. To check the exactitude and precision of a batch of pipettes.
4. To check factors attributable to the use of one pipette by several users.

Procedure¹

The procedure explained next is valid for air displacement pipettes. It includes the following steps:

1. Install a new tip on the pipette.
2. Pipet distilled water and empty into the waste container. Repeat at least 5 times in order to stabilize the humidity of the air inside the pipette.
3. Add water to the weighing receptacle until the level of liquid reaches at least 3 mm.
4. Register the temperature of the water, environmental pressure and relative humidity.
5. Cover the weighing receptacle, if this applies.
6. Register the weight shown on the balance or press tab so that the reading is zero (0).
7. Fill the pipette with water from the storage container and dispense it into the weighing receptacle expelling all the water. This is done in the same way pipettes are used on a daily basis (see step 7 of the *Correct pipetting technique*).
8. Register the new weight detected by the balance.
9. Repeat steps 7 and 8 nine (9) additional times, recording the weight registered by the balance at the end of each cycle.
10. Register the temperature of the liquid inside the weighing receptacle at the end of the tenth cycle and measure the time elapsed since the measurements started.
11. Evaluate if evaporation has been significant (this is critical when working with pipettes of very small volumes). If this is the case, an additional period of time [Ta] equal to the time used during the ten measurements must be allowed to elapse, and when completed, a new reading has to be carried out.
12. The mass of water lost by evaporation in the additional time [Ta] is divided by the total number of samples analyzed (ten). This will give an indication of the average mass of liquid lost due to evaporation per cycle. This figure must be added to each of the mass readings.

Calculations

Proceed as follows:

1. Calculate the mass of water dispensed by the pipette in each cycle. Subtract the reading registered at the end of the previous cycle to the reading registered in the current cycle. Repeat for all measurements. If appropriate, add the average mass corresponding to the calculated evaporation per cycle.

2. Convert each mass value to a volume at 20 °C, dividing the mass by the density of water adjusted to the mentioned temperature.

$$V_i = \frac{M_i}{D}$$

3. Calculate the average of the volumes calculated in step 2. (The sum of volumes, divided by the number of samples). Apply the adjustments per phenomenon such as the air pressure onto the mass (flotation). To accomplish this, multiply each mass by a correction factor [Z].

$$X = \sum V_i / n \times Z$$

4. Calculate the standard deviation of the sample.

$$SD = \sqrt{\frac{1}{n-1} \times \sum_{i=1}^n (X_i - X_{AV})^2}$$

5. Calculate the coefficient of variation.

$$[Cv] CV (\%) = \frac{S}{X_{AV}} \times 100$$

A table containing a summary of the mathematical formulae mentioned is shown next.

Table of mathematical formulae

$X = \sum V_i / n \times Z$	$[Cv] CV (\%) = \frac{S}{X_{AV}} \times 100$
$SD = \sqrt{\frac{1}{n-1} \times \sum_{i=1}^n (X_i - X_{AV})^2}$	$D \% = \frac{X_{AV} - X_{Nom}}{X_{Nom}}$
$E_s = X - V_n$	$V_i = \frac{M_i}{D}$
Conventions:	Conventions:
X = average volume SD = standard deviation Z = adjustment factor in (µl / mg) ²	CV(%) = variation coefficient D(%) = error

¹ The procedure presented is a general guide. For complete details, consult the standards BS ES ISO 8655-6:2002 or current updates.

² The values Z depend on the temperature and pressure of distilled water. Refer to specialized publications such as the Standard BS EN ISO 8655-6:2002, Attachment A.

TROUBLESHOOTING TABLE		
PROBLEM	PROBABLE CAUSE	SOLUTION
The pipette displays leaks.	The tip is placed incorrectly on the pipette.	Install the tip according to the procedure indicated by the manufacturer.
	There are foreign bodies between the tip and the adjustment cone.	Clean the joint. Remove the tip and clean the adjustment cone. Install a new tip.
	There are foreign bodies between the piston and the O-ring in the cylinder.	Disassemble and clean the cylinder/piston set. Lubricate and assemble.
	There is insufficient lubricant in the piston and/or the O-ring.	Disassemble and lubricate adequately.
	The O-ring is twisted or damaged.	Replace the O-ring. Disassemble, clean, replace gasket, lubricate and assemble.
	The piston is contaminated.	Clean the piston and lightly lubricate.
	The lower cone is slack.	Adjust the lower cone.
There are visible drops inside the pipette's tip.	There is non-homogeneous humidification of the plastic wall.	Install a new tip on the pipette.
The pipette shows inaccuracies.	Incorrect operation of the pipette.	Check the pipetting technique and correct the detected errors.
	There are foreign bodies under the activation button.	Clean the button's assembly mount.
	The pipette tip is incorrectly mounted.	Check the fit of the pipette's tip. Install a different tip suitable for the pipette's specification.
	There is interference in the calibration.	Recalibrate according to standardized procedure. Check use procedure.
	The tip is contaminated.	Use a new tip.
The tip shows inaccuracies with determined liquids.	The calibration is inadequate.	Recalibrate the pipette using standardized procedure.
		Adjust the calibration if liquids of high viscosity are used.
The control button does not move smoothly or shows high resistance to its activation.	The piston is contaminated.	Clean and lightly lubricate.
	The gasket is contaminated.	Disassemble the pipette, clean all the gaskets, or replace them if necessary. Lightly lubricate.
	The piston is damaged.	Replace the piston and the piston's gaskets. Lightly lubricate.
	Solvent vapours have entered into the pipette.	Unscrew the central joint of the pipette. Ventilate, clean and lightly lubricate the piston.

BASIC DEFINITIONS

Coefficient of variation [%CV]. A statistical parameter representing the ratio of the standard deviation of a distribution to its mean.

Density. Relationship between a body's mass and the volume which it occupies. The average density of an object is equal to its total mass divided by its total volume. It is identified by the Greek letter ρ [p]. In the International System of Units, density is measured in kilograms by cubic metres [kg/m³].

Error (of a measurement). A difference shown between the value measured and the correct value.

Exactitude. A concept related to errors shown in measurements. It is said that an instrument is exact when the value of a group of measurements are sufficiently close to the real value.

Mass. A physical property of the bodies related to the quantity of matter these contain, expressed in kilograms (kg). In physics, there are two quantities to which the name mass is given: gravitational mass which is a measure of the way a body interacts with the gravitational field (if the body's mass is small, the body experiences a weaker force than if its mass were greater) and the inertial mass, which is quantitative or numerical measure of a body's inertia, that is, of its resistance to being accelerated.

Microgram [μg]. A unit of weight equivalent to 1×10^{-6} grams (g).

Microlitre [μl]. A unit of capacity equivalent to 1×10^{-6} litres (l). One (1) μl of water weighing exactly one (1) mg and has a volume of 1 mm³.

Milligram [mg]. A unit of weight equivalent to 1×10^{-3} grams (g).

Millilitre [ml]. A unit of capacity equivalent to 1×10^{-3} litres (l). One (1) ml of water weighing exactly (1) g and has a volume of 1 cm³.

Precision. A concept related to errors shown in measurements. An instrument or method is precise when upon repeating a measurement in independent tests, the results obtained are similar.

Range. A difference between the maximum and minimum value which an instrument reads or measures.

Standard deviation [SD]. Measure of the dispersion of a set of data from its mean. The more spread apart the data is, the higher the deviation. It is used as a statistical parameter for determining the global error of a sample set.

Volume. A quantity of physical space that a mass occupies. It is calculated by dividing the mass by its average density.