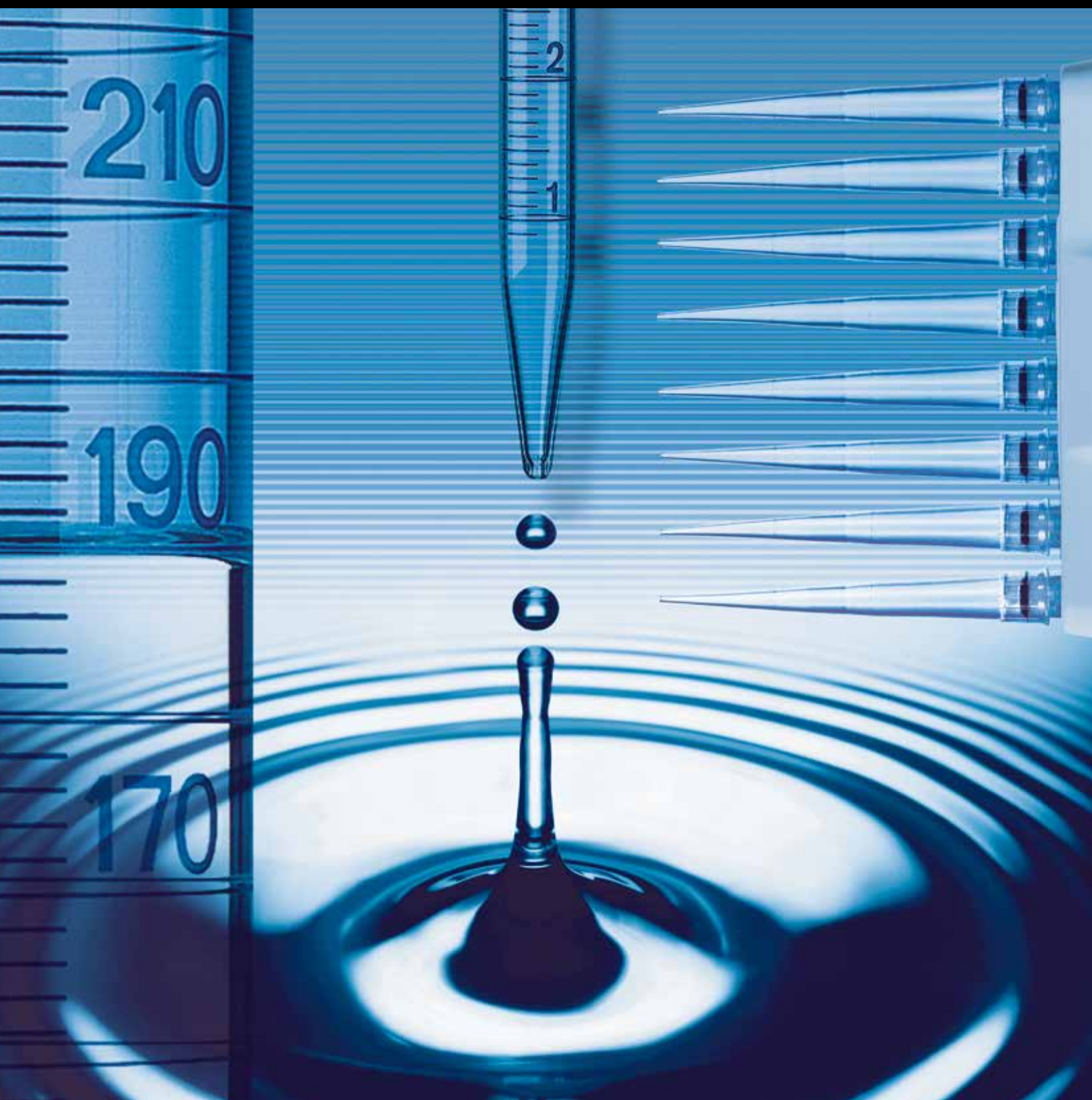


Learning the basics – how to work with
volumetric instruments.



Volumetric Measurement in the Laboratory

F I R S T C L A S S · B R A N D



Introduction

Volumetric measurement plays a central role in the laboratory. The user has to determine the degree of accuracy required for each measurement. Based on this, he can choose the appropriate volumetric instrument.

Reliable measurements require the use of precision instruments and their proper handling. To provide a better understanding of volumetric instruments and their operation, this booklet explains the most important terms for their classification and handling, and illustrates them by using BRAND laboratory equipment as examples.

The brochure 'Information on Volumetric Measurement' is designed to give the reader a quick overview of volumetric instruments. It is not intended to replace the operating manuals of the liquid handling instruments described. By all means, read the operating manuals supplied with these instruments before use – for your own safety and success.

Please do not hesitate to contact us if you have any further questions on the subject of volumetric measurement.

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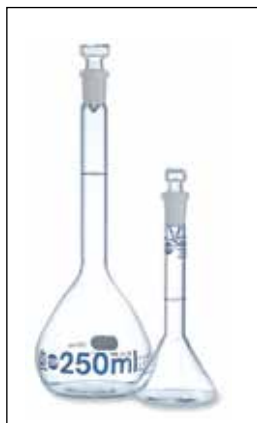
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Volumetric Instruments – an Overview

Volumetric instruments glass/plastics

The volumetric measurement of liquids is a routine operation in the laboratory. Therefore, volumetric instruments, such as volumetric flasks, bulb pipettes, graduated pipettes, graduated cylinders and burettes are standard equipment. They can be made from glass or plastic. Suppliers offer volumetric instruments in varying qualities. Graduated beakers, beakers, Erlenmeyer flasks, dropping funnels and the like are not volumetric instruments! They are not precisely calibrated, and the scale serves only as an approximate guide.

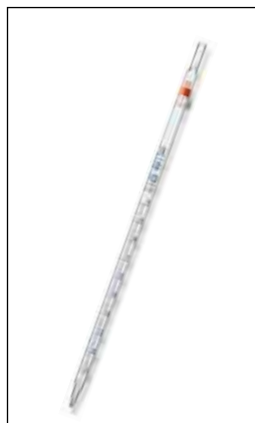
A selection of volumetric instruments by BRAND is illustrated below:



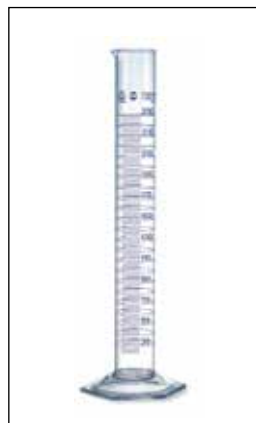
Volumetric flasks



Bulb pipette



Graduated pipette



Graduated cylinder



Burette

Liquid handling instruments

To meet the ever growing demands on volumetric measuring in the lab, such as testing in series, new devices are constantly being developed, e.g., for dispensing, pipetting and titrating. Usually, the instruments designed by different manufacturers for a particular purpose are more or less similar in functional principle. However, major differences are apparent when the focus is on constructional details and design.

This selection of liquid handling instruments by BRAND illustrates the range of instruments available:



Bottletop dispenser



Bottletop dispenser



Bottletop burette



Single-channel air interface pipettes



Multichannel air interface pipettes



Positive displacement pipette



Repetitive pipette, manual



Repetitive pipette, electronic

Manufacture of Glass Volumetric Instruments

From raw material to finished precision instrument

Blanks

The glass types used are soda-lime glass (e.g., AR-GLAS®) for bulb and graduated pipettes, and borosilicate glass (e.g., Borosilicate glass 3.3) for volumetric flasks, graduated cylinders and burettes. These glass types meet the stringent laboratory requirements for chemical and physical resilience.

High-quality blanks and strict statistical testing of the required quality characteristics are the basis for producing high-quality volumetric instruments. For example, thermal stress in the glass blanks must be eliminated by a controlled heating and cooling process.

This results in optimum mechanical stability, a requirement for keeping the volume constant despite any subsequent temperature fluctuations. Therefore, BLAUBRAND® and SILBERBRAND volumetric instruments can be heated up to 250 °C in a drying cabinet or

sterilizer without any resulting volume changes. As with all glass instruments, however it should be noted, that uneven heating or sudden temperature changes produce thermal stresses, which may result in breakage.

Therefore:
Always place glass instruments into a cold drying cabinet or sterilizer; then heat slowly. At the end of the drying or sterilizing period, allow instruments to cool off slowly inside the switched-off drying cabinet or sterilizer. Never heat volumetric instruments on a hotplate!



Blanks for graduated cylinders

Calibration

Every glass volumetric instrument is individually calibrated at BRAND, i.e. the instrument is accurately filled with a defined quantity of water, and a calibration mark is applied at the lowest point of the meniscus. In the case of graduated instruments, two calibration marks are applied. Computer-controlled systems ensure maximum precision in a fully automated production line. 'Statistical Process Control' (SPC) guarantees production of volumetric instruments with the smallest deviation from nominal capacity (accuracy) and narrow scatter of individual values (coefficient of variation).

Volumetric instruments are either calibrated 'to contain' ('In') or 'to deliver' ('Ex').

Calibration 'to contain' (TC, In):

The contained quantity of liquid corresponds to the capacity printed on the instrument. These instruments include e.g., graduated cylinders, volumetric flasks, and capillary pipettes up to 200 µl.

Calibration 'to deliver' (TD, Ex):

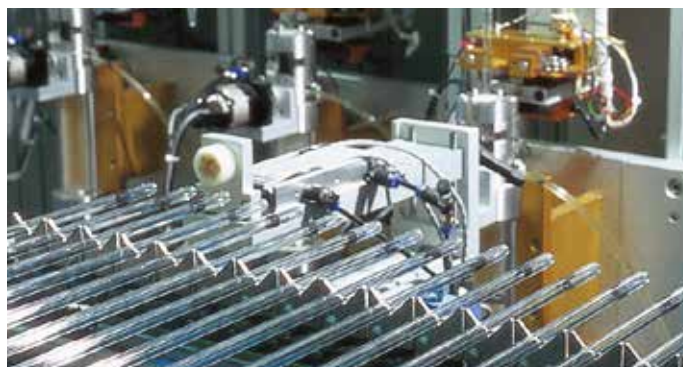
The delivered quantity of liquid corresponds to the capacity printed on the instrument. The wetting residue remaining in the instrument has already been taken into account in the calibration. These instruments include e.g., graduated and bulb pipettes, and burettes.

Reference temperature

The standard reference temperature, i.e. the temperature at which volumetric instruments will contain or dispense their volumes, is 20 °C. If the adjustment or calibration is performed at a temperature that deviates from this standard, the corresponding measurement values must be corrected.

Note:

Due to the small coefficient of expansion of the glass material, the reference temperature is of minor significance in practical use since the measurement deviations resulting from volume expansion of the measuring instrument are generally smaller than the error limit.



Calibration of graduated pipettes

Silk-screening

Calibration is followed by silk-screen printing of marks and inscriptions. BRAND uses stretchable screen stencils for all graduated pipettes, burettes, graduated cylinders, and mixing cylinders. These stencils can be stretched to match the calibration marks accurately, so that the measuring precision is maintained for all intermediate volumes.

Pipettes are additionally marked with 'color-code' rings at their upper end, which make it easier to identify similar pipette sizes clearly. The ISO 1769 industry standard defines color-coding for different nominal volumes.

Screening inks

BRAND uses quality inks manufactured especially for glass volumetric instruments:

Blue enamel:

High contrast, optimum combination of resistance and legibility. Blue enamel is used for BLAUBRAND® volumetric instruments (class A/AS).

White enamel:

White enamel is used for SILBERBRAND volumetric instruments (class B).

Amber diffusion stain:

Diffuses into the glass surface and can only be removed by abrasion. It is used for volumetric instruments that are exposed to especially aggressive cleaning conditions. An amber diffusion dye is used for BLAUBRAND® ETERNA volumetric instruments (class A/AS) as well as for SILBERBRAND ETERNA volumetric instruments (class B).



Automatic silk-screening of volumetric flasks

Firing

Firing or annealing the screened ink is the last step on the way from the blank to the finished volumetric instrument. A carefully controlled annealing process, along with the specially produced quality inks, is a prerequisite for durable graduations. This involves a gradual heating and cooling of the volumetric instruments. Depending on the type of glass, temperatures of 400 to 550 °C are reached in this process.



Silk-screened graduated cylinders before firing

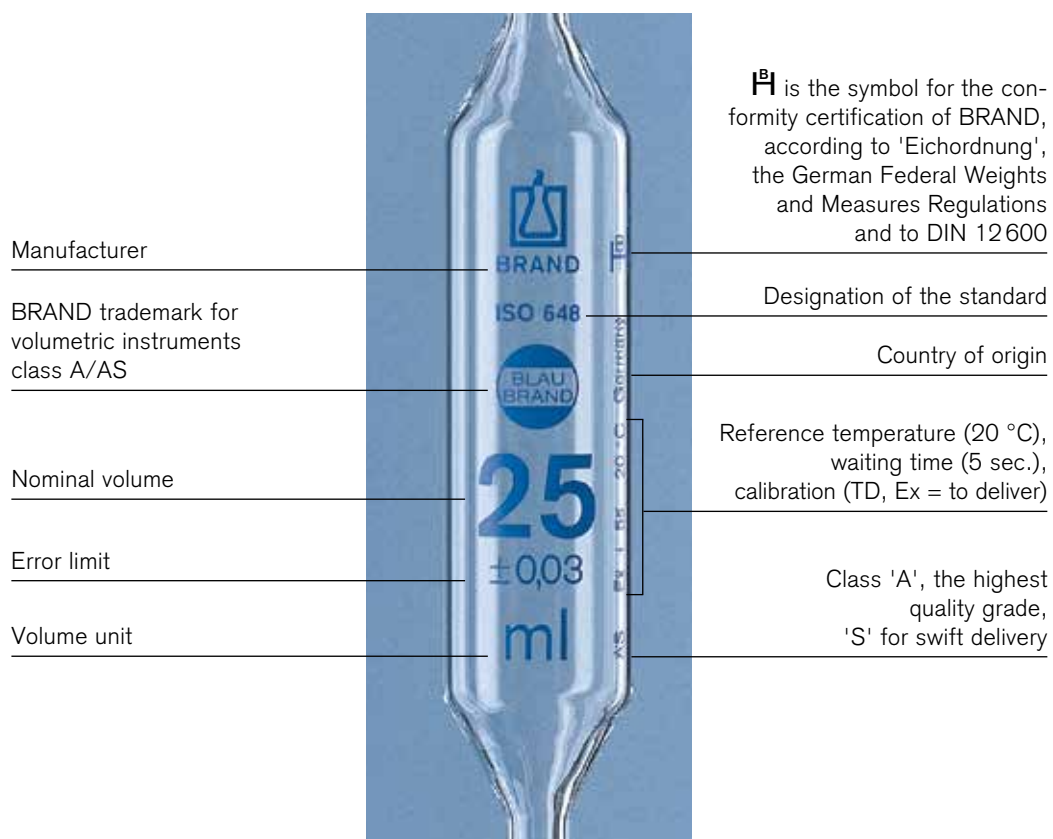
Quality assurance

Quality assurance is implemented at BRAND through ongoing testing during production and statistical testing in the final inspection. (For detailed information, see page 39.)

Identification of volumetric instruments

Batch number

Example: BLAUBRAND® bulb pipette



The labeling below **must** be printed on every volumetric instrument:

- Nominal capacity
- Unit symbol: ml or cm³
- Calibration temperature: 20 °C
- Calibration: Ex or In
- Class: A, AS or B
- Waiting time (if necessary): in the format 'Ex + 5 s'
- Manufacturer's name or logo

The following information **may** also be added:

- Country of origin
- Error limit
- Manufacturer's trademark (here: BLAUBRAND®)
- Standard, e.g., ISO 648
- Batch number

Accuracy classification

Volumetric instruments are generally available in two accuracy classes:

Class A/AS

Volumetric instruments of class A and AS have identical error limits as established by DIN EN ISO. These are generally implemented only in glass volumetric instruments. Exceptions are BRAND plastic volumetric flasks made from PFA and PMP and plastic graduated cylinders made from PMP, which are designed to meet the highest requirements and likewise correspond to class A. For class AS volumetric instruments, calibrated to deliver (TD, Ex), the additional 'S' means swift delivery.

Class AS volumetric instruments have become quite well established. The risk of clogging is low in pipettes and burettes with a larger tip opening. The delivery behavior of various liquids is compensated by observing the defined waiting time (see 'Delivery and waiting times' on page 11).



Class A/AS

- Always stands for the highest-grade accuracy
- 'S' stands for swift delivery (pipettes and burettes)
- Only class A/AS is conformity-certified
- Graduation: The long graduation marks extend over at least 90% of the tube perimeter or are present as ring markings.

Class B

Volumetric instruments of class B are available in glass or plastic. Class B instruments generally have twice the error limits of class A/AS. For class B measuring instruments calibrated to deliver (TD, Ex), no waiting time is specified.



Class B

- Generally twice the error limits of class A/AS
- Graduation: The long graduation marks extend about 20 - 40% of the tube perimeter.

The choice of volumetric instruments – glass or plastic?

There is no all-round material to meet every single requirement in the laboratory. The decision to use glass or plastic is guided by the application, product design and after considering the specific properties of the materials and economic aspects. Volumetric instruments of plastic excel by their high resistance to breakage and low weight. PP, PMP and PFA are proven materials.

The accuracy of bulb pipettes, graduated pipettes, volumetric flasks, and graduated cylinders made from PP corresponds to that of class B error limits. PMP and PFA are also used for measuring instruments that correspond to class A error limits, e.g., volumetric flasks (PMP/PFA) and graduated cylinders (PMP). Due to its higher purity, PFA is preferably used in trace analysis applications.



Graduated cylinder made of PMP, class A

Working with Volumetric Instruments

The liquid meniscus

The term meniscus describes a curvature in the surface of the liquid.

The meniscus may be curving upwards or downwards. The curvature develops as a function of the interplay between the adhesion and cohesion forces.

If the liquid molecules are attracted more strongly by the glass wall (adhesion) than by their own kind (cohesion), the meniscus is curved downwards, or concave; the edge of the liquid surface is slightly raised. This is the case, e.g., with aqueous solutions.

If the diameter of a pipette is narrow enough – as with capillary pipettes – the adhesion force is strong enough to pull up not only the edge but also the entire liquid level (capillary effect).

If the cohesion force of a fluid is stronger than the adhesion force of the glass wall, an upwardly curved (convex) meniscus is formed. This happens with mercury, for example.

Meniscus setting

Correct meniscus setting is a prerequisite for accurate volumetric measurement.



Concave meniscus in a graduated pipette.

In the case of a concave meniscus, the volume has to be read at the lowest point of the liquid level. The lowest point of the meniscus has to touch the upper edge of the graduation mark.



Convex meniscus in a graduated pipette.

In the case of a convex meniscus, the volume has to be read at the highest point of the liquid level. The highest point of the meniscus has to touch the lower edge of the graduation mark.



Appearance of the meniscus at the Schellbach stripe in a burette.

The Schellbach stripe is a narrow blue band at the center of a white stripe. Schellbach stripes are imprinted on the back of volumetric instruments to improve readability. The refraction of light causes two arrow points to appear at the meniscus. The reading point is where the two arrows meet.

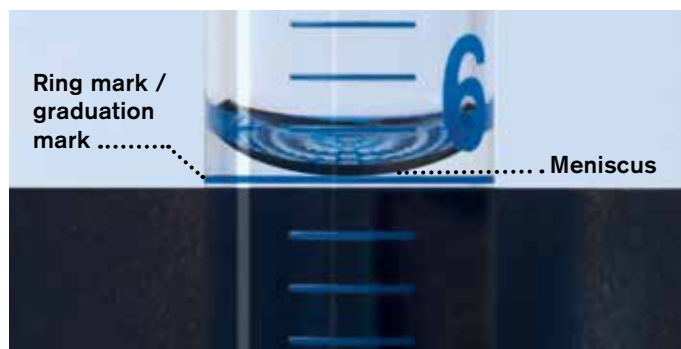
Important note:

The temperature of the liquid and the environment during use are important. While the expansion of a glass volumetric instrument is negligible, the expansion of the liquid at different temperatures must be taken into account. To minimize volume errors as much as possible, the volumes of all the liquids in contact with one another should be measured at a common (prevailing daily) temperature. Especially in the preparation of standard solutions, for example, the pipetting of the samples and the titration should be done at the same temperature to the extent possible. Significant temperature differences between the measuring instrument and the liquid should likewise be avoided.

Reading the meniscus

For parallax-free adjustment of the meniscus, the volumetric instrument is held upright and the observer's eye must be at the same height as the meniscus. In this position the ring mark will appear as a line.

The meniscus will appear darker and more easily readable in front of a light background if a piece of dark paper is held behind the instrument immediately beneath the ring mark or graduation mark.



Delivery and waiting times

In volumetric instruments for liquid delivery (calibrated to deliver, TD, Ex), the volume delivered is always smaller than the volume contained in the measuring instrument. This is caused by the fact, that a certain amount of liquid remains as a film on the inner surface of the instrument. The volume of this liquid film depends on the delivery time and should be taken into account when calibrating the measuring instrument.

Possible volume errors:

The volume delivered from a pipette or burette becomes smaller if the tip is broken off (shorter delivery time), or increases if the tip is not clean and the liquid outflow is impeded (longer delivery time). Likewise, the volume increases if the residual liquid in the tip after pipetting is blown out by mistake. (for the proper handling of pipettes, see page 13.)

Delivery time

The delivery time is defined as the period of time required for the free fall of the meniscus (discharge of water due to gravity) from the upper volume mark to reach the lower volume mark or the tip. This is connected with the defined waiting time for class AS volumetric instruments.

Waiting time

The waiting time begins when the meniscus comes to rest at the lower volume mark or in the tip. During the waiting time, residual liquid continues to flow down from the glass wall.

Waiting time for class AS:
The established waiting time of 5 s for class AS bulb and graduated pipettes is the time after which the meniscus visibly comes to rest in the tip, and before the tip can be removed from the inner surface of the receiving vessel.
The waiting time of 5 s must be indicated on the pipette by the manufacturer (see page 8).

Examples of delivery and waiting times for different classes

(25 ml bulb pipette)

Class A (conformity-certified)

Delivery time 25 - 50 sec. (no waiting time)

Class AS (conformity-certified)

Delivery time 15 - 20 sec. + Waiting time 5 sec.

Class B

Delivery time 10 - 50 sec. (no waiting time)

Working with Volumetric Instruments

Pipettes, general

Pipettes are volumetric instruments for measuring volumes of liquid and are generally calibrated 'to deliver'. During the manufacturing process, they are individually volumetrically calibrated and provided with one or more calibration marks.

We distinguish between bulb and graduated pipettes (calibrated to deliver, TD, Ex) and disposable micropipettes up to 200 µl (calibrated to contain, TC, In).



Bulb pipette with 1 mark

Bulb pipettes

- Calibration:
Class AS: 'Ex + 5 s'
Class B: 'Ex'
- Generally higher measurement accuracy than graduated pipettes
- Bulb pipette models:
The most important model is the bulb pipette with 1 mark (total delivery).
Less common is the model with 2 marks (partial delivery).

Bulb pipettes are also called single volume pipettes.



Graduated pipette, type 2, nominal volume at the top

Graduated pipettes

- Calibration:
Class AS: 'Ex + 5 s'
Class B: 'Ex'
- Scaling permits the reading of partial volumes
- Types of graduated pipettes:
Type 2 – nominal volume at top, total delivery also for partial volumes
Type 1 – nominal volume at bottom, partial delivery for all volumes
Type 3 – nominal volume at bottom, total delivery only for the nominal volume



Capillary pipettes e.g., BLAUBRAND® intraMark

- Calibrated to contain (TC, In)
- One ring mark
- Volume limited by one end and the ring mark



Capillary pipettes e.g., BLAUBRAND® intraEnd

- Calibrated to contain (TC, In)
- No ring mark
- Volume limited by both ends (end-to-end capillaries)

Handling of pipettes

Pipettes calibrated 'to deliver' ('TD, Ex')

Proper pipetting with bulb pipettes with 1 mark (here: nominal volume 25 ml) and graduated pipettes type 2, class AS (here: partial volume 3 ml)
Utility: pipetting aid (see page 18)

Filling

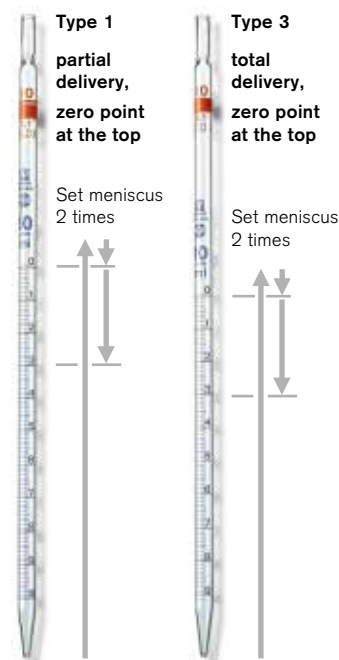
1. Fill the pipette with a pipetting aid to approx. 5 mm above the selected graduation mark.
2. Remove any liquid remaining on the outside of the tip of the pipette with a tissue.
3. Set the meniscus.
4. Wipe off any drop of liquid adhering to the tip.

Delivering

5. Hold the pipette vertically. Deliver the liquid with the tip of the pipette in contact with the inner surface of the inclined receiving vessel.
6. When the meniscus comes to a rest in the tip, the waiting time of 5 s begins (class AS only).
7. After the waiting time, draw the pipette tip upwards along the inner wall through a distance of about 10 mm to remove residual liquid.

Note:

The residual liquid still left in the tip has already been taken into account during calibration and must not be discharged into the vessel, such as by blowing out.



Type 1 and type 3

When using type 1 or type 3 graduated pipettes (zero point at top), the meniscus must

- 1.) first be set at the zero point and then the liquid be run out to slightly above the desired partial volume.
- 2.) After a waiting time of 5 s, the meniscus should be set a second time.



Fill pipette



Wipe off



Set meniscus



Deliver

Working with type 2 graduated pipettes is significantly quicker and simpler. With type 1 and type 3 pipettes there is a risk that with the always necessary second meniscus setting too much liquid is released, and that the sample needs to be prepared again (as also with bulb pipettes that have 2 marks).

Handling of pipettes

Pipettes calibrated 'to contain' (TC, In)

Correct pipetting with capillary pipettes

Utility: pipetting aid (see page 18)

Capillary pipettes are pipettes with a very narrow internal diameter. They are filled either with a pipetting aid, or automatically by capillary action. After emptying, the capillary pipette must be rinsed repeatedly with the diluting medium.

Filling

- Aspirate liquid accurately to the desired mark.
- Hold the pipette horizontally and carefully wipe off with a tissue.

Delivering

- To empty capillaries blow out the liquid with a pipetting aid, and rinse two to three times with the diluting medium (required due to calibration 'to contain').
- End-to-end capillaries are often placed directly in the dilution solution and washed out by shaking.



Fill capillary



Wipe off



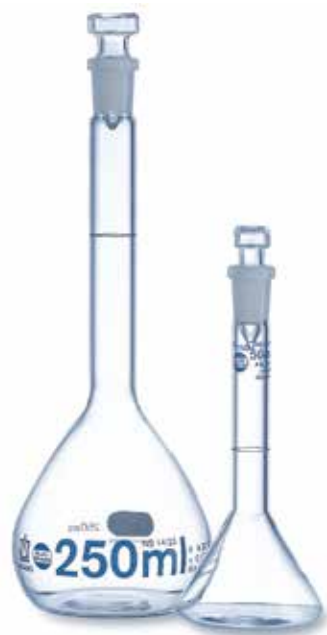
Deliver



Pipette holder with end-to-end-capillaries



Handling of volumetric flasks



Volumetric flasks, class A and B, are calibrated to contain (TC, In), and are mainly used for the preparation of highly accurate dilutions and standard solutions.

Modern analytical methods require small-capacity volumetric flasks. Small-sized conventional volumetric flasks (up to approx. 50 ml) tend to tip over easily due to their high center of gravity and their small base area. Trapezoidal volumetric flasks are much more stable. Their center of gravity is lower, and their base area is more than twice as large compared to the equivalent standard flasks.

How to prepare a standard solution with a volumetric flask:

1. Insert the precisely weighed amount of substance, or rinse in a standard solution concentrate.
2. Fill the flask with distilled water to about half. Swirl the flask to dissolve and mix the contents thoroughly.
3. Top up the flask with distilled water to just below the ring mark.
4. Top up the remaining volume, using a wash bottle or pipette, until the meniscus is exactly at the ring mark. Important: meniscus must be read at eye level. The wall of the flask must not be wetted above the mark.
5. Close the flask and shake upside down to mix contents.

Handling of graduated and mixing cylinders

Graduated cylinders:

Graduated cylinders, class A and B, are measuring instruments that are calibrated to contain (TC, In), i.e. they indicate the exact volume taken up.



Handling

- Fill with liquid.
- Set the meniscus to the required mark (read at eye level!)
- The wall of the cylinder must not be wetted above the mark.
- The indicated volume is the amount of liquid contained.

Note:

In the laboratory, graduated cylinders are frequently used just like measuring instruments that are calibrated to deliver (TD, Ex). Measurements with water showed that the dispensed volume is reduced by approximately the amount of the error limit of the graduated cylinder due to the residue amount from wetting. Prerequisite: The liquid is slowly dispensed in one portion, and to finish delivery of the liquid the cylinder is held at an incline for a further 30 s.

Mixing cylinders

The mixing cylinder is calibrated to contain (TC, In), just as the graduated cylinder. They are additionally supplied with a ground glass joint and stopper.



Mixing cylinders can be used in the preparation of standard solutions and dilutions, the same as volumetric flasks.

- After portions of various liquids have been measured out, these can then be mixed by shaking directly in the mixing cylinder.

Note:

When two liquids are mixed, a volume change may occur.

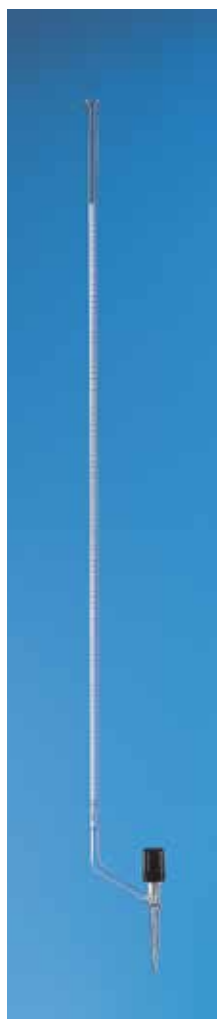
Handling of burettes

Burettes are glass volumetric instruments calibrated to deliver (TD, Ex) which are used for titration in volumetric analysis.

Note on waiting time:

Compared to pipettes, the handling of burettes is different during practical use and calibration. Typically, the volume used in a titration is less than the nominal volume, and the standard solution is added dropwise when close to the color change to avoid overtitration.

Types of burettes:



Burette with lateral stopcock



Automatic burette, Pellet pattern



Automatic burette, Dr. Schilling pattern

Calibration

Class AS: 'Ex + 30 s'

Class B: 'Ex'

In practice the time required for this dropwise addition is the same or even longer than the established waiting time. As a result, it is not necessary to observe the established waiting time of 30 s with class AS burettes during practical use.

Handling

1. Rinse the burette with the standard solution to be used, and orient it so that the burette tube stands upright. Make sure that the solution is completely homogenous, i.e., there must be no cloudiness, flocculation or precipitation present.
 2. Fill the burette slightly above the zero mark. To prime the stopcock, drain the burette no further than the nominal capacity. Should a small air bubble persist inside, hold the burette at an angle and lightly tap a finger against the location of the bubble.
 3. Refill titrant to approx. 5 mm above the zero mark. The wall of the burette should not be wetted further upwards.
 4. Drain liquid to set the zero point accurately. Important: Meniscus must be read at eye level (parallax-free level). Automatic burettes: Fill to approx. 5 mm above the zero mark. This is adjusted automatically after air release.
 5. Wipe off any drops adhering to the discharge tip.
 6. Open the stopcock and slowly add titrant to the sample (containing the indicator). The stopcock must not touch the wall of the vessel. Keep swirling the sample vessel lightly while adding titrant, or place it on a magnetic stirrer. For easiest recognition of the color change, place the sample vessel on a white surface. When the color changes, close the stopcock. The titration is finished.
 7. Read the discharged volume at eye level. The waiting time requirement (class AS: 30 s) is already fulfilled in practice during the titration process. It only needs to be taken into account during the calibration of the measuring instrument.
 8. Any drops remaining on the tip of the stopcock should be wiped against the vessel wall and rinsed down. It is part of the titrated volume.
- Before each new titration, reset the zero point and start the titration from there.

In addition to burettes, the following equipment is required for titrating: Volumetric flasks, bulb pipettes, Erlenmeyer flasks.

Handling of density bottles

Density bottles are mainly used to determine the density of liquids of moderate viscosity. They are not volumetric instruments, however, they are calibrated 'to contain' as in the case of volumetric flasks.

Types of density bottles:



Density bottle with stopper



Density bottle with thermometer and side capillary (recommended for liquids with an increased vapor pressure)

Handling

1. Determine the weight of the dry and empty density bottle.
2. Fill the density bottle with liquid, avoiding bubbles. The ground neck should be covered to about 1/3.
3. In a thermostatic bath, adjust the temperature of the bottle and contents to 20 °C.
4. Align the stopper respectively, the thermometer of the density bottle according to the marking, and insert carefully. The capillary tube fills up and the displaced liquid comes out.
5. Carefully dry the outer surfaces of the stopper (respectively, the side capillary) and the density bottle with tissue.

ATTENTION:

Be careful not to remove any liquid from the capillary. The sample liquid must be exactly level with the upper end of the capillary.

6. Determine the weight of the filled density bottle.

Calculate the density from the mass (weight) and the volume of the liquid at the reference temperature of 20 °C. The volume is engraved on the bottle. The equation is:

$$\text{Density } (\rho) = \text{Mass } (m) / \text{Volume } (V)$$

Take the buoyancy of the air into account for weighing.

Note:

Calibrated density bottles always carry a unique identification number on all component parts.
Use only parts with the same number together.

Working with pipetting aids

Pipetting aids are indispensable for working with pipettes.

Types of pipetting aids:

- motorized pipetting aids
- manual pipetting aids

Pipetting using the mouth or with a tube and mouthpiece is not allowed. A pipetting aid should always be used for this. This significantly decreases the risk of infection or injury.

Motorized pipetting aids

Motorized pipetting aids are ideal for pipetting longer series (e.g., for cell cultures).

e.g., **accu-jet® pro** by BRAND

The variable motor speed control and a special valve system enable sensitive operation with pipettes from 0.1 to 200 ml.

An integrated check valve in connection with a membrane filter effectively protects against penetrating liquids. To protect against corrosion, an active pressure compensation diverts vapors outside.

Handling

Pipetting is controlled through two large function buttons:

▲ Filling

To fill the pipette, press the upper button. Intake rate is continuously variable by trigger pressure.

▼ Delivery

Discharge rate is continuously variable by trigger pressure.

Choose:

Free delivery

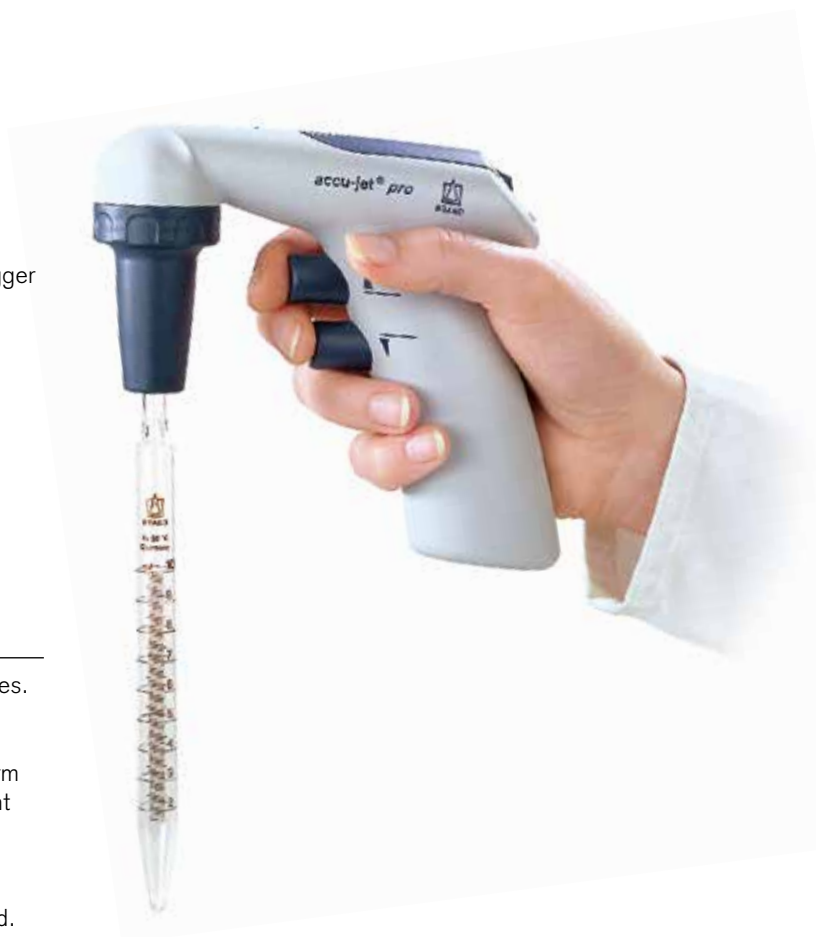
or

Power delivery

Dispensing liquid: free delivery or power delivery?

The choice of delivery modes is determined by the application. In the analytical laboratory, the **'free delivery'** mode is often preferred in order to obtain the required volumetric accuracy. To achieve the accuracy indicated on the pipettes, it is necessary to allow the liquid to run off freely, and to observe

the delivery and waiting times. In microbiology, however, volumetric accuracy is less significant. Here, the uniform and rapid delivery of nutrient solutions, etc. is of primary importance. Therefore, the **'power delivery'** mode is usually preferred in this field.



Manual pipetting aids

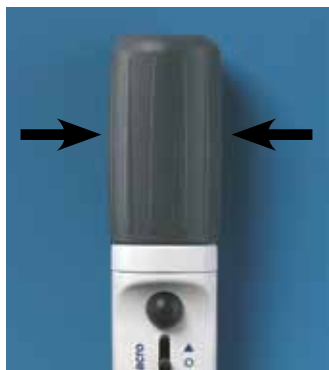
Manual pipetting aids are used when pipetting a short series, primarily in chemical laboratories.

e.g., macro pipette controller by BRAND

The macro is compatible with the full range of bulb and graduated pipettes from 0.1 to 200 ml. The special valve system allows easy

meniscus adjustment. A hydrophobic membrane filter protects the system against penetrating liquid.

Handling



Create negative pressure

Squeeze bellow.



Filling

Move pipetting lever upward. The farther up the lever is pressed, the faster the pipette will fill.



Adjust meniscus / dispensing by 'free delivery'

Press pipetting lever slightly downward. The meniscus falls – release the lever, the meniscus stops.
To drain the pipette, press the lever all the way down.
To comply with class A accuracy, do not blow out the residual liquid!

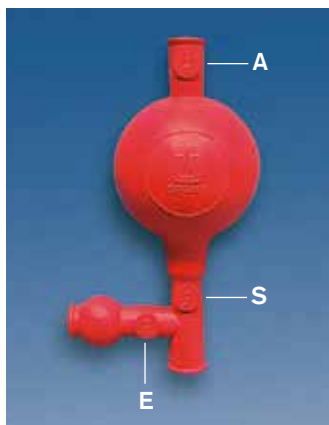


Blow out

When pipetting viscous media with 'free delivery', the pipette tip frequently does not empty completely. In these cases, blow out any remaining residues by pressing on the rubber bulb of the macro pipette controller.

Pipette filler

The classic standard pipetting aid for bulb and graduated pipettes.



Handling

1. Insert top of pipette
2. Press 'A' and squeeze the ball (create negative pressure)
3. Press 'S' to aspirate liquid just above the desired mark
4. Press 'E' to discharge liquid to the desired mark, or to drain the pipette

Blow out

To blow out viscous media, the side opening must be closed and the small ball compressed.

Attention!

The pipette filler should not be stored in the primed state, so that no liquid will be taken up!

Manual pipetting aids for small-volume pipettes up to 1 ml

Special pipetting aids have been developed for these pipettes. They are used in the medical field with capillary pipettes, blood diluting pipettes and blood sugar pipettes up to a max. of 1 ml.

Pipetting using the mouth or with a tube and mouthpiece is not allowed. A pipetting aid should always be used for this. This significantly decreases the risk of infection or injury.

e.g., micro pipette controller by BRAND

Handling



Filling / Delivery

Turn the thumb wheel to take in and deliver liquid. Pipettes calibrated to contain (TC, In) should be washed multiple times by filling and emptying with dilution solution.

Dispensing by 'free delivery'

To deliver liquid from pipettes calibrated to deliver (TD, Ex), press the air release button (observing the waiting time if necessary).

Eject

The large ejector key allows ejection of used pipettes without hand contact.

e.g., micro-classic pipette controller by BRAND

Due to its angled design, it is especially suitable for work under a microscope in IVF laboratories and in medical laboratories.

Handling



Attaching the pipette

Always attach the short end of the pipette, i.e. hold at the color code of the pipette and carefully slide it into the adaptor.

Filling

Turn the thumb wheel backward until liquid reaches the desired mark.

Delivery

Pipettes calibrated 'to contain':

Turn the thumb wheel forward until liquid is delivered. Rinse the pipette at least three times with the dilution solution.

Pipettes calibrated 'to deliver':

For 'free delivery' press the air release button until liquid has run out (observe waiting time if required).

Working with Liquid Handling Instruments

The ever increasing demands on the quality of analytical results, and the growing number of samples to be processed result in a need for volumetric instruments which help to handle the routine work in sample preparation as efficiently as possible.

Manufacturers of laboratory equipment have responded to this need by developing specialized liquid handling instruments. These appliances represent an improvement over the traditional volumetric instruments made of glass or plastic, and allow efficient work with a superior degree of precision and ease of operation.

The liquid handling instruments from most manufacturers share a similar operating principle, however the design details and materials used differ somewhat from one manufacturer to the next.

On the following pages, we will explain the functional principles and application of some of the most common liquid handling instruments, using appliances made by BRAND as examples.



Bottletop dispenser

Dispensette®



Bottletop dispenser

seripettor®



Bottletop burette

Titrette®



Single-channel air interface pipette

Transferpette® S (manual)



Multichannel air interface pipette

Transferpette® S -8/-12 (manual)



Single-channel air interface pipette

Transferpette® electronic



Multichannel air interface pipette

Transferpette®-8/-12 electronic



Positive displacement pipette

Transferpettor



Repetitive pipette

HandyStep® (manual)



Repetitive pipette

HandyStep® electronic

Dispensing with bottletop dispensers

Definition of 'dispensing':

The term 'dispensing' is understood to mean the discharge of defined quantities.

For easy, rapid and precise dispensing of reagents, bottletop dispensers are widely employed to replace poured transfers into graduated cylinders. They can be mounted directly on commercial laboratory bottles, either directly or by means of adapters. It is no longer necessary to transfer or decant chemicals. Serial dispensing in particular is greatly facilitated.



Functional principle of bottletop dispensers

By an upward movement of the piston, the preset amount of liquid is aspirated from the reagent bottle into the dispenser cylinder. By the subsequent downward movement of the

piston, the liquid is released through a valve system and the discharge tube.

There is no need to set a meniscus or to observe a waiting time.

We distinguish between bottletop dispensers with **floating** piston and with **wiping-seal** piston.



Bottletop dispensers with floating pistons

This system requires no piston seal and is therefore very durable and maintenance-friendly. The piston fits into the dispensing cylinder without contact. Piston and cylinder are separated by a gap only a few thousandths of a millimeter wide and filled with liquid. This film of liquid acts as a lubricant which makes the piston glide very smoothly.

e.g., Dispensette® by BRAND



Range of application

For dispensing aggressive reagents, e.g., concentrated acids such as H_3PO_4 , H_2SO_4 , bases like NaOH, KOH, saline solutions, as well as many organic solvents: Dispensette® III.

For dispensing organic solvents, such as chlorinated and fluorinated hydrocarbons (e.g., trichlorotrifluoroethane and dichloromethane), concentrated acids such as HCl and HNO_3 , as well as for trifluoroacetic acid (TFA), tetrahydrofuran (THF) and peroxides: Dispensette® Organic.

Not suitable for dispensing hydrofluoric acid (HF).

Materials

Depending on the requirements, parts that come into contact with liquid are made from various specially resistant materials, e.g., ceramic, platinum-iridium, tantalum, ETFE, PFA.

Safety always comes first!

When choosing a bottletop dispenser, the safety features of the instrument should be kept in mind. For example, does it reduce the risk of injury due to glass breakage? How does it avoid accidental splashing when the instrument is primed? How is contact with the medium minimized when the dispensing tube is closed?

Likewise, the suitability of the dispenser for the medium to be dispensed should be checked by the user. Information about this can generally be found in the operating manual in the chapter 'Function and limitations of use'. When in doubt, contact the manufacturer directly. Information on maintenance and monitoring of measuring instruments are also found in the operating manual.

Monitoring of measuring instruments / calibration

In regard to the monitoring of measuring instruments according to ISO and GLP guidelines, the accuracy of volumetric

instruments should be checked regularly and recalibrated if necessary (see page 33).

Bottletop dispensers with wiping-seal piston

In addition to the 'floating piston' operating principle, instruments with 'wiping-seal' piston are also used. It is frequently reported that these systems require higher operating forces and that the frictional wear can cause defective seals.

e.g., seripettor® by BRAND

The design of the system allows a replacement of the complete dispensing cartridge. The somewhat higher operating forces during filling are minimized by a spring with automatic lifting action.

The present example reflects a competitively-priced dispenser for simple dispensing tasks in the volume range of 0.2 to 25 ml.



Range of application and materials

The range of application includes the daily routine dispensing of bases, acids in low concentration, biological buffers, cell culture media, biological detergents and polar solvents.

The seripettor® *pro* dispenser is suitable for dispensing acids such as concentrated HCl, polar solvents such as acetone, essential oils and UV-sensitive media. In contrast to the seripettor® dispenser, valves made from chemically resistant materials are used in this instrument.

Titrating with bottle-top burettes

Definition of 'titration':

Titration is a volumetric method used for the quantitative analysis of a dissolved substance.

How to titrate?

Using a bulb pipette, a defined portion of a sample (liquid with an unknown fraction of dissolved material, e.g., acetic acid) is placed in an Erlenmeyer flask.

After dilution with water, 3 drops of an indicator solution are added. Then, with continuous swirling, a suitable titrant of known concentration (e.g., 0.1 M NaOH) is added from a burette until a color change in the indicator signals the endpoint of the titration.

Using the chemical equation and the volume of titrants used, the amount of substance dissolved in the sample can be calculated.



Functional principle of bottle-top burettes

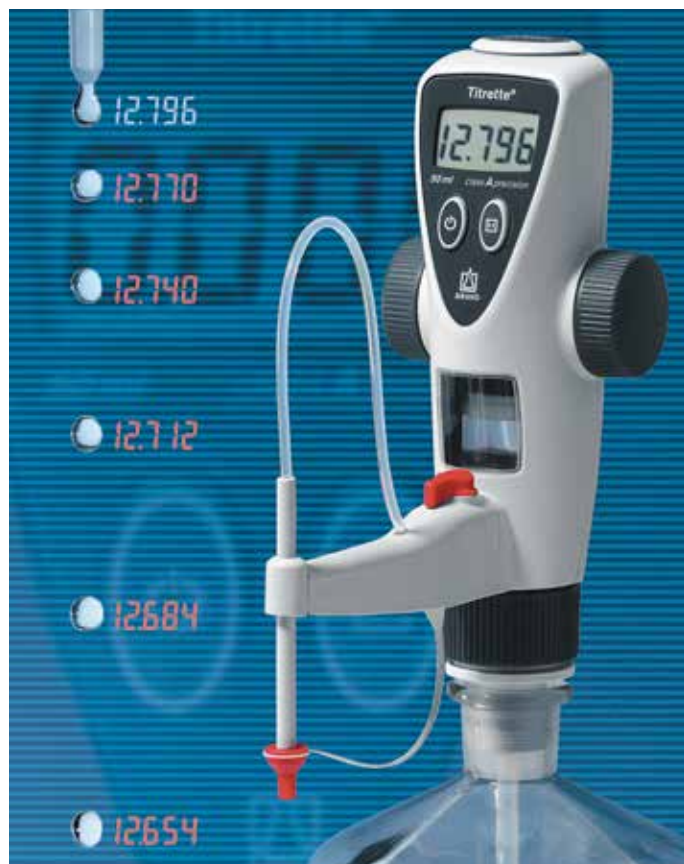
By the subsequent downward movement of the piston, the liquid is released slowly, and added to the sample through the discharge tube until the titration is finished, e.g., by change of color.

Bottle-top burettes are mounted directly upon the reservoir bottle. By the upward movement of the piston, liquid is aspirated from the reagent bottle into the burette cylinder.

Reading the volume

The discharged volume can be read directly from the display of the bottle-top burette. There are no meniscus reading errors.

e.g., Titrette® by BRAND in 10 ml, 25 ml, and 50 ml sizes



The piston moves when the hand wheels are turned, and this takes up or discharges the liquid. The electronics of the instrument automatically recognize the direction of rotation, whether filling or titration is taking place.

The liquid can be taken in quickly, and can then be delivered exactly, very slowly, drop by drop. A recirculation valve makes it possible to run the liquid back into the bottle during priming. Thus, air bubbles can be removed without loss of medium. The instrument can readily be disassembled in the laboratory for cleaning and maintenance.



Range of application

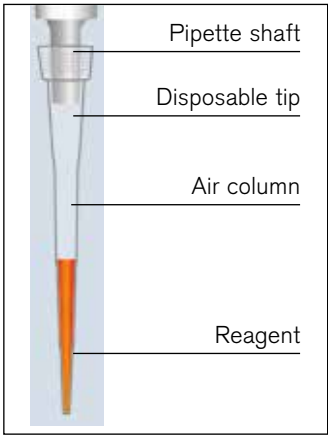
It can be used in many applications for aqueous and non-aqueous solutions (e.g., alcoholic KOH) up to 1 M.

Materials

Parts that come into contact with liquid are made from various specially resistant materials, e.g., borosilicate glass, PTFE, platinum-iridium, Al_2O_3 ceramic.

Pipetting with air interface pipettes

Definition of 'pipetting':
Pipetting is the accurate one-time uptake and delivery of liquids.
An air interface pipette is used for pipetting aqueous liquids in the microliter to milliliter range. It operates by the air interface principle.



Functional principle

The up and down movement of the piston inside the pipette shaft creates a negative or positive pressure of the air column. As a result, liquid is either aspirated into the tip or expelled from it. The air column (air interface) keeps the liquid separated from the piston.

Benefits

There is no wetting of the instrument; the liquid only enters the tip. Tips are used only once, which eliminates any carry-over. This is particularly important for applications where sterile conditions are required, or no carry-over is allowed.

Calibration

In regard to the monitoring of measuring instruments according to ISO and GLP guidelines, the accuracy of volumetric instruments should be calibrated regularly (i.e. checked) and adjusted if necessary (see page 33).

Manual single-channel pipettes

e.g., Transferpette® S by BRAND

In the routine lab and in research, precision and functionality are the standards to expect from air interface piston-operated pipettes today.

Operation



Aspirate reagent

1. Press pipetting button to the first stop. Hold the pipette vertically and immerse the tip into the liquid.
2. Allow the pipetting button to slowly slide back so that the liquid will be aspirated.

Volume range	Immersion depth in mm	Waiting time in s
0.1 µl - 1 µl	1 - 2	1
> 1 µl - 100 µl	2 - 3	1
> 100 µl - 1000 µl	2 - 4	1
> 1000 µl	3 - 6	3



Discharge reagent

1. Place the pipette tip against the wall of the vessel and press the pipetting button slowly to the first stop and hold it down.
2. The blow-out stroke empties the tip completely: press the pipetting button down to the second stop and wipe the pipette tip on the vessel wall over a distance of approx. 10 mm.



Eject tip

Press the tip ejector.

Manual multichannel pipettes

These pipettes also function by the air interface principle. They allow 8 or 12 pipetting operations to be carried out simultaneously.

Microtiter technology requires pipetting into microtiter plates of 8 x 12 cavities (96-well plates) with standardized spacing. This technology allows e.g., the detection of minute quantities of proteins. This method can only be employed efficiently with multichannel pipettes. Multichannel pipettes are ideal for the efficient transfer of samples, for serial dilutions and for washing of microtiter plates.

Areas of application

- Clinical diagnostics
- Food analysis
- Immunology
- Biochemistry
- Cell culture

Analytical techniques

- Immunofluorescence (IF)
- Radio immunoassay (RIA)
- Enzyme immunoassay (EIA, ELISA)
- Cell culture dilution



e.g., Transferpette® S-8/-12 by BRAND

Operation



Aspirate reagent

1. Press pipetting button to the first stop. Immerse the tips 2 to 3 mm into the liquid.
2. Allow the pipetting button to slowly slide back so that the liquid will be aspirated.



Discharge reagent

1. Place the pipette tips against the wall of the vessel and press the pipetting button slowly to the first stop and hold it down.
2. The blow-out stroke empties the tips completely: press the pipetting button down to the second stop and wipe the pipette tips on the vessel wall over a distance of approx. 10 mm.



Eject tips

Press the tip ejector.

Ergonomics and Strain

Intensive, repeated operations done on mechanical instruments without proper ergonomic design result in prolonged stress that can lead to a number of muscular problems, known collectively as RSI (repetitive strain injuries). At particular risk are the muscles in the neck area, shoulders, arms and thumbs. Thus, laboratory work is often accompanied by the appearance of, inter alia, tendonitis and carpal tunnel syndrome. Especially with microliter pipettes, the need for fatigue-free operation is paramount.

Electronic single- and multichannel pipettes

Operating principle

Pressing the pipetting button starts the aspiration or discharge mechanisms (incl. blow-out). The pipette's piston is moved by a motor, aspiration and

discharge are controlled by a microprocessor. Various pipetting programs can be selected with the control keys.

Advantages of electronic pipettes

The combination of motor-controlled pipettes and ergonomic design enables stress-free, fatigue-free operation. It also reduces the demands on the thumb while carrying out lengthy series which would otherwise increase the risk of RSI syndrome!

An additional advantage is the execution of pipetting programs such as the gel electrophoresis mode (with precise display of delivered volume) and a dispensing mode, which are not possible with manual pipettes.

e.g., **Transferpette®** electronic single- and multichannel pipette by BRAND



With electronic single- and multichannel pipettes, handy design, balanced weight distribution, intuitive software and clear, readily understood technical documentation should be standard.

Operation

Aspirate reagent

Immerse the tip in the reagent and press the pipetting button once – the set volume will be aspirated.

Discharge reagent

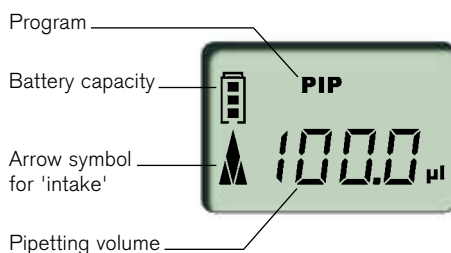
Press the pipetting button again, and the liquid will be discharged. The blow-out takes place automatically! During this process, wipe the tip on the vessel wall over a distance of approx. 10 mm.

Eject tips

Press the tip ejector.

Pipetting programs of the microliter pipette Transferpette® electronic

The display



The pipetting programs

Pipetting

The standard program. The set volume is aspirated by the pipette, and then discharged.



Mixing of Samples

Program for mixing of liquids. The sample is repeatedly aspirated and discharged, and the number of mixing cycles is displayed.



Reverse Pipetting

Program specially designed for the pipetting of liquids with a high viscosity, high vapor pressure or foamy media. For a set volume, the blow-out volume is aspirated additionally. This volume remains in the tip after delivery to prevent undefined running out, splashing, or the formation of foam or bubbles.



Pipetting with Electrophoresis

Program for loading of electrophoresis gels. The required sample volume is aspirated. During discharge, the volume being dispensed is tracked continuously, allowing the user to stop discharge to avoid over-filling sample wells. The pipette records the exact volume dispensed to ensure accuracy of sample mass calculations. GEL mode may also be used for microtitrations.



Dispensing

A program for the dispensing of liquids in a series of equal aliquots. A volume that has been aspirated is dispensed in steps.



Additional functions

Depending on the quality and design, electronic pipettes can offer further instrument-specific functions in addition to the pipetting programs.

The Transferpette® electronic, for example, offers a program to simplify and speed up the calibration of the instrument as well as a battery refresh function.

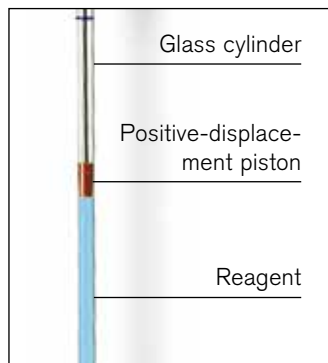
What does 'reverse' mean?

A reversal of the stops used to measure a volume. Works with mechanical pipettes as follows:

To aspirate the reagent, press the pipetting button down to the **second stop** and let it slide all the way back. Then press down just to the **first stop** to discharge the set volume.

Pipetting with positive-displacement pipettes

Positive-displacement pipettes are the ideal alternative for difficult applications where air interface pipettes reach their physical limits. They are well suited even for media of very high or low viscosity, high vapor pressure, or a tendency to foam.



Functional principle

In contrast with air interface pipettes, the positive-displacement piston is in direct contact with the pipetted liquid. The piston wipes the walls of the tips/capillaries completely clean – literally to the last drop which can be observed leaving the tip orifice. This principle always provides reproducible results, regardless of the

physical properties of the liquid. There is no need to discard tips or capillaries after each pipetting operation, since the minimal residual wetting is negligible for most applications. If carry-over is a concern, such as with infectious or radioactive media, an air interface pipette with disposable tips should be preferred.

Benefits

Highest accuracy and speedy operation. The tips or capillaries are reusable. No need for reading a meniscus for pipetting.

e.g., Transferpettor by BRAND



Range of application



Highly viscous media, such as highly concentrated protein solutions, oils, resins and fats.



Media with high vapor pressure such as alcohols, ether and hydrocarbons.



Media which tend to foam, such as tenside solutions.

Operation (similar to air interface pipettes)

Volume setting

Select desired volume by turning the volume setting knob.

Aspirate reagent

Press piston to the stop. Immerse tip into reagent and slowly let the piston return to aspirate reagent.

Discharge reagent

Place the capillary/tip against the vessel wall and press the pipetting button down a second time to the stop. Positive-displacement pipettes have no blow-out!

Dispensing with repetitive pipettes

The distribution of liquids is one of the most important and common activities in medical, pharmaceutical and biological laboratories. The most common techniques are pipetting and dispensing. Dispensing refers to the repeated discharge of identical quantities of liquid. The dispensers described in this chapter eliminate the need for repeated intake after each step – a major time-saving compared to pipetting. Since dispensing is such a common technique, the ergonomic design of the devices plays an essential role.

Dispensing tasks in the laboratory are rarely handled by fully automatic systems requiring no manual intervention. Generally, repetitive pipettes are used for these routine jobs.

Types of repetitive pipettes:

- Manual repetitive pipettes
- Motorized electronic repetitive pipettes



Functional principle

With manual repetitive pipettes, the volume delivered in each step results from the length of the stroke, defined by the number of steps on a toothed rack, and the size of the tip. Therefore, only a limited number of defined dispensing steps is available.

No intermediate volumes can be selected. A main advantage of these devices is their robustness; their drawback is their often fatiguing operation. Repetitive pipettes work on the proven positive-displacement principle. Therefore, even difficult media with high vapor

pressure, high viscosity or a tendency to foam pose no problem to the repetitive pipettes.

According to volume range, the repetitive pipette can be fitted with PD-Tips of different sizes.

Manual repetitive pipette

e.g., HandyStep® by BRAND



The repetitive pipette simplifies serial pipetting by taking up a medium once and then delivering it step by step. With one filling, up to 49 steps between 2 µl and 5 ml can be dispensed, depending on the size of the PD-Tip. The volumes and number of steps are a combined result of the setting of the volume selector key (1-5) and the size of the tip used.

Positive-displacement tips (PD-Tips) from BRAND are available in 10 different sizes, sterile or non-sterile.

Compatible tips of other manufacturers may also be used.

Available combinations with positive-displacement tips (PD-Tips) of different sizes from BRAND

Steps and volume ranges

Setting	Tip size (ml)										Steps
	0.1	0.5	1	1.25	2.5	5	10	12.5	25	50	
1	2	10	20	25	50	100	200	250	500	1000	49
1.5	3	15	30	37.5	75	150	300	375	750	1500	32
2	4	20	40	50	100	200	400	500	1000	2000	24
2.5	5	25	50	62.5	125	250	500	625	1250	2500	19
3	6	30	60	75	150	300	600	750	1500	3000	15
3.5	7	35	70	87.5	175	350	700	875	1750	3500	13
4	8	40	80	100	200	400	800	1000	2000	4000	11
4.5	9	45	90	112.5	225	450	900	1125	2250	4500	10
5	10	50	100	125	250	500	1000	1250	2500	5000	9
Volume (µl)											

Ergonomics and design

Repetitive working with hand-operated appliances can lead to a variety of muscular ailments which may particularly affect the neck, shoulders, arms and thumb. Fatigue-free operation is therefore a crucial requirement for repetitive pipettes, since they are used almost exclusively for serial dispensing. Ergonomic design is indispensable for the stress-free performance of extended pipetting operations in the same working position.

Motorized electronic repetitive pipettes



Functional principle

Intake and discharge are controlled by a single key. The piston inside the tips is driven by a motor, with a microprocessor controlling the volume and number of steps.

The ergonomic design results in fatigue-free operation. The piston wipes the walls of the tips completely clean, providing precisely reproducible results without the influences of an air interface.

The positive-displacement tips allow the dispensing of media of high density, high vapor pressure or volatility, or with a tendency to foam.

e.g., HandyStep® electronic by BRAND



The size of PD-Tips from BRAND is encoded in their piston. After inserting the tip, the size is automatically recognized and displayed. This prevents errors, and the volume to be

dispensed and the desired operating program can then be easily selected. When a new PD-Tip of the same size is inserted, all instrument settings are maintained.

In contrast to manual repetitive pipettes which only permit a limited number of volume settings, the electronic repetitive pipettes allow the continuous selection of intermediate volumes, such as 1.01 ml.

Available working modes:



Dispensing standard mode

A predefined volume is dispensed repeatedly.



Automatic Dispensing

The instrument calculates the average time interval between your three first dispensing steps, and automatically continues to work at this rhythm.



Pipetting

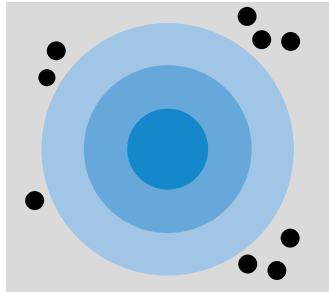
Works like a positive-displacement pipette. A predefined volume is taken up and discharged.

Precision Defined

What do 'Error Limit, Accuracy, Coefficient of Variation and Precision' mean in volumetric measuring?

An illustration of Precision and Accuracy

The dart board simulates the volume range around the centered specified value, the black dots simulate the different measured values of a specified volume.



Poor accuracy:

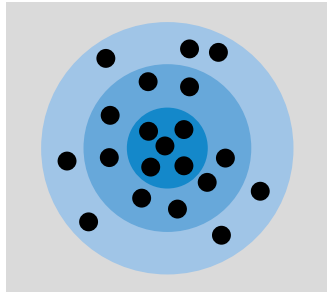
Hits far off center.

Poor reproducibility:

Hits widely scattered.

Result:

These volumetric instruments are of inferior quality.



Good accuracy:

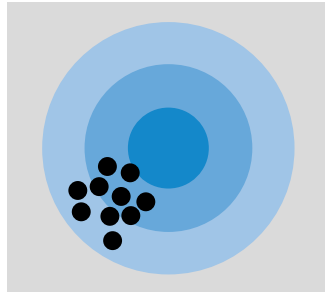
On average, hits are evenly distributed around center.

Poor reproducibility:

No gross errors, but hits widely scattered.

Result:

All deviations are 'equally probable'. Instruments exceeding the permissible limit should be removed from service.



Poor accuracy:

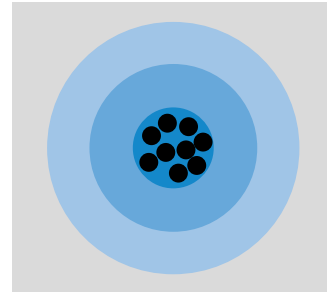
Although all hits are close together, the center (true volume) is still missed.

Good reproducibility:

All hits are close together.

Result:

Improperly controlled production, with systematic variation. Instruments exceeding the permissible limit should be removed from service.



Good accuracy:

All hits are near the center, i.e., the specified value.

Good reproducibility:

All hits are close together.

Result:

The volumetric instruments have minute systematic errors, narrow scatter; the permissible limit is not exhausted. These instruments should remain in service.

To describe accuracy, the term "Error limit" is used for glass volumetric devices, while for liquid handling devices the statistical terms "Accuracy [%]" and "Coefficient of Variation [%]" have become established.

1 Error limit

The term "Error limit" (EL) in the corresponding standards defines the maximum permissible deviation from the specified value.

2 Accuracy (A)

Accuracy (A) indicates the closeness of measured mean volume to the specified value, i.e., systematic measurement variation. Accuracy is defined as the difference between the measured mean volume (\bar{V}) and the specified value ($V_{\text{spec.}}$), related to the specified value in percent.

3 Coefficient of Variation (CV)

Coefficient of variation (CV) indicates the closeness of values of repeated measurements, i.e., random measurement variation. Coefficient of variation is defined as standard deviation in percent, related to the mean volume.

4 Partial volumes

Generally A and CV are related to the nominal volume (V_{nominal}). These values are in % and have to be converted for partial volumes ($V_{\text{part.}}$). In contrast, there is no conversion for partial volumes, if A and CV are stated in volume units (e.g., ml).

5 Error limit of A and CV

A good estimate for the error limit (EL) of the instrument, e.g., for the nominal volume (V_{nominal}), can be calculated using the values for accuracy and coefficient of variation.

6 Precision (reproducibility)

It describes the closeness in volume units between the different values in a set of measurements.

$$\begin{array}{lllll}
 \text{1} & \text{2} & \text{3} & \text{4} & \text{5} \\
 \text{EL} \geq |V_{\text{measured}} - V_{\text{spec.}}| & A [\%] = \frac{\bar{V} - V_{\text{spec.}}}{V_{\text{spec.}}} \cdot 100 & CV [\%] = \frac{s \cdot 100}{\bar{V}} & A_{\text{part.}} [\%] = \frac{V_{\text{nominal}}}{V_{\text{part.}}} \cdot A_{\text{nominal}} \% & \text{EL} \geq \frac{|A \%| + 2CV \%}{100\%} \cdot V_{\text{nominal}}
 \end{array}$$

(analog $CV_{\text{part.}} [\%]$)

Monitoring of Measuring Instruments

Which devices have to be monitored?

All measuring instruments which are used to confirm warranted product qualities are subject to monitoring.

Analytical laboratories have to verify and document the accuracy of all measuring devices used in order to achieve reliable results. This especially applies to laboratories which operate according to GLP guidelines, are accredited to DIN EN ISO/IEC 17025, or are certified to DIN EN ISO 9001. All these standards and guidelines require the availability of written instructions describing the monitoring procedure in detail.

The error limits or accuracy and coefficient of variation have also to be defined, and there must be instructions on how to proceed if the acceptable limits are exceeded.

Timing and frequency of monitoring

The instrument's accuracy and measuring uncertainty must be known and documented before its admission for use. In addition, the instrument has to be tested again at defined intervals (see DIN EN ISO 10012).

Reason:

The performance of measuring instruments may be affected by e.g., the use of aggressive chemicals, and the manner and frequency of cleaning. Since the required accuracy largely depends on the circumstances of each application, the user has to determine the intervals for routine testing. Typical intervals for liquid handling instruments and plastic volumetric instruments are every 3-12 months, for glass volumetric instruments, every 1-3 years.

Testing procedures

Volumetric instruments are tested gravimetrically. Testing for liquid handling instruments is performed according to ISO 8655; for glass volumetric instruments, ISO 4787 is applied. Many influences must be considered when carrying this out. Therefore, BRAND provides Standard Operating Procedures (SOP), including detailed testing instructions, for every type of volumetric instrument. The testing procedure is outlined step by step. To make it even easier, BRAND offers software which can perform all calculations, store them in a database, and print out a detailed test report.

Time required for testing

The monitoring of measuring devices should not become the main occupation in the laboratory; it should be limited to a reasonable extent. The demand is for simple procedures which are quick and inexpensive to follow. The combination of testing instructions (SOP), specially developed EASYCAL™ calibration software together with volumetric instruments delivered with individual or batch certificate are best suited to minimize the time needed for this procedure.

Monitoring of conformity-certified instruments

Volumetric instruments which are conformity-certified to DIN 12 600 are also subject to the monitoring procedure. There is no clear directive whether such instruments have to undergo initial testing or not. The user is responsible for answering this question. However, to be on the safe side, the initial test of a representative random sample is recommended. In addition, such a test will document the initial state in relation to subsequent tests. Another option would be the purchase of volumetric instruments with a certificate from the manufacturer.

Terms used in the monitoring of measuring devices

Calibration

Calibration in the strictest sense consists of determining the actual volume delivered. The calibration procedure should be quick and simple, eliminating potential sources of error. Therefore, BRAND provides detailed testing instructions for every type of volumetric instrument, free of charge.

Adjustment

Adjustment consists of correcting the deviation of the measured value from the nominal value.

Depending on the manufacturer, the adjustment of liquid handling instruments is generally accomplished by turning an adjustment screw. After setting, a new calibration check is required. This procedure must be repeated until the volume is within the error limits.

Procedure for volumetric testing

e.g., Microliter pipette Transferpette® adjustable volume, 20-200 µl

We recommend a calibration of the Transferpette®, as described below, once every 3-12 months. Depending on frequency of use and pipetted media, shorter testing intervals may be defined by the user.

A Preparation:

1. Verify instrument type and nominal capacity.
2. Read serial number.
3. If instrument is soiled, disassemble and clean if necessary (see operating manual).
4. Check for damage (housing, shaft tip, ejector, piston, seal). Obtain spare parts as required.
5. Place the Transferpette® pipette (unpacked) including accessories into the testing room for at least 2 hours to adjust to room temperature.

B Functional test:

1. Mount a new pipette tip.
2. Pre-rinse the tip once with testing liquid (distilled/deionized water).
3. Hold the filled pipette vertically and observe for approx. 10 seconds whether a drop is forming at the tip. Make sure the tip is not being heated, e.g., by the sun. Discharge the liquid. In case of smaller volumes (approx. < 50 µl) no drop will form even if there is a leak, due to surface tension.

A tip for finding leaks in small volume pipettes:

Discharge a small drop from the filled tip so that a small air cushion (air bubble) is present below the liquid. If, during observation, the air cushion falls, there is a leak.

C Gravimetric test:

1. Determine temperature of the liquid for testing.
2. Mount a new pipette tip.
3. Condition the instrument: aspirate and release testing liquid five times. This will improve the accuracy of the test.
4. Mount a new pipette tip and pre-rinse it once.
5. Place the weighing vessel on the balance and tare.
6. Remove weighing vessel from the balance.
7. Discharge testing liquid into weighing vessel, pressing the pipetting key to the second stop to empty tip completely.
8. Place weighing vessel on the balance. Read and note value.
9. Retare the balance.
10. Repeat steps 2. to 9. ten times. Enter values obtained at 100%, 50% and 10% of nominal volume into the test record.

Gravimetric testing values at 21.5 °C (Z = 1.0032)

Tested volume (µl):	200.0000
Specified value (mg):	199.3620
x ₁	200.2000
x ₂	199.6000
x ₃	199.4900
x ₄	199.7000
x ₅	199.7000
x ₆	199.2900
x ₇	199.3500
x ₈	199.4100
x ₉	199.2000
x ₁₀	199.1900

Values obtained by gravimetric testing only indicate the mass (weight) of the pipetted volume. To obtain the actual pipetted volume, the values have to be multiplied by a correction factor, taking into account the temperature (see below). For all BRAND liquid handling instruments detailed testing instructions are available for download at www.brand.de.

1. Calculation of the mean volume:

A mean volume (\bar{x}) of the weighing values is calculated by dividing the sum of the weighings by the number of weighings made. This mean mass is then multiplied by a correction factor (Z, units of µl/mg) to give the mean volume (\bar{V}) delivered. The factor Z combines density of water at the testing temperature and effects of atmospheric pressure. For a typical temperature of 21.5 °C and air pressure of 1013 mbar (hPa), Z = 1.0032 µl/mg.

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

$$\bar{V} = \frac{x_1 + x_2 + x_3 + \dots + x_{10}}{n} \cdot Z$$

$$\bar{V} = \frac{200.2 + 199.6 + 199.49 + \dots + 199.19}{10} \cdot 1.0032$$

$$\bar{V} = 199.513 \cdot 1.0032$$

$$\bar{V} = 200.1514$$

2. Calculation of accuracy:

$$A [\%] = \frac{\bar{V} - V_{\text{spec.}}}{V_{\text{spec.}}} \cdot 100$$

$$A [\%] = \frac{200.1514 - 200}{200} \cdot 100$$

$$A [\%] = 0.076$$

Extract from the table 'Factor Z for liquid handling Instruments'

Temperature °C	Factor z ml/g
18	1.00245
18.5	1.00255
19	1.00264
19.5	1.00274
20	1.00284
20.5	1.00294
21	1.00305
21.5	1.00316
22	1.00327
22.5	1.00338
23	1.00350
23.5	1.00362
24	1.00374
24.5	1.00386
25	1.00399
25.5	1.00412
26	1.00425

3. Calculation of the standard deviation, necessary for the determination of coefficient of variation

$$s = Z \cdot \sqrt{\frac{(x_1 - \bar{x})^2 + (x_2 - \bar{x})^2 + (x_3 - \bar{x})^2 + \dots + (x_{10} - \bar{x})^2}{n - 1}}$$

$$s = 1.0032 \cdot \sqrt{\frac{(200.2 - 199.51)^2 + (199.6 - 199.51)^2 + (199.49 - 199.51)^2 + \dots + (199.19 - 199.51)^2}{9}}$$

$$s = 1.0032 \cdot \sqrt{\frac{0.8393}{9}}$$

$$s = 0.306$$

4. Calculation of the coefficient of variation:

$$CV [\%] = \frac{s \cdot 100}{\bar{V}}$$

$$CV [\%] = \frac{0.306 \cdot 100}{200.1514}$$

$$CV [\%] = 0.153$$

The result for the calculated example is:

Results of the gravimetric testing:

Tested volume (µl):	200.0000
Mean volume (µl):	200.1514
A [%]	0.076
CV [%]	0.153
A [%] specified*	0.600
CV [%] specified*	0.200

* Error limits in operating manual.

⇒ This pipette meets specifications.

If the calculated values for Accuracy (A [%]) and Coefficient of Variation (CV [%]) are less than or equal to the error limits, the instrument is calibrated to operate within specifications.

Note:

For checking partial volumes, the values $A_{\text{nominal}} [\%]$ and $CV_{\text{nominal}} [\%]$ which are related to the nominal volume V_{nominal} must be converted.

For a partial volume of 20 µl this means:

$$A_{20 \mu\text{l}} [\%] = \frac{V_{\text{nominal}}}{V_{20 \mu\text{l}}} \cdot A_{\text{nominal}} [\%]$$

$$A_{20 \mu\text{l}} [\%] = \frac{200 \mu\text{l}}{20 \mu\text{l}} \cdot 0.5\%$$

$$A_{20 \mu\text{l}} [\%] = 5\%$$

The calculation of CV_{part} is analog.

What to do if the instrument exceeds the error limits:

1. Review the operating manual to ensure that the instrument was operated properly.
2. Follow the troubleshooting guide in the operating manual for assistance.
3. Recalibrate the instrument in accordance with the operating manual.

If despite these steps the instrument still does not meet the specifications, remove from service and contact the manufacturer for support.

Calibration software

The monitoring of measuring devices to GLP and DIN EN ISO 9001 is not exactly straightforward. Complex equations easily lead to calculating errors, and the documentation of results can be tiresome. To facilitate this tedious task, some manufacturers have developed special calibration software.

e.g., **EASYCAL™** calibration software by **BRAND**

EASYCAL™ performs all calculations and generates the complete documentation, automatically. All you need is an analytical balance, a PC, a printer (optional) and EASYCAL™ software.

A demo version of the software can be downloaded from the Internet (www.brand.de) or can be requested on CD-ROM without charge.

- Suitable for instruments from all manufacturers
- Specifications of many instruments preloaded
- Testing according to ISO 4787, ISO 8655, etc.

Monitoring made easy

EASYCAL™ calibration software facilitates the monitoring of measuring devices to GLP and DIN EN ISO 9001, both for liquid handling instruments and for volumetric instruments of glass and plastic. The software is user-friendly and intuitive to use. After determining the type of instrument to be tested, all necessary data are entered step by step on the 'Entry of measuring results' screen.

Two options are available for entering the weighing results:

manual entry, or direct import from the balance via cable, followed by automatic evaluation. After defining the error limits, EASYCAL™ performs all calculations automatically. By one push of a button, a comprehensive test record can be printed out. All results are stored in a database. The test history keeps track of all tested instruments, facilitating the monitoring over time. The test intervals determined in relation to the testing instructions (SOPs) can be defined individually.

Calibration service for liquid handling instruments

Calibration can be a time-consuming procedure. For this reason, BRAND offers a full calibration service including instrument adjustment and, if required, repair.

Instrument:		Transferpette		10 µl		Serial No.		13A8864		Thermometer:		Goldbrand		1		Balance:		Sartorius BA		3		Relative humidity:		50% ± 30%		Atmosph. pressure abs(hPa):		1013		Temperature:		22 °C / 71.60 °F		Correction factor z:		1.00328	
Results from gravimetric tests:																																					
Test volume: 10 µl																																					
Value: 9.97																																					
5 weighings per channel																																					
EX																																					
Channel-No. 1 2 3 4 5 6 7 8 9 10 11 12																																					
X 1 (mg) 9.92 9.99 9.9 9.95 9.97 9.94 9.91 9.91 9.93 9.93 9.93 9.93																																					
X 2 (mg) 9.92 9.99 9.94 9.98 9.9 9.94 9.99 9.93 9.93 9.93 9.93 9.93																																					
X 3 (mg) 9.97 9.95 9.98 9.96 9.99 9.9 9.95 9.97 9.95 9.97 9.97 9.97																																					
X 4 (mg) 9.92 9.98 9.94 9.96 9.92 9.94 9.96 9.97 9.96 9.97 9.97 9.97																																					
X 5 (mg) 9.88 9.9 9.96 9.96 9.92 9.93 9.95 9.94 9.95 9.94 9.94 9.94																																					
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X Mean (mg) 9.92 9.99 9.9 9.95 9.97 9.94 9.95 9.92 9.95 9.92 9.92 9.92																																					
V Mean (µl) 9.92 9.99 9.9 9.95 9.97 9.94 9.95 9.92 9.95 9.92 9.92 9.92																																					
A(%) Actual 0.05 0.05 0.05 0.05 0.05 0.05 0.05 0.05 0.05 0.05 0.05 0.05																																					
CV(%) Actual 0.25 0.38 0.33 0.11 0.21 0.17 0.29 0.37 0.37 0.37 0.37 0.37																																					
Result A 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0																																					
Result CV 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0																																					
Test volume: 5 µl																																					
Value: 4.98																																					
5 weighings per channel																																					
EX																																					
Channel-No. 1 2 3 4 5 6 7 8 9 10 11 12																																					
X 1 (mg) 5.05 5.05 5.05 5.05 5.05 5.05 5.05 5.05 5.05 5.05 5.05 5.05																																					
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V Mean (µl) 5.01 5.05 5.04 5.02 5.02 5.04 5.03 5.03 5.03 5.03 5.03 5.03																																					
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CV(%) Actual 0.39 0.41 0.39 0.82 0.71 0.40 0.48 0.48 0.48 0.48 0.48 0.48																																					
Result A 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0																																					
Result CV 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0																																					
The test was carried out according to ISO 8655																																					
Next test 05.2005																																					
Result: Gravimetric test ok																																					
Test date: 01.02.2005																																					
Test Operator: Andrea Smekal																																					
Signature:																																					

Test record printout

Conformity and Calibration Certificates

Types of certificates:

- Conformity certification
- Certificates of performance (works certificates)
- Calibration certificates (Eichamt, DAkkS certification)

Conformity certificates

'Eichordnung' and Conformity Certification

For volumetric instruments which are kept and used for commercial purposes, such as medical and pharmaceutical fields (manufacture and testing of medicinal products), the German Federal Weights and Measures Regulations ('Eichordnung') of Aug. 12, 1988, calls for conformity certification instead of official calibration. This also applies for volumetrically relevant accessories (e.g., pipette tips for piston-operated pipettes).

Who certifies conformity?

Conformity means: compliance of an instrument with the 'Eichordnung', the German Federal Weights and Measures Regulations, Annex 12. The conformity certification procedure is described in DIN 12600.

The conformity symbol 'H' and the manufacturer code designation (for BRAND it is 'B') or, on request the 'Eichamt' (the German State Office of Weights and Measures, with a separate conformity symbol) certifies the product as complying with the 'Eichordnung' for official certification and corresponding standards. In general the product itself carries the symbol of conformity or, with disposables, the packaging (without a separate conformity certificate).



Conformity symbol
of BRAND

Note:

Among others, blood diluting pipettes, blood cell counting chambers, thermometers, and density bottles are not conformity certified, but are still officially certified.

Certificates of performance

A quality assurance system organized according to DIN EN ISO 9001 earns the right for a manufacturer to issue certificates of performance, or factory certificates. Factory certificates are available as individual or batch certificates. All quality control results are documented and archived for a minimum of 7 years. If the batch or serial number is known, the individual results at the time of production can be accessed at any time.

Calibration certificates

Official calibration certificate

This certificate is issued by the 'Eichamt', the German State Office of Weights and Measures, and is accepted in Germany and many other countries. Both the instrument and the certificate show the individual serial number and the year of issue.

DAkkS Calibration certificate

The German Calibration Service (DKD) was founded in 1977 as a joint institution of government and industry. It verifies the conformity of measuring equipment used in industrial and research laboratories and testing institutions with the national standards administered by the PTB (German Federal Institute of Physics and Metrology). Based on the legal requirements the DKD Accreditation was successively transformed to the DAkkS Accreditation (Deutsche Akkreditierungsstelle GmbH), starting from 2010.

The DAkkS calibration certificate officially documents, at a high level, the traceability of measuring results to national and international standards, including SI units, as required by the groups of standards DIN EN ISO 9001 and ISO/IEC 17025 for the monitoring of measuring instruments.

A major difference between works calibration services and DAkkS calibration laboratories is the accurate and reliable determination of the respective uncertainty of measurement, guaranteed by the accredited laboratory and supervised by the DAkkS.

DAkkS calibration certificates are appropriate where calibrations by an accredited laboratory are required, where calibrations of the highest level are demanded and for the calibration of reference standards and for instruments used for comparative measurements.

The DAkkS is a member of the International Laboratory Accreditation Cooperation (ILAC). A multilateral agreement assures obligatory recognition of the DAkkS calibration certificate in many countries. The certificate is issued in a variety of languages.

Batch certificate

All instruments and certificates from one production batch show the same batch number. The certificate records the mean value, the standard deviation and the date of issue.

Individual certificate

Both the instrument and the certificate show an individual serial number in addition to the batch number. The certificate records the measured volume, the uncertainty of measurement and the date of issue.

BLAUBRAND® USP volumetric instruments

Companies that manufacture pharmaceutical products for the American market and are therefore audited by US agencies such as the FDA (Food and Drug Administration) are obligated to meet the requirements of USP (United States Pharmacopeia).

The current USP describes in Chapter 31 that the Class A error limits specified in the ASTM standards are required for glass volumetric instruments. These error limits are listed in tables for volumetric flasks, bulb pipettes, and burettes. For graduated pipettes up to and including the 10 mL size, the error limits are listed in the text.

Error limits

BRAND glass volumetric instruments are manufactured according to current DIN EN ISO standards. Since the construction requirements defined in the DIN EN ISO standards differ from those in the ASTM standards, different error limits result for the individual measuring instruments.

BRAND confirms with the enclosed USP certificates for all USP measuring instruments delivered that the Class A error limits corresponding to the ASTM standards are observed.



Conformity certified

All BLAUBRAND® volumetric instruments are conformity certified. With the sign , BRAND, as manufacturer, confirms that the instruments are manufactured according to 'Eichordnung', the German Federal Weights and Measures Regulations. This sign of conformity is printed directly on the instruments, according to DIN 12600.

BRAND lists all the test equipment used in each lot and individual certificate.

USP batch certificate

The mean value plus standard deviation for the batch, along with the date of issue are documented on the certificate (batch number: year of manufacture/batch).

USP individual certificate

The measured volume, measurement uncertainty, and the date of issue are documented on the certificate (individual serial number: year of manufacture/batch/consecutive instrument number).

IVD Directive

Implications and consequences



IVD Directive of EU

On December 7th, 1998, the EU directive for 'In-vitro-Diagnostic Medical Devices' (IVD Directive) was published in the Official Journal of the European Communities and became effective since June 7th, 2000.

How to define In-Vitro-Diagnostic Medical Devices (IVD)?

An 'In-Vitro-Diagnostic Medical Device' is any medical device used in-vitro for the examination of specimens, including blood and tissue donations, derived from the human body. IVD can be a reagent, calibrator, control material, kit, instrument, apparatus, equipment, system, or specimen receptacles, intended by the manufacturer to be specifically used for in-vitro diagnostic examination. IVD are mainly used to provide information

- concerning a physiological or pathological state
- concerning a congenital abnormality
- to monitor therapeutic measures.

What is a Medical Device?

The definition of a 'Medical Device' includes any instrument, apparatus, appliance, material or other article, including the software necessary for its proper application, intended by the manufacturer to be used for human beings for the purpose of:

- diagnosis, prevention, monitoring, treatment or alleviation of disease, injury or handicap
- investigation, replacement or modification of the anatomy or of a physiological process
- control of conception.

Excluded are pharmacological or immunological means, which are regulated by appropriate drug laws.

CE Marking

The CE mark is the official marking required by the European Community. It shows the user, that this product fulfills all essential safety and environmental requirements as defined in the so-called European Directives. The manufacturer marks the instrument and produces a declaration of conformity describing the instruments' fulfillment with the guidelines and technical requirements.

BRAND medical products are all included in the class of in-vitro diagnostic (IVD) devices. This includes, for example:

- blood counting chambers
- haemocytometer cover glasses
- disposable capillary pipettes
- micro haematocrit capillaries
- haematocrit sealing compound
- sample cups for analyzers
- urine beaker
- feces container
- cryogenic tubes
- pipette tips
- PD-Tips
- Transferpette® microliter pipettes
- HandyStep® repetitive pipettes

Quality Management

Quality management is indispensable. Ideally, it should already begin at project stage, and should accompany a product's design, development and manufacturing process. This guarantees the greatest possible security in working with laboratory equipment, and the reliability of analytical results.

Quality management at BRAND

Quality management is briefly described for liquid handling instruments and BLAUBRAND® volumetric instruments

Quality management at BRAND begins at product conception and continues through the design process and production. Routine checks throughout the entire manufacturing process result in volumetric instruments with the smallest possible deviation from the true volume (accuracy) and narrow scatter of individual values (coefficient of variation). The final step of this Statistical Process Control is random finished product sampling according to DIN ISO 3951.

The quality management system applied at BRAND and certified to DIN EN ISO 9001 is a combination of process monitoring and random checks. The accepted quality level (AQL) is at the very least 0.4., i.e., the limiting values are met with a statistical certainty of at least 99.6 %.

All measuring instruments used in quality control are regularly checked and are referenced to the national standards of PTB (The German Federal Institute of Physics and Metrology). Quality management according to DIN EN ISO 9001 is the basis for issuing of calibration certificates (e.g., our certificates of performance).

All test results are documented and filed for 7 years. If the batch or serial number is known, each specific test result on the date of production can be traced. As BRAND manufactures conformity certified volumetric instruments, the quality of products is automatically supervised by the 'Eichamt', the German State Office of Weights and Measures. The requirements for monitoring of measuring instruments, traceability to national standards, and staff qualification are fully met.

Cleaning of Laboratory Equipment

Manual and machine cleaning

Glass and plastic labware can be cleaned manually, in an immersion bath, or in a laboratory washing machine.

Labware should be cleaned immediately after use – at low temperatures, with brief soaking times, and low alkaline detergents. Labware which has come into contact with infectious substances should first be cleaned and afterwards, if necessary, autoclaved. This is the only way to prevent baking of the substance, and subsequent damage to the labware by any adhering chemical residues.

Note:

Carefully disinfect labware before cleaning when there is a risk of injury during cleaning procedure.

Wiping and scrubbing method

The generally accepted wiping and scrubbing method with a cloth or sponge soaked in cleaning solution is the most popular cleaning method. Labware must never be treated with abrasive scouring agents or pads which might damage the surface.

Immersion method

For the immersion method, labware is soaked in the cleaning solution for 20 to 30 minutes at room temperature, then rinsed with tap water, and finally with distilled water. Only for stubborn residues should the soaking time be extended and the temperature increased.

Ultrasonic bath

Both glass and plastic labware may be cleaned in an ultrasonic bath. However, direct contact with the sonic membranes must be avoided.

Machine cleaning

Machine cleaning with a laboratory washing machine is more gentle to labware than cleaning in an immersion bath. The labware is only exposed to the cleaning solution for the relatively short flushing periods when sprayed by the jet or ejector nozzles.

- Lightweight objects will not be tossed and damaged by the jet if they are secured in washing nets.
- Labware is protected against scratching when the wire baskets in the washing machine are plastic coated.

Glass labware

With glass labware, prolonged immersion times in alkaline media above 70 °C should be avoided. Such treatment, particularly with volumetric instruments, might cause volume changes through glass corrosion, and destruction of graduations.

Plastic labware

Plastic items generally have smooth, non-wetting surfaces and can usually be cleaned effortlessly under low alkalinity conditions. Polystyrene or polycarbonate labware, e.g., centrifuge tubes, must only be cleaned manually with neutral detergents. Prolonged exposure even to low alkaline detergents will impair their strength. The chemical resistance of these plastics should be verified in each case.

Cleaning in trace analysis

To minimize metallic traces, laboratory equipment is placed into 1N HCl or 1N HNO₃ at room temperature for not more than 6 hours. (Glass laboratory equipment is often boiled for 1 hour in 1N HNO₃.) It is then rinsed with distilled water. To minimize organic contamination, laboratory equipment can first be cleaned with alkalis, or a solvent such as alcohol.

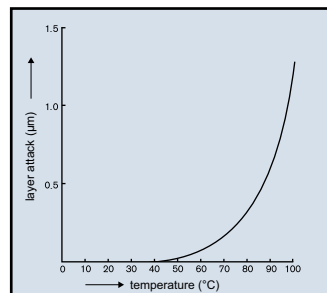
Gentle cleaning

For gentle treatment of labware, clean immediately after use – at low temperatures, with brief soaking times, and at low alkalinity. Glass volumetric instruments should not be exposed to prolonged immersion times in alkaline media above 70 °C, as such treatment causes volume changes through glass corrosion, and destruction of graduations.

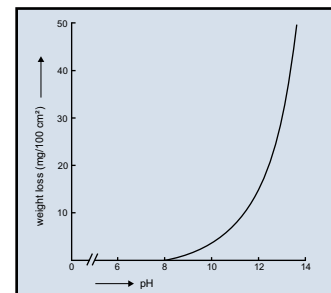
Information

At 70 °C, a 1N sodium hydroxide solution will corrode a layer of approx. 0.14 µm off the surface of Boro 3.3 (borosilicate glass 3.3) within 1 hour. However, at 100 °C, a layer of

1.4 µm, or tenfold more, will be removed. Therefore, cleaning temperatures above 70 °C should be avoided and low alkaline cleaning agents are preferable.



Alkali attack on Boro 3.3 in relation to temperature, calculated from weight loss. c (NaOH) = 1 mol/l. Exposure time: 1 hour.



Alkali attack on Boro 3.3 in relation to pH value, at 100 °C. Exposure time: 3 hours.

(Graphs are from the brochure 'Technische Gläser' by SCHOTT AG, Mainz.)

Disinfection and sterilization

Disinfection

Laboratory instruments that have come into contact with infectious material or genetically modified organisms must be disinfected prior to reuse/disposal; i.e., they must be brought to a condition in which they no longer pose a risk of infection.

Therefore laboratory instruments can be treated with disinfecting detergents for example. If necessary and suitable, the items may subsequently be sterilized (autoclaved).

Steam sterilization

Steam sterilization (autoclaving) is defined as the destruction or irreversible inactivation of all reproducible microorganisms under exposure to saturated steam at 121 °C (2 bar) according to DIN EN 285. For correct sterilization procedure, including biological security, please contact your sterilization officer.

The following points must be observed:

- Efficient steam sterilization is assured only if the steam is saturated and has unrestricted access to all contaminated areas.
- To prevent pressure build-up, containers or vessels must always be open.
- Contaminated reusable labware must be cleaned thoroughly before steam sterilization. Otherwise, residue will bake on during sterilization and microorganisms may not be effectively destroyed if they are protected by the residue. Furthermore, any adhering chemical residues may damage the surfaces due to the high temperatures.
- Not all plastics are resistant to steam sterilization. Polycarbonate, e.g., will lose its strength. Polycarbonate centrifuge tubes cannot be steam sterilized.
- During sterilization (autoclaving), plastic equipment in particular should not be mechanically stressed (e.g., do not stack). Thus, to avoid shape deformation, beakers, flasks, and graduated cylinders should be autoclaved in an upright position.

Thermal resistance

All reusable BLAUBRAND® and SILBERBRAND volumetric instruments can be heated up to 250 °C in a drying cabinet or a sterilizer, without any subsequent volume deviations. However, as with all glass instruments, irregular heating or sudden temperature changes produce thermal stresses which may result in breakage. Therefore:

- Always place glass instruments into a cold drying cabinet or sterilizer; then heat slowly.
- At the end of the drying or sterilizing period, allow instruments to cool off slowly inside the switched-off oven.
- Do not heat up volumetric instruments on a hot plate.
- Pay attention to the maximum operating temperatures of plastic instruments.

Safety Information

Handling of hazardous substances

The handling of hazardous chemicals, infectious, toxic or radioactive substances and genetically modified organisms, calls for a high degree of responsibility on the part of everyone involved, to ensure personal and environmental protection. The relevant regulations must be scrupulously observed including laboratory, professional association, environmental, radiation, waste disposal and generally accepted technical standards and guidelines (e.g., DIN or ISO).

Important information on safety

- Before use, laboratory instruments must be examined by the user for suitability and functionality.
- Always hold pipettes near the suction end, and carefully insert the pipette into the adapter of the pipette controller until it is securely and firmly seated. Do not use force. Broken glass can cause injury!
- In the course of repeated use, laboratory instruments should be examined for eventual damage, especially instruments subjected to pressure or vacuum (e.g., desiccators, filter flasks, etc.).
- The hazards of working with defective labware should never be under-estimated (e.g., cuts, burns, risk of infection). If a professional repair is not practical, properly dispose of such items.
- Instruments to be repaired must be cleansed of all residues and be sterilized, as necessary. Radioactively contaminated items must be decontaminated as prescribed by the radiation protection authorities. Volumetric glass instruments (e.g., volumetric flasks, graduated cylinders, etc.) should not be repaired when damaged. Exposure to heat may result in residual stress within the glass (greatly increasing the probability of breakage), or an uncontrolled cooling process may lead to permanent volume alterations.
- It is not permissible to simply truncate defective graduated cylinders. This shortens the distance between the upper graduation mark and the spout, as defined by DIN, resulting in an increased danger of chemicals being spilled and the operational safety is no longer guaranteed.
- Waste must be disposed of according to local laws and regulations. This applies also to disposable articles. It must not pose a hazard to human beings or the environment.
- Laboratory equipment must be disposed of according to the materials they are made of, and in a clean state, in accordance with the regulations in force. Please note that laboratory glassware is not recyclable.

Working with glass

When working with glass, it is essential to consider its limitations regarding resistance to thermal shock and to mechanical stress. Strict safety measures must be observed:

- Do not heat volumetric instruments, measuring cylinders and flasks on hot plates.
- Exothermic reactions such as diluting sulfuric acid or dissolving solid alkaline hydroxides must always be carried out while stirring and cooling the reagents, and in suitable vessels such as Erlenmeyer flasks – never in graduated cylinders or volumetric flasks!
- Glass instruments must never be exposed to sudden temperature changes. When taking them out of a drying cabinet while hot, never place on a cold or wet lab bench.
- For compressive loads, only glass instruments intended for this purpose may be used. For example, filtering flasks and desiccators may be evacuated only after confirming that they are in perfect condition.

For you notes.



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