Power of Precision: Pipetting

avantor™

Workflows Where Pipetting Plays Major Roles

HPLC/LCMS/GC/ICP/ AAS….

Cell Culture & Tissue Culture

PCR, ELISA & other Microplate Application

Microbiology & other diagnostic applications

Good Pipetting Practices are Pivotal Here

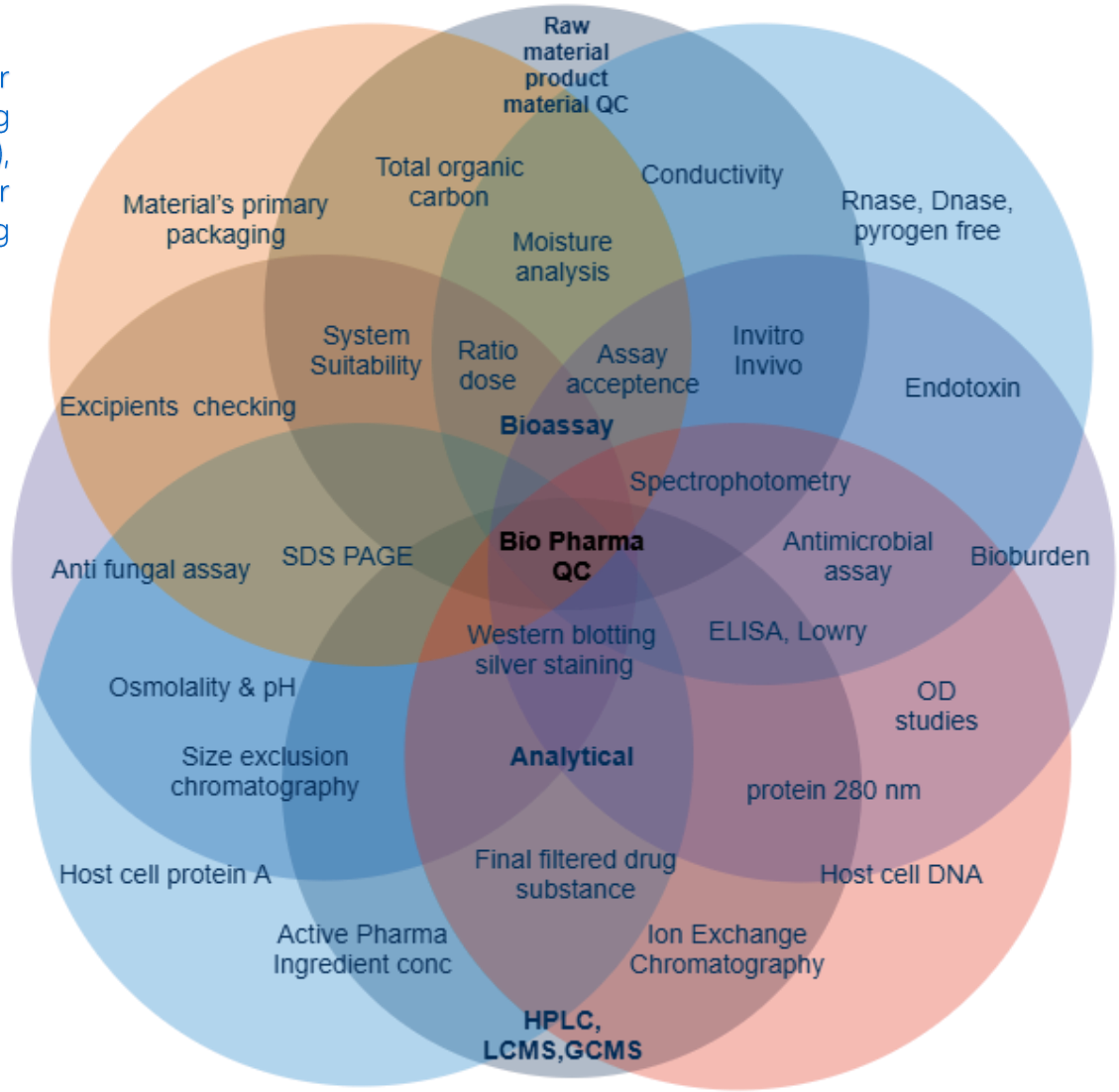
Pipetting Pharma Workflow

Quality control laboratories may perform some or all quality control activities, e.g. sampling, testing of APIs(Active Pharmaceuitical ingredient), excipients, packaging materials and/or pharmaceutical products, stability testing, testing against specifi cations and investigative testing.

Samples would be taken for:

- *One for immediate testing*
- *Second for confirmation of testing*
- *Third for retention in case of dispute.*

The result of an analysis should be traceable, when appropriate, ultimately to a primary reference substance. All calibrations or qualifi cation of instruments should be traceable to certified reference materials and to SI units (metrological traceability).

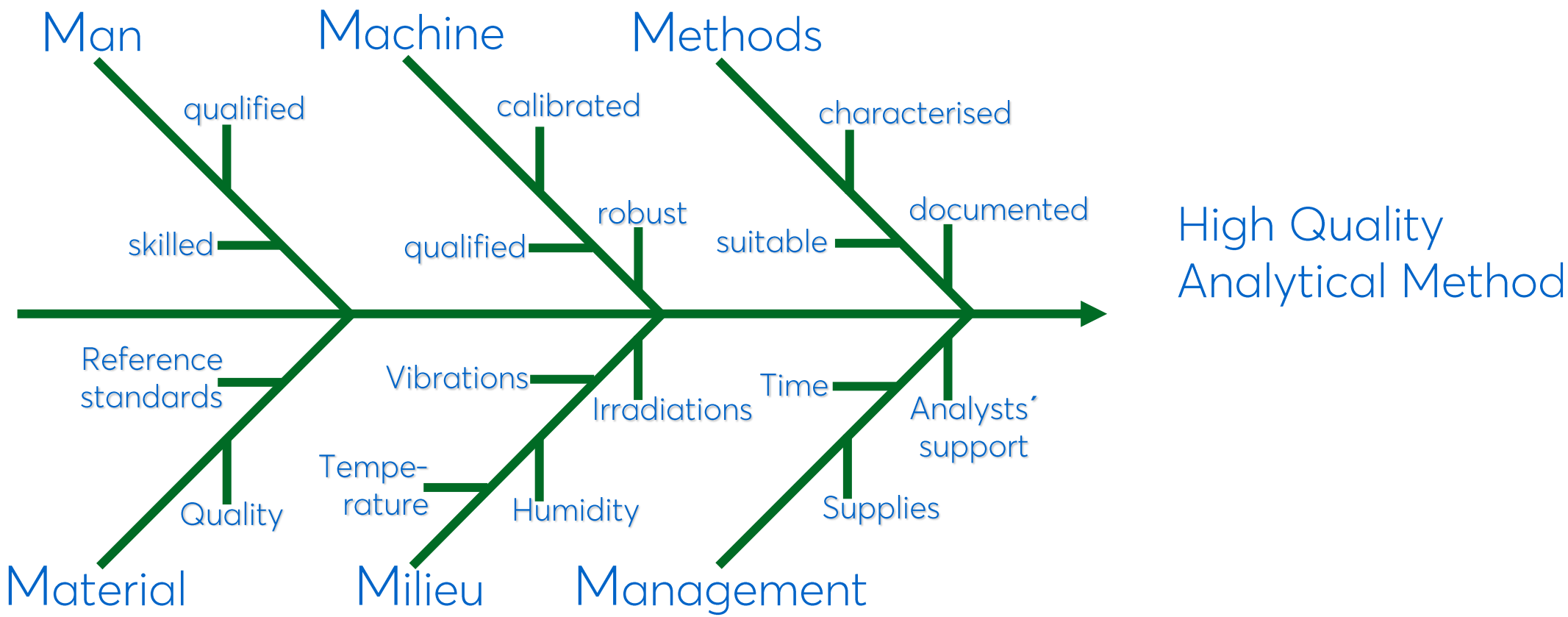


Reference substances (primary reference substances or secondary reference substances are used for the testing of a sample.

compliance testing Analysis of active pharmaceutical ingredients (APIs), pharmaceutical excipients, packaging material or pharmaceutical products according to the requirements of a pharmacopoeial monograph or a specifi cation in an approved marketing authorization.

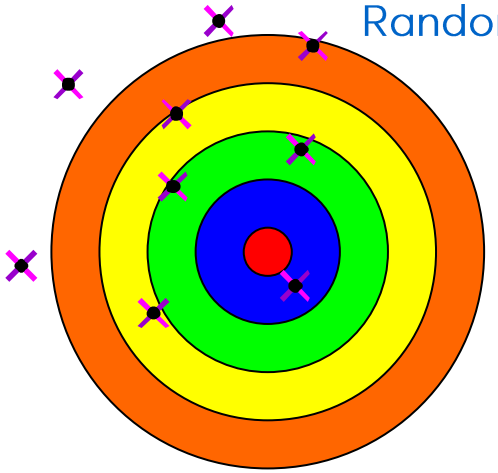
Prerequisites for an High Quality Analytical Method

The '6M' principle



Accuracy and Precision

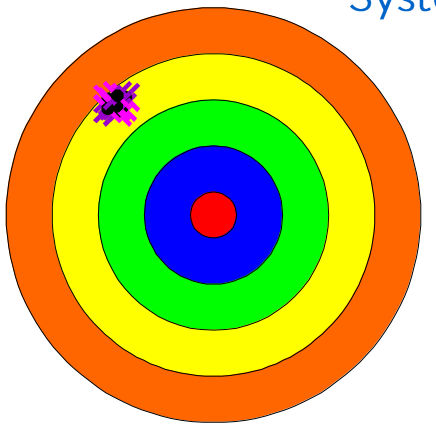
Random



- Noise
- Careless measurement
- High dead volume instrument
- Dropped digits

Low Precision
Low Accuracy

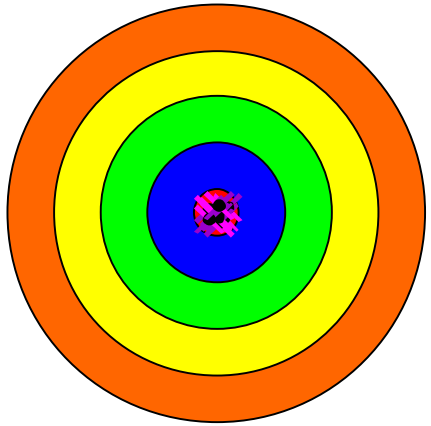
Systematic



Measured result to deviate by a fixed amount in one direction from the correct value

- Mis-calibrated instrument

High Precision
Low Accuracy



High Precision
High Accuracy

Measurements typically contain some combination of random and systematic errors.

Precision is an indication of the level of random uncertainty.

Potential source of uncertainty in pipetting

Adjustment - altering the pipette so that the dispensed volume is within the specifications.

Air Displacement Pipettes - are meant for general use with aqueous solutions. In air displacement pipettes, a certain volume of air remains between the piston and the liquid.

Aspirate - to draw up the sample.

Blow-out – to empty the tip completely.

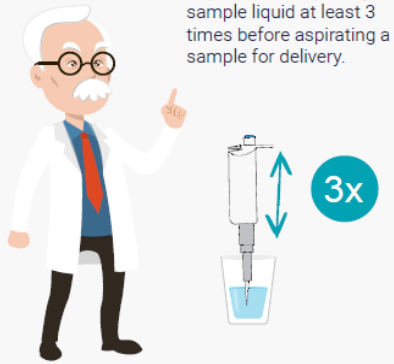
Calibration check - checking the difference between the dispensed volume and the selected volume.

Dispense - to deliver the sample.

Positive Displacement Pipettes – are used for high viscosity and volatile liquids. In positive displacement pipettes, the piston is in direct contact with the liquid

Improving pipetting skills, the 12 indispensables

01 Pre-wet the pipette tip

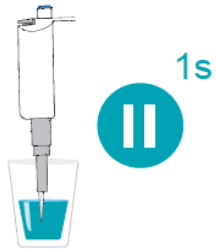


Aspirate and expel any sample liquid at least 3 times before aspirating a sample for delivery.

02 Immerse the tip to the proper depth during aspiration

Before aspirating, immerse the tip adequately below the meniscus. Large volume pipettes (1-5 mL) should be immersed to 5-6 mm, while smaller volume pipettes should be immersed to 2-3 mm.

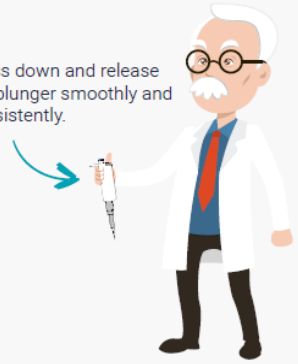
03 Pause consistently after aspiration



Leave the tip still in the liquid for about 1 second after aspirating the sample.

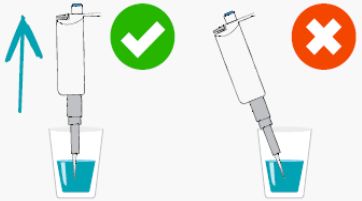
04 Use consistent plunger pressure and speed

Press down and release the plunger smoothly and consistently.

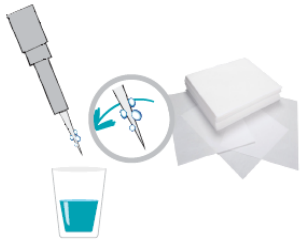


05 Pull the pipette straight out

During sample aspiration always hold the pipette vertically and avoid touching the sides of the container.



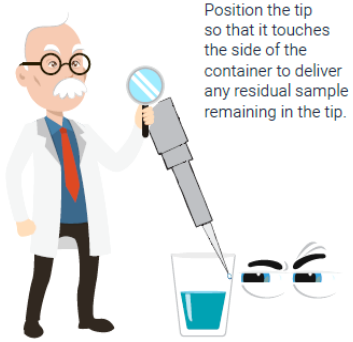
06 Examine the tip before dispensing a sample



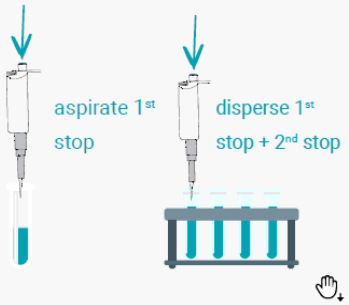
Before dispensing, carefully remove droplets on the outside of the tip with a lint-free cloth, being sure to stay clear of the tip opening.

07 Examine the tip after dispensing a sample

Position the tip so that it touches the side of the container to deliver any residual sample remaining in the tip.



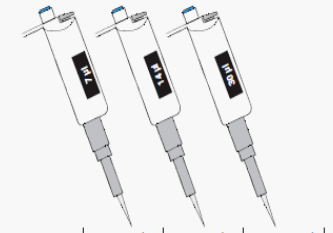
08 Use standard mode pipetting



Choose "standard (or forward) mode" pipetting rather than "reverse mode" for all aqueous samples.

09 Use the appropriate pipette

Pipette with a volume range closest to the volume you plan to aspirate and dispense.



	1-10 µl	2-20 µl	20-100 µl
1-10 µl	✓	✗	✗
2-20 µl	✗	✓	✗
20-100 µl	✗	✗	✓

10 Use the correct pipette tip

Use high quality tips intended for use with specific pipettes.

11 Work at ambient temperature equilibrium

Allow liquids and equipment to reach an equilibrium at an ambient temperature before you begin pipetting.

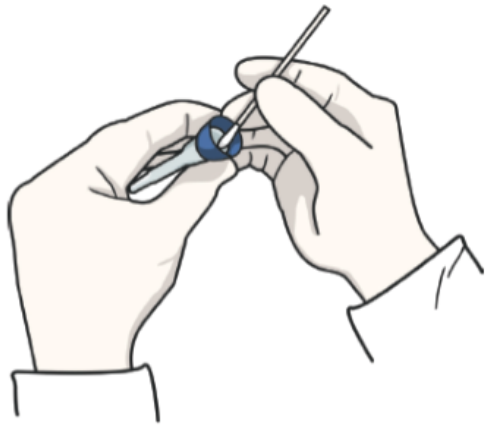
12 Minimize pipette handling

Hold the pipette loosely, return it to the pipette stand or set it down when you are not pipetting.

Wear gloves to reduce body heat transfer to the pipette.

Cleaning Pipettes

Inspect piston
Look for nicks, corrosion



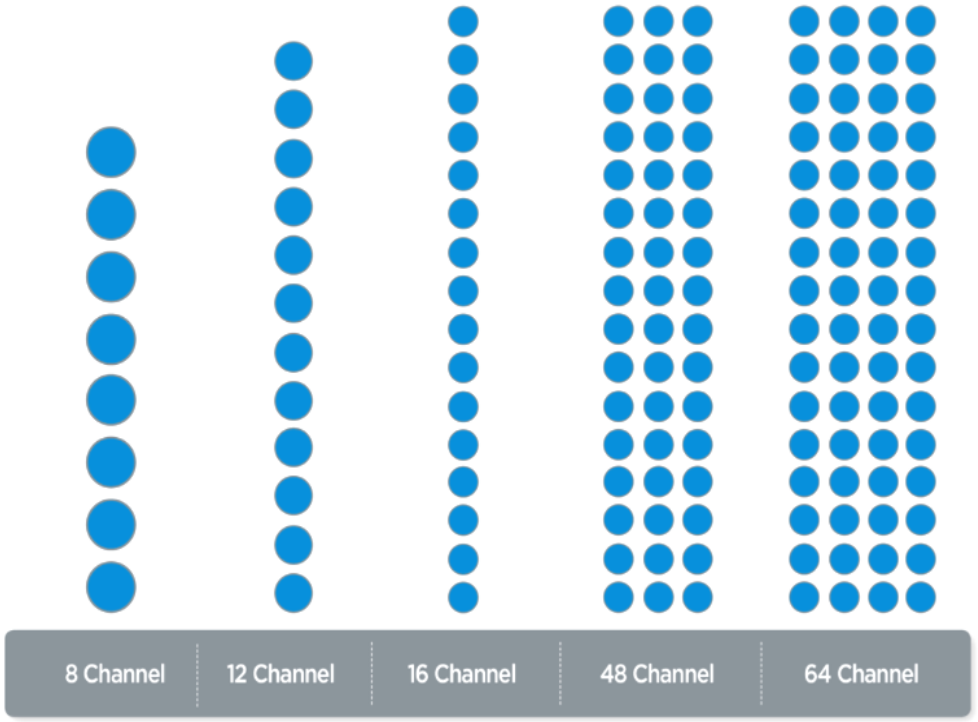
Use long-stemmed foam swab
Moisten with cleaning agent

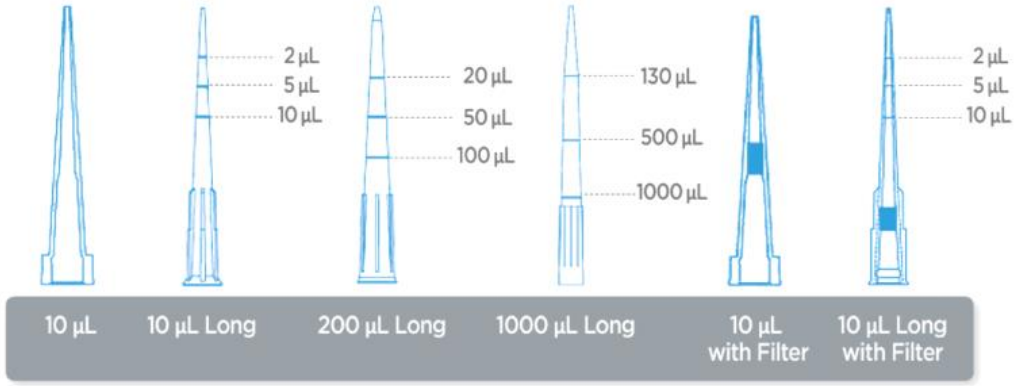
Examine shaft interior
Look for obstructions

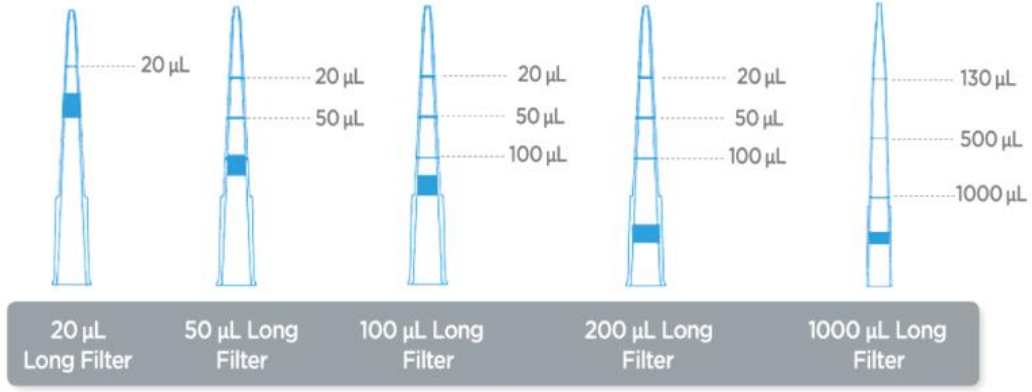
Sample	Cleanser	Action
DNA, RNA	Fresh 10% bleach solution or DNA/RNA cleanser	Immerse for 10 minutes. Rinse with distilled water. Wipe with isopropyl alcohol and allow to air dry.
Proteins	Fresh 10% bleach solution or detergent. **Not** alcohol	Immerse for 10 minutes. Rinse with distilled water and allow to air dry.
RNase	RNase decontaminant solution (RNaseAway®, RNaseZap®) or 95% EtOH followed by 3% H_2O_2	Immerse in RNase decontaminant solution for one minute, then rinse with distilled water and air dry. Alternatively, immerse briefly in 95% ethanol, rinse, then immerse in 3% hydrogen peroxide for 10 minutes. Rinse, and air dry.
Aqueous solution	Detergent solution or 70% ethanol	Immerse for 5 minutes. Rinse with distilled water, then wipe with isopropyl alcohol and allow to air dry.
Organic solvent	Detergent solution	Immerse for 5 minutes. Rinse with distilled water and allow to air dry.
Radioactive sample	High-strength radioactivity decontaminant (COUNT-OFF™ surface cleaner, Decon 90™ cleaning agent)	Immerse for 5 minutes. Rinse 3x with distilled water. Measure with Geiger counter for radioactivity. Properly dispose of gloves, liquids and all cleaning materials according to your organization's radioactive safety procedures.

Pipette Tips Types

Point to be noted: seating tips improperly and having excessive ejection force can cause damage., not to mention some ergonomic strain.







Posture Matters a Lot

SITTING

Adjust your chair before starting work, making sure your feet rest firmly and comfortably on the floor or footrest, and that you have adequate back support.

POOR

- Neck and back bent
- Elevated shoulders
- Upper arms too high
- Elbow extended
- Wrist not straight

GOOD

- Back and neck in neutral position
- Upper arm relaxed and near vertical
- Wrist and forearm straight
- Thighs parallel with floor and feet firmly supported

STANDING

Periodically rest one foot on a step or stool, and be sure to alternate your weight between feet. When standing for prolonged periods, use a padded anti-fatigue mat.

POOR

- Neck, shoulders and back are stooped
- Elbow is flexed

GOOD

- Neck, shoulders and back are upright
- Upper arm is near vertical
- Elbow at 90°
- Forearm is parallel and aligned with wrist

PIPETTING

Whether sitting or standing, be sure the bench is a comfortable height and that frequently used items are within easy reach.

More good pipetting ergonomics:

- Avoid awkward, twisting movements
- Don't over reach
- Switch hands periodically
- Keep your grip loose
- Vary pipetting with other tasks
- Take frequent breaks
- STRETCH!

POOR

- Upper arm flexed
- Elbow extended
- Wrist not aligned with forearm

- Wrist extended backward

GOOD

- Forearm parallel to the floor
- Wrist and forearm aligned

Different Pipettes for Different Applications ?

Single Channel Manual Pipette

- HPLC
- GC
- ICPMS
- Microbiology

Multi Channel Manual Pipette

- PCR
- ELISA
- Cell Culture

Electronic Pipettes (Single & Multi Channel)

- Any Liquid Handling, where repetitive dispensing is required
- Stepper Pipetting

Serological Pipettes & Pipette Controller

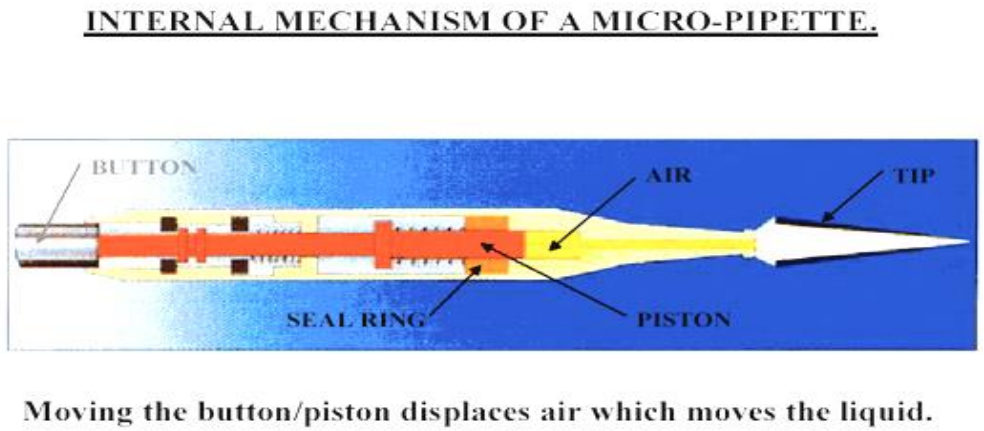
- Cell & Tissue Culture

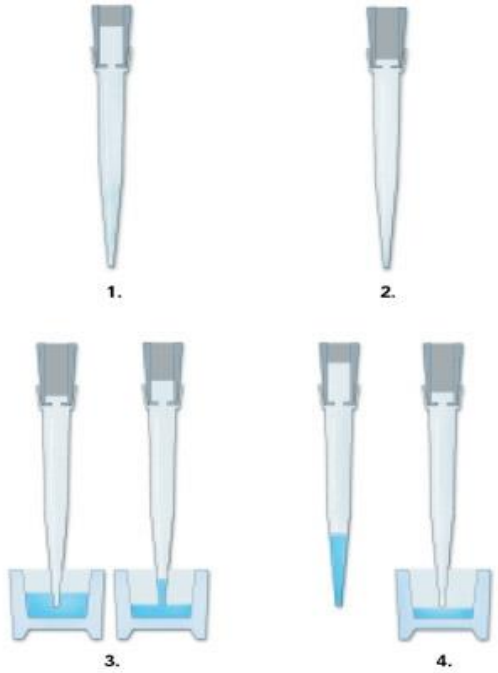
Recommendation for pipetting w.r.t solution

Solution/Compound	Examples	Pipette	Tip	Technique	Comments
Aqueous solution	Buffers, diluted salt solution	Air Displacement	Standard	Forward	
Viscous solution	Protein and nucleic solutions, glycerol, Tween 20/40/60/80	Air Displacement Pos. Displacement	Standard wide orifice Pos. Displacement	Reverse	Pipette slowly to avoid bubble formation.
Volatile compounds	Methanol, Hexane	Air Displacement Pos. Displacement	Filter Pos. Displacement	Forward	Pipette rapidly to avoid evaporation. Carbon filter tips prevent vapors from going into the Pipette.
Nucleotide solutions	Genomic DNA, PCR Products	Air Displacement Pos. Displacement	Filter or wide orifice	Forward	For genomic DNA wide orifice should be used to avoid mechanical shearing.
			Pos. Displacement		
Radioactive compounds	Carbonate, H-thymidine	Air Displacement Pos. Displacement	Filter Pos. Displacement	Forward	
Acid / Alkalis	H2SO4, HCl, NaOH	Air Displacement	Filter	Forward	
Toxic samples		Pos. Displacement	Pos. Displacement	Forward	

Air Displacement Pipettes

- ✓ Air Displacement Pipette, used for standard pipetting applications, is highly accurate.
- ✓ However, conditions such as temperature, atmospheric pressure as well as the specific gravity and viscosity of the solution may have an effect on the performance of air displacement pipettes.

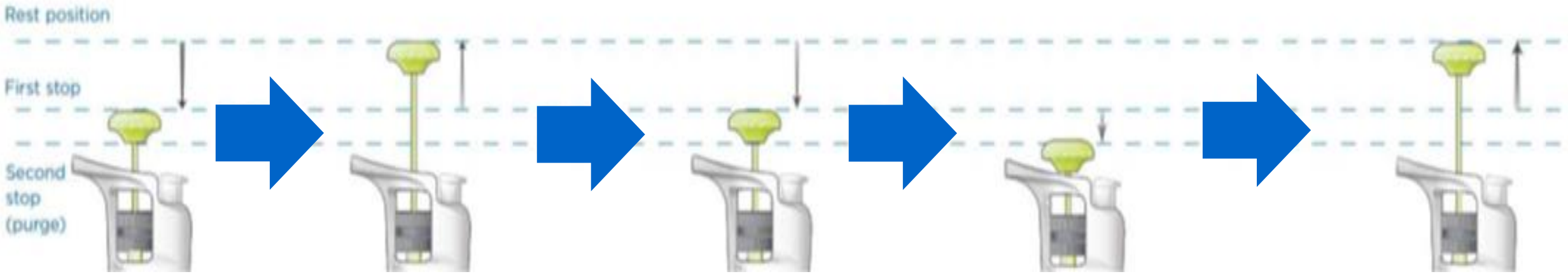




Ref: https://solutions.pipette.com/wp-content/uploads/Guide-to-Pipetting.pdf

Forward Pipetting

Preparation	Aspiration	Dispense	Purge	Home



Hold pipettes vertically and push plunger to 1st stop

Immerse the tip into the sample, release the plunger gently, so that sample gets aspirated

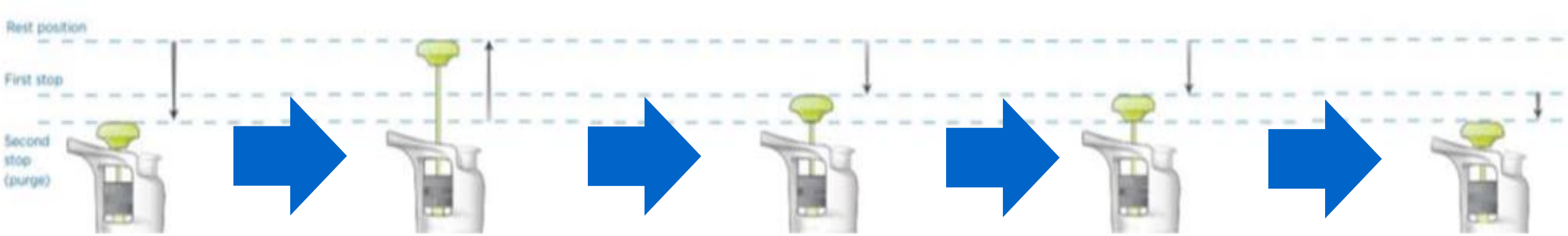
Place the pipettes tip at 10 to 45 degree to receiving vessel and push the plunger up to 1st stop to dispense sample

Wait for a second & purge the plunger to 2nd stop to blow-out residual amount of sample that was retained in the tip

Wait for couple of seconds and allow the plunger to come back to initial position

Reverse Pipetting

Preparation	Aspiration	Dispense	Re-aspiration	Complete Purge



Hold the pipette vertically & push the plunger to 2nd stop.

Immerse the pipette tip into the sample and gently release the plunger for aspiration

Set the pipette tip at 10 to 45-degree angle with the receiving vessel wall & push the plunger smoothly up till 1st stop

Restart the aspiration step for repeat pipetting if same tip is going to be used

If the same tip is not going to be used, then purge the left-over sample in the waste container & discard the tip

Factors affecting the accuracy of pipettes

Temperature (Most important for pipetting accuracy)

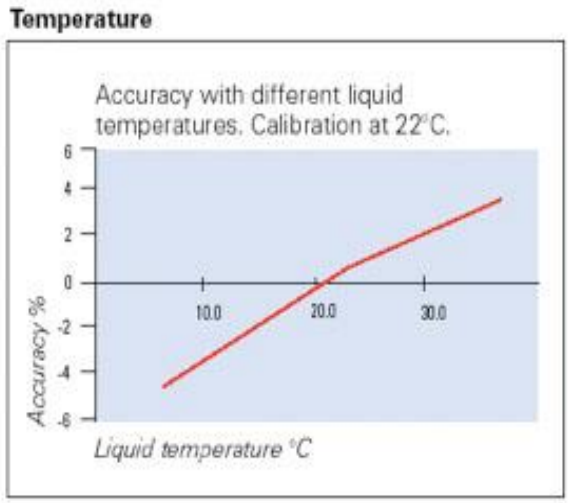
1. Change in volume happens when temperature of pipette and the dispensing liquid is different.
2. If the temperature of the liquid, pipette and air is the same, the accuracy is not significantly affected.

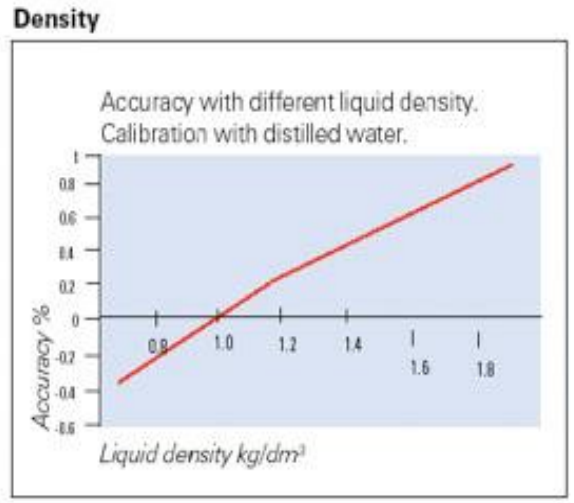
Density

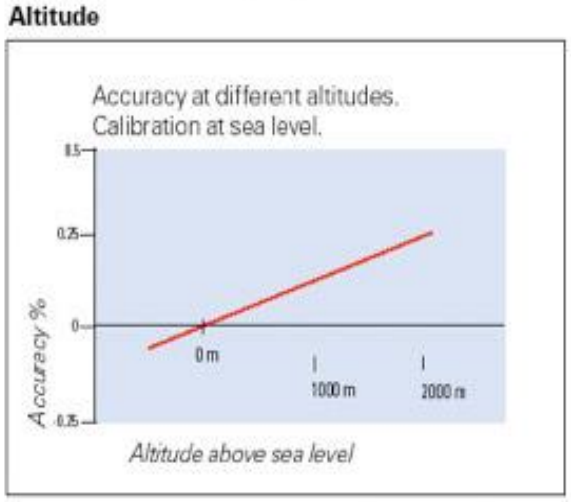
1. Density is the mass/volume ratio of the liquid. The density varies according to the temperature and air pressure.
2. Typically, the density of water is 0.996 kg/dm3, for ethanol 0.79 kg/dm3 and for sulfuric acid (H2SO4) 1.85 kg/dm3.

Altitude (Affets pipetting accuracy)

1. The air pressure decreases at higher altitudes and the conversion factor Z decreases as well.
2. The boiling point of some liquids can also change to quite close to room temperature, increasing the evaporation loss dramatically.







Ref: https://solutions.pipette.com/wp-content/uploads/Guide-to-Pipetting.pdf

Preventing cross contamination

Source of contamination	Prevention
Pipette-to-sample A contaminated pipette or contaminated tips can cause contamination of samples.	• Use sterilized tips or sterilized filter tips and if possible, autoclave the pipette. • Change the tip after pipetting of each sample.
Sample-to-pipette Samples or aerosols from samples can enter the cone of the pipette.	• Keep the pipette vertical when pipetting in order to prevent liquid from running into the pipette body. • Release the push-button slowly. • To avoid aerosol contamination, use filter tips or use a positive displacement pipette and tips. • Store the pipette vertically.
Sample-to-sample (carry-over) The remains of sample A can mix with next sample B inside the tip and may cause a false test result.	• Change the tip after each sample. • If you suspect that your pipette is contaminated, autoclave or clean your pipette

Ref: https://solutions.pipette.com/wp-content/uploads/Guide-to-Pipetting.pdf

Calibration of pipette

Definition of Calibration of Pipettes : -

- Calibration of Pipettes officially means determining the difference between the dispensed volume and the selected volume.
- Adjustment means altering the pipette so that the dispensed volume is within certain specifications.

- ❑ Liquid reservoir (Glass beaker): For test liquid
- ❑ Weighing vessel, (conical flask): Height: diameter ratio 3:1.
- ❑ Digital thermo-hygrometer: Temperature & Humidity uncertainity ≤20%.
- ❑ Barometer: Atomospheric Air pressure standard uncertainty of ≤1 kPa.
- ❑ Plastic Beaker: Dispencing washout pre-rinsing
- ❑ Plastic spanner
- ❑ Standard tips
- ❑ Glass Thermometer: standard uncertainty of ≤0.4^{0}C of test liquid
- ❑ Hand glows: cloth type Less heat transfer
- ❑ Test liquid: Water ISO 36% grade 3 aclametized 2 hours in cal room
- ❑ Calibration room constant (+/- 0.5^{0}C) temperature of 21^{0}C to 25^{0}C, Relative humidity above 50%, Atmospheric Air pressure 1013 mbar +- 15 mbar.

Minimum requirements for balance.

Volume range	Resolution mg
1 µl to 10 µl	0.001 mg
>10 µl to 100 µl	0.01 mg
>100 µl to 1000 µl	0.1 mg
>1000 µl to 10000 µl	0.1 mg

CALIBRATION REPORT

Report No :	1354830	**Report Date :**	06-Sep-2021
Prod Cat. No :	VA-500	**PipetteSerial No.:**	QI529102
Volume Range :	10-100 µl	**Bal Sensitivity :**	0.01 mg
Temperature :	25.00 °C	**Rel Humidity :**	60.00 %
Air Pressure :	1013 hPA	**Z Factor :**	1.0040 µl/mg

TEST DETAILS

Test Volume	Number of Measurements	Mean Weight	Mean Volume
10.00 µl	10	9.8500 mg	9.8894 µl
50.00 µl	10	49.4440 mg	49.6418 µl
100.00 µl	10	99.3350 mg	99.7323 µl

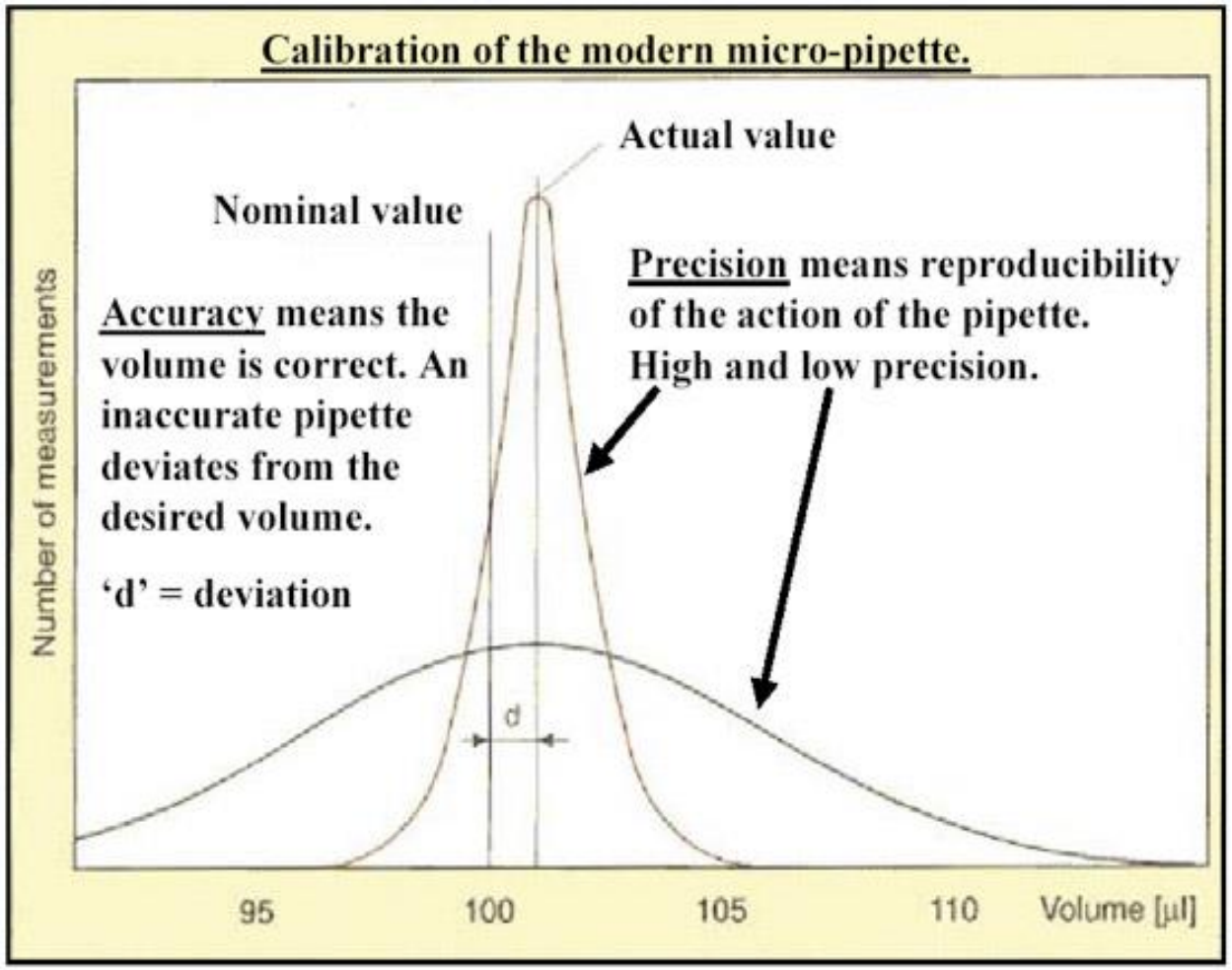
SUMMARY STATISTICS

Test Volume	SD	Inaccuracy E%			Imprecision CV%		
		Actual	Target	Status	Actual	Target	Status
10.00 µl	0.0473	-1.1060	± 3.00	PASS	0.4786	± 1.50	PASS
50.00 µl	0.0474	-0.7164	± 1.00	PASS	0.0954	± 0.50	PASS
100.00 µl	0.1084	-0.2677	± 0.80	PASS	0.1087	± 0.15	PASS

STATUS :	PASSED

Calibration Method according to ..... EN ISO 8655-6
Performed by ..... Incharge - Q.C.

Calibration of pipette



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Formulas for calculating the results

Conversion of mass to volume

$V = (w + e) \times Z$

V = volume (vl)

w = weight (mg)

e = evaporation loss (mg)

Z = conversion factor for mg/vl conversion

Accuracy (systematic error)

Accuracy is the difference between the dispensed volume and the selected volume of a pipette.

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

A = accuracy

$\overline{V}$ = mean volume

Vs = selected volume

Accuracy is expressed on the calibration certificate as a relative value:

$$ACC\% = 100\% \times \frac{A}{V_s}$$

Precision (random error)

Precision refers to the repeatability of the pipetting. It is expressed as standard deviation (s) or coefficient of variation (cv). In addition to the features of the pipette, laboratory practice and user experience are the main factors affecting precision.

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

s = standard deviation

$\overline{V}$ = mean volume

n = number of measurements

V_i = single measurement result (i = 1....n)

CV (or CV%) is the relative value of standard deviation.

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

DIN 12650 error limits for single channel pipettes

Nominal Volume	Maximum Error (F)	Relative Error (%F)
1 µL	± 0.15 µL	± 15.0%
2 µL	± 0.20 µL	± 10.0%
5 µL	± 0.30 µL	± 6.0%
10 µL	± 0.30 µL	± 3.0%
20 µL	± 0.40 µL	± 2.0%
50 µL	± 0.80 µL	± 1.6%
100 µL	± 1.50 µL	± 1.5%
200 µL	± 2.00 µL	± 1.0%
500 µL	± 5.00 µL	± 1.0%
1000 µL	± 10.00 µL	± 1.0%
2000 µL	± 20.00 µL	± 1.0%
5000 µL	± 50.00 µL	± 1.0%
10000 µL	± 100.00 µL	± 1.0%

- ISO 8655-2:2002

Note: These limits applied by manufacturers with a very controlled environment. If the tests are performed in normal lab environment the limits will be doubled

Summary

Pipettes are precision tools that have significant influence on results of scientific experiments. All laboratories working with pipettes should have in place a regular program for not only maintenance and calibration but also regular in-lab testing of pipettes, using qualified personnel and correct equipment for all work. To implement this quality program, the program should be documented and controlled with Standard Operating Procedures for laboratory personnel. The SOP should cover all aspects of the program including the continuous training of personnel to pipettes and pipetting.

To know more about Avantor's solution to handle Pipetting challenges please write to

consumable.india@avantorsciences.com

Thank You