Basics of Pipetting Handbook

Your Guide to Getting the Most Out of Your Pipette

Simplifying Progress

SARTORIUS

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Your pipette is a precision instrument. Master the basics of pipetting to get the best results from your assays through good pipetting techniques and practices.

Pipetting Needs of Different Applications

Have You Thought of What Your Pipetting Needs Are for Your Application?

Different assays have different pipetting needs. Some of the key requirement for most modern-day applications are:

- Accuracy
- Minimizing human error
- Speed
- Easy-to-clean instruments
- Precision
- Ability to decontaminate
- Avoiding contamination

With electronic pipettes, ability to save pipetting protocols on the pipette is very beneficial in repetitive pipetting work. In order to make the correct choices of pipettes and pipetting techniques, it is important to understand the pipetting needs of the assay or application being performed.

Pipetting Needs of Different Applications

Application Area	Pipetting Needs
Cell culture and cell-based assays	Speed and sterility
Nucleic acids	Accuracy and precision, easy-to-clean, avoiding contamination
Proteins	Speed, precision and accuracy
Immunology	Speed, avoiding contamination
Microbiology and virology	Avoiding contamination, easy-to-clean
Animal testing	Accuracy and precision
Analytics, quality control	Accuracy and precision, avoiding contamination

Pipetting Challenging Liquids

Have You Thought of the Properties of the Reagents In Your Assays?

Each application has its unique challenging liquids or reagents. Pipetting accurate volumes of challenging laboratory reagents requires an understanding of how the liquid's properties affect pipetting results. Different pipetting techniques and tools should be used to accommodate for the liquid properties to ensure that accurate volumes are pipetted.

Pipetting Challenging Liquids

Liquid Property	Reagents	Recommendation
Aqueous	Water, buffers	Forward pipetting
Volatile	Isopropanol, alcohol, methanol	Pre-rinse (pre-wet), reverse pipetting
Viscous	Glycerol, Tween®	Reverse pipetting
Dense	Saturated salt solution, chloroform	Volume adjustment
Foaming	Serum	Reverse pipetting, SafetySpace™ tips
Detergent-containing (low surface tension)	Triton™ X-100	Low retention pipette tips
Cold	Master mix, enzymes	Do not pre-rinse, reverse pipetting
Warm	Cell culture medium	Do not pre-rinse, reverse pipetting
Corrosive	Acids, alkalis	Filter tips
Organic	DMSO, chloroform	Low retention pipette tips, reverse pipetting
Sticky	Blood	Reverse pipetting
Radioactive	Radio-labeled proteins	Filter tips

Before You Begin Pipetting

How Do I Select My Perfect Pipette?

Below is a list of questions to ask before beginning your pipetting work:

- What kind of pipette do I need for this application?
- What kind of pipette tip is best for this reagent?
- What kind of pipetting technique best suits this liquid?
- How is my pipetting technique? Am I doing it right?
- Am I getting my best results?
- How do I take care of my pipette?

Before You Begin Pipetting

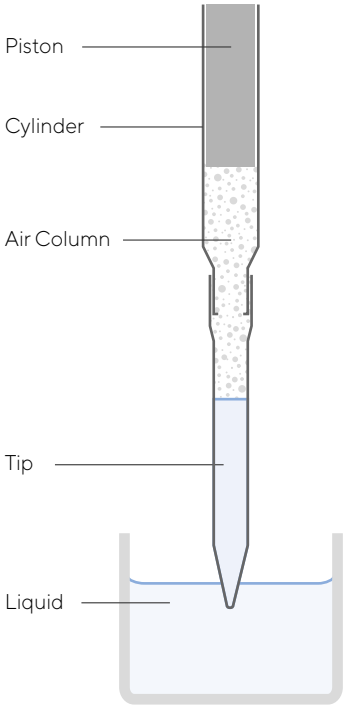
When selecting a pipette, check these features:

1. **Ergonomic Pipette Handle**
   Test that the pipette feels comfortable in your hand. Check that the finger hook suits your hand without any need to squeeze or strain your muscles.
2. **Smooth and Light Tip Ejection**
   Test that the tip ejection function is light and ergonomic to use.
3. **Light Weight**
   Test the weight of the pipette. Light weight is essential in long pipetting series to prevent muscle strain.
4. **Light Operation of the Pipette**
   Check that the pipette's plunger is light to press at both maximum and minimum volumes.
5. **Reliable Accuracy and Precision**
   Check that the pipette manufacturer complies with international standards and regulations (e.g., ISO).
6. **Easy to Read Display**
   Check that the volume is displayed in numbers, down to the last decimal.
7. **Display Visible While Working**
   Check that the volume is easy to read while working.
8. **Volume Lock**
   Check that the pipette has a volume lock to avoid accidental volume changes.
9. **Easy to Maintain**
   Check that the pipette is easy to disassamble without tools, and has only a few parts to clean.
10. **Fully Autoclavable**
    Check that the pipette is fully autoclavable. Autoclaving is the most efficient way to decontaminate a pipette.

Air Displacement Pipettes

In an air displacement pipette, there is an air column between the liquid and the piston. Air displacement pipettes are the most common types of pipette in most laboratories and are available as mechanical pipettes or electronic pipettes. When pipetting with air displacement pipettes, use of filter pipette tips or tip-cone filters helps reduce the risk of cross contamination between samples. Air displacement pipettes are suitable for pipetting aqueous liquids (water-based liquids) and are calibrated with water. Other types of liquids can also be pipetted accurately by choosing the right pipetting technique.

When working with air displacement pipettes, it is important to understand that the air column is sensitive to environmental conditions (such as temperature, air pressure, and humidity), and the accuracy and integrity of the pipetting results are affected by environmental factors, pipetting skill (e.g., how the pipette is held when aspirating, rhythm of pipetting etc.) and the chosen pipetting technique (forward or reverse pipetting or multiple dispensing).



Air Displacement Pipette

Positive Displacement Pipettes

In positive displacement pipettes, the liquid is in direct contact with the piston. Positive displacement pipettes are usually either dispensers, which deliver only one aliquot; or repeaters, which deliver multiple aliquots of the same size. Tips for positive displacement pipettes are often called dispenser tips and they are special tips with the piston inside the tip. The piston inside the tip prevents vapors and liquids from reaching the inner parts of the pipette, which effectively prevents cross-contamination between the sample and the dispenser.

Dispensers are a good choice for problematic liquids like hazardous liquids (e.g., radioactive, toxic, or corrosive materials) and can also be used for handling solvents.

Mechanical Pipettes

Mechanical pipettes are piston-operated volumetric air displacement micropipettes. These pipettes are operated manually, in most of the cases by the user's thumb movement. Mechanical pipettes are available in fixed volume and variable volume models. Variable volume pipettes have a volume range within which they should be used.

Electronic Pipettes

Electronic pipettes are also piston-driven, air displacement pipettes, which have the same basic operation principle as mechanical pipettes with the difference that the piston is moved by an electronic system. Because of this difference, electronic pipettes usually are able to offer better ergonomics, less variations between users, speeding up of the work, and can be customizable to the user's application needs.

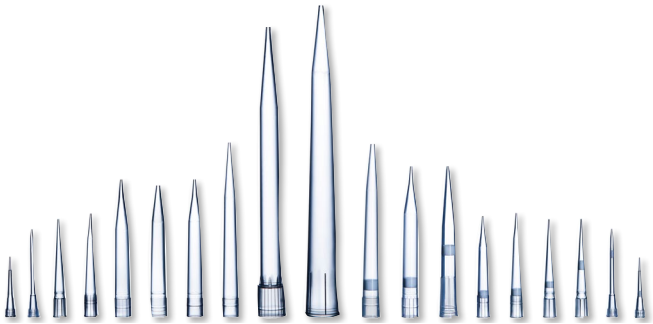
Selecting Your Pipette Tips

How Do I Select My Perfect Pipette Tips?

Pipettes and pipette tips together form a system.

The accuracy and precision of the pipetting results are dependent on the whole pipetting system, not only on the pipette or the pipette tip. Therefore, selection of the right pipette tip to go with your pipette is a very important part of the pipetting workflow.

There are a variety of pipette tips available for different pipetting needs.



Application Needs

When selecting a pipette tip, the user should consider the assay or laboratory procedure. Tips are available with various features to meet the specific needs of different applications.

Standard Tips

- Good for most laboratory work

Low Retention Tips

- Have a 'slippery' surface, suitable for liquids that stick to the standard tips

Filtered Tips

- Filter inside the tip helps the user to prevent cross-contamination and protect the pipette from damage

Extended Tips

- For reaching into long vessels, usually available as filtered and non-filtered

Sterile Tips

- Must-have for sterile work and to avoid contaminations. Usually sterilized with beta radiation

Wide-Bore Tips

- Tips with extra-large opening at the tip end (e.g., for macro molecules or liquids with high viscosity)

Important Tip Selection Criteria

Additionally, when selecting pipette tips, the user must evaluate pipette tip compatibility with the pipette, the quality and purity of the tips, and how good the tip packaging is.

Pipette Compatibility

- The tip must be compatible with the pipette. The performance you are able to get depends on the system, not the tip or pipette alone
- In most cases, the tips from the pipette manufacturer offer the best fit

Packaging

- In trays (or boxes), tips are ready to be picked, without the need for the user to touch the tips with hands
- When packaged in bulk, the tips usually need to be picked by hand and refilled into a box

Tip Quality

- The tip must be in the correct size and shape
- Any deviation, like bent tips, may cause inaccuracy and imprecision in the results

Tip Purity

- The manufacturing process is critical to the tip purity
- Tip purity is typically tested per production lot and a lot-specific certificate is written after confirming purity

Tip Certification

- Proof of manufacturing or purity processes and quality

Important Tip Selection Criteria

If you choose to use a type of pipette tip not manufactured for your pipette, you should always re-calibrate the pipette-and-pipette-tip combination. This is because there are several features that may change when using a different tip type, like size of the air column (air between liquid and piston), height of the liquid column in the tip, size of the tip hole at the bottom, and surface energy (how much does the inside wall of the tip resist the movement of the liquid).

One can achieve the desired accuracy and precision of the pipette with the new pipette tip by adjusting and calibrating the pipette-and-pipette-tip combination.

Accuracy and Precision

Accuracy is how close the measured value is to the true value. It is expressed as 'inaccuracy' or the 'systemic error' and is calculated as the difference between the mean of replicate measurements and the true value. For example, if the user sets the pipette to deliver 1000 µL but the average of replicate measurements is 995 µL, then there is a systemic error of 5 µL. High accuracy (i.e., small systematic error) means very little difference between the mean and the true value. Accuracy is achieved by adjusting or calibrating the pipette. The pipette-and-pipette-tip combination is important, as they form a system which determines the accuracy. So the pipette tip that will be used for that pipette should ideally be used for its calibration.

Precision is how close replicate measurements are to each other. It is expressed as 'imprecision' or 'random error' and is the standard deviation of the measured replicates. High precision (i.e., small imprecision or random error) means very little variation between repeated measurements of the same sample. To achieve high precision, Good Laboratory Practice must be followed—i.e., correct handling of the precision instrument | pipette, and of course the instrument must be in good condition. Additionally, tip-to-tip quality variation can lead to high random error.

- Accurate
- Precise

- Precise
- Not accurate

- Accurate
- Not precise

- Not accurate
- Not precise

Selecting Your Pipetting Technique

How Is My Pipetting Technique? Am I Doing It Right?

The goal in all pipetting work is to obtain highly accurate and highly precise results.

To obtain such results, the instrument (pipette and pipette tip) must be working well, and the user must be trained to use the pipette and must have good pipetting technique.

Four Keys to Pipetting Properly

During pipetting, the pipette user's technique during the aspiration step and during the dispensing step can affect the results. (Aspiration—when the liquid is taken into the pipette tip; dispensing—when the liquid is ejected out of the pipette tip.)

Aspiration

1 Hold the pipette in the vertical position. Vertical is the correct pipetting angle.

2 Tip should be immersed 2–3 mm with volumes 1000 µL and smaller, and 5–6 mm with larger volumes.

3 Allow enough time for the liquid to completely flow into the tip.

Dispensing

4 Touch the tip against the receiving vessel wall at an angle between 30°–45°. This will prevent drops of liquid from remaining on the pipette tip.

Forward Pipetting Technique

Forward pipetting is ideal for most pipetting needs and is best for pipetting aqueous liquids. Forward pipetting technique is the default technique used when the pipette is calibrated in the factory as well as during pipette service and calibration.

What kind of pipetting technique is the best one to use?

The pipetting technique should be selected based on the properties of the liquid being pipetted.

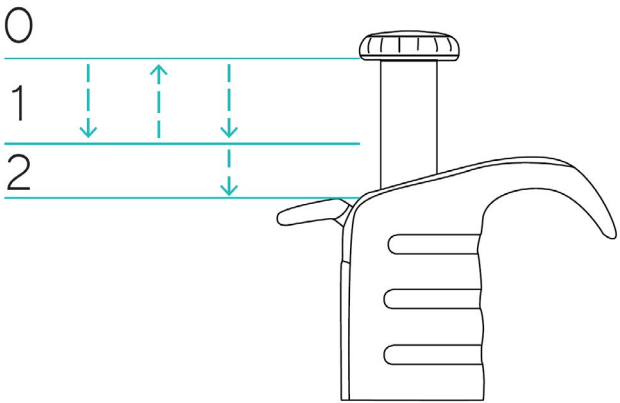
Forward Pipetting with Mechanical Pipette

1. Adjust volume of the pipette.
2. Fit the tip onto the pipette tip cone.
3. Press the operating button to the first stop.
4. Place the tip just under the surface of the liquid and smoothly release the operating button, allowing it to return to the starting position. Wait one second.
5. Carefully withdraw the tip from the liquid.
6. Dispense the liquid by pressing the operating button to the second stop to empty the tip.

Forward Pipetting Technique

Forward Pipetting With Electronic Pipette
When forward pipetting is done with an electronic pipette, the piston position is controlled by the pipette. The user presses the operating button to start aspiration and dispensing.

When to Use Forward Pipetting
Ideal for most pipetting needs, assays, and applications. Best for buffers and aqueous liquids.



Steps in Forward Pipetting

Reverse Pipetting Technique

Reverse pipetting is usually recommended when pipetting certain types of challenging liquids, such as viscous liquids and foamy liquids. In reverse pipetting technique, the pipette aspirates the selected volume plus an excess volume. After dispensing, the excess volume remains in the tip and is then discarded.

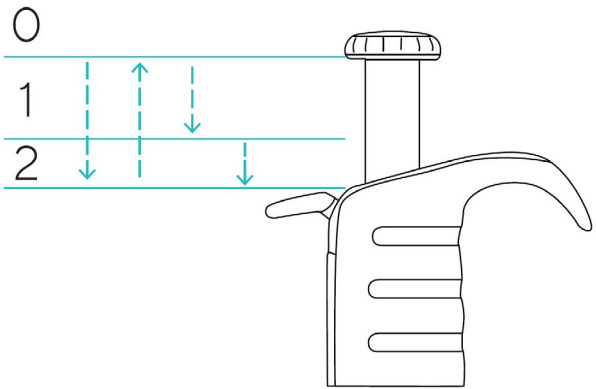
Reverse Pipetting with Mechanical Pipette

1. Adjust volume of the pipette.
2. Fit the tip onto the pipette tip cone.
3. Press the operating button all the way to the second stop.
4. Place the tip just under the surface of the liquid and smoothly release the operating button, allowing it to return to the starting position. Wait one second.
5. Carefully withdraw the tip from the liquid.
6. Press the operating button smoothly to the first stop to deliver the desired volume. The liquid that remains in the tip should not be included in the delivery.
7. Discard the remaining liquid by pressing the operating button to the second stop.

Reverse Pipetting Technique

Reverse Pipetting with Electronic Pipette
When reverse pipetting is done with an electronic pipette, the piston position is controlled by the pipette. The user presses the operating button to start aspiration and dispensing.

When to Use Reverse Pipetting
Ideal for difficult to pipette liquids, and for overcoming special needs in some assays and applications, such as bubbles that interfere with assay results, viscous liquids, volatile liquids, solvents, and in assays where it is challenging to get reproducible results in repetitive measurements (poor standard deviations or CV% in the results).



Steps in Reverse Pipetting

Pre-Rinsing

Pre-rinsing (also known as pre-wetting) means that one aspirates and dispenses a few times before actual pipetting is done. Pre-rinsing before pipetting most liquids is recommended by ISO 8655 and it is an effective way to increase the accuracy and precision of the results. Pre-rinsing increases the humidity inside the air column in the pipette and therefore less liquid evaporates from the actual pipetted sample. Less evaporation from the actual sample means better results.

To pre-rinse, aspirate and dispense 3–5 times using forward pipetting technique, then re-use the same pipette tip to aspirate the desired volume.

When to Pre-Rinse
Pre-rinsing should be done for most liquids, and especially for pipetting of volatile liquids. ISO 8655 specifies that pre-rinsing 5 times is mandatory when performing calibration.

Note: With reverse pipetting or multi-dispensing techniques, pre-rinsing is not needed, as there is always excess liquid remaining in the tip after dispensing. In cases where the liquid is significantly colder or warmer than the pipette (e.g., with liquids from the fridge), pre-wetting can actually affect the results negatively.

Tips and Tricks for Pipetting Challenging Liquids

Although air displacement pipettes are calibrated with distilled water, laboratory work is full of liquids that have completely different properties from distilled water. Some liquid properties can affect the accuracy of the pipetted volume. Some of the key properties that can affect the delivered volume include volatile liquids or solvents, highly viscous liquids, warm or cold liquid temperatures, foaming liquids, and detergent-containing liquids (liquids with low surface tension).

Other typical challenges include pipetting small volumes and pipetting liquids that stick to the inside surface of the pipette tip. In all these situations, it is important to pay attention to selection of the correct pipetting technique in order to pipette accurate volumes.

Tips and Tricks for Pipetting Challenging Liquids

Volatile Liquids

Problem:
Evaporation and dripping

Tip 1:
Pre-rinse 3–5 times to saturate the air column

Tip 2:
Use reverse pipetting technique

Examples:
Methanol, ethanol, isopropanol, hexane

Warm | Cold Liquids

Problem:
The first pipetted volume is the most accurate. If the tip is re-used, then subsequent pipetted volumes give a different volume, which is less accurate

Tip 1:
Change the tip each time, and do not pre-rinse the tip before pipetting

Tip 2:
If you want to re-use the tip, then pre-rinse the tip 3–5 times so that the first pipetted volume delivers the same amount of liquid as the subsequent pipettings

Examples:
Cell culture medium, PCR master mix, proteins (enzymes or antibodies)

Foaming Liquids (and Formation of Bubbles)

Problem:
Bubbles and foam easily form when pipetting, causing wrong volumes to be pipetted. Bubbles sometimes interfere with assay results

Tip 1:
Use reverse pipetting technique

Tip 2:
Pipette slowly

Tip 3:
Avoid sample loss if foamy liquid touches filter in tips by using long pipette tips (SafetySpace™ filter tips, extended tips)

Examples:
Serum, buffers containing detergents, protein solutions

Tips and Tricks for Pipetting Challenging Liquids

Detergent-Containing Liquids (and Liquids with Low Surface Tension)

Problem:
Tend to stick on the pipette tip, residual liquid remains on the surface of the pipette tip after dispensing, and an inaccurate volume is dispensed

Tip 1:
Use low retention tips

Tip 2:
Use reverse pipetting technique

Examples:
Tween® 20/80, Triton™ X-100, SDS solutions

Small Volumes

Problem:
How to pipette accurately

Tip 1:
Use reverse pipetting technique

Tip 2:
Avoid drops of liquid remaining on the pipette tip by touching the tip against the receiving vessel wall at an angle between 30°–45°

Liquids That Stick to Surfaces

Tip 1:
Use reverse pipetting technique (pre-rinsing is not needed)
or...

Tip 2:
Use low retention tips
or...

Tip 3:
Pre-rinse 3–5 times before pipetting if you have to use forward pipetting (if reverse pipetting is technically not possible because of the assay or application)

Examples:
Blood, concentrated proteins, serum, heterogeneous samples

Useful Pipetting Modes on Electronic Pipettes

Electronic pipettes have less dependence on the user's pipetting technique because the piston movement is electronically controlled. Electronic pipettes are very useful when one needs to do forward pipetting, reverse pipetting or mixing of samples during long pipetting series because the work goes faster and the user experiences less fatigue.

Additionally, there are many modes, such as multi-dispensing and serial dispensing, which have been developed to address specific application needs and to enable one to work faster. Therefore, electronic pipette use can significantly improve pipetting for certain types of experiments, and reduce the strain of repetitive pipetting in long pipetting series.

Useful Pipetting Modes on Electronic Pipettes

Pipetting Mode	Laboratory Applications and Assays
Plate tracker function	Used for tracking one's position when pipetting on microwell plates (96-well or 384-well plate). Useful in any application that requires addition of reagents onto microwell plates such as PCR, enzyme assays, or reagent addition to cell cultures. Increases the reliability of pipetting results in long pipetting series.
Multi-dispensing	Used for filling wells on microwell plates. Useful in PCR, cell culture, immunoassays, ELISA, and fluorescence assays (FRET, TRF).
Multi-aspiration	Used in sample pooling or removing media quickly. Useful in sample pooling (PCR, NGS, proteins), cell culture media change, ELISA, and immunoassays.
Manual pipetting	Used in checking yield (protein purification, nucleic acid isolation), and in applications where the user wants to control the piston during aspiration and dispensing (loading gels, mounting of slides for fluorescence microscopy, spotting of samples).
Sequential dispensing	Used to create calibration curves (standard curves) and for pipetting different volumes sequentially. Useful in analytical methods for spectrometry, colorimetric assays, and immunoassays.

Pipette Cleaning for Different Applications

Pipette cleaning can be divided to 2 parts: daily cleaning and full cleaning. Always follow manufacturer's recommendations for cleaning the pipette.

Daily Cleaning

1. Change tip cone filter(s), if applicable.
2. Clean the outside of the pipette using 70% ethanol.
3. Visually inspect the outside of the pipette to check for cracks, defects, or anything else that might adversely affect its correct operations.

Full Cleaning for Various Applications

Follow the manufacturer's instructions to know how to disassemble your pipette, and to know the chemical compatibility of your pipette for different cleaning or decontamination procedures.

Pipette Cleaning for Different Applications

Cleaning Agents and Their Activities

Cleaning Reagent	Radioactive Solutions	Acids and Alkalis	Organic Solvents	DNA| RNA	RNase| DNase	Proteins
Pure (type 3) water or distilled water		■				
Detergent	■		■			■
Radioactivity cleaning reagent	■					
Sodium hypochlorite				■		
Nucleic acid removal reagent				■		
Nuclease removal reagent					■	
DEPC (diethyl pyrocarbonate)					■	
UV (ultraviolet radiation)				■		

Pipette Cleaning for Different Applications

Decontamination Agents and Their Activities

Decontamination Reagent	Bacteria Spores	Vegetative Bacteria	Virus Enveloped	Virus Non-Enveloped	Fungi
Ethanol | isopropanol		■	■		
Sodium hypochlorite	■	■	■	■	■
Hydrogen peroxide	■	■	■	■	■
Vapor hydrogen peroxide	■	■	■	■	■
UV (ultraviolet radiation)	(Poor)	■	■	■	■

Pipette Cleaning for Different Applications

Autoclaving
Autoclaving is an efficient way to sterilize pipettes from bacteria, viruses, and other biological material. Before autoclaving, it is important to clean the pipette first and let the pipette dry. Additionally, any cleaning solution residue on the pipette should be removed.

Follow the manufacturer's instructions to know how to autoclave your pipette, and check which parts are autoclavable since some pipettes are fully autoclaved but others are not autoclavable. Always use the correct autoclaving settings according to manufacturers' instructions.

Pipette Checking

Pipette checking is a short test done by the pipette user. The purpose of checking is to verify the performance between calibrations. If you calibrate a pipette annually, there is a 12-month period when you do not actually know how the pipette is performing. Regular checking is important because calibration interval is usually relatively long. Pipettes are precision instruments and so regularly completed checking increases confidence in pipetting results.

Checking does not replace calibration, but is supportive action to find malfunctioning devices!

Pipette Checking

When to Check Pipettes

- If you suspect the performance of the pipette
- If something has happened to the pipette, e.g., it has been dropped
- In regular interval, e.g., from 1 day to months, depending on the application quality requirements
- If the pipette has been autoclaved or you have touched the piston of the pipette

Equipment Needed

- Analytical balance with moisture trap
- Thermometer and barometer
- Distilled water
- Documentation tool (balance with built-in functions, Excel, Notebook, etc.)

How to Check

- Test volumes, number of measurements, and limits should be selected according to the application
- 3–4 measurements at one volume is sufficient to test the correct operation of the pipette

Other Options

- There are also other possible equipment and procedures to check the pipettes. The users should consider at least uncertainty, usability, and cost of the method when selecting one

What to Do if Pipette Fails at Checking?

- Stop using the pipette
- Mark the pipette as faulty
- Inspect for broken parts
- Check if pipette adjustment is off (for adjustable pipettes)
- Send for professional repair and calibration service, if needed

Do's and Don'ts

Do's

- Select your pipette and tips according to your application needs
- Select a pipette that is light in weight and light to use, and does not strain your hand
- Select tips that fit your pipette
- Use correct pipetting techniques
- Check the pipette performance after an autoclave or if pipette is dropped
- Save time with electronic pipettes' advanced features
- Consider your working ergonomics, have comfortable working postures and habits
- Maintain and calibrate your pipettes at least annually and check regularly; this ensures that they work well for a long time and provide reliable results
- Consider the pipette as a measurement instrument
- Use tip cone filters and exchange them regularly
- Clean pipettes regularly, follow manufacturers' instructions

Don'ts

- If your muscles get tired during pipetting, something is wrong
- Don't twist or rock pipette, or use excessive force when attaching tips
- Don't work in awkward postures
- Don't pipette for too long a period of time without a break
- Don't re-use tips or over-aspirate liquid in to the pipette, this is high risk for cross-contamination
- Don't overwind pipette beyond volume range
- Don't adjust the pipette when the volume lock is on
- Don't store pipettes in horizontal position

Contact

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www.sartorius.com/pipetting-academy

Germany
Sartorius Lab Instruments GmbH & Co. KG
Otto-Brenner-Strasse 20
37079 Goettingen
Phone +49 551 308 0

USA
Sartorius Corporation
565 Johnson Avenue
Bohemia, NY 11716
Phone +1 631 254 4249
Toll-free +1 800 635 2906

For additional information,
visit www.sartorius.com