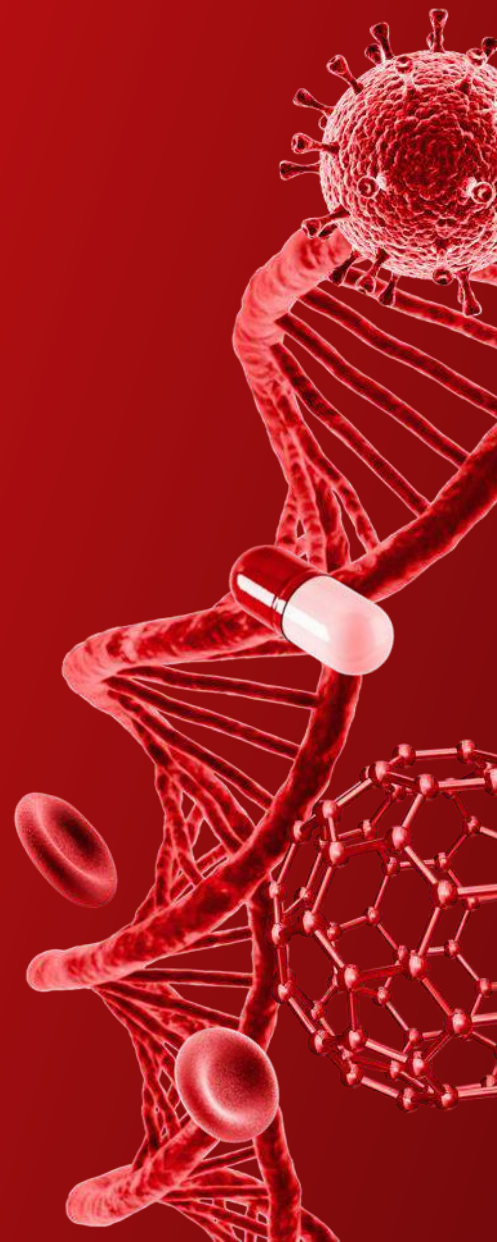
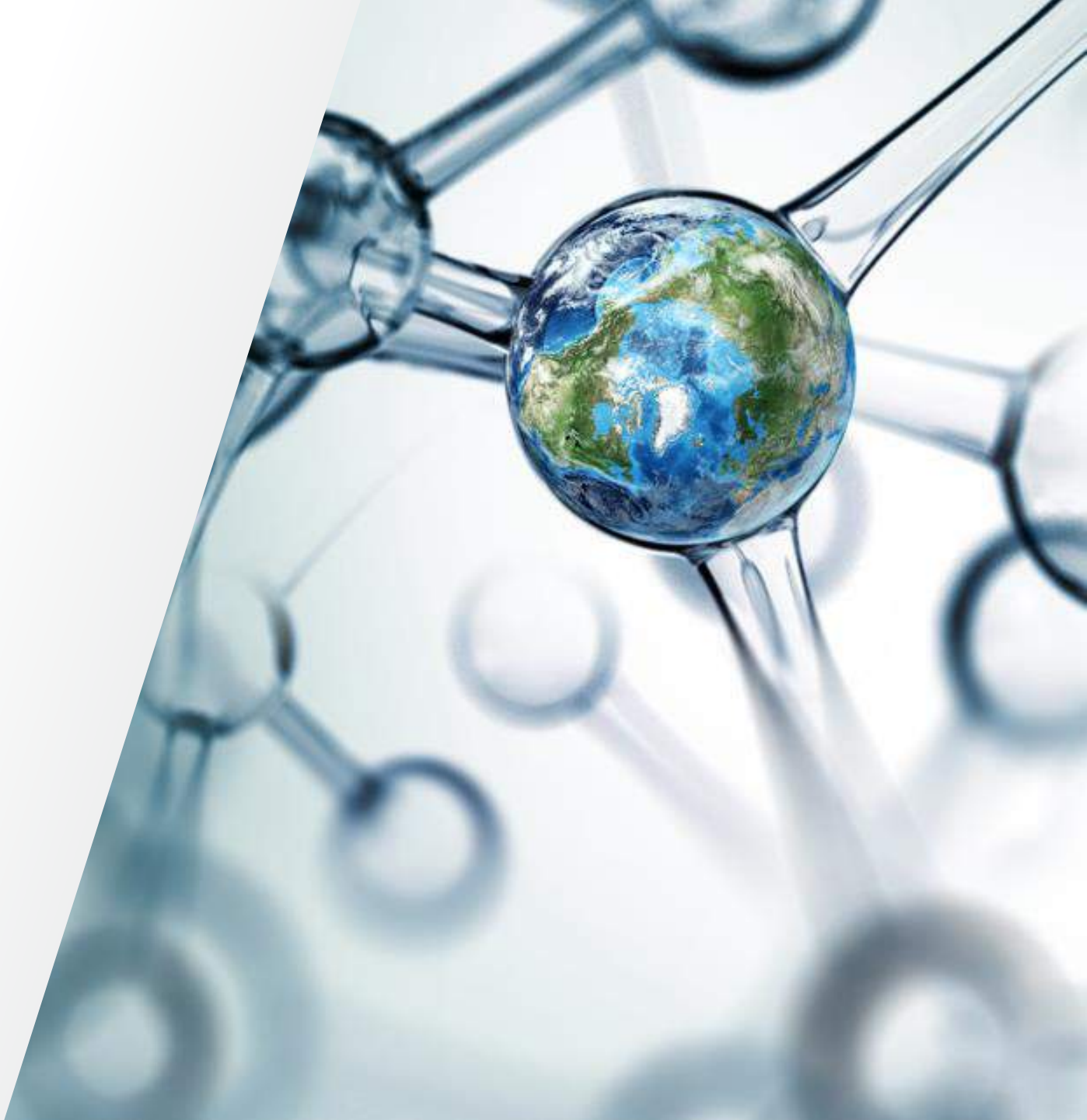
Welcome



Maria.hidesand@thermofisher.com | 2023

Pipetting Best practices

Maria Hidesand
TSS Liquid Handling Solution
Nordic



The world leader in serving science

Plan of Learning

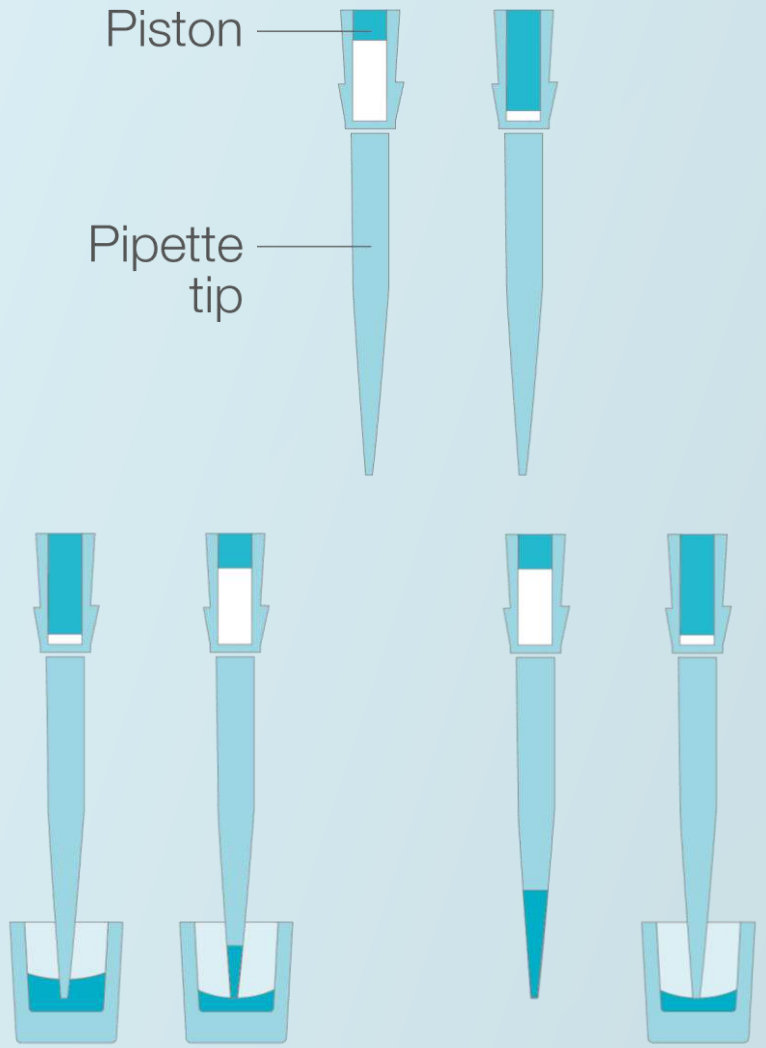
1. Air displacement vs. positive displacement pipettes
2. Pipette tip selection
3. Forward, reverse and repetitive pipetting
4. Accuracy and precision
5. Factors affecting pipetting quality
6. Cross-contamination prevention

maria.hidesand@thermofisher.com

How do air displacement pipettes work?

- **Air displacement pipettes** move liquids into and out of disposable pipette tips. They create a partial vacuum with a cushion of air between the piston and the liquid.

    1. Adjust the volume settings to move the piston to its final position
    2. Press the operating button to the first stop; this expels a volume of air equal to the volume setting
    3. Immerse the tip in the liquid and slowly release the operating button; this creates a partial vacuum that draws the liquid into the tip
    4. Press the operating button to the first stop again; this compresses the air above the liquid to force the liquid from the tip
    5. Press the operating button to the second stop to fully empty the tip, often called “blow out”



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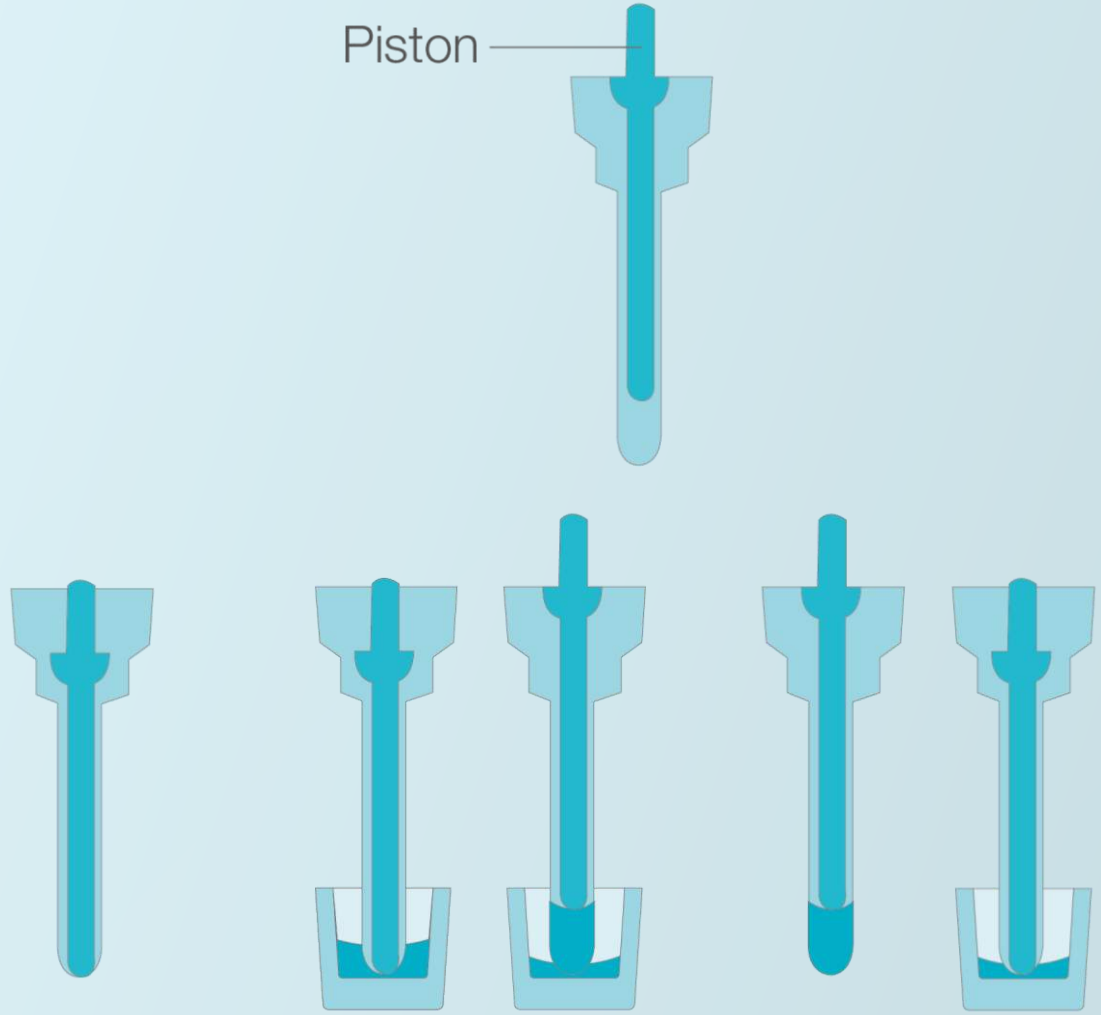
How do positive displacement pipettes work?

The piston in a **positive displacement pipette** makes direct contact with the liquid or material, which eliminates any air space between them and creates a constant aspiration force.

1. Adjust the volume settings to move the piston to its final position
2. Depress the operating button fully; this moves the piston to the end of the pipette tip
3. Immerse the tip in the material and slowly release the operating button; this draws the material into the tip
4. Depress the operating button fully again;
this moves the piston to force the sample from the tip



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Quick Tips

The pipette, tip, and liquid should all be the **same temperature** — ideally between 20°C and 25°C

Quick Tips

Pipettes are **gravimetrically calibrated** at the factory using double deionized grade water

Quick Tips

Selected tips should be **certified free of contamination** and recommended by the pipette manufacturer

Quick Tips

Pipette tips are designed for **single use**

Selecting Pipette Tips

Maria.hidesand@thermofisher.com |

Selecting Pipette Tips

Stand
Multipurpose, inclu

Standard
Multipurpose, including calibrations

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Selecting Pipette Tips

Stand
Multipurpose, inclu

Extended length
Eliminates tip cone contamination

Selecting Pipette Tips

Stand
Multipurpose, inclu

oading
oading in agarose

Gel loading

Precise sample loading in agarose

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Selecting Pipette Tips

Stand
Multipurpose, inclu

oading
oading in agarose

Stand
Free of detectable endor

Standard

Free of detectable endonuclease/RNA, for PCR

Selecting Pipette Tips

Stand
Multipurpose, inclu

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Stand
Free of detectable endor

Low retention
Reduces sample loss due to adhesion

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Selecting Pipette Tips

Stand
Multipurpose, inclu

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Stand
Free of detectable endor

Solvent safe
Carbon filters for aggressive reagents

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Selecting Pipette Tips

Star
Multipurpose, inc

Loading
e loading in agarose

Star
Free of detectable end

ent safe
aggressive reagents

Sterile filter
For microbiology, genetics, and PCR

Sterile filter
For microbiology, genetics, and PCR

Selecting Pipette Tips

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Sterile filter
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Wide orifice
Cell culture and difficult samples

Loading
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Selecting Pipette Tips

Standar
Multipurpose, includin

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Standar
Free of detectable endonucl

safe
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Sterile filter
For microbiology, genetics, and PCR

Wide orifice
Cell culture and difficult samples

Individually wrapped
Sensitive aseptic samples

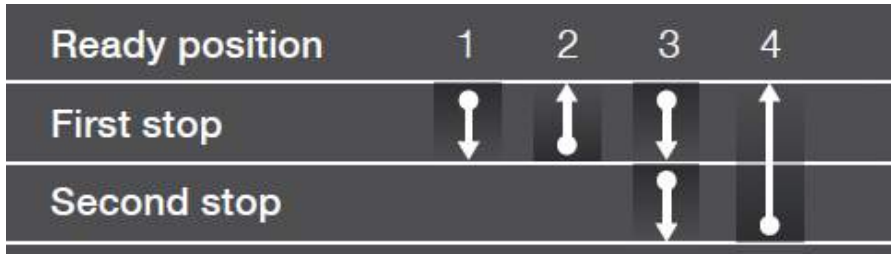
Individually wrapped
Sensitive aseptic samples

Techniques

The Forward Technique

For standard pipetting

Use this method for aqueous solutions, such as buffers, diluted acids, or alkalis. It is commonly used when pipetting and mixing a sample or reagent into another liquid.



1. Press the operating button to the first stop
2. Dip the tip to a maximum of 1 cm beneath the meniscus into the solution and slowly release the operating button. Allow the tip to fill with careful control of the operating button; hold for a minimum of 3 seconds; withdraw the tip from the liquid
3. Gently press the operating button to the first stop to dispense the liquid; wait one second and press the operating button to the second stop to completely empty the tip; then touch off the tip and remove the tip from the vessel by sliding it up the vessel wall
4. Release the operating button to the ready position and eject the tip

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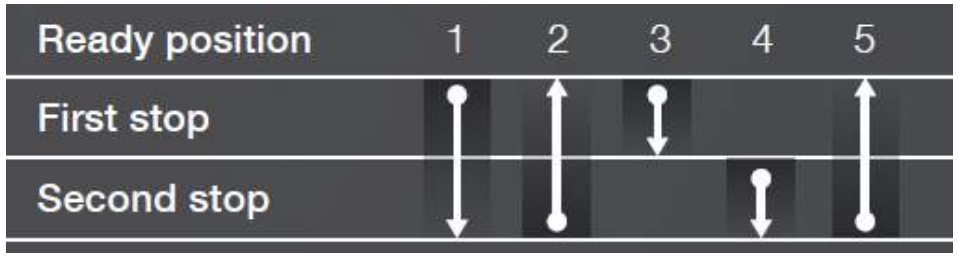
The Reverse Technique

For high-viscosity or foaming solutions



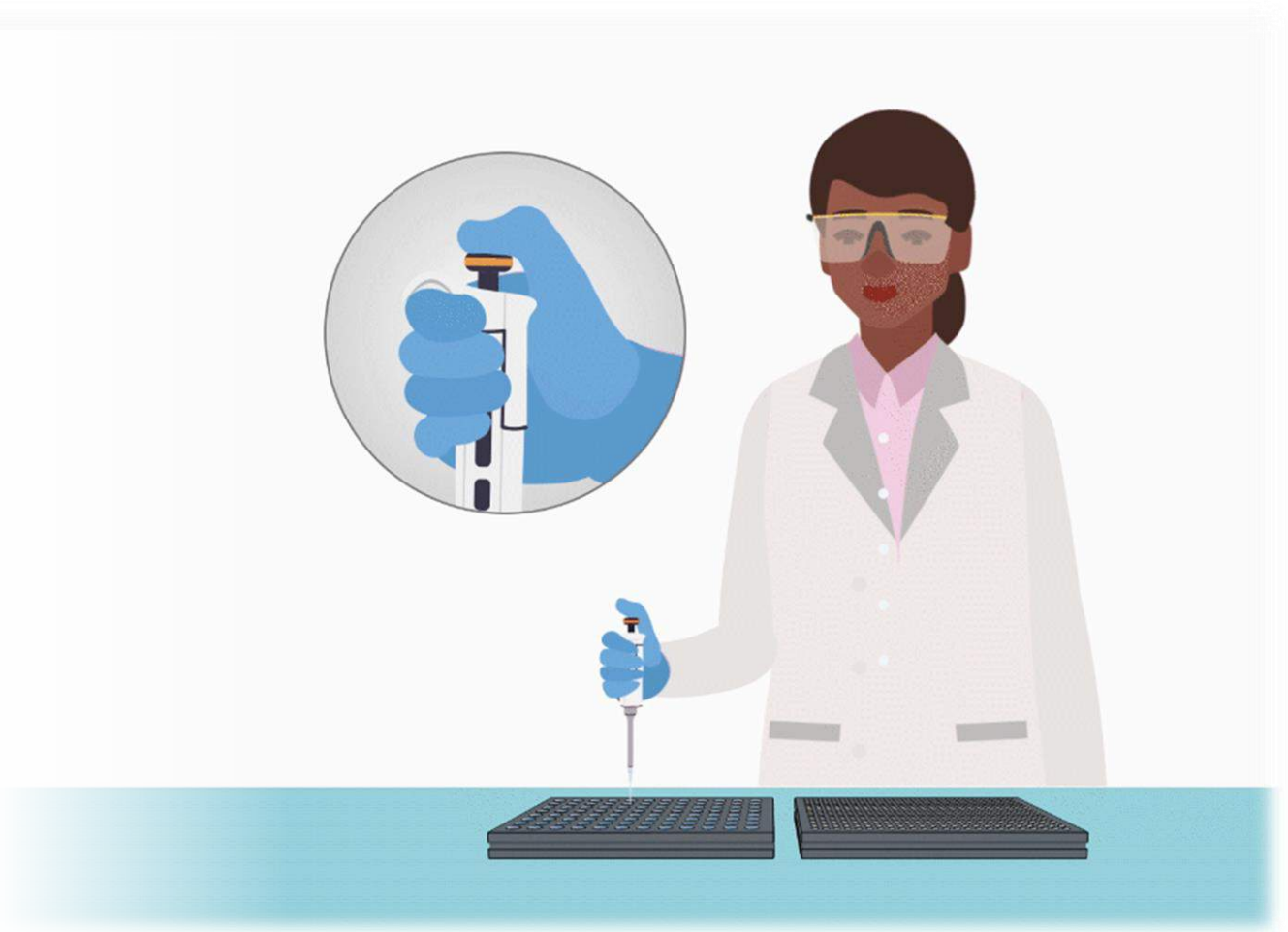
1. Press the operating button to the second stop
2. Dip the tip to a maximum of 1 cm beneath the meniscus into the solution and slowly release the operating button. Allow the tip to fill with careful control of the operating button; hold for a minimum of 3 seconds; withdraw the tip from the liquid
3. Gently press the operating button to the first stop to dispense the liquid and hold the button in position (do not dispense liquid that remains in the tip); remove the tip by sliding it along the vessel wall
4. Discard any remaining liquid along with the tip
5. Release the operating button to the ready position

Maria.hidesand@thermofisher.com |

The Repetitive Technique

For pipetting the same volume repeatedly, e.g., MasterMixes into qPCR plates

1. Press the operating button to the second stop



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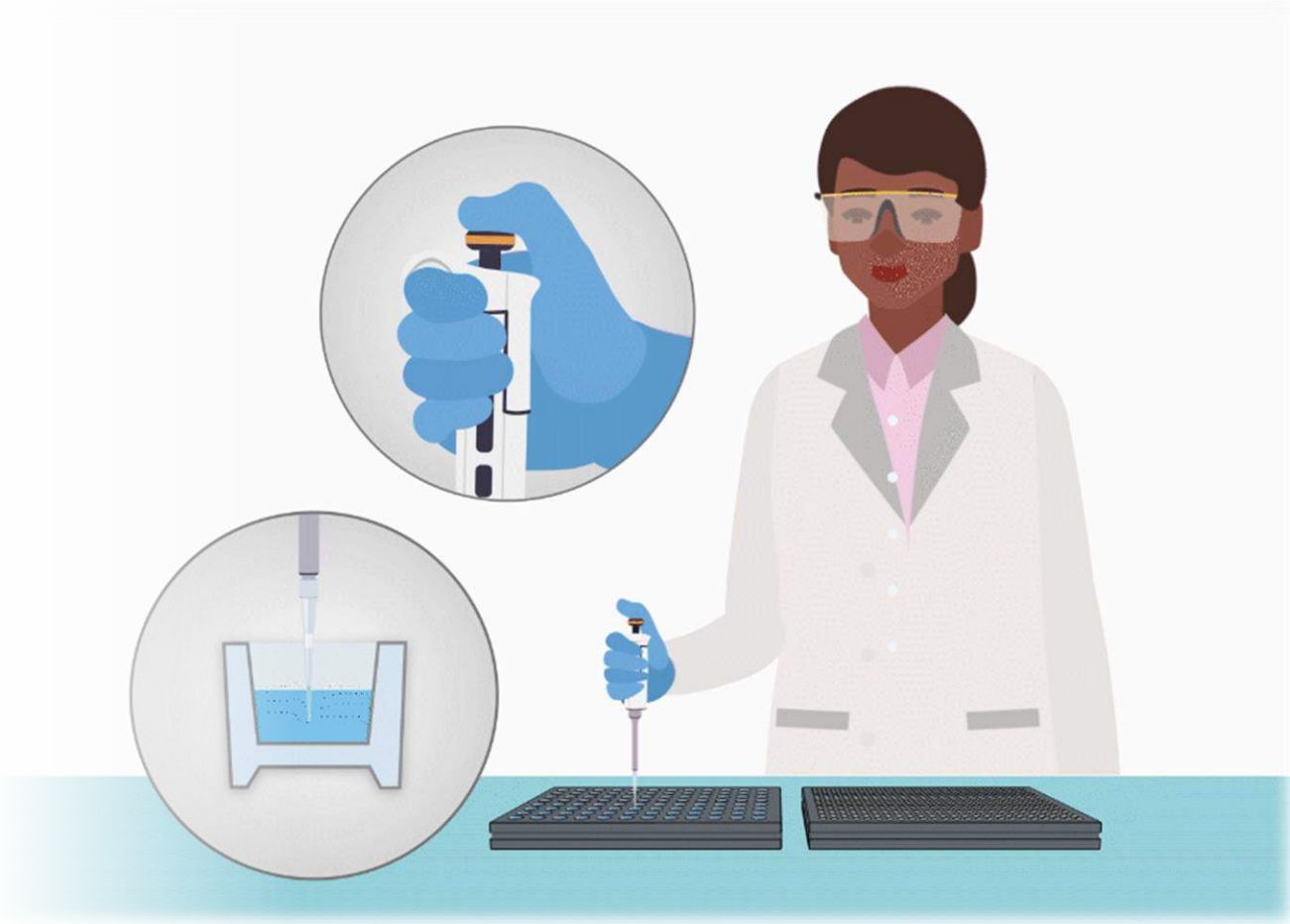
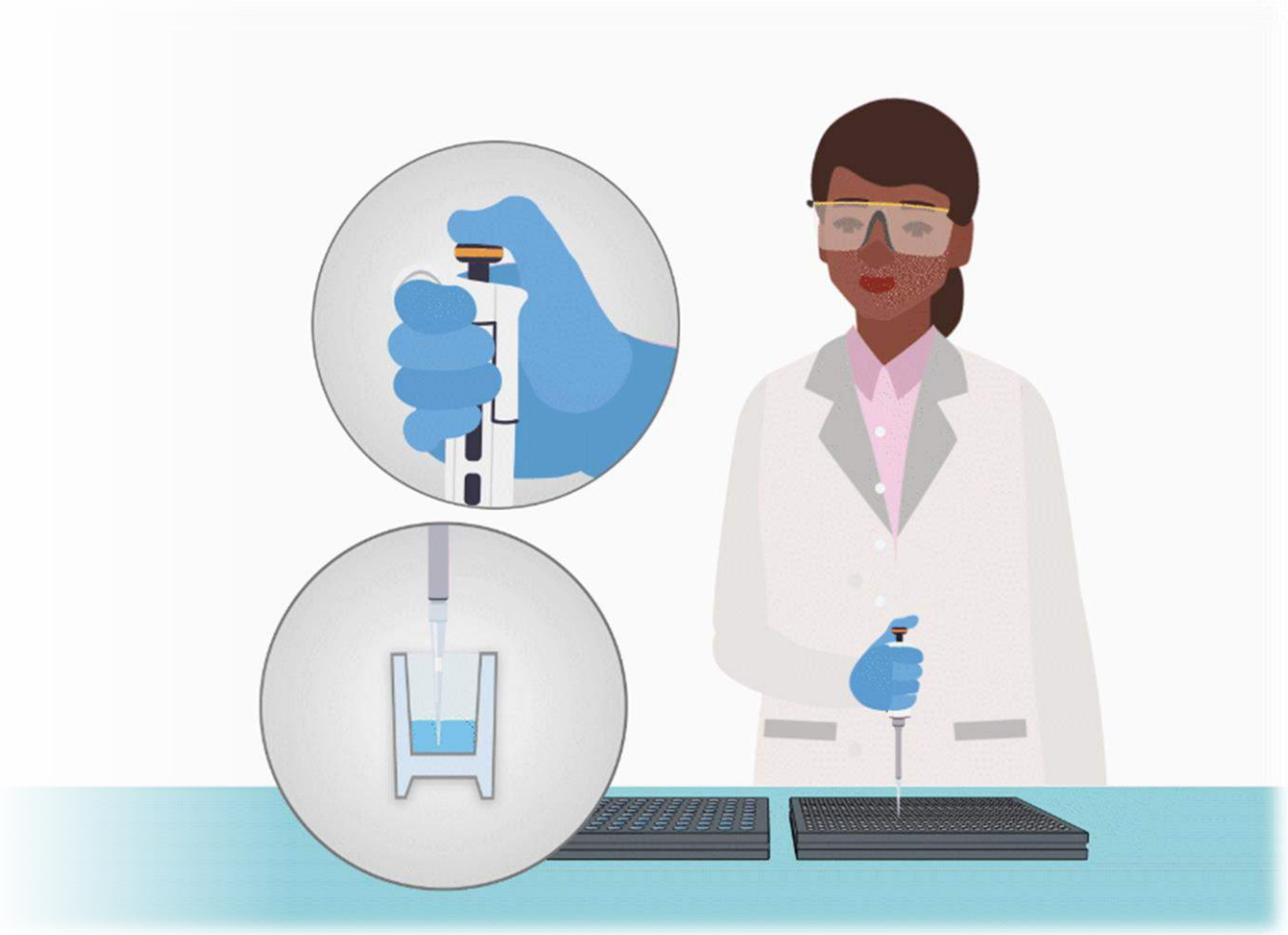


The Repetitive Technique

For pipetting the same volume repeatedly, e.g. MasterMixes into qPCR plates

1. Press the operating button to the second stop
2. Dip the tip to a maximum of 1 cm beneath the meniscus into the solution and slowly release the operating button. Allow the tip to fill with careful control of the operating button; hold for a minimum of 3 seconds for aqueous liquids and a minimum of 5 seconds for viscous liquids; withdraw the tip from the liquid
3. Gently press the operating button to the first stop to dispense liquid and hold the button in position; remove the tip by sliding it along the vessel wall



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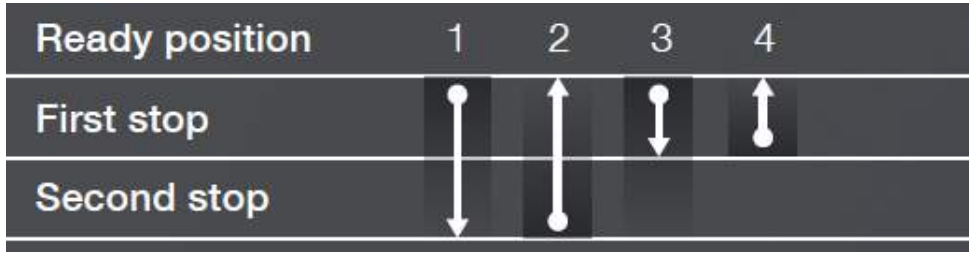

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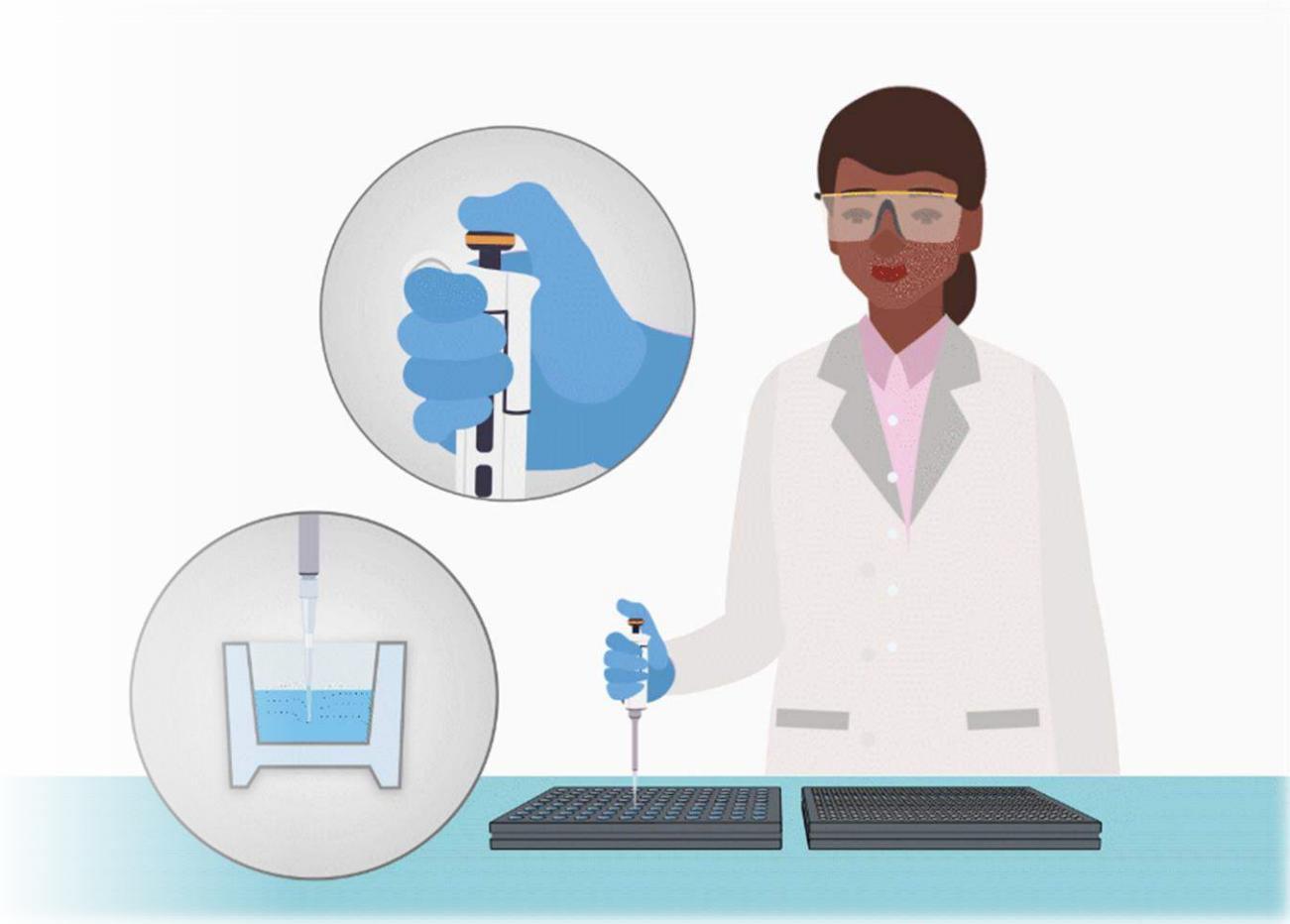
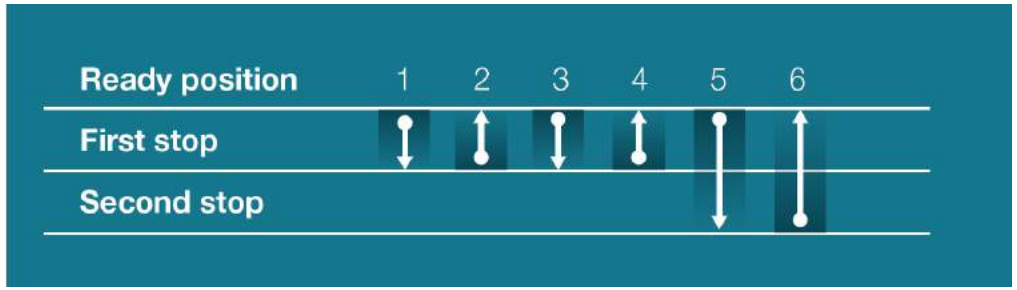
3. Gently press the operating button to the first stop to dispense liquid and hold the button in position; remove the tip by sliding it along the vessel wall
4. Repeat steps 2 and 3 to continue pipetting

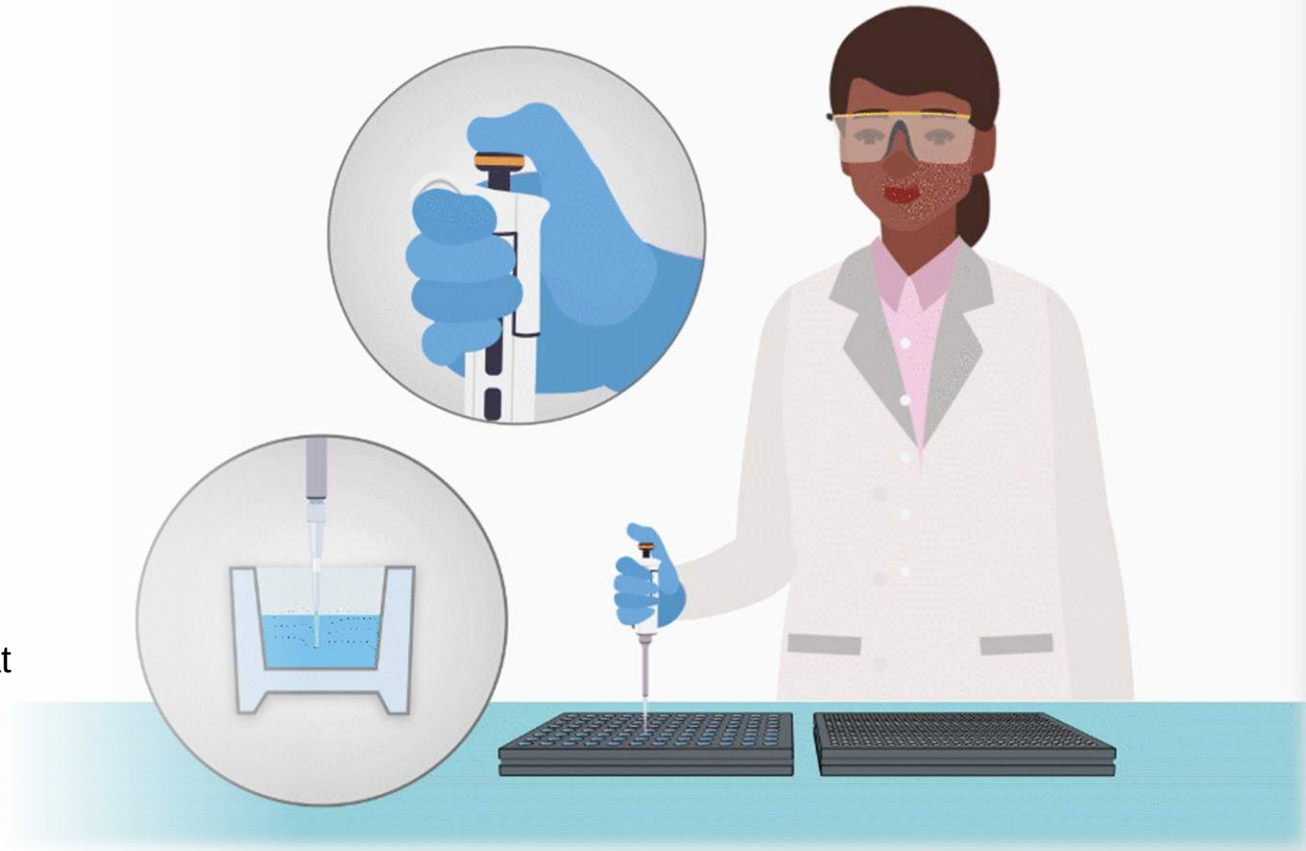


The Heterogeneous Technique

This technique is used for pipetting heterogeneous samples, such as blood or serum. Typically, pre-rinsing the tip is not possible, and the full sample should be dispensed for accurate analysis.



1. Press the operating button to the first stop. Dip the tip into the sample. Make sure the tip is sufficiently below the surface.
2. Release the operating button slowly to the ready position. This action will fill the tip with the sample. Remove the tip from the solution by sliding it along the wall of the vessel.
3. Dip the tip into the target solution. Make sure the tip is sufficiently below the surface.
4. Press the operating button to the first stop and release it slowly to the ready position. Do not remove the tip from the solution. Repeat this process until the interior wall of the tip is clear.
5. Remove the tip from the solution by sliding it along the wall of the vessel. Press the operating button to the second stop, and completely empty the tip.
6. Release the operating button to the ready position.



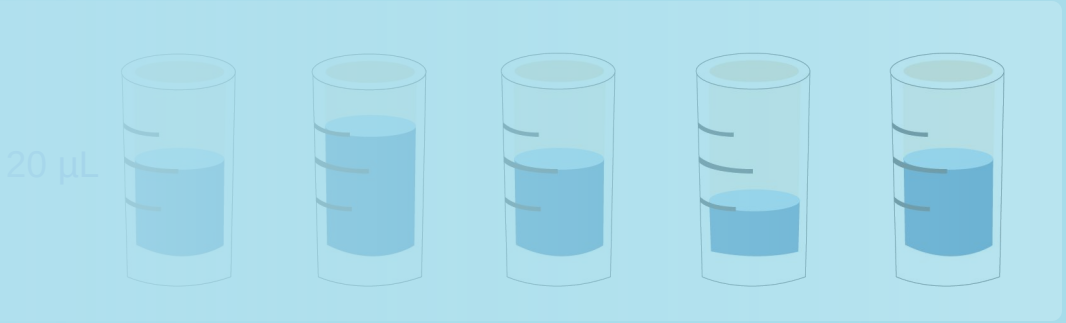
Recommendations for different compounds

Solution/ compound	Examples	Pipette displacement	Tip	Technique	Comments
Aqueous solutions	Buffers, diluted salt solution	Air	Standard	Forward	
Viscous solutions	Protein and nucleic solutions, glycerol, tween 20/40/60/80	Air	Standard, low retention or wide orifice	Reverse	Pipette slowly to avoid bubble formation
		Positive displacement			
Volatile compounds	Methanol, hexane	Air	Filter	Forward	Pipette rapidly to avoid evaporation; carbon filter pipettes prevent vapors from entering the pipette
		Positive displacement			
Nucleotide solutions	Genomic DNA, PCR products	Air	Filter or wide orifice	Forward	Use a wide orifice for genomic DNA to avoid mechanical shearing
		Positive displacement			
Radioactive compounds	(14)Carbonate, (3)H-thymidine	Air	Filter	Forward	
		Positive displacement			
Acids/alkalis	H_2SO_4, HCl, NaOH	Air	Filter	Forward	
Toxic samples		Positive displacement			

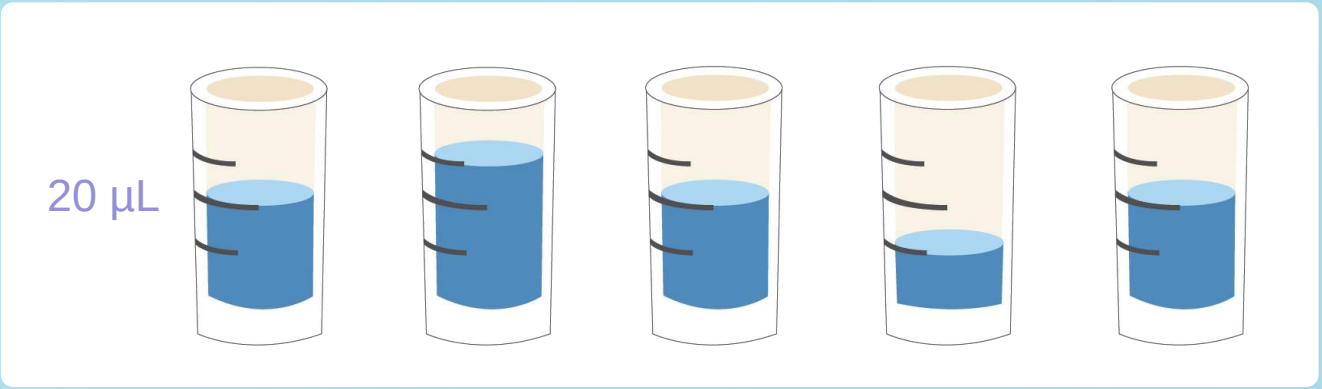
Accuracy and Precision

Accuracy and Precision

Accurate, but not precise

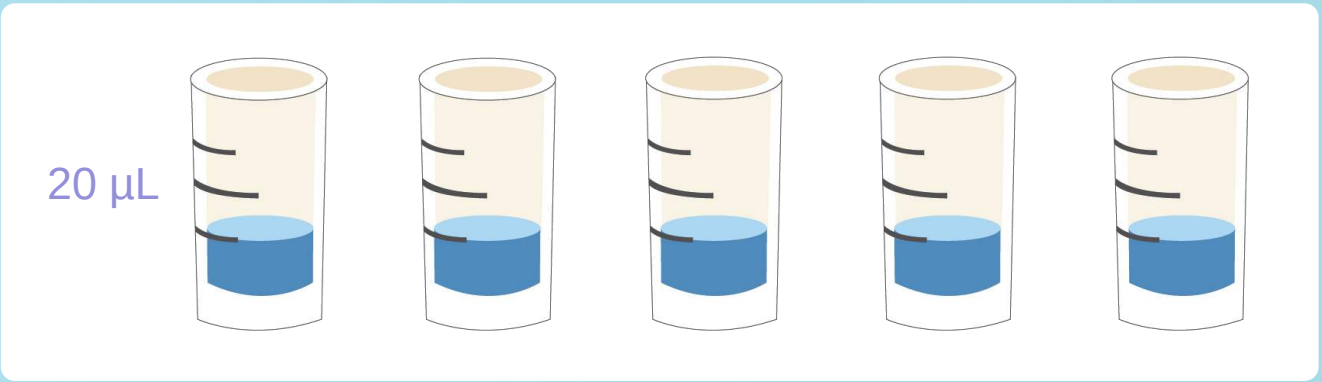


Accurate, but not precise



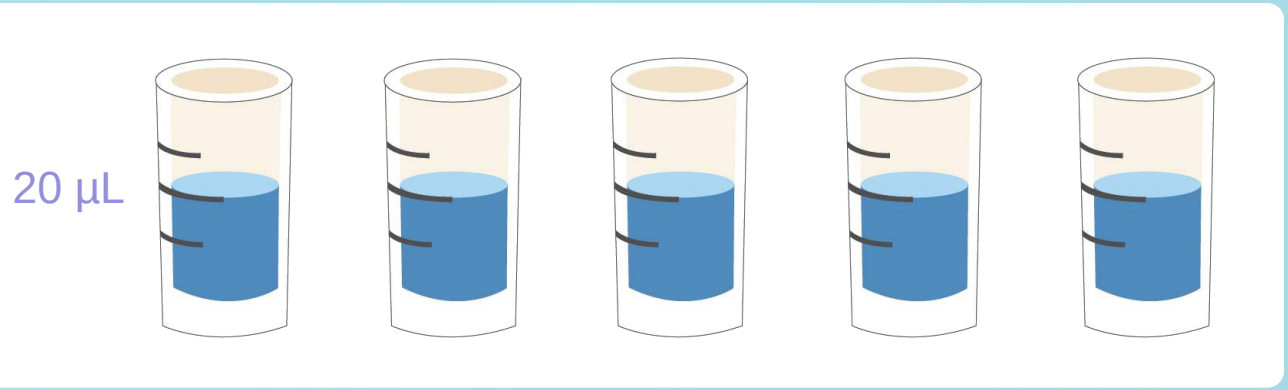
Accuracy and Precision

Precise, but not accurate



Accuracy and Precision

Accurate and precise

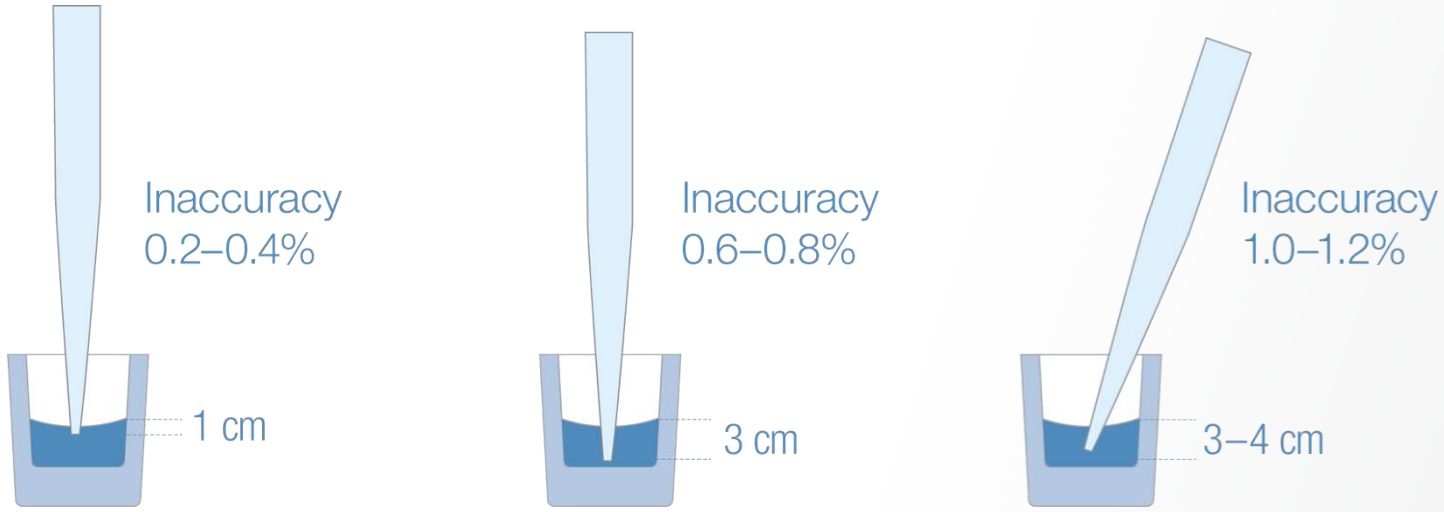


Factors affecting the accuracy of air displacement pipettes

Maria.hidesand@thermofisher.com |

Factors affecting the accuracy of air displacement pipettes

Pipetting position



1

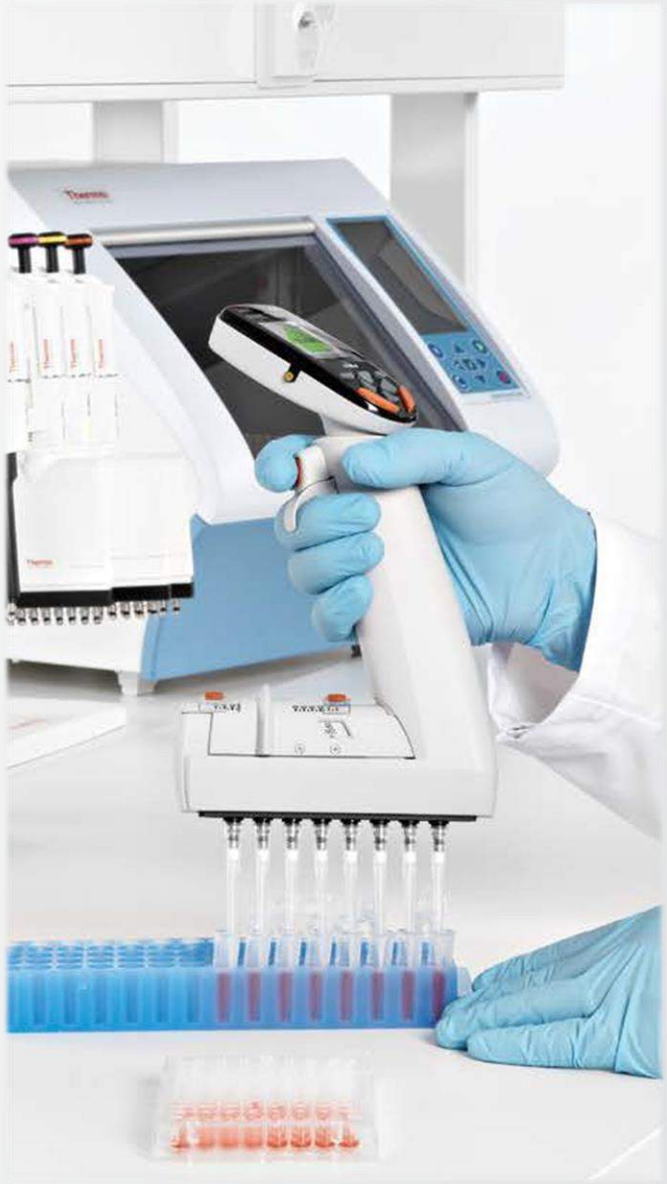
Pipette held vertically, tip immersed about 1 cm into liquid. The tip is immersed to the correct depth and correctly held vertically.

2

Pipette held vertically, tip immersed about 3 cm into liquid. Inaccuracy doubles when immersing the tip too deply.

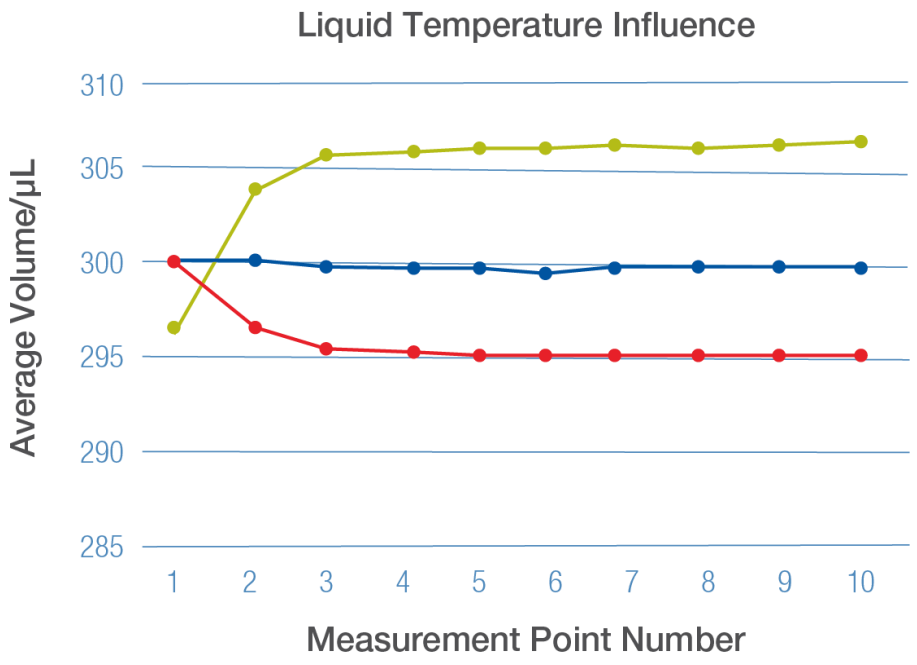
3

Pipette held at 30° to 40° angle, tip immersed about 3 to 4 cm into liquid. Inaccuracy increases 3-5 times by immersing too deeply while holding the pipette at an angle.



Factors affecting the accuracy of air displacement pipettes

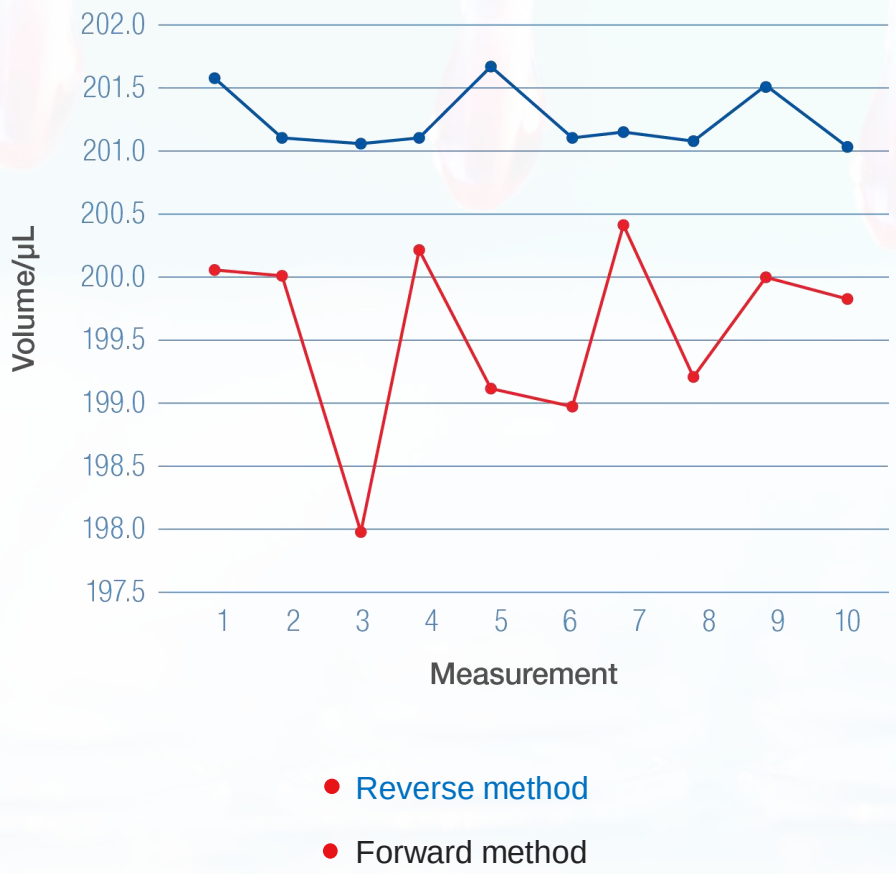
Temperature



Temperature differences cause thermal expansion/shrinking in the air space. After temperature equilibrium, the influencing factor is liquid density. Cold liquid is more dense and hot liquid is less dense than room temperature liquids.

Factors affecting the accuracy of air displacement pipettes

Viscosity



In this experiment, 200µL of viscous liquid (glycerol) was pipetted 10 times by using both forward and reverse pipetting techniques.

The pipette used was adjusted for glycerol using forward pipetting.

Using the reverse method:

- Yielded a smaller deviation between doses with greater precision
- Provided bigger doses because the liquid column in the tip was taller and pushed out a larger dose

Factors affecting the accuracy of air displacement pipettes

Density

Density (mass/volume ratio) affects the liquid volume that is aspirated into the tip, dead air volume, and earth gravity. The density of liquids also varies according to the temperature.

Typical density at 20°C

- 30% glycerol (e.g. PCR MasterMix) the density is 1.08 kg/dm3
- 100% glycerol is 1.260 kg/dm3
- Whole blood is 1.051 kg/dm3
- Ethanol is 0.789 kg/dm3

Quick Tip

Rinsing the tip 3-5 times with maximum volume with the liquid to be pipetted improves accuracy, especially with volatile solvents.

- Helps eliminate capillary action
- Provides identical contact surface for all aliquots
- Reduces sample hang up
- Helps equalize air temperature inside the tip to sample temperature

Liquid retention and inaccurate results when pipetting

Checklist for pipetting:

- ❑ Touch off of the tip.
- **After aspiration**: withdraw the tip from the liquid, touching it against the edge of the reservoir to remove excess liquid.
- **After dispensing:** Remove the tip by sliding it along the wall of the vessel. (Some liquid will remain in the tip, and this should not be dispensed.)
- ❑ Remove droplets from outside of the tip before dispensing.

- ❑ Immersion depth of tip: 1 cm (10 mm)
- ❑ Always hold pipette vertically (straight).
- ❑ Keep pipette and liquid in constant temperature (preferably room temperature).
- ❑ Use consistent plunger force and speed. Pause shortly after aspiration to allow liquid settle.

- **Note:** Finnpipettes are constructed to permit re-adjustment for other pipetting techniques or liquids of different temperature and viscosity. If pipette has been calibrated in forward mode with water, pipetting results in reverse mode with solvent cannot be the same.

Factors affecting the accuracy of pipettes

You - Pipetting gets too tedious and time consuming

- Plate numbers get high? >5-10 plates/day?
- Pipetting small volumes?
- Are you interested in improving reproducibility of your assays?
- Are you looking for an easy way to fill in your 384-well plates?
- Using a microplate dispenser minimizes Repetitive strain Injuries. There is no variation between different persons.

Solution is Automation

Preventing Cross Contamination

Pipette to sample

- Use filter tips, especially barrier tips
- Regularly clean and decontaminate
- Autoclave

Sample to pipette

- Use filter tips, especially barrier tips
- Keep the pipette vertical
- Steadily control the operating button
- Use a tip rack

Continuous Improvement of Pipetting Techniques

- Pipetting Maintenance & Calibration: Usage, smaller volume needs to be calibrated more often, regulations, pipetting errors etc.

Find out more at **thermofisher.com/pipettingtechniques**

Thank you

Maria Hidesand
Email: maria.hidesand@thermofisher.com
Mobile: +46706011556