Pipette Clinic

[redacted]
Homer Tsai

Overview

1. Principles in liquid handling
2. Guidelines to proper handling of pipettes
3. Decontamination and cleaning of pipettes

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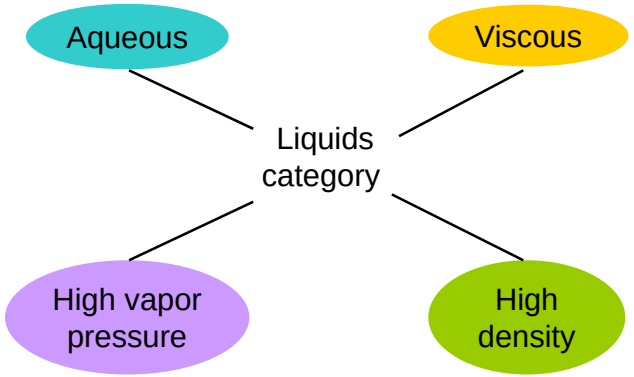
Liquids category



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Principles in liquid handling

Pipetting principles

Air-cushion
(air displacement)

- *Forward pipetting*
- *Reverse pipetting*

Positive displacement

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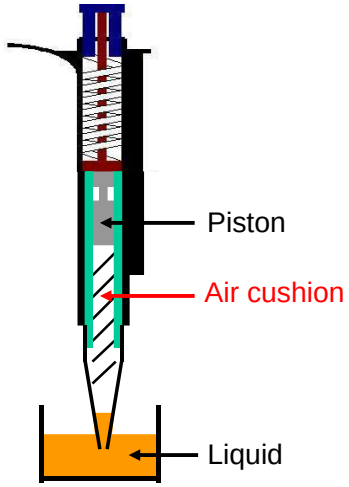
Air-cushion pipette



No contact between piston and sample

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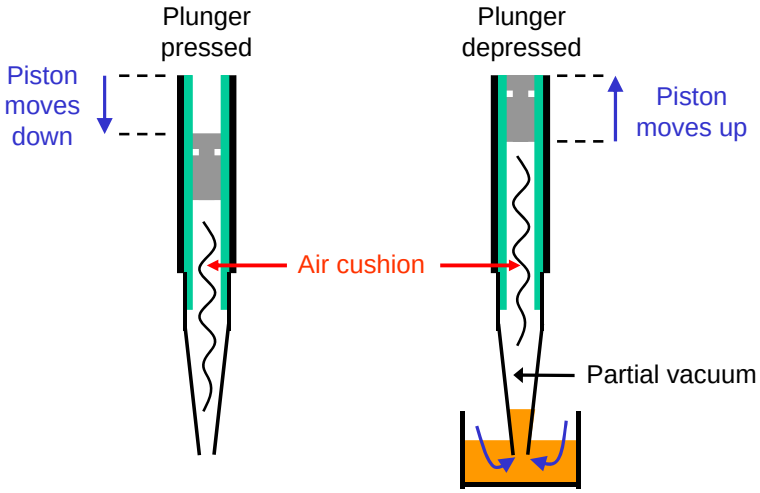
Air-cushion principle



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Air-cushion pipettes - Manual

Research®

Fixed Variable Multi-channel

Reference®

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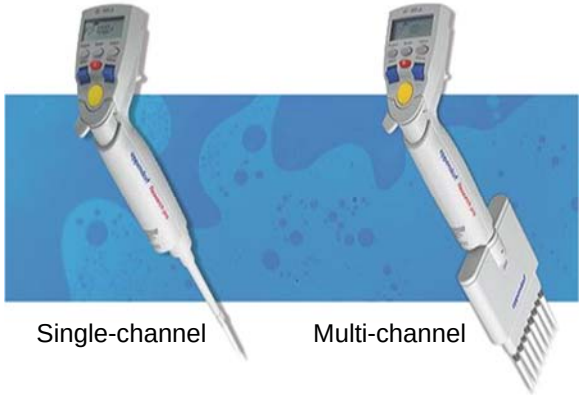
Air-cushion pipettes - Electronic

Research® pro



Single-channel Multi-channel

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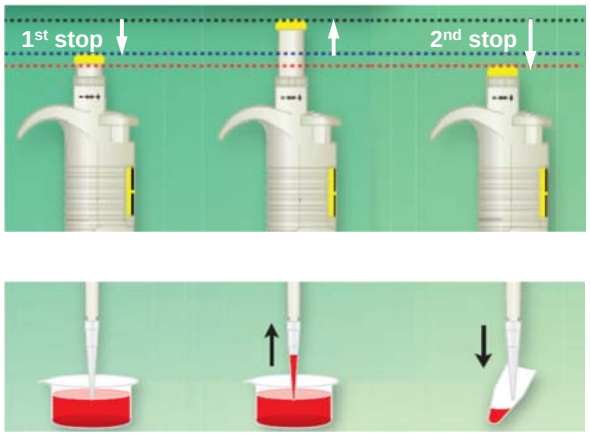
Forward pipetting – Technique



Press to **1st stop.** Immerse tip a few mm into liquid.

Release button slowly. Tip fills up.

Press to **2nd stop** to dispense.

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Forward pipetting - Applications

Application is recommended for standard *aqueous* solutions:

- Water
- Buffer
- Diluted saline solutions
- Diluted acids and alkalis

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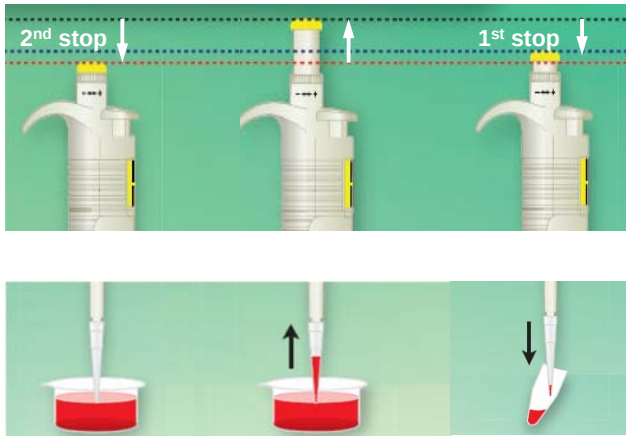
Reverse pipetting – Technique



Press to **2nd stop**. Immerse tip 3-4 mm into liquid.

Release button slowly. Tip fills up.

Press to **1st stop** to dispense. Some liquid will remain in the tip.

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Reverse pipetting – Applications

Application is recommended for:

- High vapor pressure liquids
- Viscous liquids

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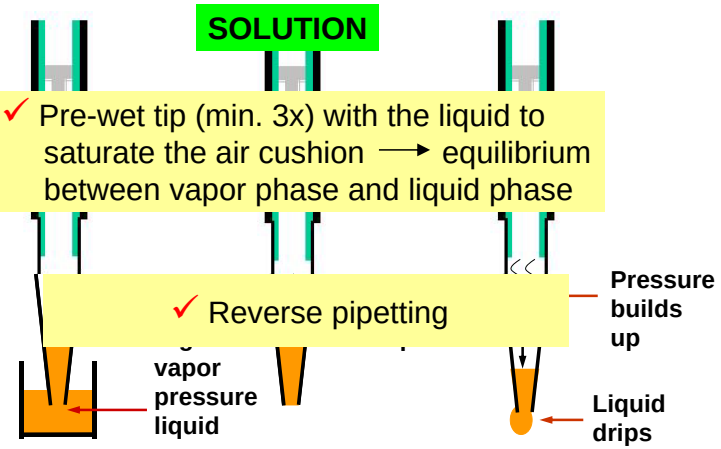
High vapor pressure liquids

SOLUTION

✓ Pre-wet tip (min. 3x) with the liquid to saturate the air cushion → equilibrium between vapor phase and liquid phase

✓ Reverse pipetting



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Viscous liquids

Problem:

- High resistance to flow
- Thin film of liquid remains in the tip

SOLUTION

✓ Slow aspiration and dispensing speed
✓ Reverse pipetting
✓ Pre-wetting

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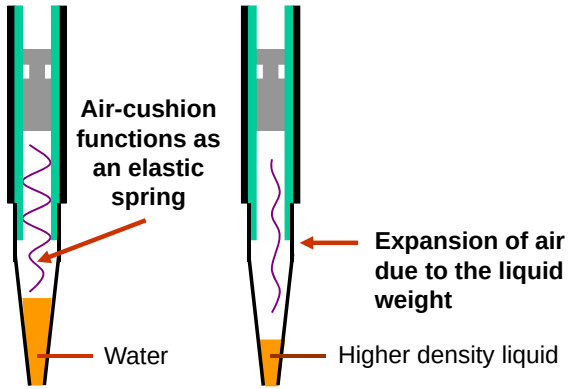
High density liquids



SOLUTION

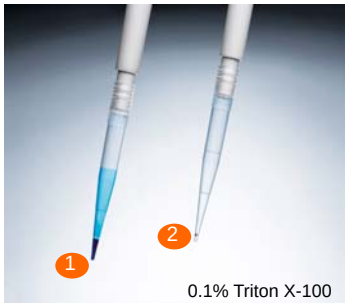
✓ Re-adjustment of air-cushion pipette →
Attention: adjustable-volume pipette fix-volume pipette

Pipetting detergent-containing liquids

Following dispensing detergent-containing liquids, a film of liquid remains on the tip surface.

- Loss of sample
- Increased reagent consumption, e.g. qPCR master mix
- Poor precision (reproducibility)

1. Substantial sample loss with standard tips
2. Maximum sample recovery with **epTIPS LoRetention**



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epT.I.P.S LoRetention

- The surface is rendered ultra-hydrophobic by modification at the molecular level without any

- coating or
- additives

which may contaminate the sample

- Minimizes loss of samples in solutions containing detergents (e.g. Triton X-100, SDS, Tween)
  - ✓ maximum sample recovery
  - ✓ improved reproducibility

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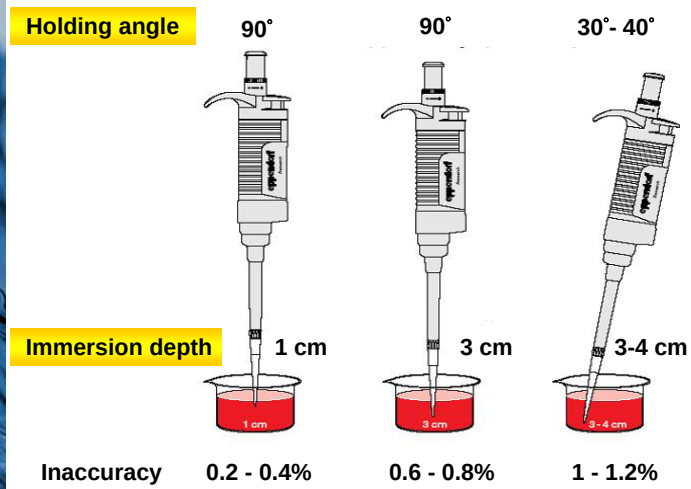
Tips to further improve pipetting accuracy



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Principles in liquid handling

Pipetting principles

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(air displacement)

- *Forward pipetting*
- *Reverse pipetting*

Positive
displacement

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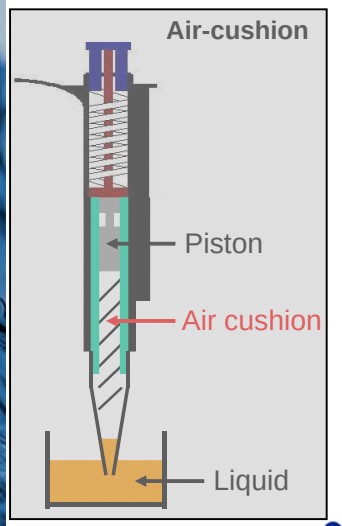
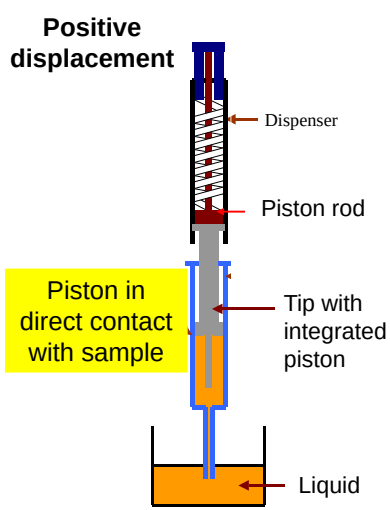
Positive displacement principle





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Positive displacement – Applications

- ✓ Ideal for “problematic liquids”:
  - High density
  - High vapor pressure
  - Viscous
  - ⇨ High accuracy and precision
- ✓ Contamination free – prevention of aerosols:
  - PCR
  - Toxic, infectious or radioactive liquids
  - Aggressive liquids (e.g. concentrated acids)

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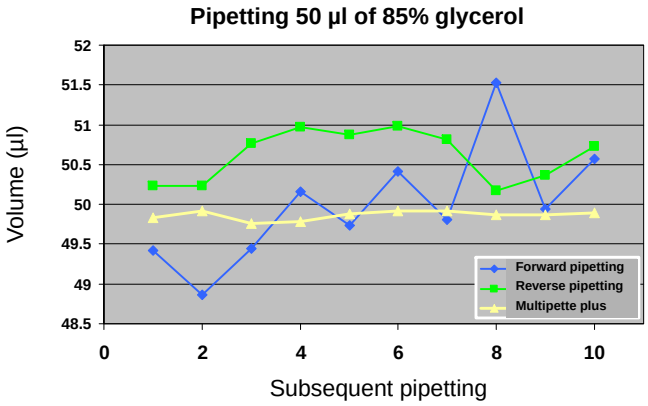
Pipetting viscous liquid

Effect of viscous liquid on pipetting accuracy and precision



Positive displacement dispensers

Multipette

stream & Xstream

Pipetting

Dispensing

Automatic dispensing

Sequential dispensing

Aspiration

Titration

Multipette plus

Combitips plus

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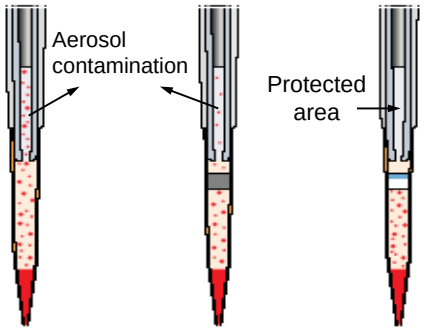
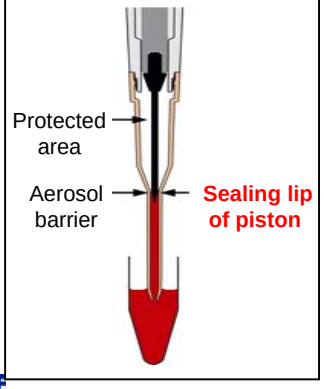
Contamination-free pipetting

Air-cushion pipette



Positive displacement
with integrated piston
in the tip



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Minimize aerosol contamination with filter tips

Blue layer binds smaller aerosols (e.g. biomolecules)

Retains drops, splashes and larger aerosols

Double protection !

Hydrophobic surface

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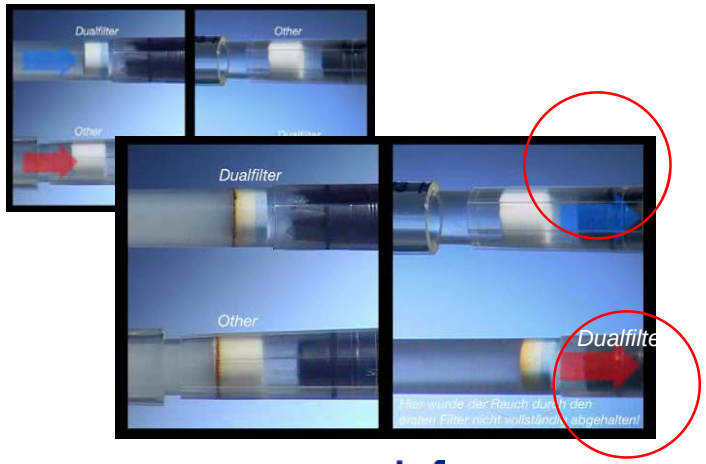
Filter tips efficiency – Smoke test



* See “Smoke Test” video

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Handling problematic liquids

Liquids category	Air-cushion pipette: Reverse pipetting	Air-cushion pipette: Pre-wetting	Air-cushion pipette: Slow aspiration and dispensing speed	Positive displacement pipette
High vapor pressure	✓	✓		✓
Viscous	✓	✓	✓	✓
High density	Re-adjustment of pipette			✓
Detergent-containing	epTIPS LoRetention			
PCR/ Infectious/ Radioactive	Dualfilter tips			✓

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Overview

1. Principles in dispensing of liquids
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Guideline #1

Know the liquid's physical properties, choose the best pipetting techniques

(forward, reverse or pre-wetting of tip)

1. ***Increases*** accuracy and reproducibility of results
2. ***Reduces*** contamination risks

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Guideline #2

Never lay a pipette down with fluid in its tip!

Prevent fluid from entering the pipette internal surfaces through the nose cone

Reduce risk of cross-contamination

Hold a pipette with a filled tip upright

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Guideline #3

DO NOT place pipettes onto the surface of the bench

- Decrease contamination risks from chemicals that have been spilled onto the bench.
- pipette cone may get chipped/bent out of shape, resulting in poor tip fitting.

Use…

Pipette carousal

Wall mount

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Guideline #4

Never release the push-button rapidly during aspiration

High aspiration speed promotes aerosol formation and leads to contamination.

Use filter tips – forms a barrier between the sample and the interior of the pipette.

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Guideline #5

Accidentally absorbed liquids SHOULD NOT be left to dry

Immediately clean the pipette

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Guideline #6

To ensure continued precision and accuracy, send the pipette for servicing and calibration.

The frequency of servicing and calibration depends on:

1. Frequency of pipette usage
2. Number of users

....but at least once a year

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Contamination scenarios

1. **Aqueous solutions, buffers, acids and alkalis**
2. **Organic solvents**
3. **Potentially infectious liquids and cell cultures**
4. **Nucleic acids**
5. **Proteins**
6. **Radioactive substances**

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Contamination scenario 1

Aqueous solutions, buffers, acids and alkalis

Disassemble pipette lower parts

⇩

Rinse contaminated lower parts with dH_2O

⇩

Allow to dry (at max. 60°C)

⇩

Lubricate piston

⇩

Assemble pipette

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Contamination scenario 2

Organic solvents

Disassemble pipette lower parts ⇨ Allow liquid to evaporate

Alternatively:

Rinse contaminated lower parts in mild detergent

⇩

Rinse well with dH_2O

⇩

Allow to dry (at max. 60°C)

⇩

Lubricate piston

⇩

Assemble pipette

• DO NOT use concentrated detergent.

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Contamination scenario 3

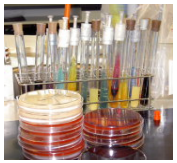
Potentially infectious liquids and cell cultures

Method A:

alcoholic disinfectants

Method B:

ultra-violet (UV) irradiation



Method C:

autoclaving

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Contamination scenario 4

Nucleic acids

Method A:

Sodium hypochlorite (5-10%)

Method B:

Glycine/HCl buffer (pH 2.0)

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Sodium hypochlorite (5-10%)

Use 5-10% fresh solution

⇩

Soak contaminated lower parts for 20 – 30 min

⇩

Rinse well with dH_2O

⇩

Allow to dry

⇩

Lubricate piston

⇩

Assemble pipette

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1X Glycine/HCl (pH 2.0)

Boil contaminated lower parts for ~ 30 min

⇩

Rinse well with dH_2O

⇩

Allow to dry

⇩

Lubricate piston

⇩

Assemble pipette

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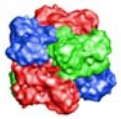
Contamination scenario 5

Proteins



Rinse contaminated lower parts with mild detergent

⇩

Rinse well with dH_2O

⇩

Allow to dry

⇩

Lubricate piston

⇩

Assemble pipette

DO NOT use alcohol ⟶ insolubility of proteins

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Contamination scenario 6

Radioactive substances

Rinse contaminated lower parts with radioactive cleaning solutions

⇩

Rinse well with dH_2O

⇩

Allow to dry

⇩

Lubricate piston

⇩

Assemble pipette

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Thank you !

Email:homertsai@hi-point.com.tw

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