Applications
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Liquid Handling No. 1
Using liquid handling systems in the laboratory

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Introduction

Dispensing very small amounts of liquid is essential in all fields of natural science and medicine. Pipetting in the microliter range is an integral part of a wide spectrum of experimental systems. New dispensing systems allowed experiments to be automated and simplified. At the same time, new technologies, such as genetics, place even higher demands on the construction and materials of the systems used. Since the late 1950s, dispensing technology in the lab has been in a constant state of development and technical improvement.

A wide range of pipetting systems is now available, including air-cushion pipettes and positive-displacement pipettes as well as manual and electronic systems. Direct pipetting, “reverse” pipetting and dispensing are all possible. New problems arise all the time in the lab. New methods or legal restrictions lead to even more complex demands being made. This issue of the “Applications” series has been designed as a lab poster, providing an overview of pipetting in the lab and of frequently-occurring problems.

Examples include:

- Which basic rules should be observed when pipetting?
- Which pipetting technique is recommended for which application?
- How can a pipette be tested and adjusted?
- Which measures should be taken for pipetting aggressive solvents?
- How can a pipette be decontaminated?

Unfortunately, it is not possible to deal with all problems in this issue. For further information, please read the instruction manual which accompanies your Eppendorf pipette.

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Construction principle of pipetting and dispensing systems

There are two fundamental principles: Air-cushion pipettes (piston-stroke pipettes) and positive-displacement systems.
The fundamental differences are shown in the two diagrams.
Piston-stroke pipettes have an air-cushion which moves between the piston and the sample liquid, and which aspirates and dispenses the sample. It functions like an elastic spring. A series of other factors must be taken into consideration, e.g. the dead volume, heat from the user's hand and the shape of the pipette tip.

This system guarantees high accuracy and precision and low costs for most applications. Filtertips can also be used to prevent contamination from aerosols.

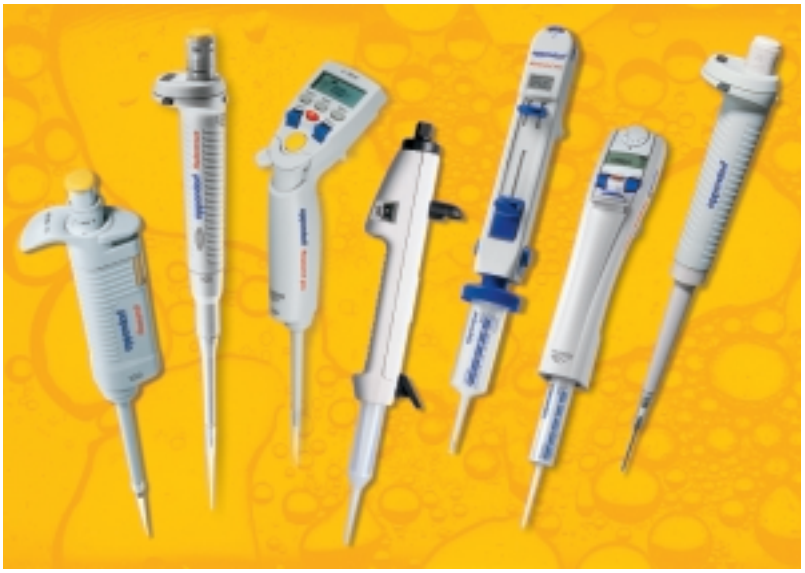
Positive-displacement systems function with virtually no air cushion, since an integrated piston in the pipette tip comes into direct contact with the sample solution. The pipette tip with the piston is replaced after every pipetting process. Many dispensers also function according to this principle. Important applications include pipetting viscous solutions, solutions with a high vapor pressure or high density, tenside solutions, as well as preventing contamination when working with radioactive or aggressive substances and biomolecules, such as nucleic acids in PCR*.

One advantage is that pipetting results are almost totally unaffected by the vapor pressure, density, viscosity or wetting behavior of the solution. Furthermore, cross-contamination as a result of aerosols being carried over is impossible.

*PCR is patented by Hoffmann-La Roche

Air-cushion pipette
The seals and spring systems are clearly visible.

Positive-displacement system
The piston which is integrated in the tip (2) and which has a leakage seal (3) is reversibly connected to the piston of the pipette (1).



Eppendorf pipette/Tip combination

Eppendorf offers the ideal pipette tips for applications in all volume ranges. Each tip has been specially developed to suit a specific volume range in order to guarantee the best possible dispensing results with the appropriate pipettes. In this way, Eppendorf pipettes combine with Eppendorf pipette tips to form a perfect system.

Pipette/epTIPS (µl)	0.1–10	0.1–20	0.5–20 L	2–200	20–300	50–1,000	50–1,250	500–2,500	100–5,000	1–10 ml/L	GELoader
Eppendorf Research®											
Fixed volume											
10 µl–100 µl				●	●						
200 µl–1,000 µl						●	●				
Adjustable volume											
0.1 µl–2.5 µl	●	●[*4]									
0.5 µl–10 µl	●	●	●								●
2 µl–20 µl				●							
10 µl–100 µl				●	●						
20 µl–200 µl				●	●						
100 µl–1,000 µl						●	●				
500 µl–5,000 µl									●		
1–10 ml										●/L[*4]	
Multi-channel											
0.5 µl–10 µl	●	●	●								
10 µl–100 µl				●	●						
30 µl–300 µl					●						
Eppendorf Reference®											
Fixed volume											
1 µl–10 µl	●	●	●								●
10 µl–100 µl				●							
200 µl–1,000 µl						●	●				
500 µl–2,500 µl								●			
Adjustable volume											
0.1 µl–2.5 µl	●	●[*4]									●
0.5 µl–10 µl	●	●	●								●
2 µl–20 µl (grey)		●	●								
2 µl–20 µl (yellow)				●							
10 µl–100 µl				●	●						
50 µl–200 µl				●	●						
50 µl–250 µl						●					
100 µl–1,000 µl						●	●				
500 µl–2,500 µl								●			
Eppendorf Research® pro											
Single-channel											
0.5 µl–10 µl	●	●	●								●
5 µl–100 µl				●							
20 µl–300 µl					●						
50 µl–1,000 µl						●	●				
100 µl–5,000 µl									●		
Multi-channel											
0.5 µl–10 µl	●	●	●								
5 µl–100 µl				●	●						
20 µl–300 µl					●						
50 µl–1,200 µl							●				
EDOS®											
Pip 10 µl	●	●	●								●
Pip 100 µl				●	●						
Pip 1,000 µl						●	●				
Multi 50 µl				●							
Multi 300 µl					●						
Multi 1,200 µl							●				

[*4] Extended inaccuracy and imprecision of the pipettes.

Eppendorf pipette/filtertip combinations

Pipette/epTIPS Filter (µl)	0.1–10 S	0.1–10 M	0.5–10 L	2–20	20–100	20–300	50–1,000	100–5.000	1–10 ml/L
Eppendorf Research®									
Fixed volume									
10 µl–100 µl				●*2	●				
200 µl–1,000 µl							●		
Adjustable volume									
0.1 µl–2.5 µl	●	●*4							
0.5 µl–10 µl	●	●	●						
2 µl–20 µl				●	●				
10 µl–100 µl					●	●			
20 µl–200 µl					●*1	●			
100 µl–1,000 µl							●		
500 µl–5,000 µl								●	
1–10 ml									●*4
Multi-channel									
0.5 µl–10 µl	●	●	●						
10 µl–100 µl					●				
30 µl–300 µl						●			
Eppendorf Reference®									
Fixed volume									
1 µl–10 µl	●	●	●						
10 µl–100 µl					●	●			
200 µl–1,000 µl							●*3		
500 µl–2,500 µl									
Adjustable volume									
0.1 µl–2.5 µl	●	●*4							
0.5 µl–10 µl	●	●	●						
2 µl–20 µl (grau)		●*1	●						
2 µl–20 µl (gelb)				●	●				
10 µl–100 µl					●	●			
50 µl–200 µl					●	●			
50 µl–250 µl									
100 µl–1,000 µl							●		
500 µl–2,500 µl									
Eppendorf Research® pro									
Single-channel									
0.5 µl–10 µl	●	●	●						
5 µl–100 µl					●	●			
20 µl–300 µl						●			
50 µl–1,000 µl							●		
100 µl–5,000 µl								●	
Multi-channel									
0.5 µl–10 µl	●	●	●						
5 µl–100 µl					●	●			
20 µl–300 µl						●			
50 µl–1,200 µl							●*1		
EDOS®									
Pip 10 µl	●	●	●						
Pip 100 µl					●	●			
Pip 1,000 µl							●		
Multi 50 µl					●	●			
Multi 300 µl						●			
Multi 1,200 µl							●*1		

*1 Restricted volume.

*2 Only for Research fix 10, 20 µl.

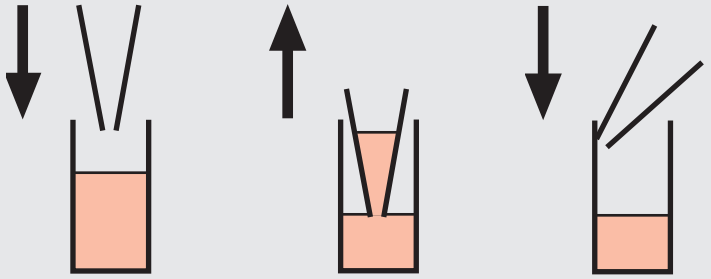
*3 Does not fit Reference fix 200 µl and 250 µl.

*4 Extended inaccuracy and imprecision of the pipettes.

1. Pipetting techniques

1.1 1.1 Air-cushion pipettes

Forward pipetting



1. Press down button to first stop. Immerse tip a few millimetres into liquid.

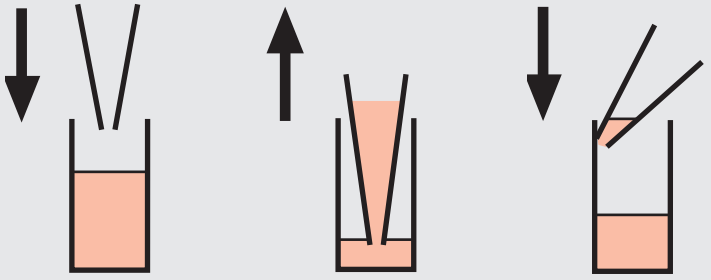
2. Release button slowly. Tip fills up.

3. Dispense liquid by pressing down button to first stop. Then blow out remaining liquid by pressing button down to second stop.

Prewet tip when working with volumes greater than 10 µl.

Application is recommended for: Standard solutions, such as water, buffer, diluted saline solutions and diluted acids and alkalis.

Reverse pipetting



1. Press down button to second stop. Immerse tip a few millimetres into liquid.

2. Release button slowly. Tip fills up.

3. Dispense liquid by pressing down button to first stop. Some liquid remains in the tip!

Application is recommended for: Viscous solutions, solutions with a high vapor pressure, wetting solvents.

1.2 Positive-displacement systems

When dispensing liquids with high vapor pressure it is preferable to use a direct-displacement system instead of reverse pipetting. For this application it is recommended to prewet the tip of the direct-displacement pipette. Dispensing hexane using the Multipette® plus/Combitips plus system can be used as an example of prewetting a positive-displacement system. Hexane is aspirated once or twice into the Combitip plus and immediately dispensed. This allows the small bubble of air remaining in the Combitip plus to be saturated with hexane vapor. Highly accurate dispensing with exact drip separation is then possible. This is very important for sample preparation in HPLC and other such applications. If the Combitip is not prewetted, the solution gradually evaporates and thus expels the hexane from the Combitip, i.e. the Combitip drips.
When pipetting with an air-cushion pipette, it is virtually impossible to saturate the entire air space above the liquid with vapor, since the volume of air is considerably larger than that in a positive-displacement tip.

2. Troubleshooting and solutions

Handling errors, damaged or contaminated devices and external factors can cause significant deviations to desired pipetting volumes. The cause of, and solutions to, some important errors are listed below.

Error	Cause	Solution
Pipette drips or leaks.	Tip is loose. Tip does not fit correctly.	Use original tip. Check tip fit.
	Nose cone is scratched.	Replace nose cone.
	Seal of nose cone leaks.	Replace nose cone or seal.
	Piston contaminated by reagent deposits.	Clean and lubricate piston. Replace seal.
	Piston damaged.	Replace piston and piston seal.
	Piston seal damaged.	Replace seal and lubricate piston.
Pushbutton does not move smoothly.	Piston scrapes and is contaminated.	Clean and lubricate piston.
	Seal swollen by reagent vapors.	Open pipette and ventilate. Lubricate piston if necessary.
	Piston visibly damaged or coated with insoluble deposit.	Replace piston and piston seal.
Volume inaccurate.	Deviating pipetting conditions.	See table on right.
	Pipette leaks.	Check tightness, then proceed as above.
	Pipette misadjusted.	Recalibrate as described.

Possible sources of error for piston pipettes with air interface			
Influencing parameter	**Effect***	**Influencable by**	**Determinable by**
Difference in density of the liquid to be pipetted versus that of the water used for adjustment	up to 1.0%	Readjustment of the pipette (observing user information)	Comparing the density of the liquid to be pipetted to that of water
Difference in vapor pressure of the liquid to be pipetted versus that of the water used for adjustment	up to 2.0%	Sufficient prewetting of the pipette tip; observing EN ISO 8655-6	Dripping tip
Uneven piston movement	up to 0.5%	Smooth operation of piston; cleaning and lubricating of the piston	Observing of one's own pipetting technique
Uneven rhythm and timing during pipetting	up to 1.5%	Even pipetting technique	Maximum permitted errors are exceeded
Depth of plunging of the pipette tip and handling angle during pipetting	up to 1.0%	Holding pipette in vertical position. Observing user information or EN ISO 8655-6	Visual control of plunging depth and handling angle
Failure to prewet pipette tip	up to 2.0%	Prewetting of pipette tip	Maximum permitted errors are exceeded
Failure to wipe pipette tip on the vessel wall	up to 3.0%	Wiping of the pipette tip on the vessel wall. Observing EN ISO 8655-6	Maximum permitted errors are exceeded
Leaky pipette tips	0.5% up to 50%	Using original or recommended pipette tips	Dripping tip or maximum permitted errors are exceeded

*Possible errors of measurement are estimates and are specified in percent of the nominal volume.

3. Checking pipettes for leaks

Testing for leaks is relatively easy with pipettes. To check it, the nominal volume of the pipette is aspired into the pipette tip (distilled degassed water) while the pipette is hold vertically. The pipette, pipette tip and test liquid should all have the same temperature. If after 1 min no distinct trop has formed on the tip, the pipette does not leak.
For volumes up to 20 µl the tip should be pre-wetted without fail.

4. Chemical stability of plastics used in Eppendorf pipettes

The parts of the pipette which may come into contact with solvent vapors are made of PP or PVDF (Polyvinylidenfluorid).

If necessary, the suitability of the pipette should be examined. Cleaning the pipette after aggressive solvents have been used is highly recommended. Due to individual differences in the conditions of use, the accuracy of the details cannot be guaranteed.

Substance	Density at 20 °C [mg/µl]	Vapor pressure at 20 °C [hpa]	Concentration [%]	Temperature [°C]	PP	PVDF
Acetic acid	1.06	15.4	96	20	+	+
Acetone	0.79	233	100	20	+	o
Ammonia	0.91	500	25	20	+	+
Butanol	0.81	6.7	100	20	+	+
Chloroform	1.47	213	100	20	o	+
Ethanol	0.79	59	98	20	+	+
Formic acid	1.23	42	10	20	+	+
Glycerine	1.26		100	60	+	+
Hydrochloric acid	1.15	213	37	20	+	+
Hydrofluoric acid	1.13		60	20	+	+
Isopropanol	0.78	42.5	99	20	+	+
Methanol	0.79	128	100	20	+	+
Nitric acid	1.41	9	65	20	-	+
Phenol	1.06		10	20	+	+
Phosphoric acid	1.71	2	85	20	+	+
Potassium hydroxide solution	1.29		30	20	+	o
Sodium hydroxide solution	1.33		30	20	+	o
Sulfuric acid	1.84	0.0016	95–97	20	o	+
Trichloroacetic acid	1.62		50	20	+	+

+ : Stable, remains unchanged even after long periods of contact
o : Partly stable, can only be used for short periods of contact
– : Unstable, changes in the material can occur after short periods of contact

5. Decontaminating air-cushion pipettes when working with various liquids

Liquid	Handling, Special features	Decontamination
Aqueous solutions and buffers	Pipette is calibrated with distilled water. Results are extremely accurate.	Open pipette, rinse contaminated parts well with distilled water, allow to dry at maximum 60 °C in drier compartment. Lubricate piston if necessary.
Inorganic acids	It is advisable to occasionally rinse the pipette lower part with distilled water if high-concentration acids are pipetted frequently. Using Filtertips is also recommended.	The plastics used in Eppendorf pipettes are acid-resistant, as are the ceramic pistons (except to hydrofluoric acid). However, aerosols from the acids can enter the pipette lower part and affect the performance of the pipette. Clean as described above in “Aqueous solutions”.
Alkalis	It is advisable to occasionally rinse the pipette lower part with distilled water if high-concentration alkalis are pipetted frequently. Using Filtertips is also recommended.	The plastics used in Eppendorf pipettes are alkali-resistant, as are the ceramic pistons. However, aerosols from the alkalis can enter the pipette lower part and affect the performance of the pipette. Clean as described above in “Aqueous solutions”.
Potentially infectious liquids	To avoid contamination, Filtertips shoud be used. Alternatively, positive-displacement systems can be used.	Autoclave the contaminated parts at 121 °C for 20 min (The Eppendorf Reference® can be completely autoclaved. It must be disassembled before by unscrewing twice) or immerse the lower parts in normal laboratory disinfectants, rinse with distilled water and allow to dry as described above.
Cell cultures	To guarantee sterility, Eppendorf Filtertips should be used.	Proceed as described above in “Potentially infectious liquids”.
Organic solvents	1. Density is different to that of water. Therefore it is necessary to adjust the pipette. 2. Pipetting should be carried out rapidly, due to the high vapor pressure and the changes in the wetting behaviour. 3. After pipetting has been finished, open the pipette and allow the liquid to evaporate.	This evaporation process is normally sufficient for liquids with a high vapor pressure. Alternatively, immerse the contaminated parts in detergent, rinse well with distilled water and dry as described above.
Radioactive solutions	To avoid contamination, Filtertips should be used. An alternative would be to use positive-displacement systems.	Open pipette and place contaminated parts in complex solutions or special cleaning solutions, rinse well with distilled water and dry as described above.
Proteins/ Nucleic acids	To avoid contamination, Filtertips should be used. An alternative would be to use positive-displacement systems.	1. Proteins: Open pipette, rinse pipette with detergent. Rinse and dry as described above. Lightly lubricate piston. 2. Nucleic acids: Decontaminate by boiling in glycine/HCl buffer (pH 2) for 10 minutes (this ensures that no more DNA can be detected on an agarose gel). Rinse well with distilled water and dry as described above. Lightly lubricate piston. 3. Clean with sodium hypochlorite (5%), rinse well with distilled water and dry as described above. Lightly lubricate piston.

6. Testing the systematic and random measurement deviation of pipetting systems

A. Gravimetric testing
The process is described in detail in the Eppendorf "Standard Operating Procedure" (SOP), which is available free-of-charge. Download under www.eppendorf.de/SOP. The following preconditions must be fulfilled for gravimetric testing (see photo):

- Evaporation protection for the weighing chamber, with the aid of moist blotting paper or an evaporation trap.
- No drafts, direct sunlight or heat.
- Ion free and degassed water must be used as a sample solution.
- The temperature of the room, the water and the pipette must be maintained at a constant 15–30 °C, ± 0.5 °C.

The mass of the water is obtained by weighing; this must be converted into the volume (volume = mass/density). Temperature and density of the test liquid are also factors in this conversion.
Example: Mass obtained: 99.3 mg. At 23 °C, the density ρ of water is 0.997 mg/µl. The actual volume is 99.6 µl. Further details can be found in the Eppendorf SOP.

B. Photometric pipette test
Testing pipettes with a volume of less than 1 µl is virtually impossible using an analytical balance. A photometric test has been developed for these pipettes, which is described in detail in the special application (no. 25) from Eppendorf. (www.eppendorf.de/application)

Standard values for gravimetric testing of pipettes

Random measurement deviation (Imprecision)	Scale graduation (balance)	Typ of balance
up to 0.01 µl	0.001 mg	Micro balance
up to 0.1 µl	0.01 mg	Semi-micro balance
up to 1 µl	0.1 mg	Analytical balance