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Essential tips for good results

The Science of Pipetting to Perfection

A guide to expert pipetting

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A book from **Eppendorf SE**

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Foreword

Scientific findings are the basis for far-reaching decisions. It is essential to ask ourselves: how are these results accomplished? What are the hurdles that scientists must overcome to ensure the integrity of their data when their experiments involve the handling of liquids in the lab?

Since the 1950s, we at Eppendorf have listened to our customers – our mission was, and still is to this day, »to improve people's living conditions.« With this book, we have combined our six decades of experience in liquid handling with our customers' feedback to create a comprehensive pipetting guide that supports you in carrying out good science and helps you to fulfill your mission with pride.

Do you sometimes ask yourself which pipette and pipette tip combination is the right one for your applications? Or what could improve your pipetting skills when working with difficult and volatile liquids? There are several lab practices that can help you achieve accurate results, and this pipetting guide will walk you through them.

You can learn more about the everyday and unique challenges in routine pipetting and how to deal with them. We discuss which pipette systems are suitable for which application, and provide solutions for more precise pipetting results.
We also shine a light on the benefits of regular maintenance of your pipetting systems, and the effects of calibration and adjustments.
In today's day and age, it is unthinkable to not address the topic of sustainability when working in a lab. This pipetting guide also focuses on how you can use your resources responsibly and how you can create better lab practices that positively impact the environment.

The book furthermore provides insights into how to improve ergonomics in the lab to ensure that the most significant resource in science is also safeguarded: you, the scientist.

Thank you for pushing science forward with your research – we hope this book will not only help to improve your results but also your overall pipetting experience.

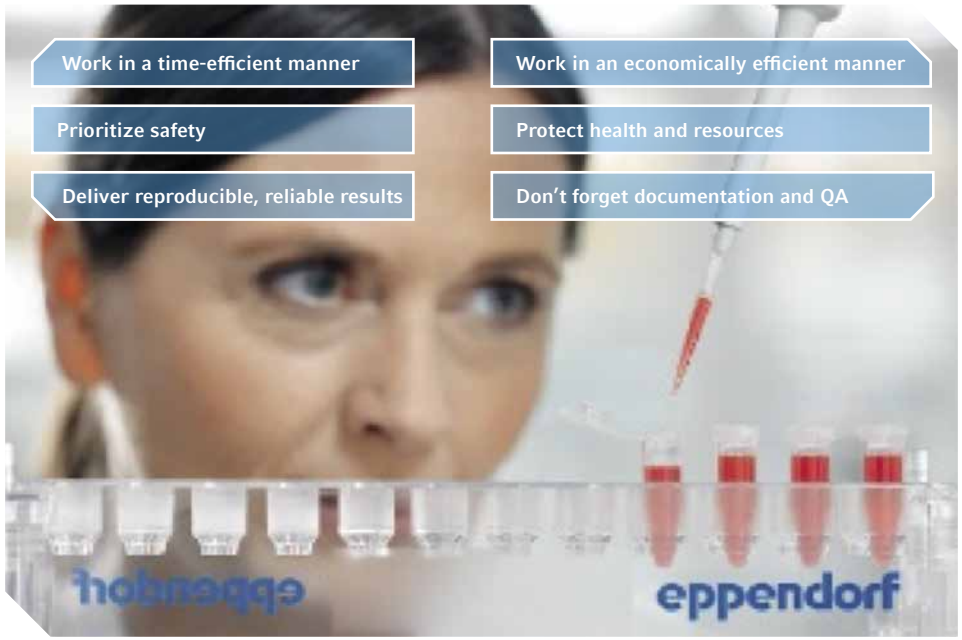
Your expert partners for liquid handling at Eppendorf

1. The science of pipetting

1.1. Science counts – but what counts in science?

The standards in modern-day scientific and industrial laboratories are high, which means the performance has to be professional. Important decisions depend on the outcome of sample analyses within medical, molecular biological, and pharmaceutical applications and proteomics. Companies have to withstand competition, and scientists have to perform premium research in order to publish quality science and be recognized by the community.

The need for high-throughput assays and nucleic acid analyses is constantly increasing in all areas, from microbiome and cancer research to pharmacogenomics. This leads to sample processing being carried out in high throughput formats, requiring smaller and more numerous reaction vessels. Today's scientists and laboratory staff bear a significant responsibility and their work requirements are complex and demanding.



1.2. The science of pipetting: success factor or pitfall

Quality is a prerequisite for successfully mastering today's standards. In order to maintain high quality at all times, the smallest detail counts. One such detail is the science of pipetting. Although it seems like a minor factor, it can have an immense impact. Being the most frequently performed step in any experimental process, pipetting is directly responsible for critical criteria which define quality and continuous excellence. **Efficient** technical performance will safeguard **reliable** and **reproducible** results, the **safety** and integrity of the samples as well as the safety of the operator. Pipetting is so substantial that it can be both a success factor and pitfall!

Time-efficiency

BENEFITS

Time-efficiency means optimizing your equipment, workflow, and daily practices in order to save valuable time. Organization of your work space is paramount. Proper storage and accessibility of your pipettes help streamline your workflow.

NOTE

Old habits can become counterproductive. New formats and technologies can increase time-efficiency. A few examples:

> Filling microplates with 8-, 12-, 16- and 24-channel pipettes instead of using single-channel pipettes

> Repetitive dispensing of volumes in long series such as tube or plate filling, and aliquoting reagents with dispensers or electronic pipettes instead of using single-channel pipettes

> Sample transfer between different formats with multi-channel pipettes with adjustable tip spacing instead of using mechanical single-channel pipettes (makes you up to 3x faster)

> Considering digital solutions for quicker setting of parameters or to support documentation quality

Reliability & Reproducibility

Reliability means that a result can be repeatedly validated, reflecting the true nature of the sample. A result is reliable only after it has been reproduced consistently. **Reproducibility** is most likely the most important characteristic of research and analysis results. A rising number of experiments published cannot be reproduced by other groups within the scientific community. Pipetting may be one reason for this.

Accuracy and precision are the basis for obtaining good results. Accuracy means that the pipette delivers the volume which has been selected. You can increase accuracy through minimizing the risk of error by choosing a complete pipetting system as specified in ISO 8655*[1]. This should consist of high-quality pipettes that are professionally maintained, calibrated and used with the recommended quality matched tips. Accuracy is greatly influenced by conditions which impact air cushion size such as, for example, temperature differences between pipette and sample. The air cushion between liquid and piston experiences thermal expansions which either reduces or increases the amount of liquid aspirated. Air cushion size also depends on the density of the liquid as well as on geographic altitude as air pressure decreases in higher altitudes compared to sea level.

Precision is a quality term. Precision means that individual measurements are close in value with no deviation between them. Precision can be affected by laboratory practice. Pipetting technique, correct pipetting angle and speed, and smooth, consistent pipetting rhythm as well as concentration on the task contribute to precision. Precision is further improved by the use of multi-channel pipettes, automation as well as electronic pipettes and dispensers, which minimize user bias.

*[1] ISO 8655:2002 parts 1–6: Piston-operated volumetric apparatus. www.iso.org

NOTE

Many factors can affect the precision and accuracy of pipetting and therefore the reproducibility and reliability of results. Some examples:
> Wrong pipette or technique for challenging liquids
> Tips which do not fit the pipette
> Wrong pipetting technique
> Leachables which interfere with reactions and assays
> Aerosol contamination
> Fatigue
> Poor cleaning, maintenance, calibration and adjustment of pipettes

NOTE

The largest variable in scientific experiments is the experimenter. User-to-user variances, pipetting speed, inconsistent pipetting rhythms and different pressure placed on the operating button affect the reliability of results (when using mechanical pipettes instead of electronic pipettes or automated systems).

Safety

The term safety can be applied to the **sample**, the **user,** and **legal compliance**.

Sample safety means protecting your sample from contamination, which include PCR inhibitor additives, aerosols and biomolecules (e.g. DNA fragments, DNase, RNase). Proper careful handling to maintain **sample integrity** is equally essential. High-quality consumables are critical; consumables made from low-quality plastic carry a risk of poor sample recovery. Furthermore, leachables and slip agents used in the manufacturing process of low-quality plastic consumables may distort analysis results. Suboptimal properties of the plastic surface lead to poor sample recovery, leachables, or slip agents that can falsify results. Autoclavable pipettes protect the sample from contamination and special pipette tips (or using handheld dispensers working with the positive displacement principle) prevent aerosol contamination.

User safety corresponds to the physical and mental health of the operator. It requires the safe transfer of infectious, radioactive and toxic substances, as well as ergonomic working conditions which will help avoid physical strain and fatigue. Ergonomic equipment, posture and the arrangement of the equipment within the laboratory contribute significantly to maintaining health. Adequate laboratory clothing protects against contamination. Tips significantly influence both sample and user safety. Contamination of sample and pipette can be prevented by using filter tips; a positive displacement pipette is the instrument of choice when it comes to handling toxic, radioactive or infectious materials. In all cases, the equipment and the consumables should be suited to the application.

Legal safety means compliance with guidelines for clinical applications, ISO8655 or ISO/IEC 17025. Applicable guidelines and regulations depend on your region/country and application.

Avoid the following for optimal conditions to achieve good results:
> Usage of suboptimal equipment for cost reasons
> Poor maintenance of lab equipment
> Inefficient daily practices
> No personal protective equipment
> Excessive effort, high operating forces and repeated physical strain
> Limited time to exercise caution and complete documentation
> Not enough opportunities for rest and relaxation of muscles

NOTE

Quality

Quality assurance requires regular performance checks of pipettes including calibration according to ISO 8655, replacing worn parts regularly, depending on use, and completing documentation in compliance with traceability requirements.

NOTE

The risks of not meeting quality standards include the potential disqualification of batches or entire studies, resulting in significant loss of time and money.

1.3. The holistic expert approach

The science of pipetting involves a holistic approach. It is the synergistic interplay between pipetting equipment, technique, workflow and working conditions. The type of sample and the application determine the choice of equipment and handling.

2. Benefits of this book

This book is aimed at all experts who work on complex and demanding projects in the laboratory as well as those who aim to become experts. No matter whether you are an experienced researcher or this is your first day in the lab; whether you are the lab manager and/or the person in charge of health and safety – with this book, you will have the opportunity to learn the science of pipetting from the ground up, allowing you to consolidate your professional knowledge and receive useful information.

The knowledge in this book will help you to:

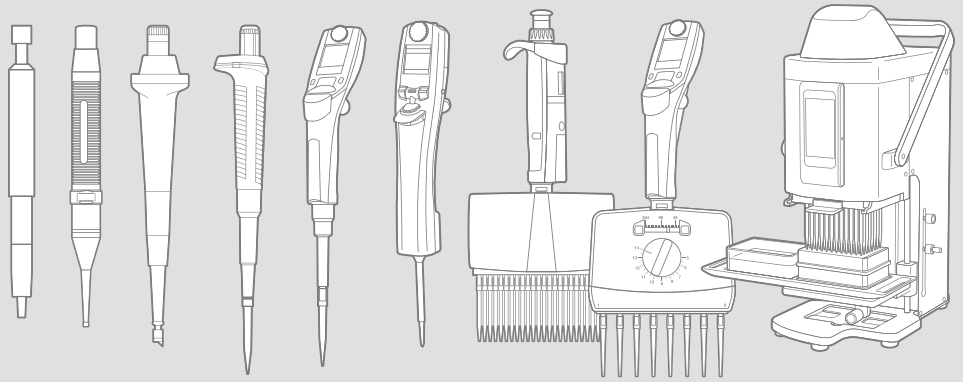
> Maintain and increase reproducibility & reliability of results
> Streamline workflows and increase efficiency
> Perform your work in a safe manner
> Create ergonomic health-promoting working conditions

3. The first piston-stroke pipette

The invention of the pipette proves that genius and madness lie close together. Before 1958, samples were laboriously pipetted by mouth. Who does not know the old stories about how mercaptoethanol was accidentally ingested? Dr. Heinrich Schnitger, a very talented German doctor from the University of Marburg, changed everything. Schnitger was an enthusiastic tinkerer. After suffering from tuberculosis as a soldier in World War II, Heinrich Schnitger decided to study medicine for his own health. Deeply frustrated by pipetting by mouth, he disappeared from the laboratory for a few days and returned with a self-designed tool for pipetting microliter volumes. Schnitger »converted« a tuberculin syringe by adding a spring to the plunger, which hit a stop at the top to define the pipetting volume. Initially without commercial interest, he created the first prototype of a piston-stroke pipette for his lab. He eventually collaborated with the founders of Eppendorf, Netheler and Hinz, who at the time were developing industrially manufactured pipette tips. Their collaboration resulted in the development of a functional microliter system. The »Marburg« pipette, launched in 1961, was so innovative, that it already contained elements that are still used today.

In the following years, Eppendorf changed the nature of pipetting forever. The well-known microliter system of today was developed, consisting of a microliter pipette, tips and the first standardized plastic vessels with mounted closures, the Eppendorf Tubes®. »Eppis®« are now world-famous and indispensable in the laboratory.

Today, electronic and automated dosing devices are available on the market – the evolution of the pipette continues!



4. Pipettes

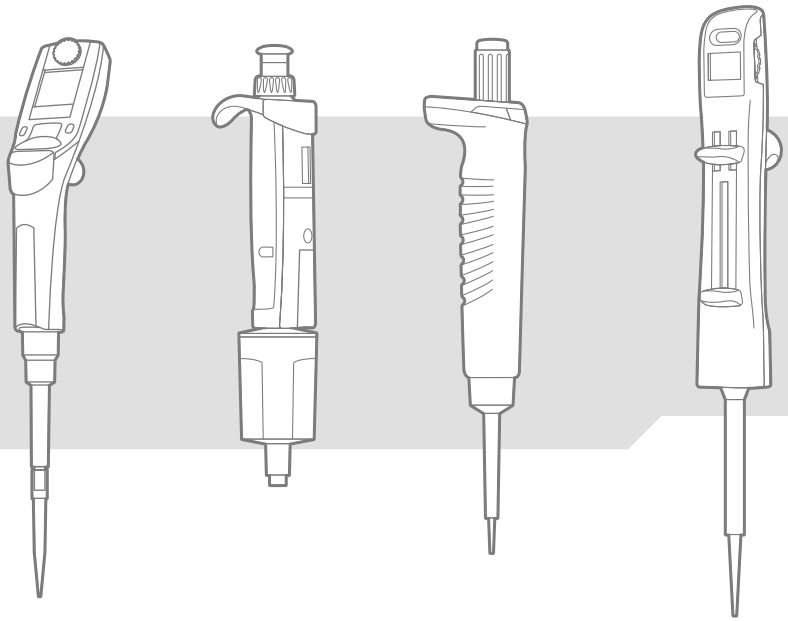
The pipette is the workhorse of the laboratory, and challenging applications have to be mastered every day. These applications range from difficult liquids to pipetting into a multitude of reaction vessels. Several »secret recipes« are circulating throughout the world: pipetting any kind of liquid, with a set of 3 pipettes, cutting off tips for viscous liquids or pipetting volatile liquids as quickly as possible to avoid loss. Error-prone workarounds like filling rows and columns of 384-well plates alternately with 8- or 12-channel pipettes are another example.
Fortunately, challenges can often be easily met with the right pipette for your application.

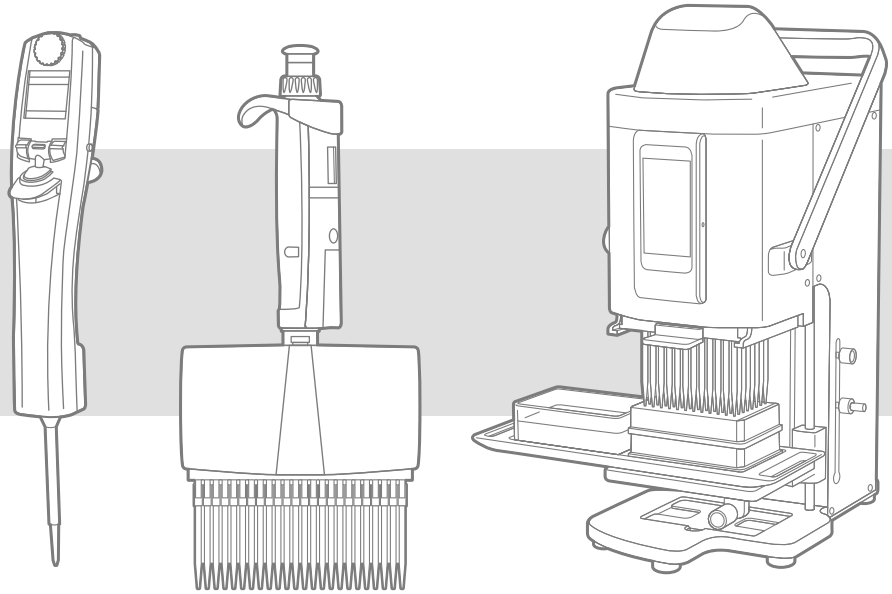
NOTE

The pipette affects the quality of your application in terms of reproducibility, time-efficiency, economic efficiency, user & sample safety, and health.
The right pipette allows you to optimize the complete workflow in every respect: a) to perform precise and accurate work, b) to achieve the best possible reproducible and reliable results, c) to process analyses efficiently in the shortest time, d) to work safely and ergonomically.

The choice of your pipette depends on various factors:

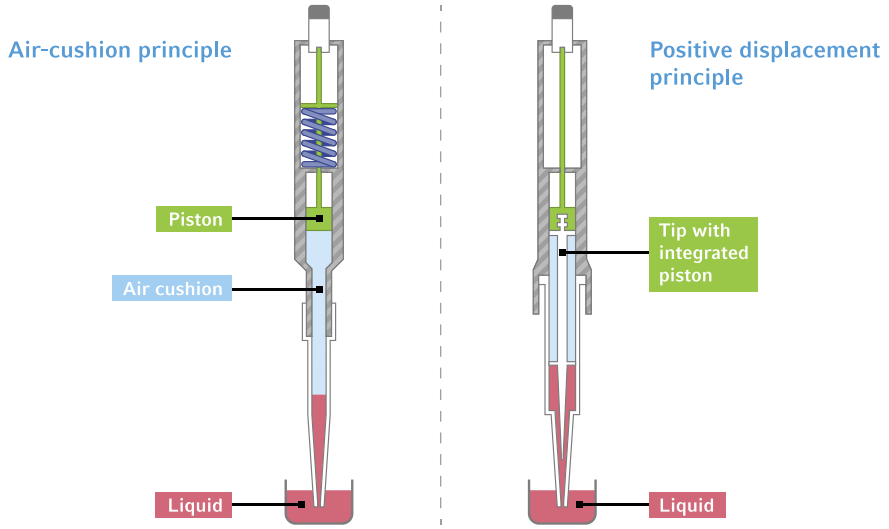
1. **Type of liquid:** Air-cushion pipettes are the optimal choice when working with aqueous solutions. However, different types of liquids are regularly in use, each of them requiring special consideration. Some liquids have physical properties which differ from those of water. Often, more precise results are achieved with alternative devices which work according to the positive displacement principle. This applies e.g. for viscous liquids which have a relatively high resistance to flow, and volatile liquids which often start dripping due to their high vapor pressure. Liquids containing detergents display reduced surface tension, and they tend to stick to the inner wall of the tip. Additional problematic liquids include those which foam and liquids at extreme temperatures.
2. **Volume options:** Accurate and precise pipetting techniques rely on more than the right pipette. The (maximum) nominal volume should be as close as possible to the desired transfer volume. Thus, the air cushion remains as small as possible, which is important because it reacts to external influences and can therefore influence the volume dosed.
3. **Type of reaction vessel:** Pipetting of single tubes, strips or microplates characterized by a multitude of vessels or specifically shaped vessels is faster and easier with the appropriate tool.
4. **The number of samples:** Processing samples in high-throughput format requires time-efficient tools.





4.1. Air-cushion pipettes or positive displacement instruments?

Dispensing systems function according to **two different physical principles:** dispensing via an **air cushion** or by **positive displacement**. Air-cushion piston-stroke pipettes are the »normal« pipettes we know from everyday laboratory use. The underlying principle is based on the presence of air between the liquid in the tip and the piston in the pipette. In positive displacement instruments, the piston is integrated into the tip and in direct contact with the liquid.



Reliability & reproducibility:
Worldwide, air-cushion pipettes are most commonly used in laboratories to achieve high-precision results for most pipetting requirements. However, the accuracy and reproducibility of pipetting results can be compromised if liquids are pipetted that have physical properties different from water, including viscosity (e.g. glycerol), volatility (e.g. ethanol, acetone), surface tension or density. In these cases, positive displacement instruments simplify the pipetting process and provide more accurate results.

Time-efficiency:
Dispensers are used to reduce the number of individual pipetting processes. Dispensers enable faster processing of long test series with high dispensing accuracy and provide great flexibility regarding applications and sample volumes. Dispensing of a single volume into a large number of vessels and plates using a hand-held dispenser is the preferred option if automation is not possible or would be too costly. Dispensers usually operate according to the positive displacement principle.

Safety:
Positive displacement instruments reduce the risk of cross-contamination and protect both the user and the pipette from hazardous liquids. The liquid is contained within a hermetically sealed system. Dispensers have a significant beneficial effect on ergonomics. Ergonomically shaped levers and easy-to-reach dials enable comfortable dispensing. A hand rest supports a relaxed hand position and ease of use.

Recommended applications for air-cushion pipettes and positive displacement instruments

	Air-cushion pipette	Positive displacement instrument
Application	**Aqueous liquids**	
	Challenging liquids* can be pipetted using some tricks, but accuracy and reproducibility can be compromised; special tips might be needed	Challenging liquids*
		Applications requiring the absence of aerosols for the prevention of cross-contamination, e.g. PCR.
Characteristics	**High precision and accuracy for aqueous liquids**	
		High precision and accuracy for challenging liquids*; Simplifies the pipetting process
		Reduces risk of cross-contamination and protects user and pipette from hazardous liquids

* Challenging liquids: non-aqueous liquids like viscous, dense, volatile, infectious, radioactive or foaming liquids and detergents

TIP

> Don't cut off pipette tips for the purpose of pipetting viscous liquids. Dripping pipettes do not usually mean that the pipette is damaged. Often such problems can be easily solved with a positive displacement instrument and the right techniques.
> Don't get confused when performing many single pipetting operations one after the other! Use a dispenser, electronic, or multi-channel pipette when automation is not possible.

More information:

Liquid Guide
https://eppendorf.group/liquid-guide

Fundamentals of dispensing
https://eppendorf.group/userguide-19

Pipetting of challenging liquids
https://eppendorf.group/challenging-liquids

4.1.1. Positive displacement instruments

In positive displacement systems, the **piston** is **integrated into the tip and in direct contact with the liquid.** Compared to the air-cushion principle, the flow properties and the wetting behavior of the liquids to be dispensed are of lesser importance so that these devices are also **suitable for liquids and applications** which can be seen as **critical in conjunction with air-cushion systems.**
Such applications include:

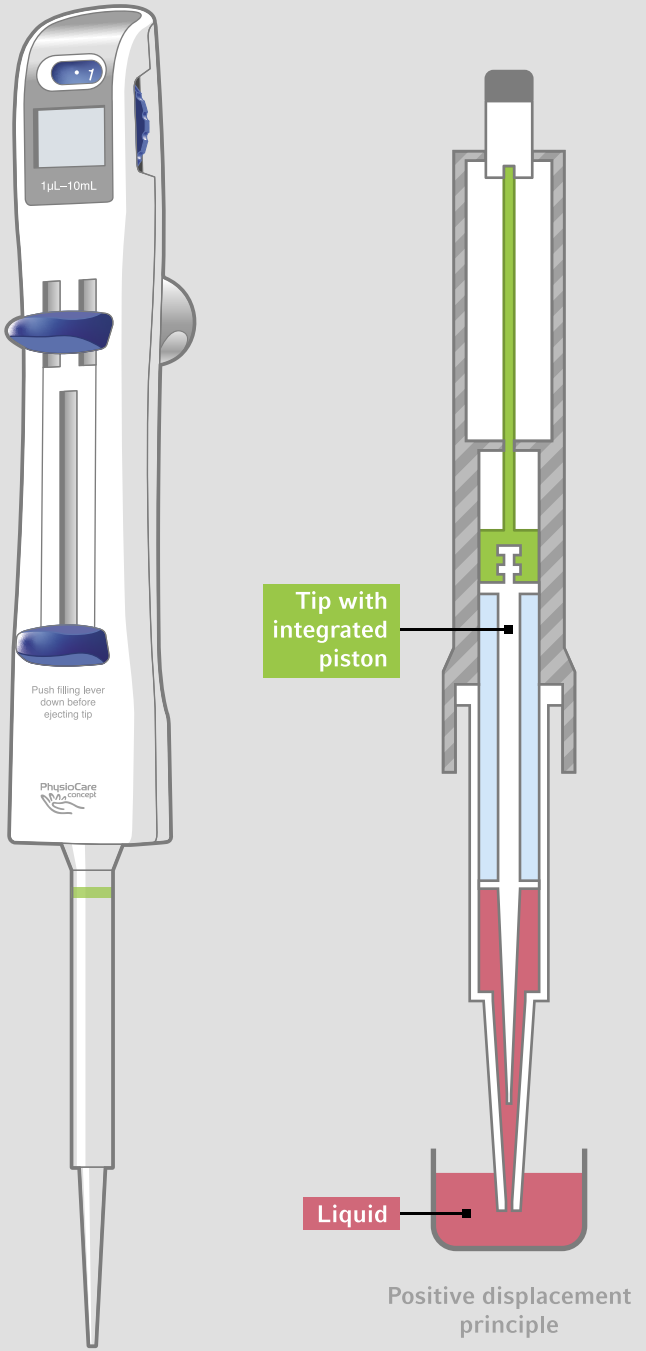
> Liquids with high vapor pressure, high viscosity or high density whose properties differ widely from the corresponding values for water
> Hot or cold liquids
> Applications in molecular biology such as PCR or hazardous liquids, which call for the absence of aerosols to prevent contamination or cross-contamination

The dispensing accuracy of positive displacement dispensing systems depends on the disposable plastic tip to an even greater extent than air-cushion systems do, and tips are specially designed. Most handheld dispensers work according to the positive displacement principle.

Dispensers can be used to reduce the number of individual pipetting processes; therefore the ergonomic properties of the instrument play a key role. A certain volume is repeatedly dispensed from a previously aspirated volume, depending on the selected setting of the dispenser. Every press of the thumb on the pipetting lever results in a mechanical stepped feed which is transferred to the piston of the syringe-like plastic tip. A selection of cylinder sizes offers a large number of different sample volumes ranging from 1 µL to 50 mL.

TIP

Modern dispensers reduce user error by displaying the pre-selected dispensing volume on an integrated display.



Positive displacement principle

4.1.2. Air-cushion pipettes

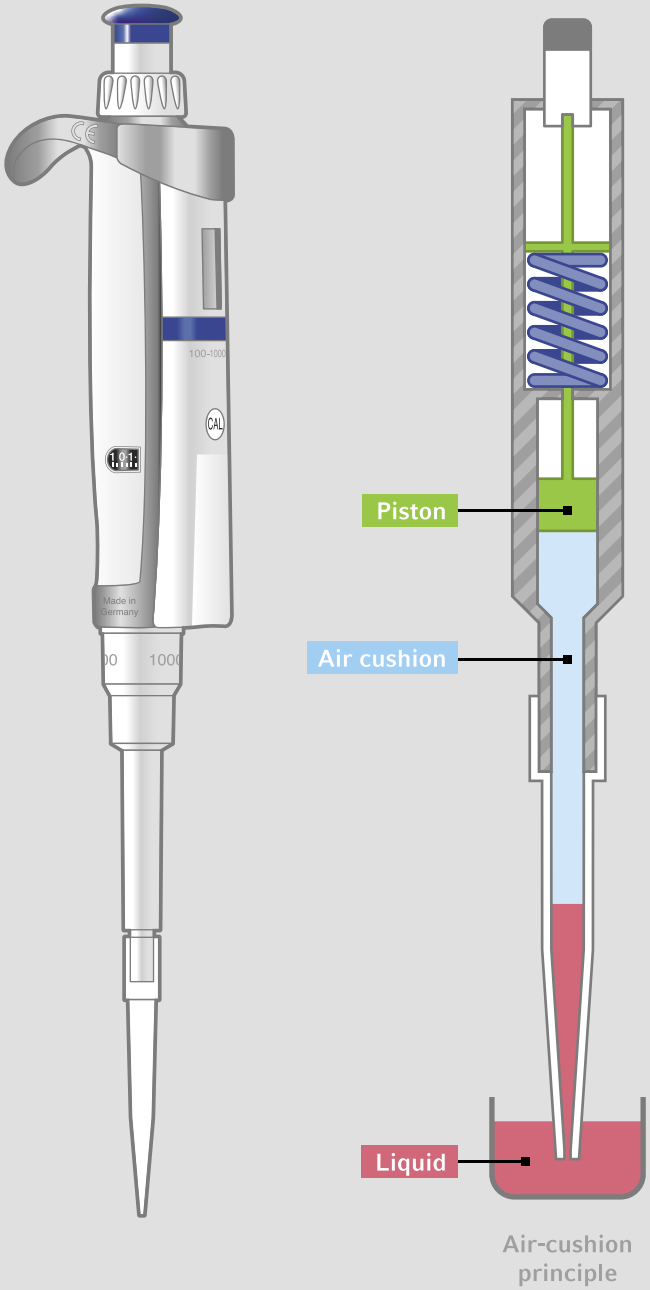
Air-cushion pipettes have an air cushion between the piston and the liquid. They consist of a piston-cylinder system which performs the actual measurement. The air cushion separates the sample aspirated into a plastic tip from the piston inside the pipette. The upward movement of the piston produces a partial vacuum in the tip, causing the liquid to be drawn into the tip. The air cushion moved by the piston acts like an elastic spring from which the volume of liquid in the tip is suspended. To minimize the effects of temperature, air pressure and humidity, air-cushion pipettes are designed in a special way that minimizes the effects of these factors on dosing accuracy.

The right volume version

When humidity or temperature change, the air cushion expands or contracts. This affects the volume of liquid being pipetted. Precision can be greatly improved if the air space between the piston and the sample is kept as small as possible.

TIP

The smaller the pipette tip, the smaller the air volume and the higher the accuracy of the results. The volume variant of the pipette selected should be as small as possible while accommodating the sample volume required.



Air-cushion principle

Single-channel pipettes can be divided into fixed-volume pipettes and variable-volume pipettes which cover a range of volumes.

Variable-volume pipettes are perfect for precise individual dispensing volumes. Depending on the respective pipette volumes, ranges like 0.5 µL to 10 µL, from 20 µL to 200 µL, from 500 µL to 2.500 µL or from 1 mL to 10 mL can be flexibly and accurately adjusted to the required volume. You need fewer devices compared to fixed-volume single-channel pipettes. However, the volume must be set individually, and calibration is more complex.

In contrast, **fixed-volume pipettes** are perfect for beginners and laboratories with limited budgets. They allow for faster work since the volume (e.g. 100 µL, 500 µL or 1.000 µL) cannot be changed. Compared to adjustable pipettes, the risk of making mistakes is reduced and calibration is much easier and faster. However, you need to buy more pipettes if different volumes are needed.

More information:

Influence of physical parameters on the dispensed volume of the air-cushion pipette
https://eppendorf.group/userguide-21

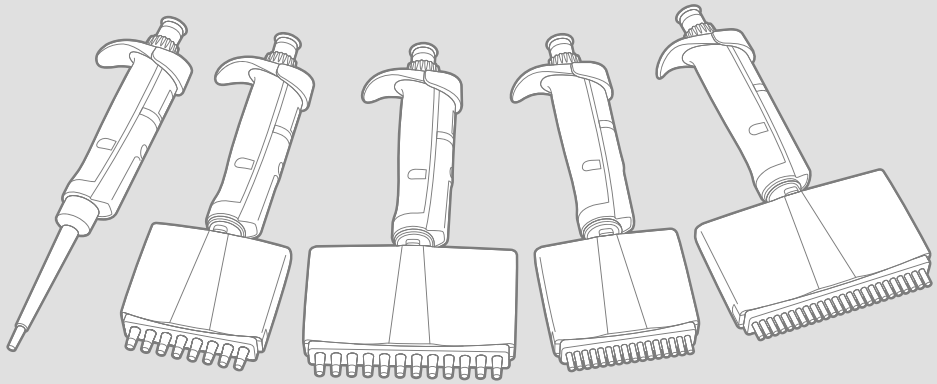
Single-channel or multi-channel pipettes

The choice of a pipette also depends on the **reaction vessel** and **the number of samples** to be processed. While **single-channel pipettes** are suitable for the use of single tubes (e.g. 0.2 / 0.5 / 1.5 / 2.0 / 15 / 50 mL tubes), **multi-channel pipettes** are optimal for PCR strips and microplates.

Multi-channel pipettes enable the loading of entire rows and columns of microplates. Accordingly, even 384-well plates can be quickly and accurately processed with 16- or 24-channel pipettes.

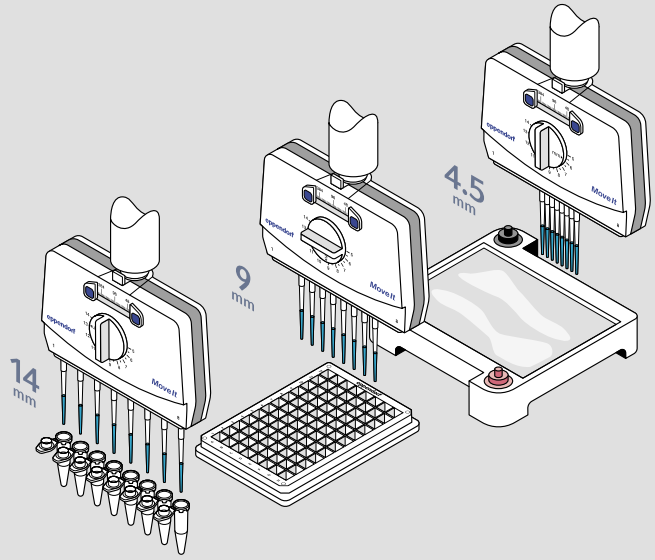
TIP

Multi-channel pipettes considerably reduce the number of pipetting steps during loading of microplates or PCR strips, facilitating simultaneous loading of entire rows and columns and thus a more convenient and efficient operation with significant time-saving. Moreover, reactions can be started within an entire row or column simultaneously. For your safety, look for a feature enabling successive tip ejection. During a single tip ejection stroke, the outside tips eject before the inside tips. This significantly reduces operating forces.



Multi-channel pipettes with fixed vs. adjustable tip spacing

Every day, samples are pipetted back and forth between different vessel formats. From 1.5 mL reaction vessels into 96-well plates. From 96-well plates to 384-well plates. From reaction tubes or PCR strips into the wells of an agarose gel. And many more applications. Multi-channel pipettes with fixed tip spacing cannot be used for these application, making it very labor-intensive to transfer hundreds of samples using a single-channel pipette. Often, single-channel pipettes are used for multiple sample transfers from one vessel type to another. This can be time-consuming and inconvenient, especially when throughput increases. **Multi-channel pipettes with variable tip spacing can be adjusted manually with pre-defined settings for different plate formats.**

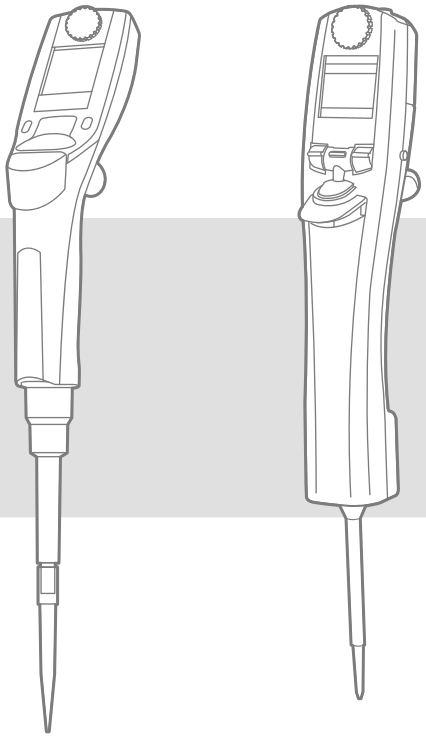


Multi-channel pipettes with fixed vs. adjustable tip spacings

Fixed tip spacing	Adjustable tip spacing
Sample transfer between identical formats in larger scale or between reservoir and plate	Transfer of individual samples between different formats in larger scale
Optimally matched to the well spacing of 96- or 384-well plates	Adjustable with pre-defined settings for different plate formats
Available as 8-, 12-, 16- and 24-channel pipettes	Available as 4-, 6-, 8-, and 12-channel pipettes

4.2. Electronic pipettes and dispensers

Electronic pipettes and dispensers are semi-automatic systems and suitable for a variety of applications besides standard pipetting.



Time-efficiency:
Electronic pipettes usually feature various operation modes such as dispensing, reverse pipetting, diluting and mixing. Besides pipetting, titrating and quantification of liquid residues, entire microplates can be filled rapidly with electronic dispensers. Also, these functions improve reproducibility and simplify workflows. Routine procedures can be performed in a time-saving manner. Functions and volumes can be programmed individually and stored in the pipette's memory for additional time savings when performing routine procedures. Efficient rechargeable, durable and exchangeable batteries enable relaxed work in the laboratory all day long.

Reliability & reproducibility:
Setting a constant dispensing speed facilitates higher reproducibility and reliability than working with mechanical pipettes. Liquid uptake and release are always triggered the same way, at the touch of the button, as well as pipetting speed, independent of the user whose speed is subject to variability on a day-to-day basis. Much more precise pipetting can be achieved compared to mechanical pipettes. Moreover, the risk of repeating analyses and experiments is minimized which saves time and reduces cost.

User safety:
User-friendliness and ergonomics play an important role when it comes to reducing fatigue caused by repetitive movements, especially in the wrist and thumb, and preventing negative health effects such as repetitive strain injuries. Light and well-balanced models can be operated easily and contribute considerably to maintaining health.

TIP

Electronic pipettes allow for a high level of reproducibility, significant time savings and reduced pipetting forces. A wide range of applications can be addressed via multifunctional modes.

NOTE

The lab of the future is digital. Electronic pipettes are an essential element of new digital lab solutions allowing you to work faster, more accurate and in collaboration with your fellow researchers.

More info at www.eppendorf.com/VisioNize

Selected operation modes of electronic pipettes & dispensers (using the examples of the Eppendorf Xplorer® pipette and Multipette®/Repeater® E3 multi-dispenser families)

Eppendorf Xplorer (plus) & Multipette/Repeater E3x

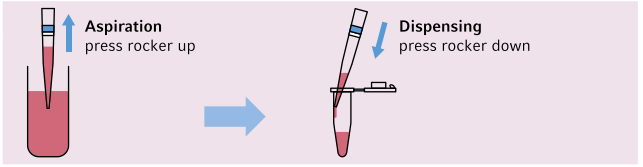
Eppendorf Xplorer (plus)

Multipette E3x

Modes of operation

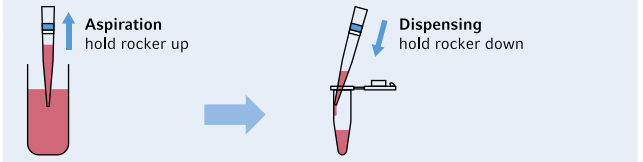
Pipetting

Aspirate set volume – Dispense complete volume



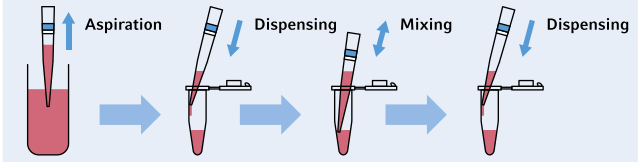
Manual pipetting

Aspirate as long as rocker is held up – Dispense complete volume



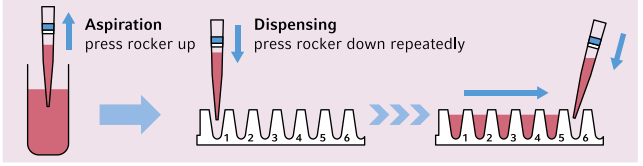
Pipetting and mixing

Aspirate once – Dispense complete volume and mix



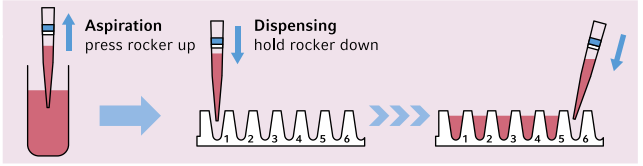
Dispensing

Aspirate only once – Dispense many times same volume by pressing rocker down repeatedly



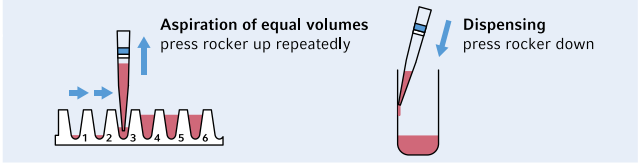
Automatic dispensing

Aspirate only once – Dispense many times same volume by keeping rocker down



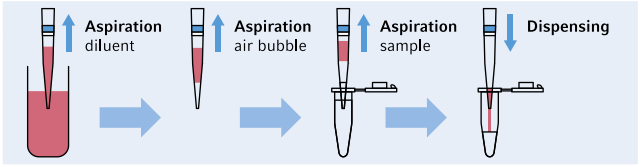
Multi-aspiration

Consecutive aspiration for pooling into one pipette tip



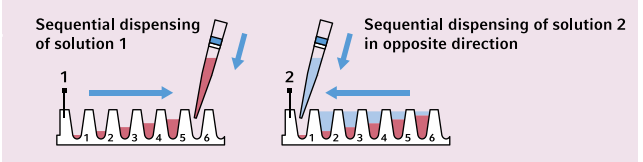
Diluting

Aspirate of a diluent and a sample separated by an air bubble



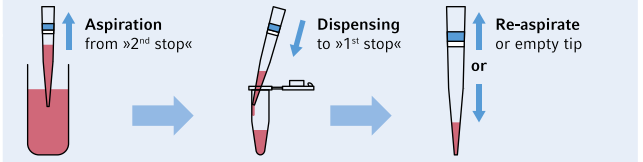
Sequential dispensing

Aspiration of liquid followed by dispensing it in up to 10 partial volume steps in sequential order



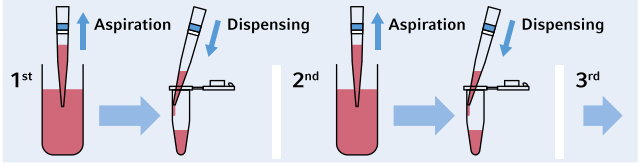
Reverse pipetting

Over-aspiration of liquid follow by a regular dispensing step



Sequential pipetting

Pipetting of up to 10 user-defined volume steps which each can be up to the nominal volume.

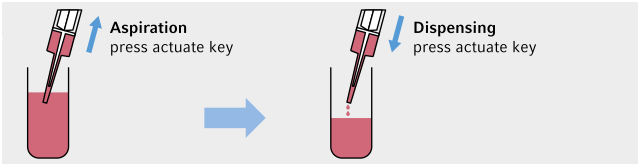


Exemplary operation modes of electronic dispensers

Modes of operation

Titration

Aspiration of liquid followed by controlled dropwise dispensing (e.g. for concentration determination)



4.3. Automated systems

Particularly in the life sciences, sample volumes are reduced and/or the number of samples to be processed is increased. Microplates with 96 or 384 wells require many dispensing steps. Pipetting or dispensing liquids precisely and error-free into the wells in a manual fashion is time-consuming and exhausting, and it requires high concentration. Furthermore, manual pipetting is prone to errors, and it can lead to repetitive strain on the arm muscles. Only the automation of such processes allows a high number of dispensing steps to be carried out reliably and reproducibly with small sample volumes.

Reliability & reproducibility:
Automated pipetting systems guarantee higher precision and accuracy than manual pipetting systems, simply because the systematic error is lower and the risk of introducing errors is minimized. By limiting variances between different users and eliminating random errors, automated pipetting systems additionally increase reproducibility.

Time-efficiency:
Entire protocols can be programmed and run automatically, freeing time for other tasks. Additional options to map temperature incubation, mixing steps and other processes that are not directly connected with the movement of liquids, relieve from the typical interventions. Electronic notification, for example by email, enables remote work, without having to look at the device.

Costs:
Higher throughput, fewer sample failures, and the ability of highly skilled worker to perform more important tasks will offset the initial investment costs.

Safety:
The user is freed from tedious pipetting series and protected from typical occupational illnesses caused by frequent pipetting.

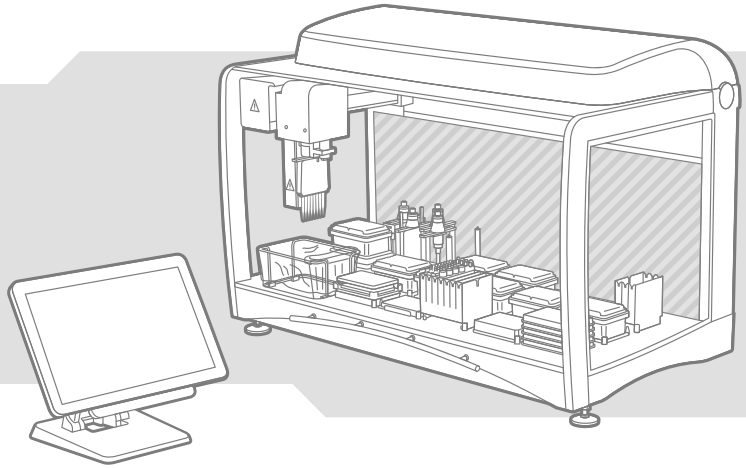
Quality:
Documentation is carried out automatically and electronically, as well as the recording of pipetting protocols for tracking deviations or detecting errors. Automated pipetting systems support the complete documentation of all sample movements and work steps in the laboratory. An indispensable advantage, especially in regulated fields.

An automated pipetting system generally consists of a robotic arm (XYZ transmission system for positioning) and dispensing devices, which are either permanently installed or can optionally be exchanged according to volume and number of channels. Large systems have space for several permanently installed pipetting heads or can replace them independently. Processes that have been previously carried out manually can be quickly and easily transferred to automatic systems. As the sequence of movement is freely programmable, it can be used for a whole range of tasks.

Applications
> Routine applications such as serial dilution, aliquoting, cherry picking and normalization, as well as reformatting
> PCR and qPCR setup, purification of nucleic acids
> Automated cell seeding, media exchange, cell-based assays, ELISA and cytotoxic testing

TIP

**Manual precise and error-free pipetting of liquids into microplates is time-consuming, exhausting, prone to errors and stressful for arm muscles. Pipetting robots allow for automation and standardization of routine pipetting work.
A higher number of accurate and precise dispensing steps can be carried out using automated systems than by using manual pipettes. Automated pipetting systems offer increased reproducibility by minimizing typical sources of error. Running entire protocols automatically without supervision frees up valuable time.**



#RD2587 0324 6625

5. Pipette tips

Pipette tips are key components of the dispensing system. When was the last time you were puzzled by non-reproducible results, for example in a real-time PCR? You checked all the variables in your experiment but were still unable to find the cause. During pipetting, you have always used a pipette with a »fitting« tip – a system of two components whose precision and accuracy you considered reliable throughout the workflow. However, you should put this perceived reliability to the test! Indeed, the shape of the tip, its material properties and its actual fit significantly impact the accuracy of the dispensing process.

5.1. Pipette tips influence results

Reliability & reproducibility:
Pipette tips may lead to non-reproducible analysis results. The causes are manifold and include leachables or inhibitors in the tip material which interfere with the experiment. In addition, banana-shaped tips used for multi-channel pipettes, or drops of liquid hanging on to the outside of the tip when pipetting volumes below 1 µL cause problems. A bad tip fit leads to the uptake of varying sample volumes, dripping or tips that dislodge at random. Liquid retention in tips results in loss of sample, increased reagent consumption and lack of accuracy, precision and, as a result, reproducibility. Other issues are not that obvious. Using tips not recommended by the pipette supplier leads to the problems mentioned above. This issue often remains unnoticed since problems with analysis results are typically linked to reagents, methods or the pipette. Rarely is the consumable the focus of investigation. Moreover, calibrations are mostly understood as »checking the pipette« instead of »checking the system«. In fact, in accordance with ISO 8655, only tips recommended by the pipette manufacturer should be used for the calibration process.

Safety:
Genetic material can be degraded by RNases and DNases. In addition, contamination of plastic consumables with nucleic acids may lead to cross-contamination and false positive results. If no filter tips are used, both pipette and sample can be exposed to contamination from previous samples through aerosol formation within the pipette tip and pipette itself. In these cases, radioactive, toxic or infectious substances are of particular concern.

Ergonomics:
Powerful pushing of tips onto the pipette tip cone, sometimes used in combination with sideways or rotating movements to achieve a good tip fit, subjects your joints and muscles to repetitive strain.

Within the scientific community, a rising number of experiments published cannot be reproduced by other groups. Besides the use of inadequate pipettes by operators who are not trained in proper pipetting technique, a further source of error is often overlooked: tips. A broad survey has found that the main influencing factors are design/shape, production quality and material. While the influence of these factors on single pipetting results are obvious, their systemic impact on calibration steps performed with different tips, and results altered by the use of autoclaved tips, must also be taken seriously.

NOTE

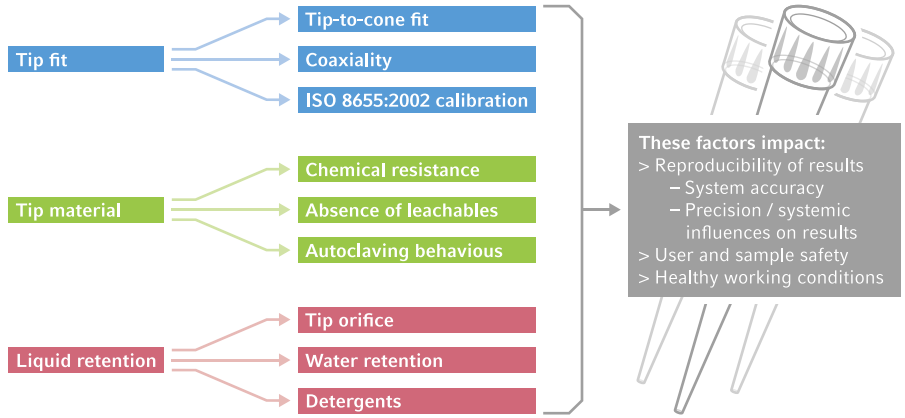
Adapt the environment of your samples to their specific needs. Besides choosing the right pipette, the purity grade of the tips is important. Care should be taken to verify the stability, reliability and geometry of consumables, as well as the absence of certain substances from the tip material.

TIP

More information:

The Tip of the Iceberg: How Pipette Tips Influence Results
https://eppendorf.group/appnote-354

What to consider when selecting pipette tips



NOTE

Pipette tips must ensure pipetting accuracy in the microliter range; for this reason, their design must ensure that even the smallest droplets are dispensed accurately. If these requirements are not met, errors will occur early, during the preparation step. Errors that occur early on will carry forward into later analysis steps.

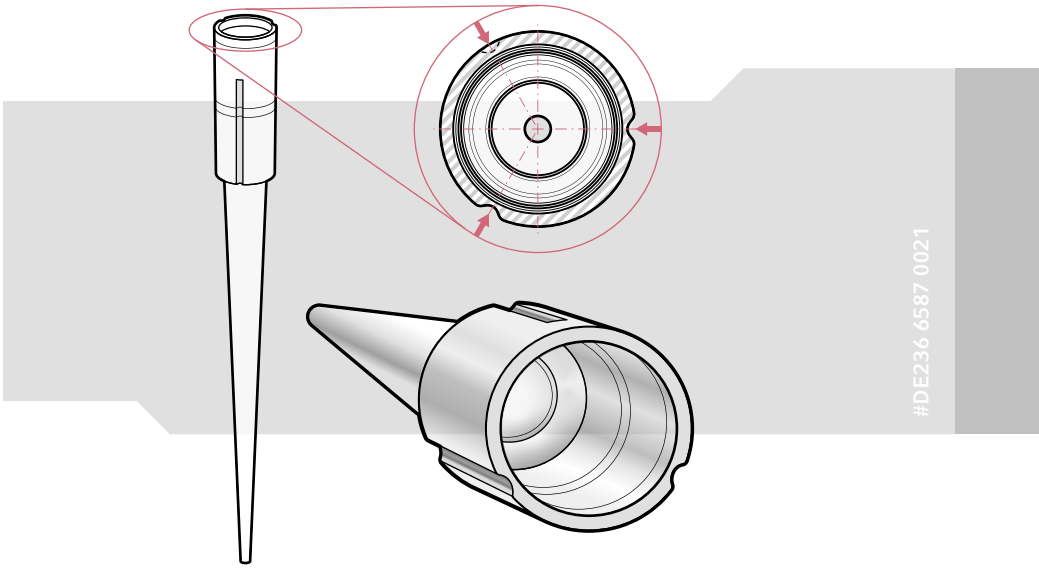
TIP

Use tips with features that ensure a perfect tip-to-cone fit. For example, elastic forming groves allow the tips to stretch as much as necessary to enable a perfect seal. Tips become easily attachable and ejectable; they fit reliably, and your operating forces will be greatly reduced. Tips and tip cones should form a perfect system.

5.1.1. Tip fit

It is a basic requirement of a tip to fit tightly onto the pipette tip cone to generate an air-tight system. If the tip fit is not tight, the system will not aspirate the set amount of liquid, and it may even leak. While in the worst case, liquid may drip from the tip, an incomplete seal is often not recognized during the daily laboratory routine. ISO 8655 benchmarks the influence of non-tight pipette tips with 0.5–50% of nominal volume. This means, for example, that the error of a pipette with 1,000 µL nominal volume is 5 µL–500 µL due to leaky tips.

In multi-channel pipettes, tip and pipette need to be perfectly aligned in a row **(tip coaxiality)**; otherwise, precise targeting of complete rows or columns of microplates cannot be achieved.

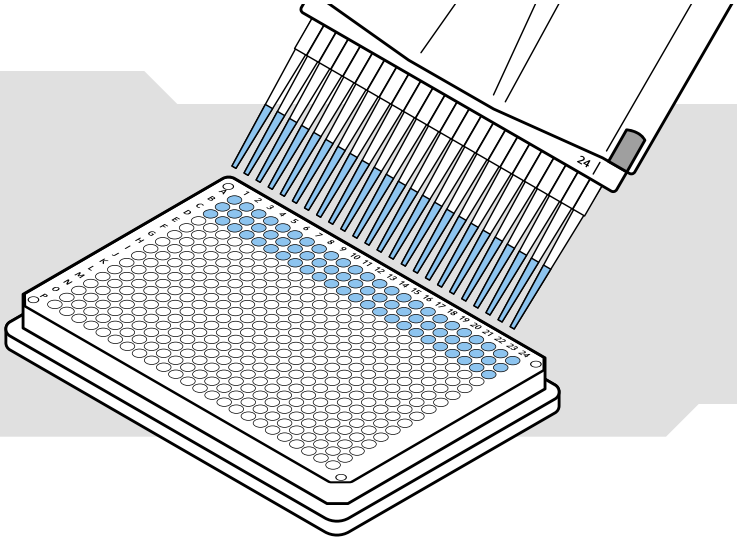


NOTE

Tips have to ensure a complete seal between tip and pipette. Leaky tips lead to the uptake of varying sample volumes. Especially with small volumes, this increases the relative error of your analysis. Similarly, tips that drip or dislodge completely from the pipette harbor the risk of potential cross-contamination and sample loss, leading to non-reproducible results.

TIP

Coaxially and finely shaped tips will ensure the safe transfer of liquid into the small wells of microplates.



Tips are specific to the pipette. At the same time, pipettes are adjusted to a certain air cushion size and filling height within the tip. Using tips other than those for which the pipette was adjusted causes the size of the air cushion to vary in size, which in turn leads to variable filling levels and increased systematic error*[2]. Accordingly, the international standard ISO 8655 defines the pipette and tip to be a system. The standard stipulates that an extra calibration step is required when alternative »universal« tips from different manufacturers are used. It was shown that the pipetting error increases by up to 0.6% systematic error and 0.8% random error with generic tips*[3].

*[1] Lochner KH, Ballweg T, Fahrenkrog HH. Untersuchungen zur Meßungenauigkeit von Kolbenhubpipetten mit Luftpolster. J Lab Med 1996; 20 (7/8): 430-440
*[2] The Tip of the Iceberg: How Pipette Tips Influence Results https://eppendorf.group/appnote-354
*[3] Carle AB, Rodrigues G, Rumery D. Best Practices for the Use of Micropipettes. Poster. www.artel-usa.com

NOTE

Only if pipette and tip are produced by the same manufacturer can the customer be certain that each batch of pipette tips will correspond exactly to the calibration requirements described in ISO 8655. Non-system suppliers are unable to offer the same guarantee as they do not possess system specifications for the pipettes manufactured by the multiple companies. In these cases, the operator is required to calibrate and, if necessary, adjust the pipette to the foreign tips – every time a new batch of tips is used.

5.1.2. Tip material

Chemical resistance

Many applications require the pipetting of **harsh substances such as acids, bases, solvents, or saline solutions** which may influence the chemical resistance of the materials they come in contact with. Their physical properties can thus lead to **dosing errors.** To avoid this, pipette or dispenser tips should be tight during dosing despite the effect of chemicals, and the devices should be chemically resistant over a long period of time. The tips are usually made of polypropylene (PP) and/or polyethylene (PE), which are proven to be highly resistant to chemicals*[4].
Considering the relatively short contact times between liquid and material during pipetting, it is sometimes possible to use tips made from PP or PE for dosing of organic acids or various organic solvents such as acetone, diethyl ether, chloroform or toluene – even though PP and PE only exhibits a limited chemical resistance against these liquids. Prerequisites include keeping contact time as short as possible (i.e. no uninterrupted serial dispensing), and not heating the liquid.
In case of doubt, a test should be carried out.
Regarding the resistance of pipette and dispenser tips: **the longer the disposable article is used for the above-mentioned chemicals, the higher the expected dosing error.**

*[4] Carlowitz, B.: Kunststofftabellen. 4. Aufl. München: Hanser, 1995.– ISBN 3-446-17603-9.

TIP

If you are unsure if your tips are resistant to a specific chemical, you can find information about chemical stability in the user manual.

More information:

Chemical Stability of Consumables
https://eppendorf.group/userguide-23

Leachables

Chemicals such as lubricants, plasticizers and biocides, which are used as additives in the manufacturing process, can leach from the plastic into the sample and interfere with a broad range of biological assays. For example, they can significantly hinder enzymatic assays and binding studies, slow down evaporation, distort absorbance values, and lead to incorrect DNA quantification. Leachables can also cause alterations and growth reduction in various cell culture systems.

TIP

Ensure that your tip supplier avoids the use of additives like plasticizers, biocides and slip agents. Additives ease production processes but are known to disturb biological assays.

More information:

Leachables: Minimizing the Influence of Plastic Consumables on the Laboratory Workflows
https://eppendorf.group/whitepaper-26

Autoclaving behavior

Users who need sterile products have **two options to achieve sterility:** purchase sterile products or decontaminate the pipette tips themselves (e.g. autoclaving). The first choice is safeguarded by professional quality assurance. For the second choice, it can be difficult for users to set up comparable quality assurance. Instead of testing the effectiveness of the decontamination method, in most cases, an established method becomes »trusted« and is not further scrutinized. Thus, sterility is not guaranteed. In case autoclaving conditions differ from standard methods (121 °C, 2 bar total, 20 min), for example if shorter cycles are applied than those specified by the tip manufacturer, a test for autoclaving effectiveness is very important. Pipette tips without a filter are – if not declared otherwise – usually autoclavable. However, it has to be taken into account that PP, based on its composition becomes soft at approximately 110–120 °C (melting temperature is approximately 160–180 °C).

NOTE

Autoclaving according to standard methods stresses the material. In the worst case scenario, the orifices of the tips can be fused shut following autoclaving.

5.1.3. Liquid retention

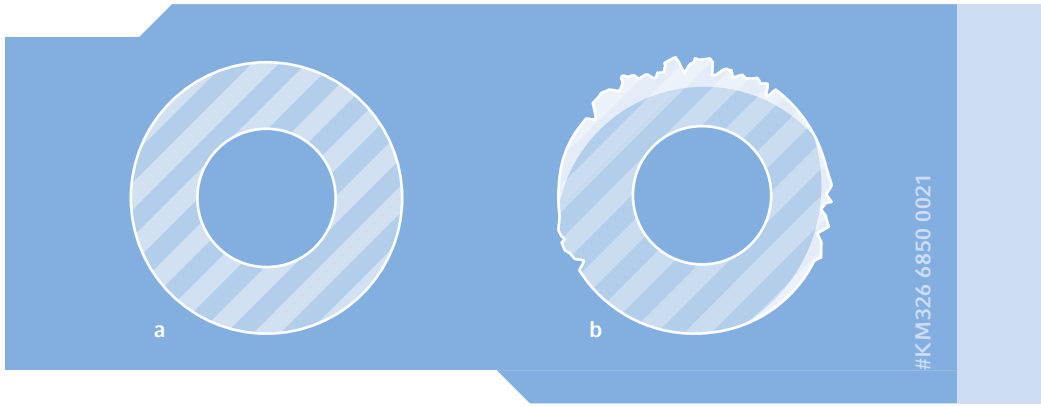
Retention of liquid inside the tip results in **loss of material, increased reagent consumption and lack of accuracy, precision and reproducibility.** The quality of the tip orifice as well as the material and inner surface are important factors to consider.

Tip orifice

The zone where the liquid leaves the tip during dispensing is very important for the accuracy of pipetting. Here, at the tip orifice, the cut-off of the drop occurs. Any imperfection in geometry or shape, for example those caused by production failures, may lead to liquid retention. A poor drop cut-off may not only **impair the pipetting result** but can also make it **impossible to dispense small volumes:** sometimes a drop leaves the tip, sometimes it doesn't.

TIP

Work with tip suppliers who guarantee minimum production tolerances and consistent quality.



Tip orifices of a) high quality, b) low quality

More information:

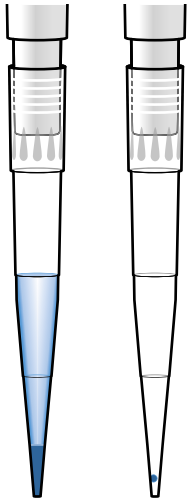
The Tip of the Iceberg: How Pipette Tips Influence Results
https://eppendorf.group/appnote-354

Water retention

Water retention, also known as **»wetting«** is not only influenced by the tip's orifice but also by its material and inner surface. If the inner surface is uneven or the tip is made of an unfavorable material, the liquid will be retained on the surface inside the tip. However, a completely smooth inner surface is not the only solution for minimizing water-retaining effects. The combination of material composition and surface structure decides on water retention. Tips are generally made from a plastic called polypropylene (PP). However, not all PP is created equal. Each tip manufacturer has its secret formula for the PP it uses to produce pipette tips. The mixture of ingredients determines the water repellent characteristics. This can be observed by pipetting drops of water onto different surfaces (e.g. glass and plastic). **The rounder the drop, the higher the surface energy and the less pronounced the wetting effect.**

Detergents

Liquids with a lower surface tension than water (detergents) coat polypropylene surfaces and **leave a liquid film in pipette tips** after pipetting. Since the lower surface tension of detergents has a marked influence on the dosing properties of samples, it is noticeably more **difficult to perform accurate work** with these liquids.
Low retention pipette tips with ultra-hydrophobic and homogeneous surfaces repel detergent solutions and allow maximal dosing of the liquid. Adhesion forces between liquid and surface are reduced. Liquids roll off completely so that only a tiny drop remains in the tip. In comparison, detergents adhere to the inside walls of standard tips, which leads to more liquid being retained.



TIP

Liquid retention results in loss of material, increased reagent consumption, and lack of precision and accuracy. Detergent-containing liquids, including expensive master mixes, enzyme solutions, and reagents, as well as samples prepared for protein analysis, tend to adhere to the inner surface of the pipette tip. Select tips which ensure minimal liquid retention for applications where minimized liquid retention is crucial and the highest reproducibility of results is needed. Ensure that the tips are uncoated, that they are free from additives and that their material does not leach into the sample.

5.1.4. Purity

PCR and many other biological methods require disposable products to meet purity criteria. The reasons are manifold: The smallest amounts of genetic material must be protected from degrading enzymes (DNases and RNases); PCR-inhibitors interfere with detection reactions, and contaminating nucleic acids, including human DNA, can enter tips and reaction vessels during the production process. Consequently, **disposable articles must be free of these impurities.**

Overview and relevance of purity criteria

Sterility
> Sterile products are required whenever the presence of germs may have a negative effect; for example, to prevent microbial contamination of samples
> Per definition, a sterile product does not harbor any organisms on its surface. The degree of sterilization is expressed as residual probability of contamination. This probability is translated into a »Sterility Assurance Level« (SAL). Thus, a SAL value of 10^{-6} indicates the probability of occurrence of one non-sterile item among 10^{6} (1,000,000) sterilized items

Pyrogen-free (endotoxin-free)
> The absence of pyrogen prevents endotoxin-based contamination in cell culture, pharmaceutical, and medical research laboratories
> Thermostable substances (glycoproteins) from the outer membrane of bacteria and other microorganisms can cause fever in humans and impair the growth of cell cultures

Bacterial DNA-free (E. coli)
> The presence of DNA contamination could lead to false-positive results for different applications involving DNA

Human DNA-free
> Contamination of the sample with DNA can lead to false-positive results.
> Contamination is a major concern for DNA analysis

DNase-free and RNase-free

> DNases are enzymes that degrade DNA. DNase contaminations can severely affect DNA analysis

> RNases are enzymes that degrade RNA. These enzymes are extremely resistant, and they will withstand autoclaving and irradiation. RNase-free products are an absolute must in the field of molecular biology because RNA is highly sensitive and can be destroyed very quickly by RNases

ATP-free

> ATP is a part of all living cells; therefore, its presence can indicate biological contamination

> The test procedure for the quantitative and qualitative detection of ATP is already an integral part of hygiene monitoring, e.g. in the pharmaceutical industry

PCR inhibitor-free

> PCR – the amplification of nucleic acids – has been established as one of the most important and commonplace molecular biology methods used in almost all fields of the life sciences where DNA is analyzed. Since certain substances impair this reaction, laboratory products must be free of PCR-inhibitors

> The consumables used must contain no impurities that could adversely affect PCR*[1]. This is particularly crucial if only low amounts of template DNA are available

*[1] Bucher, H.; Ewald, K.: Inhibitorische Effekte von Filterspitzen auf PCR und real-time PCR (Eppendorf SE 2011).

NOTE

Traces of DNA are not removed by applying higher temperatures or autoclaving.

Manufacturers of disposables have developed different levels of purity which can be selected according to individual requirements.

Exemplary purity grades for disposables

	Eppendorf Quality™	Sterile*	PCR clean	PCR clean and Sterile*	Forensic DNA Grade*[1]	Biopur®*[1]
Continuous quality control for the following relevant criteria						
Function, tightness, precision	■	■	■	■	■	■
Low wetting	■	■	■	■	■	■
High chemical resistance	■	■	■	■	■	■
High thermal resistance	■	■	■	■	■	■
High centrifugation stability*[2]	■	■	■	■	■	■
High transparency	■	■	■	■	■	■
Each lot certified	■	■	■	■	■	■
Each lot certified the following purity criteria						
Human DNA-free*[3]			■	■	■	■
DNA-free*[3] (bacterial DNA)						■
DNase-free*[3]			■	■	■	■
RNase-free*[3]			■	■	■	■
PCR inhibitor-free*[3]			■	■	■	■
ATP-free*[3]						■
Pyrogen-free*[3] (endotoxin-free)		■		■		■
Sterile (Ph.Eur./USP)		■		■		■
Methods (Examples)						
Applications requiring high general quality, but no checked special purities	■					
Bacterial and yeast cultures		■		■		■
Cell and tissue culture		■		■		■■
Isolation and storage of DNA			■■	■	■■	■
Isolation and storage of RNA			■	■	■	■■
DNA analysis (PCR, restriction analysis, hybridization, sequencing , NGS)			■■	■	■■	■
Application Area (Examples)						
Routine application	■					
Molecular biology			■■	■	■■	■
Microbiology		■		■		■
Cell technology > Stem cell research > Transgenic animals / plants		■		■		■■
Research > Medical research > Agriculture & aquaculture research		■	■	■		■■
Quality control > Food and beverage > Water supply > Environmental monitoring		■	■	■		■■
Forensic			■	■	■■	■

■ Recommended ■■ Highly recommended

*[1] Increased safety due to availability of individually packaged / single-blistered products.

*[2] For accurate details regarding centrifugation stability, please refer to the individual instructions for use of the product.

*[3] The suffix »-free« in connection with the purity classes means that the test showed conformity within the detection limits (see general purity certificate or lot-specific certificate)

5.1.5. Contamination

Movement of liquids containing biomolecules (e.g. DNA fragments, DNase, RNase), microorganisms or PCR inhibitors leads to the formation of aerosols. Handling infectious, radioactive or toxic liquids is inherently hazardous. The consequences of aerosol-contamination may include sample inhibition, introduction of foreign material and degradation of the sample by DNAses or RNAses – as well as danger to the operator.

For prevention:

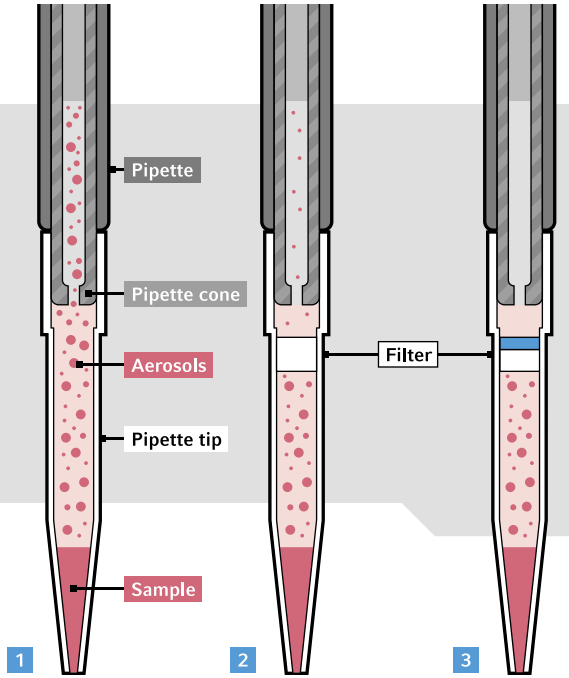
1. Use of **filter tips with two-phase filters** to prevent penetration
2. Use of **positive displacement systems** for hazardous liquids and to prevent aerosol formation. The liquid is hermetically sealed inside the tip and no aerosol is formed.

NOTE

Conventional single-layer filters may not fully block particles and molecules.

TIP

Use filter tips with two-phase filter protection and an additional liquid and moisture barrier. During use, the tip seals immediately when the liquid passes the first hydrophobic layer. It is designed to retain drops and splashes. The sample then enters a second filter layer, which forms a reliable barrier against the liquid and thus protects the pipette from contamination. These tips provide both a reliable barrier against aqueous liquid in case of accidental over-pipetting and retain 100% of all droplets, splashes, aerosols and biomolecules. (Accidental over-pipetting is a frequent pitfall when combining pipettes with filter tip sizes whose nominal volume is less than that of the pipette, leading to a contamination of the pipette if the sample penetrates the filter.)



Aerosols are formed during the movement of liquids. Without a filter, 1 the pipette is exposed to contamination by samples and aerosols. Conventional single-layer filters 2 do not fully block particles and molecules. Only dual filter tips 3 provide reliable protection even against the finest impurities.

5.1.6. Tip ergonomics

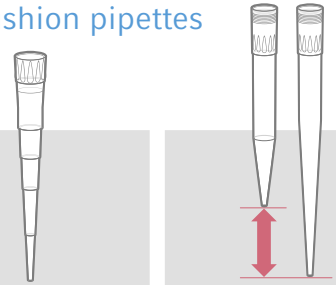
Tips must fit reliably, and they must not dislodge. In multi-channel pipettes, they must align in a perfect row. In practice, excessive force and rotating motions are often used to attach tips firmly. If this is done routinely, joints and muscles will suffer from repetitive strain. Ejecting the tips is often similarly laborious. Multi-channel pipettes are especially challenging: More pipette tip cones, higher attachment, and more ejection forces.

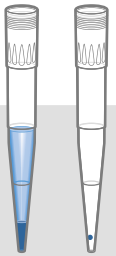
Use tips which ensure reduced tip attachment and ejection forces. Pipette tip cones with haptic feedback (e.g. spring-loaded tip cone) indicate a safe tip fit. Tips with features like elastic forming groves allow the tips to stretch as much as necessary to enable a perfect seal due to a perfect tip-to-cone fit. Tips become easily attachable and ejectable; they will fit reliably and help reduce operating forces.

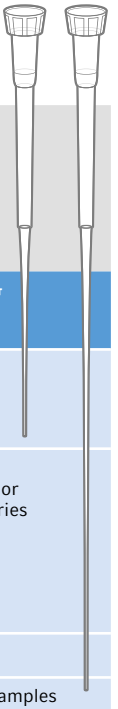
TIP

5.2. Types of tips

5.2.1. Tips for air-cushion pipettes







	Standard tips	Long-distance tips	Filter tips Dual filter tips	Dual filter tips with additional barrier	Low retention tips	Gel loader & microloader tips	Serological pipettes
Design	Standard design	Elongated tips	Tips with two-phase filter	Tips with two-phase filter protection and an additional liquid and moisture barrier	Ultra hydrophobic, uncoated tips, free from additives	Flexible, long and narrow tips	Long pipettes
Description	Standard pipette tips	Free access to larger vessels with reduced risk of touching the sides of tubes or wells	Dual protection for pipette and sample from aerosols and biomolecules	Closes immediately when in contact with liquid	Maximum sample recovery of detergent-containing solutions	Tips for loading gels or microcapillaries	Length of pipette affords increased distance to the cell culture and thus increased safety. Serological pipettes also hold larger volumes.
				100% blocking of liquid			
Application	For tubes up to 10 mL	Conical tubes	Applications where highest reliability of DNA/RNA analysis results are needed: DNA-applications, e.g. PCR, NGS library preparation RNA-applications, e.g. gene expression analysis Protein-applications, e.g. antibody research	Applications with hazardous sample material	Routine protein applications: isolation, purification, denaturation	Loading of samples onto polyacrylamide gels	Cell culture applications, e.g. medium exchange, cell seeding, cell splitting, handling of large flasks
	Pipetting and mixing of liquids in vessels of standard depth, e.g. 12- or 96-well microplates	Tall reagent vessels, Erlenmeyer flasks, graduated cylinders	Cell culture media and reagents	Applications where limited sample amounts are available	Liquids which tend to adhere to the inner surface of the tip (wetting effect), which include master mixes, NGS reagents and enzyme solutions such as restriction enzymes, ligases and DNases, as well as DNA ladders for gel electrophoresis	Applications which require additional immersion depth while pipetting microliter volumes	
	Loading of electrophoresis gels	Narrow deep vessels, e.g. 384-well microplates	Applications with hazardous substances	Applications where sample preparation is especially time-consuming and costly	Not suited for aqueous solutions	Recovery of surplus solution from the capillary	
	Removal of supernatants and phase extraction					Filling of microcapillaries for microinjection	

TIP

Select tips that work well with the respective reaction vessels. Long-distance tips are the better choice for narrow deep vessels. For example, the wells of a 384-well microplate are difficult to reach with standard tips, and there is a risk of touching the vessel walls during pipetting, which, in turn, may lead to cross-contamination. Similarly, tips which are too short cannot ensure that all liquid is removed. Double-layer filter tips are suitable for contamination protection of both sample and pipette. Low retention tips are perfect for the transfer of detergent-containing solutions.

5.2.2. Tips for positive displacement instruments

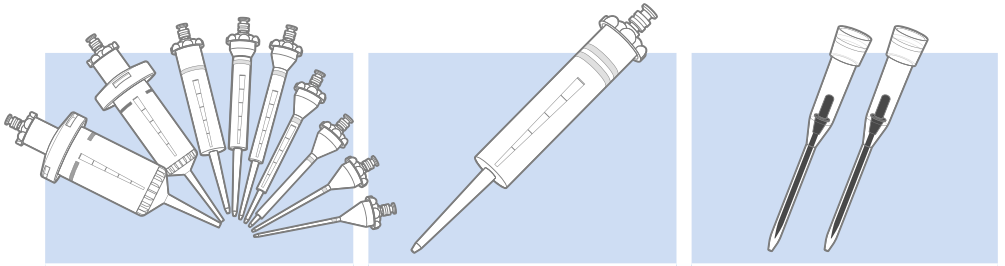
The dispensing accuracy of positive displacement dispensing systems depends on the disposable pipette tip to an even greater extent than in air-cushion systems. Each tip used in a positive displacement system features an integrated piston which pushes the liquid out of the tip – comparable to a syringe. Positive displacement tips are specific to the system for which they were designed and are not inter-changeable with tips foreign to the system.

NOTE

Tips for positive displacement pipettes and dispensers prevent aerosol contamination and protect from infectious, radioactive and toxic substances. The hermetically sealed piston ensures that liquids remain completely contained.

TIP

Positive displacement tips can handle liquids with a viscosity of up to 200 mPa*s (e.g. ~86% glycerol) successfully. For highly viscous liquids like liquid honey, glycerol 99.5%, Tween® 20, Triton® X-100, or liquid detergents, we recommend the use of specialized tips which are optimized to handle this challenge. Such tips enable dispensing with reduced operating force and can significantly increase your working speed.



Combitips® advanced	ViscoTip®	Mastertip®
Syringe-style tip with integrated piston for positive displacement dispensers		Syringe-style tip with integrated piston for positive displacement pipettes
Dispensing or pipetting of any liquid type (aqueous and challenging, non-aqueous liquids	Dispensing or pipetting of challenging high viscous liquids with viscosities of 200 to 14,000 mPa*s	Pipetting of aqueous and viscous solutions or liquids with high-vapor pressure. Suitable for high, narrow-mouth vessels

6. Expert pipetting techniques

The pipetting technique is equally important as an accurate pipette and high-quality tips in order to meet accuracy and precision standards. To this end, the pipette must be handled correctly and deployed within its physical limits.

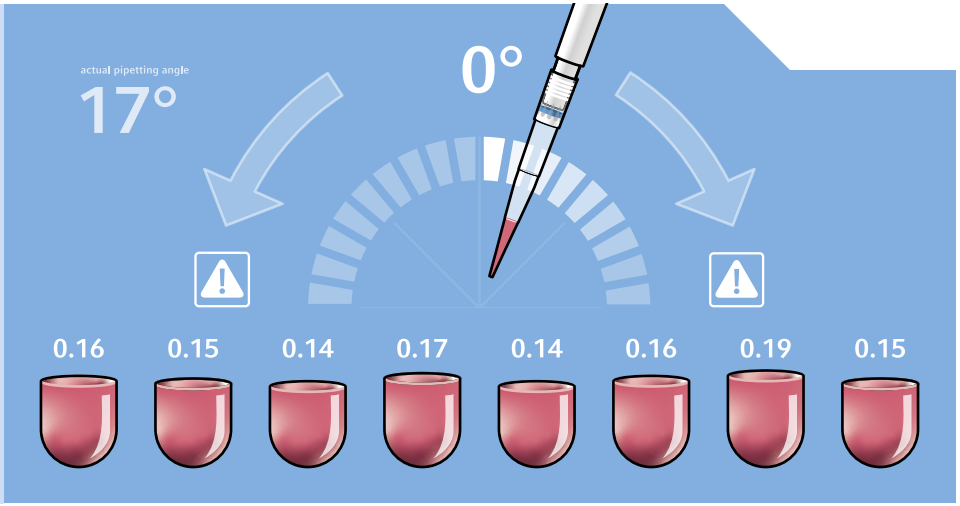
Reliability & reproducibility:
Several factors affect precise pipetting, and thus the reliability of the pipetting result. This is particularly true for air-cushion pipettes: physical factors such as temperature, humidity and air pressure influence the dispensed volume as well as the properties of the liquid. Not every liquid can be pipetted equally well and accurately. Incorrect handling can quickly influence dispensing accuracy, leading to considerable problems and non-reproducible results.
In this context, **the basic techniques are often the ones that cause most of the problems** because they are so widely used. A correct pipetting technique and the handling of problematic liquids are essential for obtaining reliable, correct and reproducible results.

6.1. Expert tips

Here are some easy ways to improve your pipetting skills and to avoid problems. These tips and tricks will help you to get better and obtain more reproducible results.

6.1.1. Vertical pipette posture for liquid uptake

Hold the pipette as vertical as possible to the liquid during immersion, ideally at an angle of 90°. The greater the inclination angle of the pipette when aspirating liquid, the lower the hydrostatic pressure of the liquid in the tip, and the larger the volume aspirated.



The volume deviation at different inclination angles is shown here as an example. Each sample has a different volume.

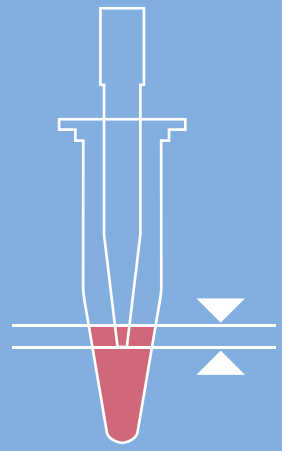
6.1.2. A small air cushion reduces errors

Reduce errors by choosing a pipette/tip combination with the smallest possible air cushion. Pipetting a volume of 100 µL will be more accurate when choosing a 100 µL pipette rather than a 1,000 µL pipette.

6.1.3. Correct immersion depth

The immersion depth of the pipette tip has a significant effect on the result. Immerse as little as possible but deep enough to prevent air uptake. To aspirate the liquid, the tip should only be immersed a few millimeters into the medium. If the tip is immersed too deeply in the liquid, this changes the hydrostatic pressure which can falsify the dispensing volume.

Volume in µL	Depth in mm
0.1 – 1	1
1 – 100	2 – 3
100 – 1,000	2 – 4
1,000 – 10,000	3 – 6



6.1.4. Pre-wetting = saturation of air cushion

To increase accuracy, the pipette tip should be pre-wetted by taking up and discharging the liquid several times before transferring the volume. Prewet the tip at least three times to equilibrate the air to the liquid. Depending on the liquid type and volume, more pre-wetting steps might be helpful.

6.1.5. Correct uptake of liquid

The liquid should be aspirated slowly and evenly. A waiting time of 1 to 3 seconds for the liquid to rise in the tip should be considered depending on the liquid type and volume.

6.1.6. Correct liquid discharge

The liquid should be discharged by touching the vessel wall so that the tip is completely emptied with the help of the adhesion force of the liquid.
Tip: Discharge volumes below 10 µL directly into the liquid.

6.1.7. Blow-out

A blow-out at the end of the pipetting process will ensure that all liquid is discharged from the tip.

More information:

Infographic and tutorial video: Stay on top of techniques
www.eppendorf.com/stayinformed

How to pipette correctly – a short step-by-step introduction to proper pipetting
https://eppendorf.group/how-to-pipette-video

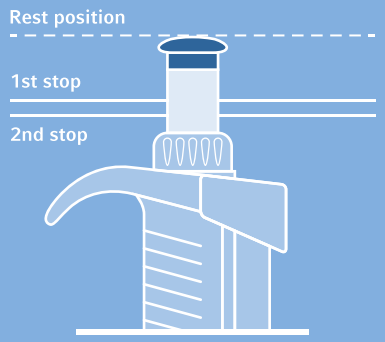
6.2. Forward & reverse pipetting

6.2.1. Forward pipetting

The most frequently used technique when dosing liquids with mechanical pipettes is forward pipetting which is suitable for aqueous solutions.

Liquid uptake: Press the control button down to the first stop. Immerse the tip into the liquid and allow the control button to move upwards completely for liquid uptake.

Liquid discharge: Press the control button down to first stop.
Blow out: To empty the tip, push the button to the second stop and hold; lift the pipette, while the tip touches the inner vessel wall.



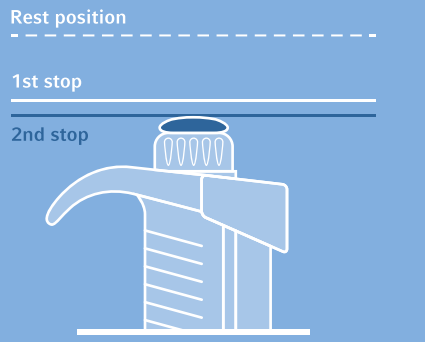
Forward & reverse pipetting		
Forward	**Liquid uptake:**	1. Press to 1st stop 2. Move up completely
	Liquid discharge:	Press via 1st stop down to 2nd stop
Reverse	**Liquid uptake:**	1. Press to 2nd stop 2. Move up completely
	Liquid discharge:	Press to1st stop

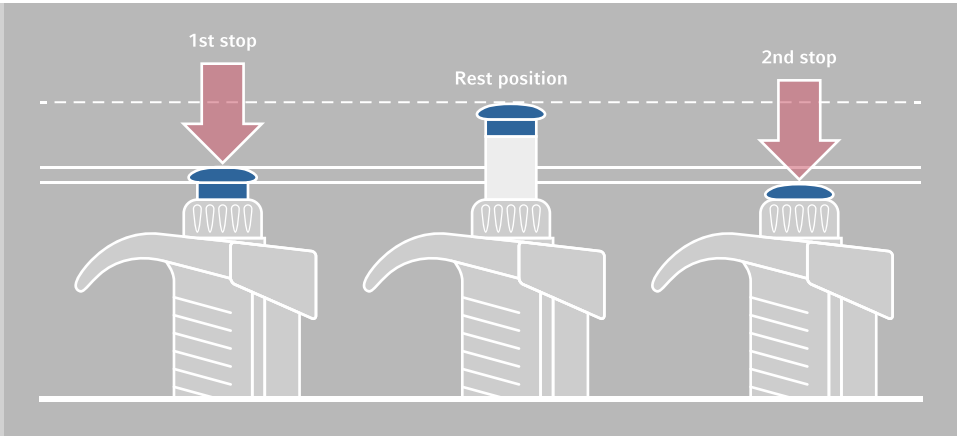
6.2.2. Reverse pipetting

Compared to forward pipetting, reverse pipetting is more suitable for many liquids that are difficult to pipette, such as viscous or foaming liquids. In principle, more liquid is initially aspirated than it is required.

Liquid uptake: Press the control button down to the second stop. Immerse the tip in the liquid and move control button up for liquid uptake. Now, the actual volume as well as the blow-out volume are held inside the tip.

Liquid discharge: Press the control button down to first stop and pull out the pipette along the vessel wall. An excess of liquid remains in the tip, which is subsequently discarded or returned to the vessel.





6.3. Physical influences on dispensing volume

Influences of temperature, humidity and air pressure can lead to inaccurately aspirated and dispensed volumes and should therefore be minimized.

6.3.1. Relative humidity

Despite the pre-wetting of the pipette tip, the pipetted volume is dependent on the humidity of the room air. If the relative humidity of the room air is changed from 80% to 20%, the volume released will be reduced by:

- 2.1 to 3.5% for 10 µL pipettes
- 0.3 to 0.6% for 200 µL pipettes

TIP

If you observe pipetting inaccuracy, be aware of the influence of relative humidity.

6.3.2. System temperature, temperature differences, and gradients

Reliability & reproducibility: As long as there is no temperature difference within the pipette-liquid-room air system, the volume delivered is almost independent of the system temperature (Lochner et al. 1996). However, due to the high coefficient of thermal expansion of air, even small temperature changes in the air cushion during the period when the pipette tip is immersed in the liquid can cause relatively high errors.

Such temperature changes are caused by differences in temperature between the pipette, the liquid and the surrounding air. The hand heat of the pipette operator also plays an important role.

> If the pipette is warmer than the liquid and room air, the air which is aspirated during liquid uptake is heated and expands. As a result, liquid is displaced from the tip, and the volume aspirated, and subsequently dispensed, will be reduced.

> Conversely, if the pipette and ambient temperature are colder than that of the liquid, an opposite temperature gradient occurs, and a correspondingly larger volume of liquid is detected.

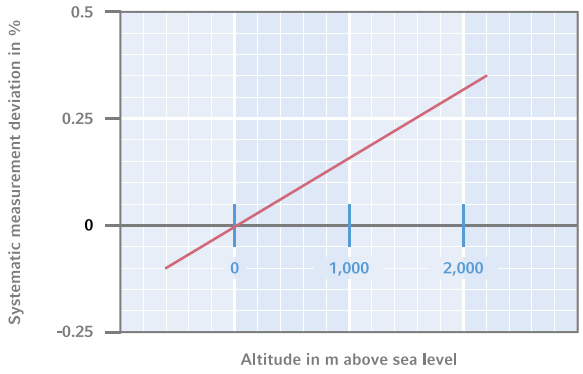
To prevent temperature changes in the air cushion during pipetting, **the temperatures of the pipette and the liquid should match the ambient temperature**. The smaller the temperature differences, the more accurate the results. However, the ideal case in which the temperatures of all components involved are identical is an exception in laboratory practice, especially when clinical or biochemical applications require the pipetting of ice-cold or body-warm liquids. This often unavoidable error should be determined by control measurements and taken into account in the evaluation.

If systematic measurement deviations associated with temperature fluctuations represent a challenge for your everyday laboratory work, it may be time to consider positive displacement systems

TIP

6.3.3. Air pressure and sea level

The mean air pressure of a particular location depends on its altitude above sea level. If, for example, a pipette is adjusted in Hamburg and used in Munich, the annual average air pressure difference is -63 mbar due to the different altitudes above sea level. This results in a 0.064% smaller volume (0.64 µL) for a 1,000 µL pipette, or 0.14% (0.07 µL) for a 50 µL pipette, respectively. If the air pressure fluctuates by ±25 mbar during strong weather changes, this results in an additional difference of ±0.024% for a 1,000 µL pipette and ±0.056% for a 50 µL pipette.



TIP

When calibrating pipettes, air pressure fluctuations should be taken into account.

6.4. Pipetting of challenging liquids

In addition to aqueous solutions, many other liquids with different physical properties are used daily in the laboratory. At times pipetting is difficult and leads to inaccurate results. In some cases, it is simply not possible to pipette the liquid with an air-cushion pipette. In these cases, positive displacement pipettes, which operate without an air cushion, are the instrument of choice. They are largely independent of the properties of liquids and external physical influences. However, if you only have an air-cushion system at your disposal, there are a few tips and tricks on how to achieve the best possible dosing result. In most cases, it is advisable to adjust the pipette to the respective liquid. Further options include electronic pipettes or automated systems, where liquid types can often be programmed and parameters such as pipetting mode or speed can be set. Tools like the VisioNize® pipette manager from Eppendorf even offer pre-defined settings for different liquid types when working with connected electronic pipettes.

Reliability & reproducibility: Pipetting of non-aqueous liquid is challenging as unequal volumes may be aspirated, liquid can remain inside the tip after dispensing or may be lost due to dripping. Cutting off pipette tips for pipetting viscous liquids or pipetting as fast you can to avoid dripping tips in the case of volatile liquids lead to inaccurately aspirated and dispensed volumes. In some cases, the imprecise dosing of liquids might be tolerated. If accuracy is crucial, you should adapt your tools and techniques to the physical properties of your liquid and surrounding conditions.

NOTE

Dangerous liquids can corrode the pipette, and they may also be harmful to the operator. The user, the pipette and the samples must be protected.

TIP

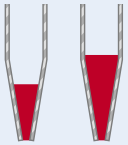
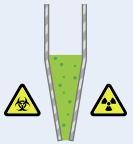
A reliable way to accurately pipette difficult liquids is to use positive displacement pipettes without air cushions. If no positive displacement pipette is available, or if pipetting with air-cushion pipettes is required, some tricks might help you (see below).

More information:

Infographic and tutorial video: Stay on top of techniques
https://eppendorf.group/stay-informed

Video: How to pipette correctly – a short step-by-step introduction to proper pipetting
https://eppendorf.group/how-to-pipette-video

Recommendations to master any type of liquid

		Water	Viscous e.g. glycerol, oil	Dense e.g. sulfuric acid, caesium chloride	Volatile e.g. acetone, ethanol	Infectious / radioactive e.g. biohazard material	Detergent (containing) e.g. Tween® 20, Triton® X-100	Foaming e.g. protein-containing liquids	Eppendorf Solutions Mechanical systems	Eppendorf Solutions Electronic systems
Type of Liquid										
Potential problems	Observations	> Air-cushion pipettes are optimized to the physical properties of water	> High resistance to flow > Liquid residues stay attached to inside tip wall > Imprecise results	> Influence on size of air-cushion > Dispensed volume too low or too high	> Air-cushion expands > Liquid drips out of the tip > Imprecise results	> Aerosols contaminate pipette > Threat to human health and sample safety	> Reduced surface tension > Liquid residues stick to the inner wall of the tip > Imprecise results	> Foam is created > Liquid residues remain in the tip > Imprecise results	**Advantages** > Easy to clean > Economical > Lightweight	**Advantages** > High reproducibility > Ergonomic working > Multifunctionality
Workaround	Air-cushion pipettes	> Optimally suitable for the use of water > No adaptation necessary	> Work slowly > Reverse pipetting > Adjust to liquid type*1	> Adjust pipette to liquid density > Adjust to liquid type*1	> Prewet at least 5 times > Reverse pipetting > Adjust to liquid type*1	> Use filter tips > Automated systems protect user and sample	> Use tips with low retention effect > Adjust to liquid type*1	> Reverse pipetting	> Eppendorf Research® plus > Eppendorf Reference® 2 > Research plus Move It® > Pipet Helper® 	> Eppendorf Xplorer® (plus) > VisioNize® pipette manager > Xplorer plus Move It® > Easypet® 3 > epMotion® 
Recommendations	Positive displacement dispenser	> Serial pipetting for multiple samples and vessel formats	> Higher precision regardless of physical properties of liquid > Serial dispensing > No adjustment to liquid type needed	> Higher precision regardless of physical properties of liquid > Serial dispensing > No adjustment to liquid type needed	> Higher precision regardless of physical properties of liquid > Serial dispensing > No adjustment to liquid type needed	> Higher precision regardless of physical properties of liquid > Serial dispensing	> Higher precision regardless of physical properties of liquid > Serial dispensing	> Higher precision regardless of physical properties of liquid > Serial dispensing	> Multipette® M4 	> Multipette® E3/E3x 
	Positive displacement pipettes	> Varitip® S system*2 allows accurate pipetting from large bottles and narrow vessels	> Varitip® P*2 allows accurate pipetting, for example from beakers	> Varitip® P*2 allows accurate pipetting, for example from beakers	> Varitip® P*2 allows accurate pipetting, for example from beakers > Varitip® S*2 system and valve for drip-free dispensing	> Varitip® P*2 allows accurate pipetting, for example from beakers	> Varitip® P*2 allows accurate pipetting, for example from beakers	> Varitip® P*2 allows accurate pipetting, for example from beakers	> Varipette® 4720 	
	Bottletop dispenser & burets	> Liquid dispensing directly from supply bottles	> Liquid dispensing directly from supply bottles (with Varispenser® 2/2x up to a viscosity of 500 mm²/s)	> Liquid dispensing directly from supply bottles (with Varispenser® 2/2x or Eppendorf Top Buret up to a density of of 2.2 g/cm³)	> Liquid dispensing directly from supply bottles	> Liquid dispensing directly from supply bottles	> Liquid dispensing directly from supply bottles (with Varispenser® 2/2x up to a viscosity of 500 mm²/s)	> Liquid dispensing directly from supply bottles	> Varispenser® 2/2x for dispensing large volumes 	> Eppendorf Top Buret™ for titration 

*1 This option is only available on automated systems and electronic pipettes.
*2 Varitips S and Varitips P are designed to be used with Varipette large volume pipette models from Eppendorf.

More information:

Precise and Reproducible Pipetting of Problem Liquids
https://eppendorf.group/challenging-liquids-video

Pipetting of challenging liquids
https://eppendorf.group/challenging-liquids

Dispensing of highly viscous liquids
https://eppendorf.group/appnote-211

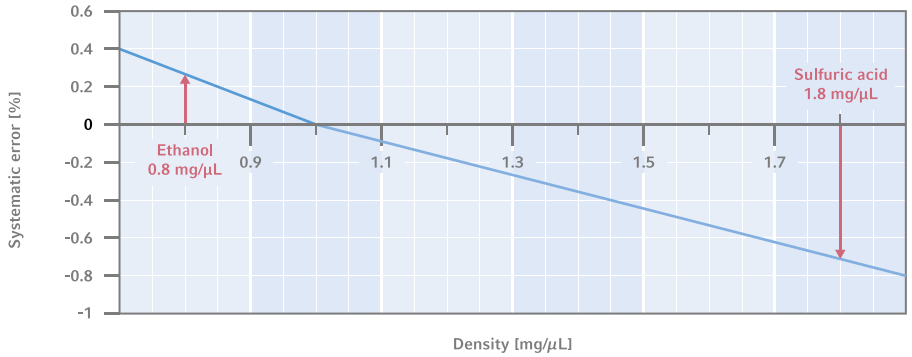
6.4.1. Liquids with higher or lower density than water

Challenging liquids often exhibit densities which are different from that of water.

> Sulfuric acid
> Caesium chloride
> Ethanol

Reliability & reproducibility: Liquid density influences the size of the air cushion. Depending on the density of the liquid, the air volume above the liquid expands differently. Accordingly, the volumes pipettes may be either too high or too low. Air-cushion pipettes are primarily designed for aqueous solutions; they are adjusted using distilled water. For example, if a liquid of a density that is higher than that of water is pipetted with an air-cushion pipette, the volume that is aspirated into the tip will be too small. For this reason, pipettes should be adjusted to the density of the respective liquid.

Systematic error of different liquid densities, pipette calibrated with water



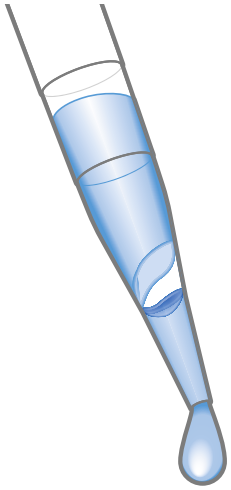
TIP

Difficult liquids can be dosed correctly with air-cushion pipettes if the density error is compensated, for example by adjustment. However, this correction then only applies to this particular value and not to the total volume range of the pipette. Hence, it is more convenient to go for positive displacement dispensers where the problem does not occur because there is no air cushion.

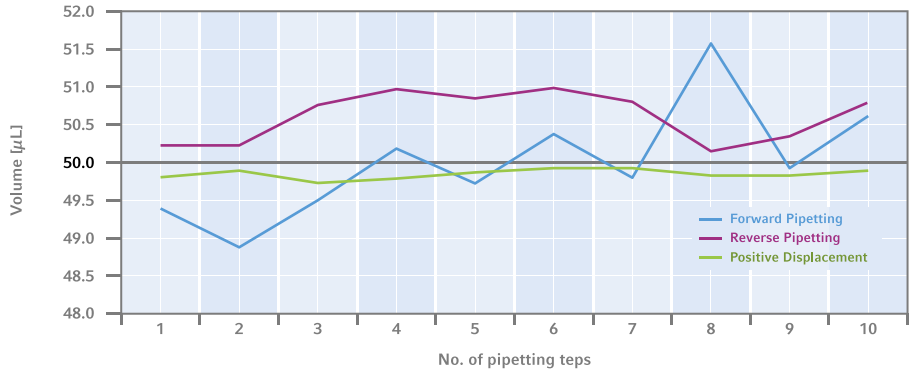
6.4.2. Viscous liquids

Numerous analyses and protocols require pipetting of viscous liquids such as:

> Glycerol 99.5%
> Tween® 20, Triton® X-100
> Ointments, oils, nail polish, creams, shampoos, collagen
> Engine oil, paints
> Liquid honey, mustard, tomato sauce
> Molasses 83°BX



Air-cushion pipette: Viscous liquids have a negative effect on accuracy and precision (Pipetting 50 µL of 85 % glycerol)



Reliability & reproducibility: These liquids do not flow easily into or out of the pipette tip. Their viscous flow characteristics are noticeable during liquid dispensing in the form of residues inside the pipette tip and very slow liquid uptake and release. Usually, air bubbles are also sucked into the pipette tip, because the pipette tip does not remain inside the liquid long enough for the complete volume to be aspirated. This means that too little liquid is aspirated and a considerable proportion of the volume remains inside the tip.

NOTE

Is cutting off tips one of your »secret recipes«?
In laboratories worldwide, this is a common practice to facilitate the uptake and discharge of liquids. However, significant errors in the volume of liquid pipetted result from this technique. Avoid this suboptimal habit and learn how to pipette like an expert.

TIP

1. If you use an air-cushion pipette
> Be sure that you pipette slowly and use reverse pipetting.
> The pipette needs to be adjusted because the densities of viscous liquids often differ from that of water.
> Use low retention pipette tips which repel liquids and allow maximal transfer of the liquid aspirated.
> Use electronic air-cushion pipettes, digital pipette managers or automated systems which can be programmed to accommodate liquids up to a certain viscosity. Adapt your pipetting habits to maintain accuracy.

However, when dispensing with air-cushion pipettes, a considerable dispensing error may occur, even if an adapted pipetting technique is used and/or the pipette has been adjusted.

2. Use positive displacement dispensers or pipettes
Dispensers offer the best dosing accuracy for viscous liquids due to the piston integrated into the tip. The sealing lip of the piston removes all liquid residue from the tip. Electronic positive displacement systems significantly reduce the effort required for dosing viscous liquids.
Dispensers require special positive displacement tips for dispensing liquids regardless of the physical properties

3. Use a special tip for highly viscous liquids, such as creams or liquid honey.

NOTE

You can still use an air-cushion pipette with some limitations if you work slowly, while employing the reverse pipetting technique. Several electronic air-cushion pipettes and automated systems can be easy programmed and adapted to support the user when pipetting challenging liquids. However, positive displacement dispensers are a better way to deal with viscous liquids and safeguard precision and accuracy. The sealing lip of the piston removes residues from the tip.

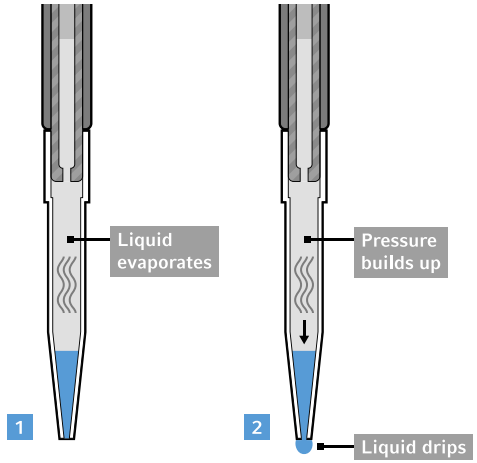
More information:

Dispensing of highly viscous liquids
https://eppendorf.group/appnote-211

6.4.3. Volatile liquids

Pipetting volatile liquids is one of the most frequently encountered tasks in the laboratory:

> Ethanol
> Acetone
> Phenol
> Chloroform
> Trichloroacetic acid



Reliability & reproducibility: Volatile liquids evaporate inside the tip. Since the air cushion of the pipette is still »unsaturated«, the vapor rises into the air cushion. This creates pressure in the air cushion which pushes the liquid out of the tip (dripping). In addition, volatile liquids often feature low surface tension and density, which facilitates liquid being pushed from the tip. Volatile liquids cannot be precisely pipetted with air-cushion pipettes without adapting the technique.

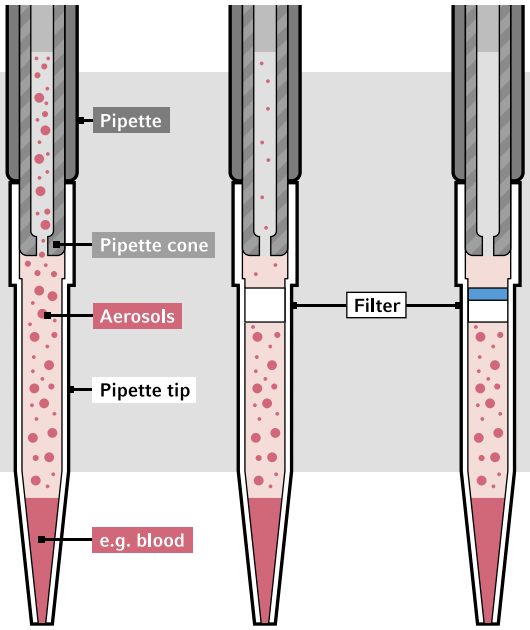
TIP

Pre-wetting of the tip when pipetting water or aqueous solutions by repeated pipetting up and down (filling and emptying) causes an accumulation of moisture in the tip and inside the pipette by increasing water vapor saturation. This prevents the expansion of air by evaporation of the volatile liquid and liquid loss.

In the case of aqueous solutions, a single pre-wetting step of the new tip is sufficient to keep evaporation to a minimum during subsequent tip changes. In the case of liquids with high vapor pressure, each tip should be pre-wetted even after the change.

Pre-wet the pipette tip to prevent liquid loss by saturating the air cushion. For this purpose, the tip should be pre-wetted by repeated fluid uptake and release. Pre-wetting is not necessary with positive displacement systems. As no air cushion is present, the problem does not occur. A new development in the field of electronic pipettes are liquid management features (as e.g. found in the VisioNize® pipette manager by Eppendorf) which can also simplify accurate pipetting by automatically applying the right pipetting technique depending on the set liquid type and volume.

6.4.4. Hazardous liquids



Hazardous liquids can be:
> Infectious
> Radioactive
> Toxic

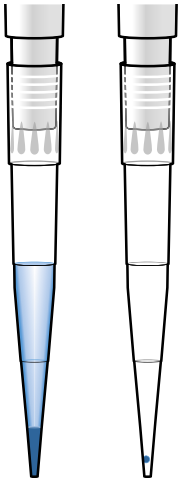
NOTE

Dangerous liquids may corrode the pipette material, and they pose a hazard to the operator. Droplets with microparticles of these liquids evaporate and form aerosols which rise inside the pipette and thus pose a risk to future samples as well as the operator.

TIP

If you use air-cushion pipettes, we recommend filter tips with 2-phase filters that prevent the passage of micro-droplets and tiny particles. Filter tips with 2-phase filters and additional liquid and moisture barrier can completely prevent contamination of the pipette by aerosols and biomolecules if the sample enters the filter due to »overpipetting«. Overpipetting occurs when pipettes are combined with filter tip sizes whose nominal volume is less than that of the pipette. For safety and peace of mind, positive displacement instruments which seal the liquid inside the tip and thus prevent aerosol formation are recommended for hazardous liquids.

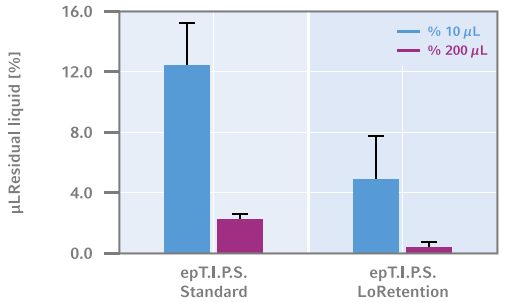
6.4.5. Detergent containing liquids



Pipetting liquids containing detergents is ubiquitous in modern laboratory processes. Common detergents are:
> SDS (protein denaturation, solubilization, isolation, buffer)
> CTAB (protein gel electrophoresis, precipitation of plant DNA)
> Triton® X-100 (lysis buffer, protein denaturation, enzyme buffer)
> PCR master mixes, real-time PCR master mixes
> Tween® 20 (buffer, hybridization reactions)
> Brij® 35 (protein solubilization, isolation of membrane proteins and native proteins)
> CHAPS (protein solubilization, isolation of native proteins)

Reliability & reproducibility: Detergents reduce the surface tension of the liquid, leaving a residual volume in the tip. Detergents do not flow easily, but they remain »sticky«. Since these liquids are mostly colorless, and are pipetted in small volumes, it may not be possible to determine the actual amount of detergent transferred. Such loss leads to a lack of accuracy, precision and reproducibility.

Higher sample recovery of PCR Mastermix when using epT.I.P.S.® LoRetention

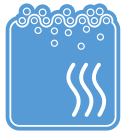


Costs: Loss of reagents due to remnants left behind inside the tip may increase consumption of detergent-containing solutions such as, for example, qPCR master mixes.

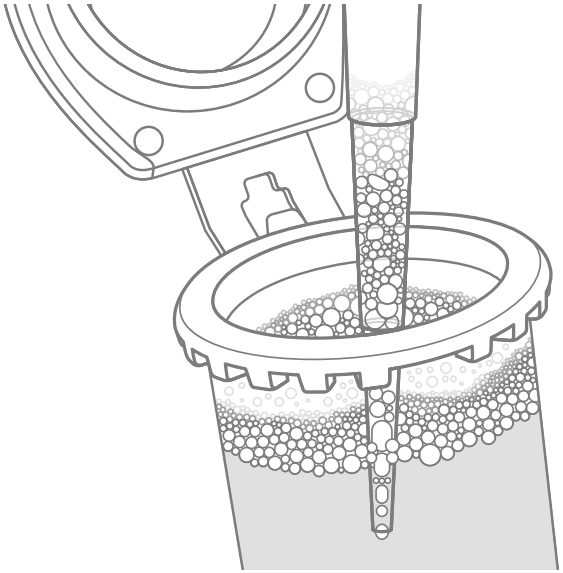
TIP

Use tips with low adhesion to facilitate low liquid retention. Low-retention tip surfaces have a special ultra-hydrophobic surface so that the liquid flows down and is discharged. Positive displacement systems are also great to pipette detergent-containing liquids; the piston of regular positive displacement tips feature a sealing lip which removes all liquid from the tip when dispensing. With electronic pipettes, digital lab assistants, like the VisioNize® pipette manager, can set the ideal pipetting settings for different liquid classes.

6.4.6. Foaming liquids



Liquids with a tendency to foam during pipetting:
> Detergents
> Protein-containing liquids, e.g. cell culture media



Reliability & reproducibility: Foam formation during pipetting is usually caused by a high protein or detergent content, making precise pipetting difficult. Foam also leads to splashes, which results in a higher contamination risk.

TIP

Prevent foam formation due to high protein content in the sample by working slowly while employing the reverse pipetting technique when working with air-cushion pipettes. For detergent-containing liquids, it's best to use tips with low adhesion facilitating low liquid retention. The tip surfaces are specifically treated so that the liquid can flow down and be discharged. If positive displacement systems and automated systems with integrated adjustment for liquid classes are used, no change of the pipetting technique is necessary. The sealing lip of the piston removes residues from the tip.

7. Pipetting under sterile conditions

Contamination is the most prominent challenge when working under sterile conditions as it is the case in a cell culture lab. In general, we find two major types of contaminants: microbial and eukaryotic. The microbial contaminants are fungi and yeast, bacteria, and mycoplasms.
Eukaryotic contaminants are not easy to spot as they constitute cross-contaminants originating from other eukaryotic cell lines. This phenomenon was first described in 1967 and to date, it is estimated that 15–30% of cell lines used in labs worldwide are either cross-contaminated or misidentified. No matter what type of contamination, there are many unwanted consequences:

Time-efficiency: Loss of time since experiments must be repeated.

Costs: Increase in cost due to increase usage of consumables (materials, reagents, etc.)

Reliability & reproducibility: Loss of reproducibility due to different cellular responses when you work with contaminated versus non-contaminated cells. The worst case scenario: a loss of significance when contamination remains unnoticed and you work with the wrong cells for years.

NOTE

Maintaining a sterile environment has priority in a cell culture lab. This involves preventive hygiene, personal protective equipment, being organized inside the biosafety cabinet, and appropriate pipetting techniques.

More information:

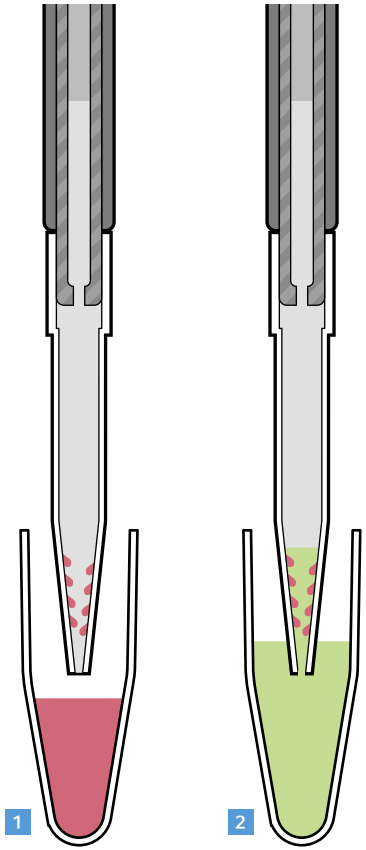
Webinar: How liquid handling affects your cell culture
https://eppendorf.group/cell-protection-webinar

Maintaining a reduced germ count in the environment takes absolute priority. **Preventive hygiene**, which includes regular cleaning of the laboratory, as well as incubators, water baths and biosafety cabinets, plays a major role in achieving this goal. Likewise, a dedicated lab coat should be available which is only worn inside the cell culture laboratory. Your workspace in the biosafety cabinet is the place of action and the place where most mistakes can happen. All preventive hygiene, personal protective equipment and organization will be in vain if **aseptic technique** is not followed when handling cells, reagents and pipettes.

7.1. Expert tips – Prevent contamination

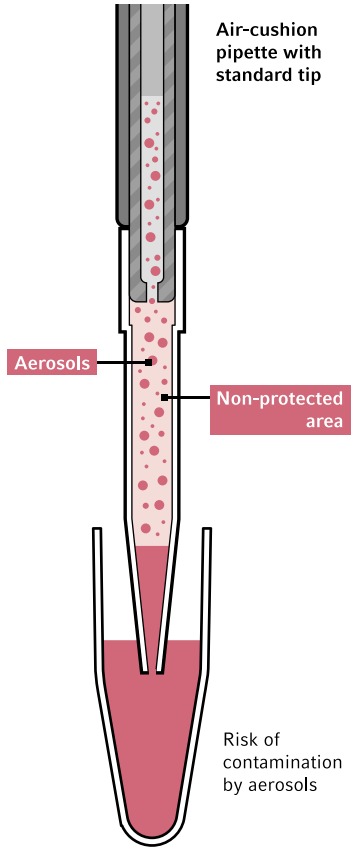
Three types of contamination regarding pipetting can be distinguished:

Pipette to sample
Caused by contaminated pipette tip or pipette
> Use dedicated set of pipettes only for cell culture lab
> Do not move hands over open vessels
> Ensure an organized workspace
> Disinfect before placing pipettes in the biosafety cabinet; place a sticker on each pipette and label it »cell culture«.
> Disassemble the pipettes to clean them from the inside
> Use sterile pipette tips
> Use filter tips
> Clean or autoclave the pipette
> Use positive displacement instruments
> Select pipettes and tip length to ensure that the tip cone of the pipette does not touch the inner wall of the vessels



1 Residues of sample A in the pipette tip
2 Contamination of sample B

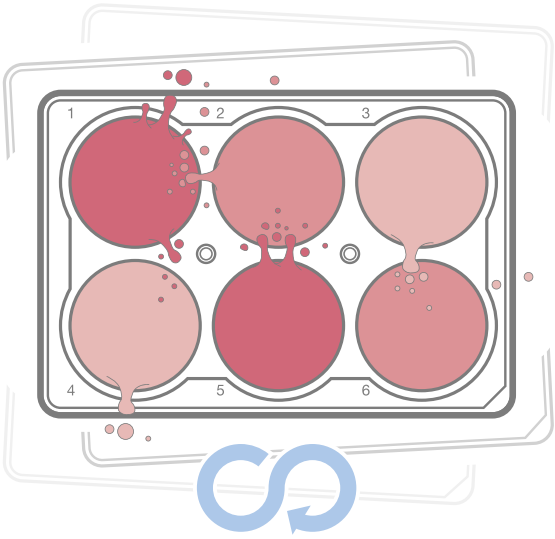
Sample to pipette
Caused by movement of the sample or its aerosols into the inside of the pipette
> Use filter tips
> Keep pipette hanging in the rack
> Aspirate slowly (to prevent liquid from splashing up inside the tip, alternatively use electronic pipettes to ensure uniform aspiration)
> Use positive displacement pipettes
> Select pipettes and tip length to ensure that the tip cone of the pipette does not touch the inner wall of the vessels



In order to prevent aerosol contamination:
> Mix the sample by rolling rather than shaking
> Place vessels directly next to each other to avoid large distances
> Open vessels slowly
> Use filter tips
> Pipette directly into the liquids, or onto a surface to avoid splashing and bubbling
> Use positive displacement instruments (no aerosols)

TIP

Sample to sample
Caused by carrying over sample material
> Replace pipette tip after each sample dose
> Pipette slowly to avoid splashes
> Use your own medium bottle and reagents



NOTE

The most effective protection against contamination is provided by positive displacement systems; their tips feature a piston with an integrated sealing lip. This prevents aerosol formation and contamination of the instrument.

7.2. Expert tips – Use the right pipette

Electronic pipetting aids
Electronic pipetting aids and serological pipettes are an option during steps for which precision is not paramount – for example, washing steps and medium changes. Since determination of the volume inside a serological pipette depends on the interpretation of the liquid meniscus by the operator, precision cannot be guaranteed. The great advantage of serological pipettes is their length. This allows working with more distance to cell cultures. More distance to the cells = more safety.

Mechanical pipettes
Mechanical pipettes are more accurate for aqueous solutions; however, technique and speed are controlled by the operator.

Electronic pipettes
Among other advantages compared to mechanical pipettes, electronic pipettes dispense equal volumes at the same speed at each stroke; only the technique is the precision-limiting factor. Be aware that accuracy and precision may be affected when non-aqueous liquids are pipetted.

Dispensers
A dispenser that works according to the positive displacement principle offers the highest precision for all types of liquids. Unfortunately, the recommended pipetting technique can be difficult to follow when working inside a biosafety cabinet. By using a dispenser instead of a multi-channel pipette for dispensing assay reagents or seeding cells, immersion depth and pipetting angle will not influence accuracy.

The plastic material of which the pipettes are made should be UV-resistant, so that pipettes can remain safely in vaccine cabinets or biosafety cabinets without deleterious effects on the pipette and its functionality.

NOTE

7.3. Expert tips – Manage equipment safely

Have a dedicated set of pipettes and tips in the cell culture lab. If you choose a new pipette for cell culture, consider one that is easy to clean with a smooth surface that does not have many rough edges and grooves.

> Use pipettes exclusively in the biosafety cabinet
All pipettes or pipetting accessories should be restricted for use in the cell culture laboratory, e.g. labeled »cell culture laboratory«. Put a sticker on each pipette and label it with »cell culture«

> Disinfect each pipette, pipette rack and tip box by wiping them down before starting work
Disinfect the pipettes each time before you place them in the hood. Do it thoroughly by wiping with a disinfectant to allow a complete wetting of the surface. Please refer to the user manual of the pipette manufacturer for chemical compatibility

> Disassemble and clean the pipette regularly; autoclave if possible.
You can also disassemble the pipettes to clean them from the inside if necessary. Autoclavable pipettes provide extra safety

Check the sterility assurance level (SAL) of serological pipettes and consumables. A value of 10^{-6} is ideal; it means that a maximum of 1 out of every million products used may potentially be contaminated. A SAL value of 10^{-3} is usual on the market.

TIP

8. Maintenance of pipetting systems

A scientist's pipette is like an artist's brush. Precision and accuracy of the pipettes are important to ensure the quality of your results. Regular maintenance, calibration and adjustment ensure that your pipettes will continue to generate reproducible results.

NOTE

Keeping a pipette clean, calibrated and serviced guarantees a long lifetime and accurate results. You can perform many pipette maintenance steps yourself, right in your lab!

More information:

Standard operating procedure for manual dispensing tools
https://eppendorf.group/SOP-pipettes

8.1. Cleaning and maintenance

Clean instruments – correct results. Modern quality management in the laboratory requires regular cleaning and inspection of the pipetting systems. How often a pipette should be cleaned and checked depends on the respective frequency of use, number of users of the instrument, the aggressiveness of the liquid to be pipetted, and the acceptable error limits set by the user.

Safety: A pipette should be cleaned regularly to guarantee contamination-free work as well as sample and user safety.

Reliability & reproducibility: Regularly checking the pipette status is a necessary procedure to guarantee accuracy and precision for all pipetting activities.

8.1.1. Decontamination and cleaning

Decontamination is the reduction or the removal of contaminants. No matter whether you use mechanical or electronic pipettes, both can get contaminated from all the different liquids and solutions they are exposed to. Common contamination points are the outside of the pipette as well as the inside of the lower part.

Cause of external contamination

> Contact with the inner wall of the vessel or tube: bacteria, blood, DNA, patient samples, etc. may contaminate fresh tubes

> Contact with contaminated gloves: contamination of other pipettes

Cause of internal contamination

> Overpipetting (when more liquid is aspirated than the tip volume allows) or upside-down placement of pipette with filled tip: liquid enters tip cone and contaminates other samples

> Contaminating aerosols rise inside the tip cone, contaminate subsequent samples and potentially ruin entire applications

External contamination can be removed by suitable agents listed in the respective operating manuals. Generally, the components must be wiped with de-ionized water and dried afterwards.

Accidentally absorbed liquids (internal contamination) should not be allowed to dry. The lower part of the pipette should be disassembled and cleaned according to manufacturer's specifications. Depending on the cleaning agent treatment with a suitable lubricant might be necessary.

Before cleaning your pipette, please be familiar with chemical compatibility charts provided by the pipette manufacturer.

Type of Liquid	Note	Tips for decontamination and cleaning
Inorganic acids	Use pipettes made of acid-resistant plastic and piston. However, acid aerosols can still get into the lower part and impair function.	If concentrated acids are pipetted frequently: open pipette and clean lower pipette part with purified water, let the parts dry (at max. 60 °C in a drying cabinet); lubricate piston if necessary.
Alkaline solutions	Use pipettes made of alkaline-resistant plastic and piston. However, alkaline aerosols can still get into the lower part and impair function. Use filter tips.	If alkaline solutions are pipetted frequently: occasionally, open pipette and clean lower pipette part as recommended above in »Inorganic acids«.
Satured solutions	Precipitation of aerosols can cause crystals which destroy the seal. Regular cleaning of the piston and checking of the piston seal are recommended.	Clean piston with solvent solution to solubilize the crystals (be aware of chemical compatibility), rinse with purified water, let dry and lightly lubricate the piston.
Potentially infectious liquids	Positive displacement instruments can be used to prevent contamination, since the liquid is hermetically sealed inside the tip. Use filter tips if you decide to use air-cushion pipettes.	Autoclave contaminated parts (121 °C, 20 min). Optionally, open pipette and place lower pipette parts in standard laboratory cleaning agents, rinse, dry and lubricate as described above.
Cell culture	Use filter tips.	See above »potentially infectious liquids«.
Organic solvents	Adjustment due to altered liquid density is obligatory. Consider pipetting techniques for volatile liquids with high vapor pressure. Open pipette to allow evaporation of liquids after pipetting.	Usually evaporation is sufficient. Optionally, open pipette and place contaminated pipette parts in standard laboratory cleaning agents, rinse, dry and lubricate as described above.
Radioactive liquids	Positive displacement instruments can be used to prevent contamination, since the liquid is hermetically sealed inside the tip. Use filter tips with air-cushion pipettes.	Open pipette, place contaminated pipette parts in complex-forming liquids or special cleaning agents, rinse, dry and lubricate as described above.
Proteins and nucleic acids	Positive displacement instruments can be used to prevent contamination, since the liquid is hermetically sealed inside the tip. Use filter tips with air-cushion pipettes.	**Proteins:** Open the pipette. Rinse with or immerse in detergent. Afterwards rinse, dry and lubricate (if needed) as described above. **Nucleic acids:** **1)** Use commercial decontaminating reagent if materials are chemically resistant. Apply as recommended by manufacturer. Rinse, dry and lubricate (if needed) as described above. **2)** Decontaminate with sodium hypochlorite (max. 4 %). Rinse thoroughly, dry and lubricate as described above.

NOTE

Upon delivery, all pipettes (mechanical and electronic) come properly lubricated by the manufacturer. Re-lubrication is only necessary when cleaning agents such as soap, liquid detergent or organic solvents have been used and the lubricant was actively removed. Autoclaving, on the other hand, does not affect the lubricant; therefore instruments do not need to be lubricated after autoclaving.

TIP

Pipettes should be cleaned regularly to make sure that all hidden contamination is removed. The lower part of pipettes should be easily removable and cleanable. The pipette should be resistant to common cleaning agents and DNA/RNA cleaning solutions to make sure the pipette can be wiped from the outside and rinsed on the inside. Most contamination can be removed by wiping and rinsing. If sterility is an issue, choose a pipette that can be autoclaved without disassembling to simplify pipette maintenance.

More information:

Videos: Pipette cleaning tutorials
https://eppendorf.group/pipetting-cleaning-video

Videos: Pipette cleaning tutorials
https://eppendorf.group/pipette-maintenance-video

Video: Pipette lubrication
https://eppendorf.group/pipette-grease-video

Standard operating procedure for manual dispensing tools
https://eppendorf.group/SOP-pipettes

8.1.2. Disinfection

Disinfection is a process that is designed to kill actively growing and vegetative microbial microorganisms. Following the manufacturer's specifications, the outer surfaces of the pipettes can be carefully wiped with disinfectant or 70% isopropanol. 4% sodium hypochlorite can be used for external cleaning of the tip cone and ejection tube. Following exposure, the sodium hypochlorite solution must be thoroughly removed with demineralized water.

8.1.3. Autoclaving

When pipettes suffer from contamination by bacteria, fungi and mycoplasma, autoclaving is a secure method of inactivating the contamination*[1].

Today's pipettes can either be completely autoclaved or (due to electronics in the upper part), the lower parts can be autoclaved. Thus, residual doubts with regard to sterility can be eliminated. Autoclaving of air-cushion pipettes as well as the pipette tips is usually carried out at 121 °C at an overpressure of 1 bar (100 kPa) for a period of 20 minutes. After autoclaving, the autoclaved parts must be dried and cooled down at ambient temperature. If it is necessary to disassemble pipettes before autoclaving, it is important to wait with assembly until they have cooled completely, otherwise plastic parts may be overstretched and damaged. Check the manufacturer's specifications to see if it is necessary to re-grease the pipette piston after autoclaving. Filter tips should not be autoclaved. Instead, sterile tips can be purchased.

TIP

When regular autoclaving of pipettes to maintain sterility and cleanliness is required, autoclavable pipettes without disassembling facilitate the process. Autoclavable materials do not swell or change configurations during heat-sterilization, therefore, calibration remains unaffected.

8.1.4. Decontamination using UV light

UV light is used to kill or inactivate microorganisms by destroying nucleic acids and disrupting their DNA, leaving them unable to perform vital cellular functions. In cell culture, safety cabinets and clean benches are UV-decontaminated. The UV-resistance of the pipette material is therefore of central importance. UV-resistant pipettes can remain safely in safety cabinets and clean benches because the UV light used to disinfect the bench does not have a negative effect on the pipette material and thus on the function of the pipette.

Decontamination using UV light should be carried out with a 30-watt low-pressure mercury vapor lamp at a wavelength of 254 nm. The optimum distance between lamp and pipette is approximately 60 cm.

8.1.5. Regular check of pipette status

Precise and correct pipetting of samples and reagents is crucial. Checking a pipette's performance at regular intervals is necessary to ensure that it is functioning correctly.

In-house guidelines established by the user should require regular inspection of pipettes and dispensers as well as the tools used for inspection. It is recommended that they are checked at least once a year (ISO 8655). Depending on the user's specifications and the usage of the pipette with chemicals, more frequent calibration may be necessary.

NOTE

Leak tightness test

To check leak tightness, aspirate an aqueous-like solution with the pipette's nominal volume (largest volume adjustable by the user and determined by the manufacturer) into the pipette tip and hold the pipette vertically. Use distilled, degassed water and make sure that the pipette, pipette tip, and test liquid are at the same temperature. If after 15 seconds no clear drop has formed at the tip, the pipette is tight. For volumes up to 20 μL the tip should be pre-wetted. If the pipette is leaking, you should disassemble and reassemble the lower parts. If this does not work, call for service.

Visual inspection

A visual check for leaks, broken parts and contamination should be performed daily.

*[1] Uphoff CC, Drexler HG. CELL CULTURE MYCOPLASMAS. DSMZ – German Collection of Microorganisms and Cell Cultures

Quick check

The quick check should be performed monthly or after major changes (e. g. a new batch of pipette tips or after replacement of volume-determining parts) to roughly check random and systematic error. It is a shortened calibration with 4 measurements per volume. However, since four measurements do not ensure statistical certainty, the quick check is not considered a substitute for a standard calibration of 10 measured values per volume. If the measurement results fall outside the specified tolerances, the pipette has to be cleaned, adjusted and calibrated.

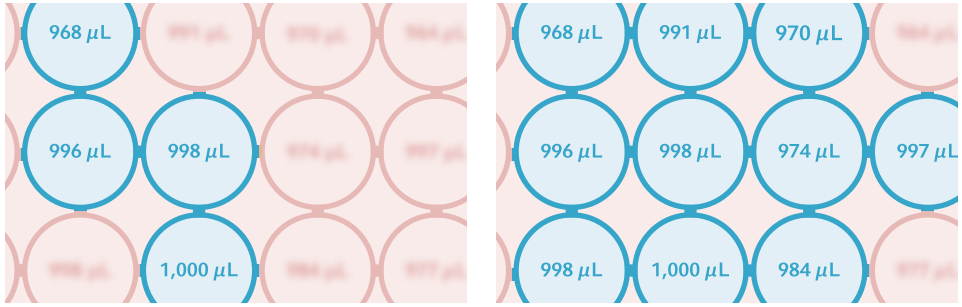


Figure: Quick check using 4 measured values and calibration using 10 measured values. A higher number of measurements provides higher significance, whereas a lower number of measurements may in fact give false results.

8.2. Calibration and adjustment

Calibration of pipettes and dispensers is of great importance for accurate and precise pipetting results. Calibration is an evaluation of the capability of the pipette's performance which states its current accuracy and precision. A complete calibration with a positive result confirms that the errors of measurement of a pipette are within the specified tolerances. It can be realized by using a gravimetric, photometric, or titrimetric test method. The gravimetric test according to ISO 8655 is currently the most accurate and recommended method.

Reliability & reproducibility: Calibration of pipettes is a necessary procedure to guarantee accuracy and precision of all pipetting activities.

There is no general rule or basis of calculation for determining sensible time intervals. Calibration results documented over a longer period can be used to draw conclusions regarding reasonable calibration frequency. Test intervals can be set according to the specifications of the laboratory. ISO 8655 requires at least annual calibration.

Calibration is the measurement to check the device's current status (no changes are made to the device). In contrast, adjustment involves making changes to the device with respect to the size of the air cushion. Adjustments need to be performed if the pipette failed calibration or when liquids with a density different from that of water are used.

NOTE

8.2.1. Precision & accuracy

Precision & imprecision (random error)

A pipette is precise if it always pipettes the same volume as selected. In other words, a pipette is imprecise if individual volumes pipetted differ from one another. The term precision refers to the fact that there is no deviation between repeated measurements with regard to special error specifications (e.g. by manufacturer, ISO 8655 or internal specifications). Vice versa, imprecision is a measure of the variation between individual measured values, also known as random error.

Imprecision (random error) is caused by:

> Handling
> Temperature changes

Accuracy & inaccuracy (systematic error)

Put simply, the volume a pipette aspirates can be right (accurate) or wrong (inaccurate). A pipette works correctly (accurately) if the volume delivered equals the volume selected. A pipette works incorrectly (inaccurately) if the delivered volume deviates from the selected volume. Inaccuracy measures the deviation of the mean delivered volumes from the selected volume, also known as systematic error.

Inaccuracy (systematic error) is caused by:

> Handling
> Environment
> Tips
> Defective or misadjusted device

Imprecision (random error) vs. inaccuracy (systematic error)

Evaluating a calibration, there are different possibilities in which a pipette is prone to random and/or systematic error and which can influence the results in different ways:

a) Accurate and precise: the ideal pipette status
b) Inaccurate and precise
c) Accurate and imprecise
d) Inaccurate and imprecise: the worst pipette status delivering wrong and non-reproducible results

A good calibration can be compared to a target where the bull's eye is hit every time. This represents a precise and accurate pipette. Each dot is a single pipetting event. The more distant each dot is from the bull's eye, the less accurate is the pipette. And the more scattered the dots are, the less precise is the pipette.

Random and systematic error

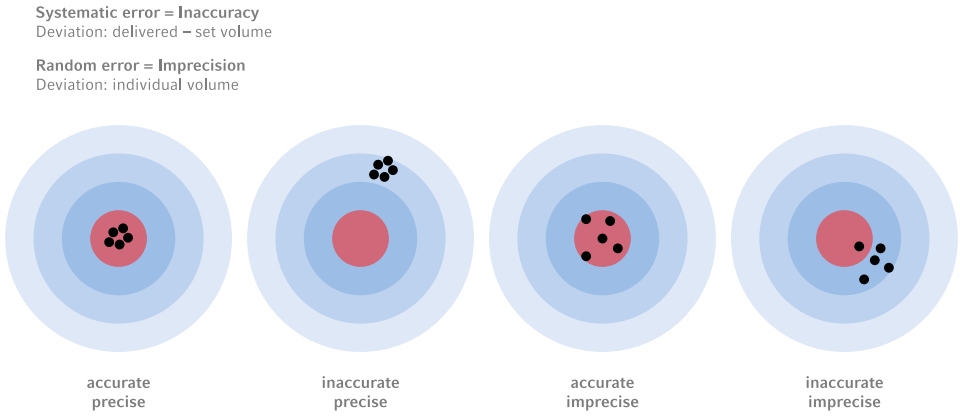


Figure: Imprecision (random error) and inaccuracy (systematic error)

NOTE

Inaccuracy and imprecision, i.e. systematic and random error, respectively, develop gradually over time. This process is especially accelerated by aggressive chemicals.

8.2.2. Gravimetric calibration according to DIN EN ISO 8655

The test norm most commonly used for pipettes and dispensers is the standard DIN EN ISO 8655. This standard lays down the maximum permissible random and systematic measurement deviations for the various liquid handling systems. The error limits specified in the standard always refer to the overall system, consisting of the dispensing device and accessories such as the pipette tip. Under specified ambient conditions a defined volume of distilled, degassed water dispensed from a dispensing system should have a certain expected weight. This involves determining the correlation between the dispensed volume and the selected volume of a dispensing system.

NOTE

Compliance with specified error limits must be verified by the user by way of inspection, measurement and testing of equipment, or analytical quality assurance, at least annually. The user is thus free to specify shorter intervals and to determine the limits within the ISO specification. The frequency of usage, number of users of the dispensing system, handling, and the aggressiveness of the liquids to be dispensed play a key role in this decision. In calibration laboratories, tests are typically performed in accordance with the manufacturer's specifications.

The gravimetric test procedure requires an analytical balance. For particularly small volumes, the evaporation loss during the weighing procedure should be kept as low as possible. Executing a proper calibration according to ISO 8655 requires special ambient conditions:

> Draught-free room
> Constant temperature of 15–30 °C (±0.5 °C)
> Air humidity of ~50%
> Before the test, pipette, tips and test liquid must have been kept in the room for a sufficient amount of time (at least two hours) to achieve equilibrium with the room conditions
> Test cycles shall be as constant as possible to keep the same measurement duration within each cycle and from cycle to cycle in order to be able to compensate for evaporation effects during a series of measurements
> Degassed, distilled or ion-free water as the test liquid

Three series of 10 measurements are used for the calibration of a variable-volume pipette, and one series of 10 measurements for a fixed-volume pipette. For variable pipettes, the three test volumes comprise:
> Nominal volume (largest volume adjustable by the user and determined by the manufacturer)
> 50% of the nominal volume
> 10% of the nominal volume

If the calibration results of mechanical pipettes exceed the permissible limits, an adjustment must be performed on the pipette or, alternatively, the pipette needs to be serviced.

More information:

Calibration and adjustment of dispensing systems in the laboratory
https://eppendorf.group/userguide-25

Standard operating procedure for manual dispensing tools
https://eppendorf.group/SOP-pipettes

Video: Pipette greasing
https://eppendorf.group/pipette-grease-video

8.2.3. Adjustment

An adjustment is an active change or reset of the pipette, where the air cushion size is changed. An adjustment is necessary in case of:
> Failed calibration
> Pipetting liquids with different densities than water
> Use of tips which differ in geometry from tips recommended by the manufacturer
> Deviating conditions (air pressure in extremely high or low-altitude locations)

NOTE

If a positive displacement instrument does not operate within the error limits, it must be serviced. The user cannot perform adjustments.

Electronic pipettes should offer the possibility of adjustment in order to compensate for physical influences easily and without problems. Depending on the application, individual adjustment options can be used.
An adjustment does not affect imprecision (random error). The random error can be reduced by exchanging worn parts.

NOTE

If there is doubt about the accuracy of the pipetting volume, the pipette should not be readjusted prematurely. Instead, check for leak tightness, which is often the cause of inaccuracy. If the pipette continues to show deviation in either direction, all measurement conditions should be checked again. Consider tips (pipette and tip comprise a system), as well as the temperature of the sample, the pipette, air and weight to volume conversion (mg to µL)

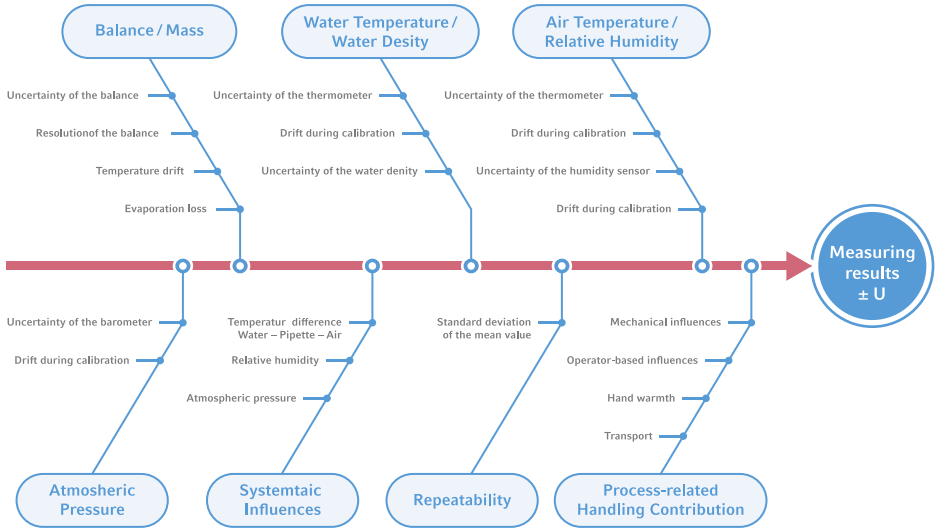


Figure: Overview of external influences on the measurement result (adapted from DKD-R8-1 guideline). Not all of them should and can be compensated for by adjustment.

Adjustment has to be performed as follows:

> Pipette, tip (as recommended by manufacturer), and the liquid must be at the same temperature (15 °C to 30 °C, ±0.5 °C)
> Desired target volume is pipetted and weighed ten times.
> The mean value of these measurements, corrected to account for the density of the liquid at the respective temperature, gives the actual volume of the pipette
> If the target volume after adjustment does not match the result of the measurements, adjustment is carried out according to the manufacturer's instructions (see operating manual of the respective pipette) e.g. by using the special key supplied
> Repeat the steps above until the target volume is met

Adjustment affects the entire measuring range. For variable volume pipettes, all volumes specified in the technical manual must be tested without fail (generally 10%, 50% and 100% of the nominal volume).
Pipettes whose factory settings have been altered by adjustment must have clear visible identification according to DIN EN ISO 8655.

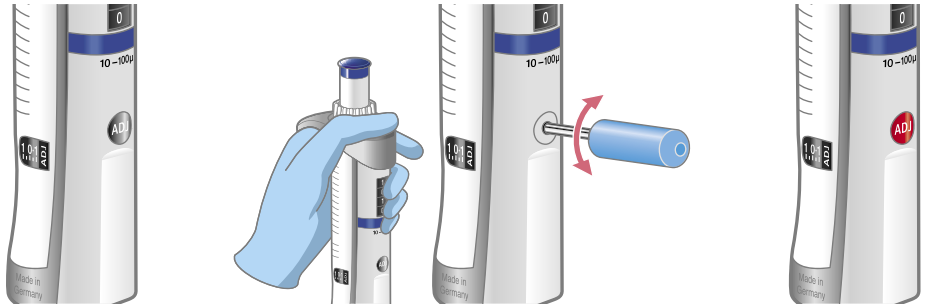


Figure: Adjustment of an air-cushion pipette und clear visible identification (sticker).

Liquids with a density other than water

Upon manufacturing, pipettes are calibrated to distilled water under the specified measurement conditions. Air-cushion pipettes can be adjusted to a certain volume of a liquid with a density other than water so that the selected volume corresponds to the delivered volume. The density of the respective liquid must be taken into account during weighing and volume calculation. The adjusted pipette only delivers the correct volume for the liquid which was used for the adjustment, as well as the volumes that were tested. Consequently, the adjusted pipette must be marked as a »fixed-volume pipette for solution X«.

Many air-cushion pipettes have an additional adjustment window for this purpose, where the exact adjustment can be seen at a glance. The adjustment window also makes it easy to reset a re-adjustment, if necessary.

Errors associated with liquids having an increased vapor pressure (e.g. organic solvents) cannot be compensated for re-adjustment. Instead, the use of a positive displacement instrument is recommended.

NOTE

Figure: Adjustment window of an air-cushion pipette

More information:

Calibration and adjustment of dispensing systems in the laboratory
https://eppendorf.group/userguide-25

Video: Pipette greasing
https://eppendorf.group/pipette-grease-video

8.3. Service

Reliability & reproducibility: Regular service prevents uncontrolled downtime or unnoticed dispensing errors and can contribute to better results. Pipettes are precision instruments that can wear out through use and then become imprecise. Parts subject to wear, such as O-rings, must be replaced regularly, and the pipette must be re-calibrated. Only regular, thorough external and internal maintenance of the pipette guarantees a long service life, continuous reliability, and reliable results.

Sample safety & costs: Cost and liability risks associated with inaccurate pipettes can be significantly reduced through preventive maintenance and calibration. Experiments with expensive reagents or valuable samples do not have to be repeated due to incorrect pipetting results.

User safety: A well-maintained pipette also prevents health problems that may be caused by repeated pipetting with pipettes in unmaintained, poor condition.

Before any maintenance activities an »As-Found calibration« is done to document how accurate and precise the pipette has performed this far. An »As-Left calibration« often includes a cleaning and maintenance service before the calibration itself is carried out. The combination of an As-Found and an As-Left calibration delivers seamless documentation and provides renewed certification of conformity. Calibration does not require intervention which modifies the dispensing system on a permanent basis.

TIP

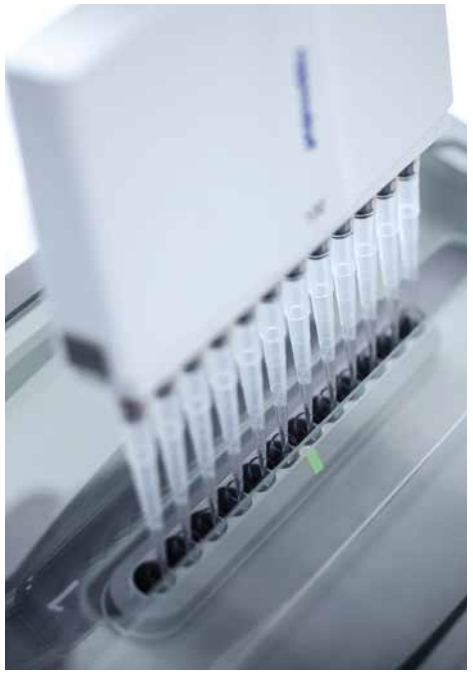
Pipette exchange programs are a good option for pipettes where the repair effort is not economical. The customer can buy a new pipette for a special price instead of having their old pipette repaired.

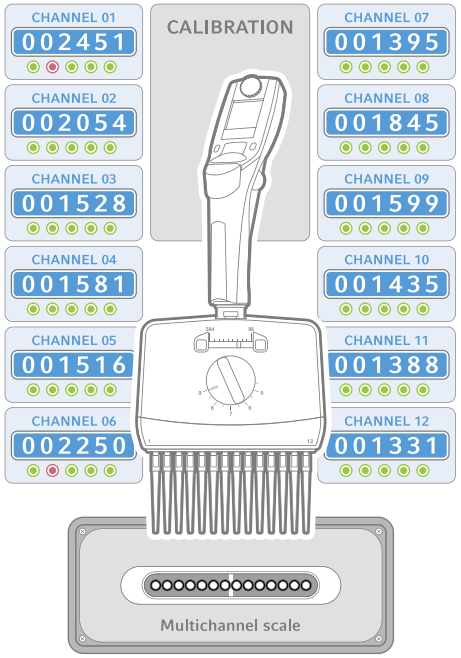
Criteria for choosing a professional pipetting service

> Calibration processes according to manufacturer's specification and according to ISO 8655
> Conformity of calibration records or certificates, with ISO 8655 to support GLP documentation
> Usage of a scale with 4, 5 or even 6 decimal places according to ISO 8655 for small-volume standard pipettes with a nominal volume of 10 µL or less
> Usage of a multi-channel scale for multi-channel pipettes to fulfill requirements of ISO 8655
> Replacements only with original spare parts
> Professionally trained and qualified calibration technicians. If possible, choose an ISO 17025 accredited pipette service laboratory which employs processes validated in accordance with ISO 17025:2017, using validated calibration software. In addition, the service laboratory should offer validated environmental conditions, a defined quality management system, comprehensive traceability and recurrent external monitoring.

More information:

Video: Example of a pipette service process
https://eppendorf.group/pipette-service-video





8.4. Common errors

Sources of error and solutions

Problem	Cause	Solution
Pipette is dripping	> Pipette tip is loose. > Incorrect pipette tip used. > Piston is damaged. > Seal is damaged. > Liquid with slightly increased vapor pressure used. > Liquid with high vapor pressure used. > O-ring at cone is worn out (only multi-channel pipettes)	> Re-attach pipette tip. > Use original manufacturer pipette tip. > Exchange piston and piston seal. > Pre-wet pipette tip several times. > Use positive displacement device. > Replace O-ring(s) at cone.
Control button is stiff	> Piston is contaminated. > Seal is contaminated. > Seal is damaged. > Piston is damaged. > Solvent vapors entered the pipette.	> Clean and grease piston. > Clean seal. > Exchange seal. > Replace piston. > Remove and disassemble lower part. > Clean and grease piston.
Uneven piston movement	> Piston is contaminated. > Solvent vapors entered the pipette.	> Clean and grease piston.
Wrong dispensing volume of air-cushion pipettes	> Incorrect/foreign tip is used without pipette/tip calibration. > Temperature between instrument, consumables, environment and liquid is not consistent. > Air humidity or air pressure are not considered.	> Use original manufacturer pipette tip or adjust pipette/tip system > Ensure temperature consistency between pipette/tip system, the environment and the liquid. > Adjust the pipette/tip system to air pressure or use postitive displacement device.
Wrong dispensing volume of multi-dispensers	> Dispensing tip is not leak-tight. > Dispensing tip has been autoclaved for re-use.	> Use new dispensing tip. > Due to their construction with a piston inside the tip, positive displacement tips (e.g. Combitips® advanced) are not autoclavable. Use new tip.

Eppendorf transparency on test methods and test specifications

As a premium supplier, Eppendorf places great importance on product quality, and complies with global quality standards. Eppendorf testing procedures are validated, and as part of method validation, limit of detection (LOD) is determined. LOD constitutes a regulatory requirement in the United States Pharmacopeia (USP), the International Conference on Harmonization (ICH) and the ISO 17025 standard. The limit of detection indicates the lowest analyte concentration at which a reliable distinction can be made between a signal from a sample containing analyte and the background noise of a sample without analyte (blank).

To increase transparency, we have listed the Eppendorf portfolio of test methods and test specifications in the table below for a better overview:

Test parameter	Human DNA	Bacterial DNA	RNase	DNase	PCR inhibitors	Sterility	Endotoxins	ATP
Test method	Real-time PCR	Real-time PCR	RNA digestion	DNA digestion	Real-time PCR	Irradiation or gassing with ethylene oxide	LAL* test	Bioburden determination
LOD	< 2 pg	< 50 fg	1.0 x10^{-9} Kunitz units	1.0 x10^{-6} Kunitz units	Fewer than 10 targets amplifiable	SAL 10^{-6}	< 0.001 EU/mL	< 5.5 x 10^{-12} mg

* Limulus amebocyte lysate

A major advantage which distinguishes Eppendorf quality is that the tests for the listed contaminants are carried out and certified by an independent external laboratory on a lot-specific basis. In addition to being GLP-compliant, the independent testing laboratory is ISO 9001-certified and ISO 17025-accredited – assuring that all testing methods are validated for those specific applications which require ISO 17025-compliance.

9. Sustainability in the laboratory

Sustainability in the lab is easier than you might think. Responsible use of resources not only supports the environment, but it also reduces sample volumes, reagents and consumables.

9.1. Sustainability in the context of research and lab

Until the early 1960s, laboratory samples were still pipetted with glass tubes. Exact dosing and maintaining reproducibility were difficult. In addition, the glass tubes were reused, which involved time-consuming cleaning. The carry-over of sample material and the contamination of subsequent experiments could not always be avoided, and reliability of test results could not be guaranteed. An analytical method with an appropriate analysis system was needed to both examine many small sample quantities and eliminate the risk of cross-contamination leading to false results. Disposables such as »Eppis®« and tips were part of the microliter analysis system invented at that time, which is still used today. Disposables eliminate the risk of carry-over and contamination, protecting valuable samples as well as the operator and thus ensuring reliable results.
The requirements for a reliable analysis system are same today as they were then. For this reason, a sustainability assessment that aims only to avoid plastic has its limitations. It is worth taking a closer look at the meaning of sustainability, and what a meaningful sustainability analysis entails.

Acting sustainably generally means reconciling economical, ecological and social issues:

Economic aspects, which play an important role in research work, comprise the costs associated with one's work. These include, for example, costs for laboratory operation, reagents, or equipment. Likewise, samples are valuable and expensive, sometimes even irreplaceable. Experiments can therefore not always be repeated, and care must be taken that they work the first time. Consequently, the quality of the materials used should be high to ensure the quality of the results, their reproducibility, and reliability.

Ecological aspects aim to minimize the consumption of resources. These include the energy consumption caused by the use of equipment as well as the consumption of consumables, chemicals and reagents. The reduction and prevention of waste is a major aspect in this context.

Social aspects aim to create good working conditions in the laboratory. Safety has priority. Similarly, occupational health and safety measures as laid down in laboratory guidelines and operating manuals must be considered. Ergonomics is also an aspect that must be applied to health protection and should not be neglected.

9.2. 3Rs for more sustainability

Reduce, Reuse, and Recycle are the 3 R's to make laboratory work more sustainable in practice:

Reduce – Use fewer resources and less material
Reuse – Reuse resources and use them as effectively as possible
Recycle – Use reusable materials that can be recycled where possible

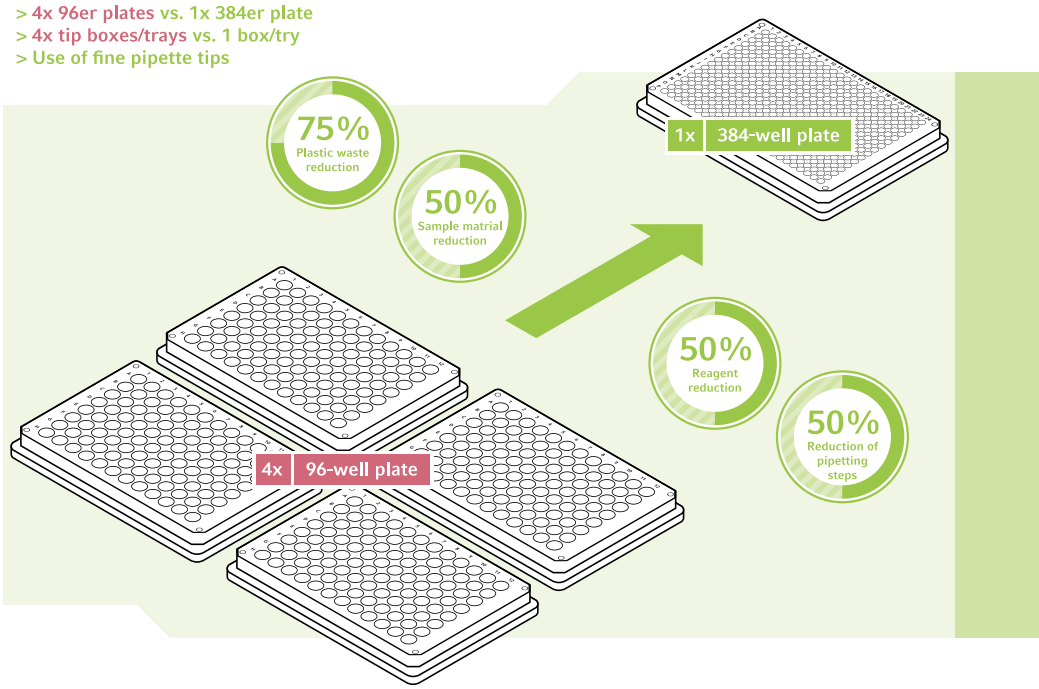
9.3. Laboratory sustainability guide

Reduce – Waste reduction through miniaturization and planning ahead

> Miniaturize reaction volume

Smaller reaction volumes are an effective way to save resources. Miniaturization enables waste reduction while at the same time saving reagents and sample material.

Use one 384-well plate instead of four 96-well plates, for example. Switching over from 96-well plates to 384-well plates significantly reduces sample volumes, as well as reagents and consumables, and it reduces waste. The cherry on the top: By switching to 16- or 24-channel pipettes, the number of pipetting steps can be reduced by 50%.



Reduce packing waste

Use refill systems for pipette tips. These are available either loose in a bag or as a replaceable reload system, which is placed on top of a box. By the way, a box is quite durable and can be autoclaved up to 100 times, a rack up to 10 times.

TIP

With the newest generation of refill systems you can easily save more than 70% plastic waste compared to racks.

Plan experiments, choose consumables wisely

Use chemicals, reagents and consumables of high quality and purity to prevent distortion of results and the need to repeat experiments.

Choose premium quality tips, tubes and plates that are made of high-purity polypropylene (PP). PP is particularly advantageous due to its inertness to a variety of solvents, and has proven itself over the years. However, only high-purity polypropylene can guarantee that no undesirable additives such as plasticizers, lubricants and biocides are present. Make sure that the manufacturer guarantees high quality based on lot-specific tests and controls.

Use tips and tubes adapted to different methods and applications, which are offered in different degrees of purity. Bacterial and cell cultures, for example, require sterile and pyrogen-free materials. For molecular biological methods, DNase and RNase-free materials are essential. In special application areas such as stem cell research or quality control, even higher purity grades are required.

Select coordinated devices and consumables

Pipette and pipette tips are often developed by the manufacturers as a system and are perfectly matched. One advantage of such systems is, for example, that tips are more reliably attached to the pipette tip cone. In this case, you do not have to worry about tips that might fall off. This minimizes sample loss and unnecessary waste.

Reuse – Effectiveness and longevity

Consider the life cycle costs of devices

The longer the life of a device, the more favorable for users and the environment. The price often plays a decisive role when purchasing equipment. More meaningful are the life cycle costs – that is, investment, maintenance, and service costs for a device throughout its entire life.

Pay attention at the point of purchase

Consider the following aspects during purchasing:
> Quality of the device and the individual components
> Ease of repair of the device

Extend the life of your device

Extend the life of your device by:
> Regular maintenance
> Repair and exchange of spare parts instead of purchasing new equipment
> Regular cleaning and inspection
> Replaceable batteries or rechargeable batteries prevent premature disposal and thus ensure the longest possible service life

Calibrate regularly

Regular calibration and qualification (IQ/OQ) of pipettes guarantees precise and accurate results through verified functional quality. Accurate and precise pipettes and devices guarantee that tests do not have to be repeated unnecessarily to obtain correct results. This saves reagents and sample material but also reduces the consumption of consumables. Installation qualification (IQ) and operational qualification (OQ) services verify and document your instrument's ability to meet the manufacturer's design specifications with respect to performance.

Keep the packing of devices and pipettes in case they need to be sent in for calibration, qualification, maintenance or repair.

TIP

Go for second-hand and share
Not every purchase necessarily has to be a new device, even used devices can be an option. You can find them at specialized dealers, but you should make sure that a technical check is performed and that the instrument is fully functional. A 24-month warranty for the device should be included.

The sharing of equipment in institutes and research facilities not only saves costs but also resources. Large devices have been shared by institutes for a long time, but it can also be worthwhile for smaller instruments, especially if a department does not necessarily need them every day.

Recycle – Close the loop

Separate waste according to type
Most of the materials used in the laboratory are made of high-quality plastic. If not contaminated, this reusable material may recycled.

Trays and top racks, for example, are made of polyethylene terephthalate (PET) or polycarbonate (PC), depending on the manufacturer. Both plastics can be recycled relatively easily and reused for other products. Similarly, all packing materials should be separated according to type.

NOTE

Recycling is not always possible. With used pipette tips, tubes and other consumables, safety is paramount. Also tip boxes and racks which are used at the bench are potentially contaminated. Recycling is currently only possible if a hazard risk can be excluded. Which consumables come into contact with potentially hazardous material is often not definable and appropriate separation is almost impossible to implement in practice. Thermal recycling is therefore currently the norm.

Sustainable procurement

When assessing the sustainability of a product, it is helpful to look at its entire life cycle and the possible environmental impacts at the various stages of the life cycle. Not only utilization should be considered, but also other factors such as transport and production.

Selection of suppliers
Check if the manufacturer works sustainably. Are environmental aspects considered, such as resource-efficient production or the use of renewable energies? Choose companies that are certified according to DIN EN ISO 14001 or EMAS. These companies have established an environmental management system and work continuously to improve their own environmental performance. You can usually find this information on the manufacturers' websites.

Logistics
Find out how the products are shipped. Shipment by sea generates ten times fewer CO_2 emissions than shipment by air. A company should therefore consider shipping goods by sea whenever possible.

Avoid single orders
Collective orders not only save transport costs but also reduce packing material and emissions.

More information:

Learn more about sustainability at Eppendorf
https://eppendorf.group/sustainability

10. Ergonomics and prevention

Healthy working conditions become increasingly important and the pipette takes center stage in critical ergonomic considerations. Workflows in today's laboratories get more and more condensed. The strain is constantly increasing, and the daily workload is demanding for body and mind. Air-conditioned rooms, heavy, unwieldy equipment, and constant repetitive movements put a strain on the musculoskeletal system. Mental well-being is also negatively affected by high pressure and stress during daily work.

Responsible organizations already realized the value of personal health for their company culture, and that it is a prerequisite for long term competitive advantage. As a result, the ergonomics of laboratory devices and the entire work environment command a higher priority.

Ergonomics: Pipette design and handling can play a role in the development of health-issues.

Time-saving: Efficiency suffers from non-ergonomic pipetting. Vice versa, ergonomic pipetting is very efficient, which saves time.

Costs: Even though at first glance conflicting, economic conditions often benefit from a holistic ergonomic approach.

Reliability & reproducibility: Ergonomic pipetting enables a relaxed and fatigue-free working style, resulting in fewer mistakes.

RSI-syndrome
Up to 10,000 pipetting operations per day are quite common in some labs. Therefore, the RSI-syndrome (Repetitive Strain Injury Syndrome) is widespread among pipette users in laboratories. The RSI-syndrome comprises different symptoms including the neck, shoulder, arm, and hand complaints, which occur after frequent repetitive physical strain. Likewise, sensory disturbances or loss of strength or muscle cramps in the forearm are common. A well-known example is the painful carpal tunnel syndrome, an entrapment of the metacarpal nerve in the carpal tunnel of the wrist.

More information:

Tips and useful knowledge about ergonomic working conditions
https://eppendorf.group/ergonomics

Ergonomics in the lab
https://eppendorf.group/physiocare

White papers regarding ergonomic working conditions
https://eppendorf.group/physiocare-whitepapers

10.1. Influencing factors

Health problems are caused by long term physical strain over months and years. Ergonomic working conditions start with the pipette itself: the shape, the weight, operating forces, the concept of operation.

Impact of tool design

Balance & weight
A pipette with an unfavorable center of gravity can be poorly balanced in the hand. This requires a stronger grip, which in turn places unnecessary strain on the hand and fingers. A lower pipette weight of 40 g results in 4 kg if lifted 100 times a day and 1,000 kg for 250 working days per year. Thus, a pipette intended for daily use should be as light as possible.

Operational forces
Repetitive up and down movements that require a high degree of effort, with constant changing of the position of the thumb during each pipetting stroke, puts stress on muscles of hand and fingers.

Volume adjustment of a mechanical pipette with gloves requires manual dexterity. The repeated thumb movements require strong muscle activity.
Precision tasks such as gel loading and removal of supernatants are associated with increased physical strain on the thumb muscles due to static muscle activity, which is particularly harmful.

High tip attachment forces impede tip fitting and ejection. Repeated pushing of the pipette tip cone onto the tip by high force during tip fitting is particularly harmful, as it is associated with increased physical strain and simultaneous repetitive movement and vibration.
Easily operable buttons and volume dial ring, easy and comfortable tip-fitting and ejection lower the operational forces. Based on these facts, electronic pipettes and automation devices offer certain advantages over mechanical pipettes.

Button and display positioning
If the volume display is covered by the hand or otherwise inaccessible due to your working position, awkward movements will be necessary to view the volume setting. The volume display should be readable equally well for both left and right-handed people. Likewise, buttons should be ergonomically arranged and easily accessible without changing fingers, irrespective of hand size.

NOTE

A user should never be forced to stress certain muscles or parts of the body excessively or to make unnatural movements.

Impact of an intuitive interface

Intuitive operation
The pipette display should be intuitive with the set volume to be read at a glance. For electronic pipettes, the menu should not be divided into too many submenus, and programming should be easy. If it is not intuitive, you will have to spend extra energy »to think about it«.

Cognitive ergonomics requires the design of an intuitive interface between the instrument and the user. The intuitive design of the interface of a pipette may be reflected in the volume display, the ease of reach and intelligibility of buttons, and the practicality of software.

Impact of pipetting posture

Ergonomically correct pipetting is not limited to the ergonomic pipette; the user should avoid issues like **a round back or an unnatural sitting position**. A proper pipetting posture prevents muscle strain during work.

Impact of laboratory organization

The **right storage and easy accessibility** of your pipette help make your workflow easy and effective. It allows you to keep all required instruments in view and within reach, therefore ensuring a smooth workflow.

More information:

User Guide: The Eppendorf PhysioCare Concept® – 3 Spheres Model
https://eppendorf.group/userguide-46

10.2. Prevention checklist

Physical and mental relaxation are core elements of a successful prevention concept. The field of ergonomics extends far beyond ergonomic chairs.

More information:

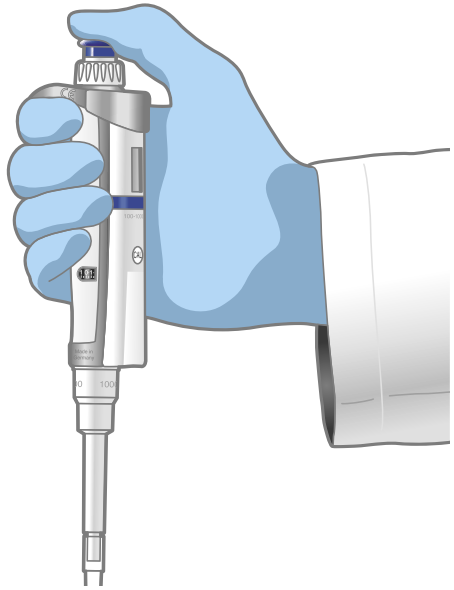
Lab workflows: How can I improve my ergonomics in daily work?
https://eppendorf.group/physiocare-workflows

10.2.1. Relax your body

Use ergonomic equipment to relax the body – especially the arm, hand, fingers, back, and shoulders. Likewise, pay attention to correct pipetting with good posture, but also remember to stretch the muscles after work to relax them again.

Ergonomic equipment
When selecting new laboratory equipment, you should consider the ergonomic properties of the instrument of interest.

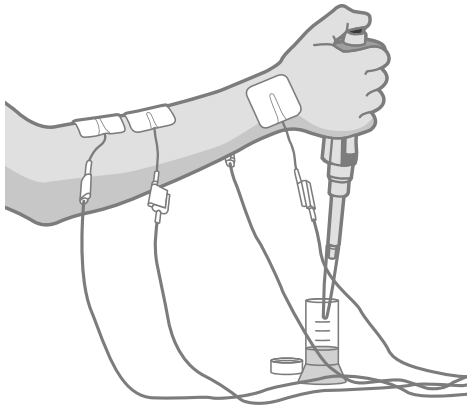
Does the pipette follow the shape of your hand?
The hand-grip of a pipette should fit the user's hand. After all, human hands come in assorted shapes and sizes, and a good pipette handle accommodates them all. Ergonomic design of this part of the pipette is crucial for stress-free work over many hours.



TIP

The control button, ejector and hand rest need to be conveniently positioned for hand and fingers. The distance between the control button and ejector (thumb constantly changes back and forth) should be as short as possible; maximum comfort is provided as there is no need to twist the thumb.

Suffering from old, heavy pipettes?
For many scientists, the pipette is the most important tool in the laboratory. You spend several hours a day with your pipettes, involving thousands of movements. For this reason, limiting the weight of a pipette is crucial to lessening the impact on your fingers and the muscles in your arms. Every gram counts.

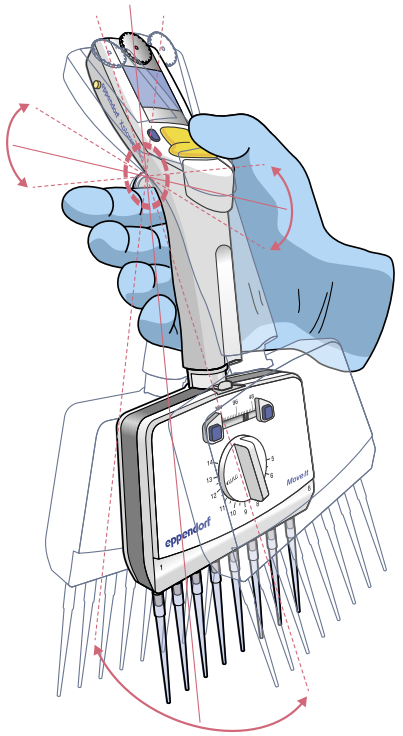


Painful thumbs?
Besides the weight of the pipette, which stresses arm and finger muscles, your thumb needs to constantly work – up and down, up and down. With a pipette in your hand, it is always at work. And if you own a two-button pipette system, you also have to move your thumb from the major button to the ejector button and back again. The lower the forces required to operate the control buttons, the less strain on your thumb.

TIP

Electronic pipettes and automation devices require the lowest operational forces for pipetting.

The right balance in your hand?
Do you need a break? Many electronic pipettes are top-heavy due to the battery. With a natural and relaxed hand position the demand for a break decreases.



TIP

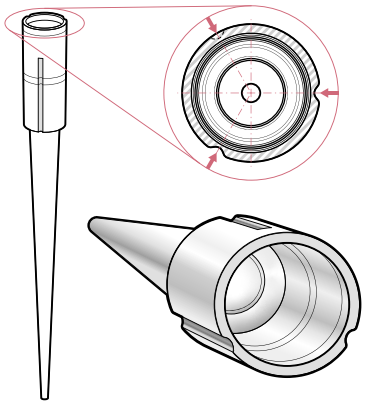
A well-balanced overall geometry ensures comfortable handling of the electronic pipette and helps the user avoid stress – all day long.

Forcing your tip onto the tip cone?
In general, non-system tips tend to require increased forces for attachment to the tip cone. Many users literally »hammer« their pipette into the tip box; as a result, their pipette, as well as their wrist, will soon suffer damage.

TIPP

A cushion function built into the tip cone, such as those employed in spring-loaded cones, limits the amount of force you have to apply to attach the tip. Gently put the tip cone on the tip, slightly lower the pipette tip cone, and easily attach the tip to the tip cone. Done.

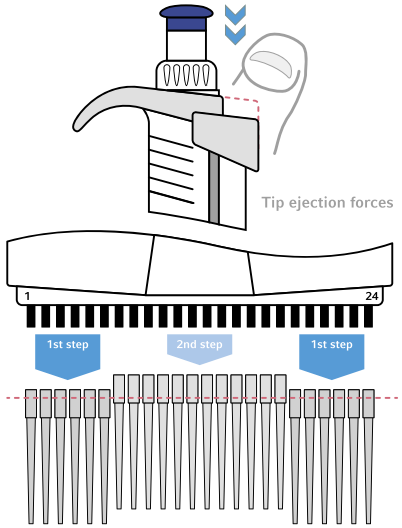
Extendable pipette tips reduce attachment and ejection forces
Pipette tips can contribute significantly to reducing attachment and ejection forces.



TIP

Use tips with elastic forming grooves, which expand when fitted to the tip cone and adapt exactly to the shape of the tip cone. A light press on the pipette is enough and the tip fits perfectly – without hammering. Used in combination with spring-loaded tip cones, this system ensures a perfect seal while at the same time reducing operating forces to a minimum.

Reduce ejection forces in multi-channel pipettes
The more channels a multi-channel pipette features, the greater the total ejection forces.



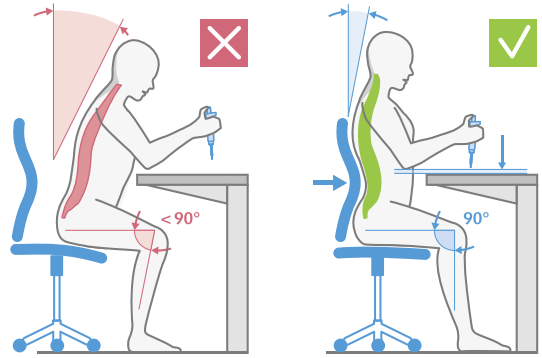
TIP

Use multi-channel pipettes with successive tip ejection. During a single tip ejection stroke, the outside tips eject before the inside tips. This will reduce your operating forces by 50%.

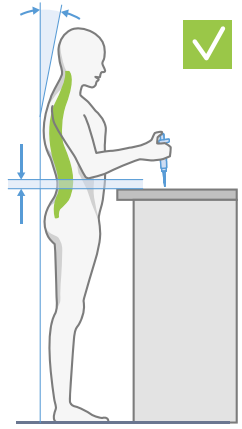
Ergonomic pipetting

Do you always think about good posture?
In reality, correct sitting and standing depend on the particular task as well as the given laboratory furniture. Here are some recommendations that will help you reduce tension even further:

Tips for correct sitting
> Adjust the height of the chair, with the knees at a 90° angle
> Chair with backrest – the lower back is in contact with the backrest
> Don't perch on the end of the seat – use the entire chair!
> Straight neck and back



Tips for correct standing
> Use sit-stand aid
> Workbench at least waist-high
> Straight back
> Slightly bent neck
> Flat shoes
> Elbows slightly higher than the table



Do you schedule breaks and stretching exercises?
Insufficiently stretched muscles can result in stiffness, fatigue and restricted movements in the long run. Stretching increases blood circulation and thus the transport of oxygen, waking you up with an extra boost of energy. The overall benefits of exercise include the improvement of flexibility and range of motion.

Only a few minutes of rest per day is sufficient. Once in the morning, after lunch, and in the afternoon. Also, relaxed muscles reduce the risk of developing RSI.

TIP

Ergonomic environment

Do you care about an optimal work environment?
Temperature, lighting, noise and air quality can contribute to a comfortable workplace. Those factors are largely determined by the design of the lab itself and to some extent by the placement of equipment.

Find more information at:
https://eppendorf.group/ergonomics-environment

Are you concerned with ergonomic workflows?
Use a personal set of pipettes, store small devices that are not in constant use in drawers, keep the necessary equipment and consumables within easy reach, and keep your personal workspace clean and tidy.

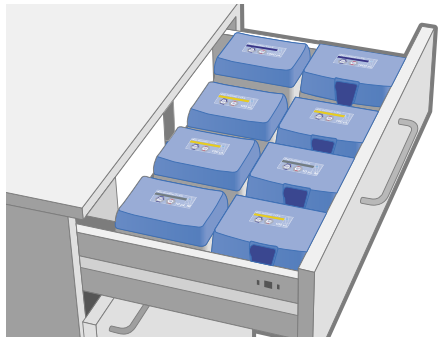
10.2.2. Relax your mind

Intuitive products that are easy to use reduce stress levels, save valuable time, and can contribute to »peace of mind«.

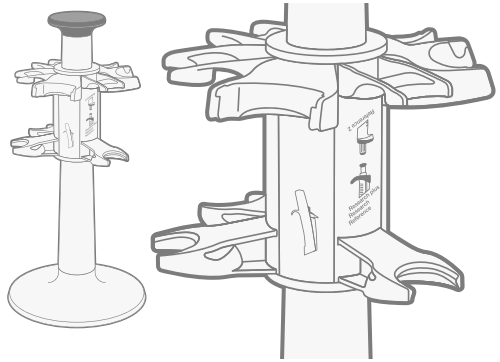
Colors help recognize volume classes in an instant
Volume classes can be identified quickly and easily with a standardized specific color code on pipettes.
For example, blue indicates a pipette of the volume class of 1,000 µL, whereas yellow indicates the volume class of 100 µL.

TIP
Use pipettes with color-coding.

Identify the correct tip at a glance
Standardized color codes on tip boxes, which are matched to the color codes of the respective pipettes, allow easy and quick identification of the corresponding volume class.



Pictograms for easy organization in the carousel
The pipette holding systems within a pipette carousel are designed for the respective pipette types (air cushion, positive displacement, electronic and mechanical pipettes). Forcing a pipette or dispenser into the wrong holder can damage both in the long run.



TIP
Clear pictograms attached to the holders help find the correct and safe position for your devices.

Intuitive software programs in electronic pipettes
Every electronic pipette programming is slightly different. Some require IT training, while others can be programmed without opening an instruction manual.

TIP
Setting up an electronic pipette should be simple and intuitive – science is challenging enough.

10.3. The Eppendorf PhysioCare Concept®

The ideal ergonomic laboratory product concept offers ergonomic product design, optimal integration into an ergonomic workplace, and the ability to optimally integrate into an entire laboratory workflow. Therefore, ergonomics is created by a holistic concept based on three areas to reconcile laboratory workflow with health and well-being. There are three spheres in the lab: Each of the three spheres has different ergonomic foci and requirements.

Sphere 1 – The user

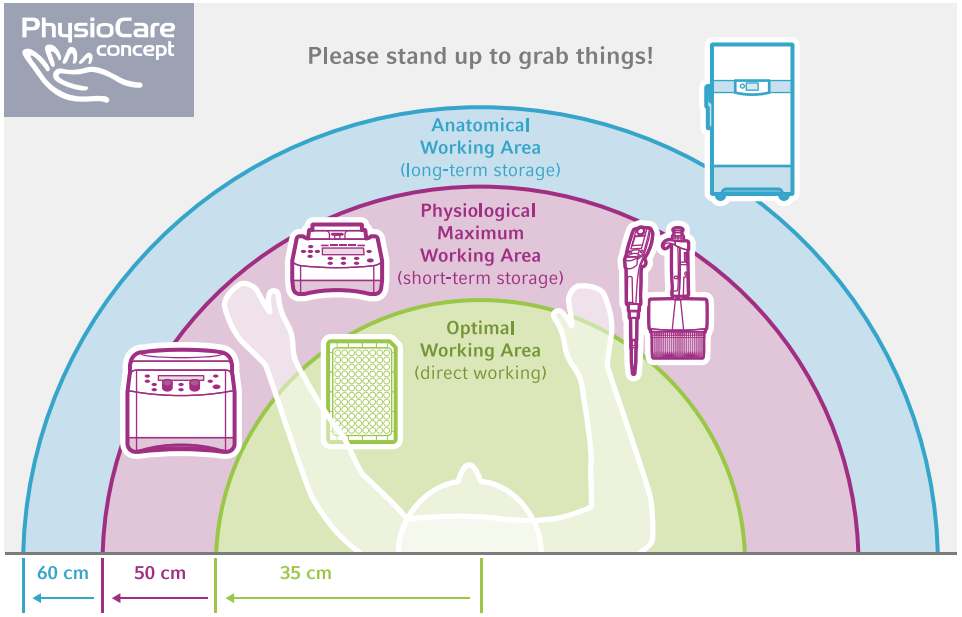
Sphere 1 is your immediate work environment. The equipment should be selected based on your needs concerning ergonomic product design and performance.

Sphere 2 – The bench

Sphere 2 comprises medium-term storage products. It aims to integrate products in harmony with the specific requirements of the workplace to create an ergonomic set-up of your personal bench for an ergonomic workflow.

Sphere 3 – The workflow

Sphere 3 comprises instruments and storage products within the entire laboratory. The goal is to design your complete workflow processes ergonomically to improve your work in the lab and thus the results achieved by the entire company or institute. The third sphere also includes soft aspects like training or documentation.



More information:

The Eppendorf PhysioCare Concept® – 3 Spheres Model
https://eppendorf.group/userguide-46

Make Your Lab a Better Place - The Eppendorf PhysioCare Concept
https://eppendorf.group/physiocare-video

11. Glossary & weblinks

11.1. Technical terms and abbreviations

Aerosol
A »mist« of very fine droplets of liquid which remains in the air, e.g. when thrown up from the surface of a liquid. Aerosols can be produced in the pipette when pipetting with piston-stroke pipettes (> contamination). Positive-displacement pipettes rule out aerosol formation in the pipette.

ATP
Adenosine triphosphate. One of the four components of RNA (> nucleotide), and a compound which allows the cell to store energy chemically and release it when needed. ATP thus serves as the »source of energy« to the cell for all metabolic processes.

Autoclaving
A physical method of sterilization. A thermal process used to destroy microorganisms and inactivate viruses as well as most enzymes. Pipettes to be autoclaved are stored in a pressure vessel at 121 °C at an overpressure of 100 kPa in water vapor for 20 min. DNA is not always entirely destroyed by autoclaving.

Carry-over effect
In the context of the PCR technique, it is the carry-over of DNA from one sample to the next; possible cause of false positive results.

Contamination
Soiling, exposure of objects, e.g. to microorganisms, radioactive sub stances, biologically active compounds.

DNA
Desoxyribo**n**ucleic **a**cid, also known as genetic material. Consists of four different components, the > nucleotides. DNA is the carrier of the genetic information in all living creatures. This information is found in all living creatures encoded according to the same system (the genetic code is universal).

DNase
DNA catabolic enzyme.

ELISA
Enzyme-**L**inked **I**mmuno **S**orbent **A**ssay. A »sandwich« ELISA operates according to the following principle: the surface of microtiter plates is coated with antibodies to the protein to be detected. The protein bound to this is detected by a second enzyme-marked antibody; the enzyme linked to the second antibody enables a dye reaction.

Endotoxin
Part of the bacterial cell wall of germs, it causes symptoms of illness such as fever (> pyrogens).

Gene
Scientific term for a unit of information of the genetic material. A gene contains the information for an > RNA or a protein (> DNA).

Gravimetric volume check
Determination of the mass of a pipetted volume. From the mass m of a liquid, the value for the density p of this liquid at a specific temperature T is used to calculate the associated volume. The volume V is calculated according to the for mula V = m/p(T).

Liquid handling
The handling, transport preparation of liquid samples.

Nucleotides
Components of nucleic acids (> DNA and > RNA) consisting of three components: a sugar molecule, a phosphate and a base. When abbreviating the names of nucleotides, generally only the initial letters f the bases are used (A, C, G, T, U). Nucleotides are required as reagents, e.g., for sequencing and with > PCR.

Nucleic acid
Collective term for > DNA and > RNA.

PCR
Polymerase **C**hain **R**eaction: cyclic process typically involving three different temperature steps with the aim of reproducing > DNA. A typical PCR can produce 10^9 to 10^{12} molecules starting, theoretically, from only one DNA molecule.
(PCR is a term that has been patented by the Hoffmann-La Roche company.)

PCR inhibitors
Substances inhibiting > PCR. Laboratory articles which are used for PCR must therefore be free of these inhibitors. This is particularly important with the amplification of tiny amounts of genetic material and with quantitative PCR.

Pipetting
The conventional pipetting method is used for the majority of all applications in the field of liquid handling. Here, the push-button of the pipette is pressed to the first pressure point (measuring stroke), the pipette tip is immersed a few millimeters vertically in the liquid and the liquid is drawn into the tip slowly and evenly by allowing the push-button to return in a controlled manner. To dispense liquid, the pipette tip is held against the wall of the vessel and the push button is then pressed to the first pressure point. Any remaining liquid is then blown out by pressing the button to the second pr point (blow-out).

Positive displacement
The positive displacement principle is based upon the concept that a pipette tip contains a moveable piston that contacts the liquid being used.

Pyrogens
Heat-stable substances (glycoproteins) contained in the outer membrane of bacteria and other microorganisms, causing fever in humans and inhibiting growth in cell cultures.

Reverse pipetting
In contrast to conventional pipetting, when aspirating liquid with reverse pipetting, the button is pressed to the second pressure point and a greater volume is aspirated into the tip. However, when dispensing liquid, pipette blow-out is not used;
the residual liquid remains in the tip. This technique offers benefits in terms of accuracy and precision depending on the nature of the pipetting liquid. It may be useful when liquids with a high viscosity or liquids that tend to produce foam or bubbles are to be pipetted.

RNA
Ribonucleic acid.

RNase
Enzyme that breaks down > RNA into the individual components, i.e. catabolizes it. RNase can be found on the surface of the skin, particularly on the hands. The enzyme RNase is extremely stable and can only be destroyed by autoclaving or treatment with soda solution (1 M).

Sterility
By definition, a sterile product has no living organisms on its surface. Sterility must be defined as the probability of an unsterile object occurring in a quantity of sterile objects. A probability of 1 to 1 million for residual contamination with living microorganisms following sterilization is seen as an adequate degree for reliable sterilization. This corresponds to a SAL (**S**terility **A**ssurance **L**evel) of 10^{-6} which means: in a sample of 10^6 (1 000 000) microorganisms, one single microorganism will survive, or in a quantity of 10^6 objects, there is only one single unsterile object following sterilization. For sterilization using β-irradiation, many regulations provide for a radiation dose of 25 kGy (kilogrey) to achieve a SAL of 10^{-6}. This also applies, e.g. to medical devices which come into direct contact with the human body (in vivo).

Sterilization
is understood to mean the destruction of all forms of microorganisms capable of living and reproducing.

Titration
Determination of the amount of a dissolved substance by adding another substance drop by drop (e.g. neutralization of lye with an acid).

UV light
Ultraviolet light: Light with a wavelength of approx. 250 nanometers used for > sterilization.

11.2. Weblinks

www.eppendorf.com/liquidhandling
Information on the liquid handling product portfolio from Eppendorf.

www.eppendorf.com/consumables
Information on pipette tips and other laboratory consumables from Eppendorf.

www.eppendorf.com/service-support
Information on pipette services and support from Eppendorf.

www.eppendorf.com/physiocare
Information on a holistic solution to harmonize workflows in the lab with your health and well-being.

www.eppendorf.com/applications
Articles, white papers and userguides on pipetting tools and techniques.

www.eppendorf.com/SOP
Standard operating procedure for Eppendorf pipettes and multi-dispensers.

www.eppendorf.com/certificates
General and Lot-number-specific quality certificates from Eppendorf.

www.eppendorf.com/sustainability
Information on sustainability at Eppendorf.

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