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What Really Matters: Manual Liquid Handling Tools for Healthcare Applications

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Abstract

Do you work in healthcare and pick up a pipette every day to perform your analyses? Have you ever stopped and thought about your particular choice? Could your selection have an impact on your analyses' results? The answer to that question is simple and clear: Yes! When you have chosen a pipette, you have decided if you will move the liquid mechanically or electronically. You have also decided to apply one of two general operating principles for liquid handling tools – two principles that can't be equally applied for all liquids. And the decision you've made governs how you'll be able to clean and decontaminate your liquid handling tool. In addition, the design and properties of the pipette or dispenser you have selected will determine its resistance to the chemicals you will use for your analyses. In short, you have several issues to consider when choosing a liquid handling system to achieve success when you perform diagnostic tests that involve dispensing blood or biological, immunological, and MALDI-TOF analyses. Due to the sheer diversity of requirements for performing analyses, no one liquid handling tool meets all the requirements for every experiment. But carefully assessing the requirements for performing an analysis makes it easier to choose the most appropriate tool for your particular application. This article summarizes analysis requirements and provides guidance on selecting the right tools and routines for contributing to the reproducibility of diagnostic analyses in the work you do each day in the laboratory.

Introduction

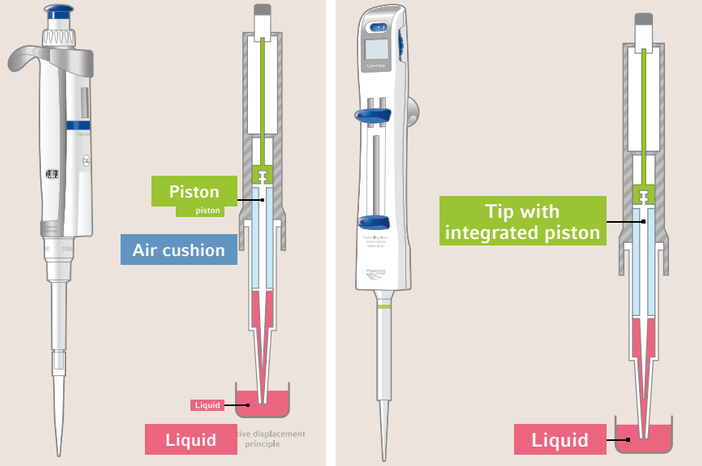
Diagnostic work in healthcare includes applying various methods of analysis. The commercial kits typically used in this work sometimes place very high demands on liquid handling systems, but these must be met for the diagnostic analyses to be successful. These demands include the liquid handling system's dosing accuracy, its decontamination properties, and its chemical resistance as well as the prevention of contamination and leachables in the samples. These issues are considered in table 1 for various healthcare-related analyses. The analyses and detections use a wide variety of sample materials that result in key method-specific issues related to the requirements for liquid handling systems.

Table 1: Various healthcare-related analyses' requirements for liquid handling systems.

Requirement	Blood dosage	Molecular biological analyses	Immunological analyses (quantitative ELISA)	MALDI-TOF analyses
Dosing accuracy	(x)	x	x	
Contamination prevention	x	x	x	(x)
Decontamination	x	x	x	x
Chemical resistance		x	x	x
Leachables			x	x

In accordance with ISO 8655 [1], the pipette or dispenser and tip are to be understood as a complete system – hereinafter referred to as the pipetting system. Liquid handling tools in general can differ in their handling, which can be mechanical or electronic, and in their operating principle, which can involve an air-cushion system or a positive displacement system (see information box 1). Both the handling and the functional principle influence implementation of the analyses considered here. This means that when you choose a liquid handling tool for specific healthcare diagnositic work, you always need to balance the key requirements of the analysis. The following discussion, including the overview of Eppendorf product recommendations is intended to help you to make the right choice.

INFORMATION BOX 1



Air-cushion pipettes and positive displacement systems

Liquid handling systems work according to two different functional principles: using an air-cushion or by positive displacement. Air-cushion piston-stroke pipettes have air between the liquid in the tip and the piston in the pipette with the air operating like a spring. In positive displacement instruments, the piston is integrated into the tip and comes into direct contact with the liquid. The sample is hermetically sealed within the tip. While air-cushion pipettes are most suitable for dosing aqueous solutions, positive displacement systems support the transfer of challenging solutions that differ physically from water.

Dosing Accuracy

How accurate are your diagnostic analyses' pipetting results? In addition to the pipetting technique you use (see information box 2), dosing accuracy strongly correlates with the pipetting system's performance, control over the liquid's movement, and the type of liquid handled.

Ensuring the Pipetting System's Performance

Liquid handling tools and their tips should always be understood as a system in the sense of ISO 8655, which means the tools and their tips should be regularly checked and calibrated together as a system. Air-cushion system pipette tips can have a considerable influence on dispensing results [2]. The tip's shape, for instance, influences the size of the air-cushion and thus the dispensing accuracy of the system. Manufacturers adjust their pipettes to meet the limit values that are valid for the specific tip type used. They also clearly define the test tip in the operating manual or in the technical data for the liquid handling tool. If pipettes are sent to a calibration laboratory (as, for example, Eppendorf offers through its epServices) to be checked without further specifying, the pipettes will be calibrated according to ISO 8655 [1] or DKD R 8-1 [3] with the original tips designated by the pipette manufacturer. If, however, you use alternative pipette tips for your analyses, you may discover large deviations from the calibration results due to the different tip shapes (see figure 1). You cannot apply the results from a calibration with original tips to the systematic and random measurement deviation of your alternative pipette tips. It is therefore better to use the original tips from the manufacturer of your liquid handling tool. Otherwise, you will also have to have your pipetting system tested with the alternative pipette tips from your laboratory according to ISO 8655 and have the system adjusted to them if necessary [1]. In addition, you should also check the quality of the alternative pipette tips you use to see if they vary within a box or among different batches in order to ensure reproducible results.

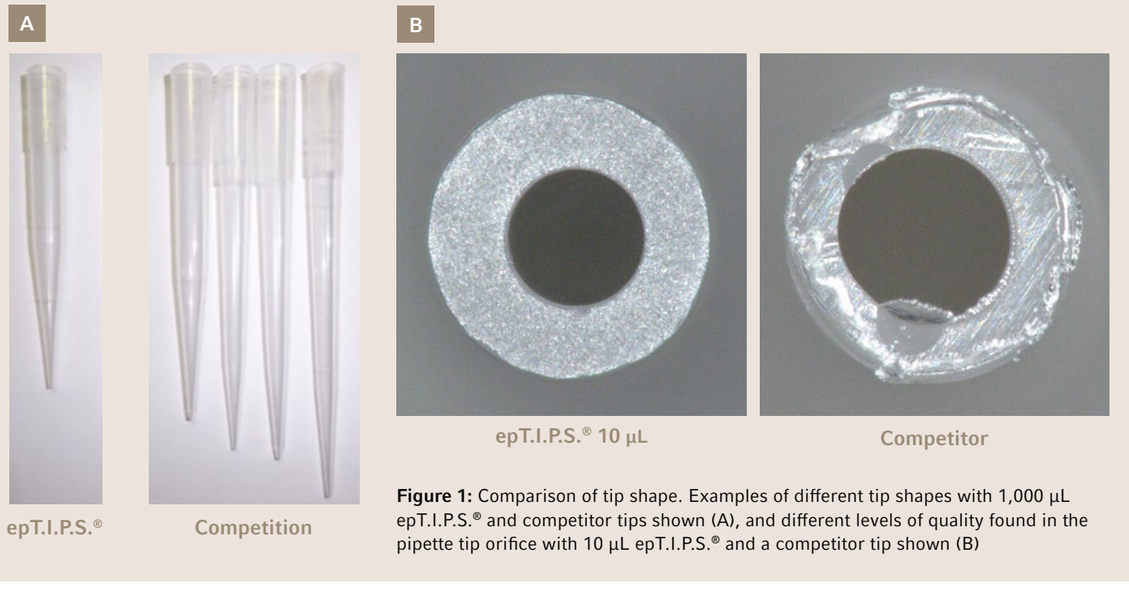


Figure 1: Comparison of tip shape. Examples of different tip shapes with 1,000 µL epT.I.P.S.® and competitor tips shown (A), and different levels of quality found in the pipette tip orifice with 10 µL epT.I.P.S.® and a competitor tip shown (B)

Control Over the Liquid Movement

Does the removal of individual phases after sample centrifugation sometimes cause you trouble? Are you concerned about splashes or cross-contaminations from multiple liquid aspirations and releases? If so, take a critical look at the liquid movement! Maintaining maximum control over the liquid's movement with the liquid handling tool not only influences pipetting accuracy, it also facilitates removing individual phases after centrifugation without stirring up other phases. Make sure the liquid handling tool is light in your hand, requires low operating forces, and has optimal stroke distance. And just what is stroke distance all about? If the stroke distance is too short, a large volume of liquid will be moved with minimum operation of the control knob, which leads to inaccurate liquid uptake. The resulting high aspiration speed can unintentionally stir up another phase. If the stroke distance is too long, the ergonomics, and thus control, also suffer because your thumb has to cover a long distance and will tire easily.

Accurate Dispensing of Challenging Liquids

Due to its operating principle, the ideal liquid handling system for accurately handling liquids with physical properties different than water in healthcare analyses (e.g., foaming PCR mastermix, viscous blood, etc.) is a positive displacement system (e.g., Multipette®/Combitips®; see information box 1). You can, however, increase the accuracy of air-cushion systems to a level acceptable for many applications when you take electronic pipettes into account. Employing preprogrammed adjustments for certain types of liquid and a dedicated reverse pipetting mode or by managing settings for different types of liquid with connected pipettes using new tools like the VisioNize® pipette manager, an electronic pipette such as the Eppendorf Xplorer® could be your tool of choice as well.

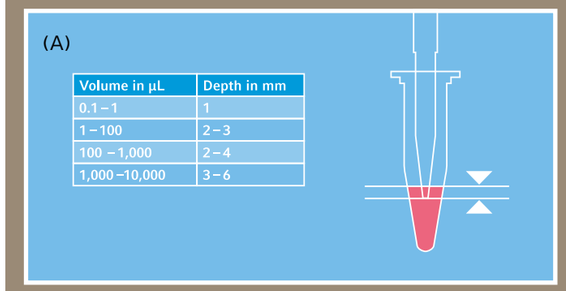
INFORMATION BOX 2

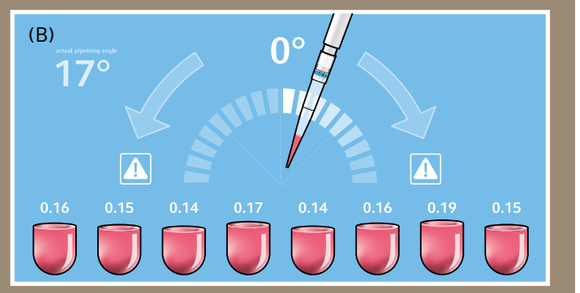
Good pipetting technique for air-cushion systems

Your pipetting skills form the basis for reproducible diagnostic results. Consider these points:

> Select a pipette with the smallest possible air-cushion for your pipetting volume because the systematic error of the pipette increases with the size of the air-cushion.
> Observe a small immersion depth of the tip into the liquid you're pipetting (A). The ISO 8655 provides recommendations.
> Hold the pipette vertically during liquid aspiration (B).
> Pre-wet the tip with the liquid you're going to pipette in order to saturate the air-cushion and moisten the inner surfaces of the pipette tip.
> Make sure to aspirate slowly and evenly.
> Wait a moment (one to three seconds) for the liquid to rise in the tip – even longer for large volumes (≥ 5 mL).
> For liquid dispensing, place the tip against the wall or immerse it into the liquid in the target vessel.

Tip: Consider a positive displacement system – it's less prone to pipetting errors!





Contamination prevention

Contamination in healthcare diagnostic work means a loss of both time and money. Your analyses have to be interrupted and the laboratory decontaminated at great expense. In addition, you must document when the contamination occurred and whether it led to false-positive or false-negative diagnostic results. And, of course, the potential impact on patient treatment and the loss of reputation associated with a recall cannot be quantified. All reasons enough to focus on the prevention of contamination – it will pay off in the long run!

Contamination can occur with any liquid movement: If not through direct liquid transfer (such as splashing or touching a vessel wall), then through aerosols, which can transport contaminating or even infectious particles. These particles are then drawn into the pipette cone during the pipetting process and settle there. During the next dosing, they can be mobilized again and distributed through the air stream (see figure 2).

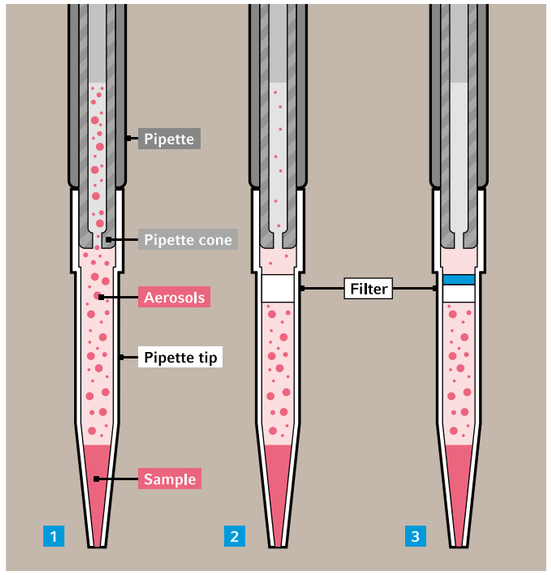


Figure 2: Aerosol formation in the air-cushion system and the protection provided by filter tips
Aerosols containing contaminating particles can form during any liquid movement. These rise with the air flow into the pipette cone and settle there (1). During the next dispensing, they can be mobilized again and distributed through the air flow. Single-layer filter tips can partly block particles and molecules (2), while filter tips with two layers provide a reliable barrier (3).

The key therefore is this: Avoid this risk altogether by using a positive displacement system. It does not contain an air-cushion in which aerosols can accumulate.
This system employs hermetically sealed dispensing tips so aerosols cannot enter the device and thus endanger subsequent liquid dispensing.

If you use a pipette with an air-cushion system, the following applies: The slower your dispensing speed, the lower the aerosol formation. With mechanical pipettes, it's too easy to work too fast. Take advantage of electronic pipetting systems such as the Eppendorf Xplorer® family: Select suitable dispensing speeds for the motor-operated liquid aspiration and dispensing to control the movement of liquid. If you use mechanical pipettes, consider a single-button-operated pipette such as the Eppendorf Reference® 2, which reduces the risk of contamination thanks to its mode of operation: You eject the pipette tip immediately after dispensing by activating the same operating button. As a result, the piston – and with it the air stream – experiences only one upward movement, which minimizes aerosol formation and thus the risk of contamination. Another sensible step to preventing contamination is to use filter tips: Good filters trap splashes as well as the finest aerosols (see figure 2).

If you use filter tips, make sure to use ones that have a filter efficiency of class E12 according to EN ISO 1822 (equivalent to a filter efficiency of class ISO 25 E according to DIN EN ISO 29463-5) such as the ep Dualfilter T.I.P.S.® and ep Dualfilter T.I.P.S.® SealMax® [4]. If you do not use filter tips, make sure you regularly clean the pipettes.

In addition, when you purchase pipette and dispenser tips that have the degree of purity you require you need to expect that the manufacturer will maintain the promised degree of purity. Make sure the manufacturer does not only test their consumables internally but rather via an external, preferably ISO 17025 accredited, laboratory. Accredited laboratories are under the supervision of an competent authority and offer full traceability of each test result. Certificates confirming purity should ideally show detection limits as well as be lot specific and easily obtainable from the manufacturer's website (e.g., www.eppendorf.com/certificates). If you only need a few pipette tips, you may find it makes more sense to use individually sterile-packed pipette tips like epT.I.P.S.® singles or single-blistered Combitips® advanced rather than potentially contaminating the rest of a tip box. Taking these steps will help you, the user, to ensure maximum security and documentation.

Decontamination
Decontamination of used materials through autoclaving and chemical disinfection contributes significantly to contamination prevention in healthcare diagnostic work. Autoclaving does, however, only eliminate living organisms; it does not destroy other contaminating molecules from these organisms (e.g., nucleic acids) that can negatively impact further testing such as molecular biological analyses. For this reason, a combination of both autoclaving and chemical disinfection is the most effective form of decontamination. This approach cannot, however, be used for all liquid handling tools due to the tools' properties and materials. The following is recommended:

> Select pipetting systems (pipette/dispenser, tips, boxes) with as many autoclavable components as possible. It is also helpful if you can autoclave these components as one piece.
> Use liquid handling tools made of materials resistant to common decontamination agents.
> Choose liquid handling tools with covers for large openings (e.g., for temporary adjustments, USB ports, etc.) and as few recesses or gaps as possible.
> Prefer wipe disinfection to avoid inhalation of aerosols during spraying and to ensure effective decontamination through complete wetting. Keep in mind that displays are often less resistant to aggressive decontaminants. In the case of spray disinfection, always wipe after spraying [5].
> If your routine protocol calls for chemical disinfection followed by autoclaving, be sure to wipe the materials with a water-soaked tissue before autoclaving. If you do not wipe, substances from the disinfectant will remain on the surface. Many decontamination agents contain catalysts. If energy is applied (e.g., UV radiation under the clean bench or heat in the autoclave), these agents can trigger severe reactions on the surface and cause the surface material to roughen, which in turn will reduce the efficiency of future decontaminations.

Wherever possible, Eppendorf incorporates materials in its liquid handling tools that are both autoclavable and resistant to as many of the aggressive chemicals commonly used in laboratories as possible. This means you can fully autoclave all mechanical air-cushion pipettes from Eppendorf (e.g., Reference® 2 and Research® plus) without having to disassemble them. These pipettes also have a very broad chemical resistance to common decontamination agents as their surface contains PTFE – a very robust, durable material that also reduces the adhesion of contaminating particles. Other liquid handling tools do, however, reach their limits in this regard. In particular, liquid handling tools with built-in electronics, displays, or other technology are more sensitive and therefore you can only autoclave selected parts or not at all. You can, though, easily detach all the lower parts (even those with adjustable cones) of Eppendorf Xplorer® (plus) electronic pipettes and autoclave them. The use of (aggressive) decontamination agents on electronic devices is also somewhat limited, as vapors or entering liquid can damage the electronics, displays, or other manufactured materials. In these cases, alcohol (isopropanol 70 %, ethanol 70 %) is recommended for decontamination.

Chemical Resistance
Decontamination is not the only factor that plays a role in requiring pipetting systems to be resistant to chemicals. The reagents commonly used in such healthcare diagnostic work as ELISA and MALDI-TOF analyses – chemicals like hydrochloric acid, sulfuric acid, phosphoric acid, and organic solvents – corrode metals. For this reason, Eppendorf strives to limit the addition of metals to pipetting systems: Where possible, Eppendorf does not employ metal for pistons, but instead uses plastics that are robustly resistant to chemicals. Also, metals should not be present in areas that may come into contact with splashes of liquid or its vapors (e.g., the tip cone and tip ejector sleeve). This is a feature of most models of the Eppendorf Reference® 2, Research® plus and Xplorer® pipette families (for detailed information on the various models, see table 1): Their lower parts do not contain any metals that could come into contact with liquid or its vapors. When considering the issue of chemical resistance, keep the following in mind: No material is resistant to all existing chemicals, their concentrations, and mixtures. Consult the chemical-resistance lists of manufacturers, which estimate the resistance of their equipment to chemicals [6-8]. As an alternative approach, Eppendorf offers a lower part with advanced chemical resistance – e.g., 100 % resistance to trifluoroacetic acid (TFA) – for the most frequently used volume ranges of the Eppendorf Reference® 2 and Research® plus single-channel pipettes. For these products, Eppendorf uses robust PTFE for the piston and piston seal. Due to other material components, these lower parts are not autoclavable however. Positive displacement systems, on the other hand, offer you the option of transferring aggressive substances in hermetically sealed pipette tips [9]. In any case, you should use liquid handling tools with built-in electronics, displays, or other sensitive technology carefully around chemicals: Aggressive chemical vapors, in particular, can damage sensors and electronics.

If you increasingly dispense aggressive chemicals, have your pipettes and dispensers serviced more frequently. Especially for pipettes, regularly cleaning the internal lower part can prevent crystals from forming in the piston area due to chemical vapors, thus ensuring the integrity of the piston seal for a longer period of time. ISO 8655 also recommends shortened maintenance and inspection intervals when working with aggressive liquids [1].

Leachables
Pipette tips are made of polypropylene, which has good chemical resistance. Chemicals, and especially organic solvents, can, however, cause the transfer of leachables – plastic additives added to safeguard certain product functions and to simplify or even reduce the cost of manufacturing it. However, leachables can significantly interfere with analyses [10]. When using pipette tips, a cumulative effect can occur if protocols require, for example, mixing the sample using multiple up and down pipetting steps, which significantly increases the contact time of the pipette tip and sample compared with single dosing [10]. For this reason, manufacturers should reduce the amount of additives used in their production of plastic articles to the minimum necessary to ensure certain properties of the product such as stabilizers for durable resistance. They should altogether avoid using additives that make production easier and thus cheaper. These dispensable additives include plasticizers, biocides, and slip agents. Eppendorf deliberately allows for higher production costs, completely dispensing with these three groups of additives, and certifies this.
Eppendorf reduces all other additives necessary for production to a minimum, so Eppendorf consumables do not interfere with biological analyses [2,11]. When you buy tips and other consumables, make sure the manufacturer of the products you select can prove they do not use any of the above-mentioned dispensable additives and keep all other additives, including trace metals, to a minimum.

Summary

You should consider a number of factors when you select manual liquid handling tools for healthcare diagnostic work. Ask yourself what is most important in your current analysis: Is it dispensing accuracy? Minimizing contamination risk? Or are you transferring mainly aggressive substances and you want to protect yourself and the liquid handling tool from them? Diagnostic analyses often have several of these requirements; prioritize them and choose the liquid handling tool most appropriate for your analysis. Also consider whether your analysis is qualitative, semi-quantitative, or quantitative; in many cases, there is no one single tool that meets all the requirements for blood dosing or even molecular biological, immunological, and MALDI-TOF analyses. And when the time comes for you to address these challenges and select the best liquid handling tool to meet your requirements, know that Eppendorf is there to support you with its wide range of products for healthcare diagnostic work (see table 2).

Eppendorf product solutions
The table summarizes the requirements for dosing accuracy of liquid handling systems, their decontamination and chemical resistance, and the prevention of contamination and leachables in the samples for various healthcare applications. Recommendations for individual Eppendorf products are noted for the respective key aspects. Select the appropriate Eppendorf liquid handling tool based on your application and associated priorities.

Tab. 2: Eppendorf Manual Liquid Handling product recommendations for healthcare applications.

Requirement	Key aspect	Recommended Eppendorf product		Healthcare diagnostic work			
		Liquid handling system: Air-cushion	**Liquid handling system: Positive displacement**	**Blood Dosage**	**Molecular Biological Analyses**	**Immunological Analyses (Quantitative ELISA)**	**MALDI-TOF Analyses**
Dosing accuracy	**Maintenance and calibration:** Close-meshed inspection ensures high dosing accuracy.	> epServices for all pipettes	> epServices for all Multipette® multi-dispensers				
	Manual control of liquid movement: Smooth and balanced stroke of the operating button allows precise dispensing control.	> Eppendorf Reference® 2 family > Eppendorf Research® plus family	> Multipette® M4				
	Electronic control of liquid movement: Electronically controlled liquid aspirations and dispensings at predefined speeds allow maximum control.	> Eppendorf Xplorer® family	> Multipette® E3(x)				
	Challenging liquids: Increased accuracy for safe transfer of challenging liquids (foaming, viscous, aggressive)	> Eppendorf Xplorer® family with liquid adjustment > Eppendorf Xplorer® family with liquid types managed via VisioNize® pipette manager	> Multipette® M4 > Multipette® E3(x)				
	Liquid handling device and tips are a system: Using original pipette or dispenser tips enhances the reproducibility of pipetting results with maximum precision and accuracy.	> epT.I.P.S.® pipette tips	> Combitips® advanced dispenser tips				
	Dispenser tips: Integrated piston wipes the liquid from the inner surface of the tips during dispensing.	*not applicable due to functional principle (air-cushion)*	> Multipette® M4 > Multipette® E3(x)				
Contamination prevention	**Aerosol accumulation in the pipette cone:** Single-button operation of pipettes reduces aerosol-carrying air flow into the pipette cone.	> Eppendorf Reference® 2 family	*not necessary: sample is hermetically sealed within Combitips® advanced due to functional principle (positive displacement)*				
	Dispenser tips: Sample is hermetically sealed within the dispenser tip without aerosol formation.	*not applicable due to functional principle (air-cushion)*	Combitips® advanced for > Multipette® M4 > Multipette® E3(x)				
	Long-distance pipette tips: Select tip shape according to the vessel (e.g. Vacutainer®) to ensure easy access to your sample when working with deep, slim vessels.	> epT.I.P.S.® 5 mL L > epT.I.P.S.® 1,250 µL L	*not available*				
	Tips wrapping: Using individually sterile-packed tips helps to avoid contaminating the rest of a tip box.	> epT.I.P.S.® Singles	> individually blister-wrapped Combitips® advanced				
	Pipette filter tips: Filters of EPA class 12 according to ISO 1822 (equivalent to ISO 25 E according to DIN EN ISO 29463-5) prevent the entry of aerosols and biomolecules into the pipette cone.	> ep Dualfilter T.I.P.S.® > ep Dualfilter T.I.P.S.® SealMax®	*not necessary: sample is hermetically sealed within Combitips® advanced due to functional principle (positive displacement)*				
	Manual control of liquid movement: Smooth and balanced stroke of the operating button allows precise dispensing control.	> Eppendorf Reference® 2 family > Eppendorf Research® plus family	> Multipette® M4				
	Electronic control of liquid movement: Electronically controlled liquid aspirations and dispensings at predefined speeds allow maximum control.	> Eppendorf Xplorer® family	> Multipette® E3(x)				
	Purity of pipette and dispenser tips: Purchasing tips in required and externally certified purity from manufacturers ensures sample safety.	> epT.I.P.S.® in: PCR clean, PCR clean and sterile > ep Dualfilter® T.I.P.S. in: PCR clean and sterile > ep Dualfilter® T.I.P.S. SealMax® in: PCR clean and sterile	Combitips® advanced dispenser tips in > PCR clean > Biopur				
Decontamination	**Pipette/Dispenser autoclavable**	> Eppendorf Reference® 2 family > Eppendorf Research® plus family > All lower parts of Eppendorf Xplorer® family	*not applicable*				
	Pipette/Dispenser tips autoclavable	> epT.I.P.S.®	*not applicable*				
	Tips box/rack autoclavable	> epT.I.P.S.® Box (2.0)	> Combitips® advanced Rack (without consumables)				
	Using decontamination agents: Broad chemical resistance to common decontamination agents facilitates decontamination of devices.	> Eppendorf Reference® 2 family > Eppendorf Research® plus family > All lower parts of Eppendorf Xplorer® family	Decontamination with alcohol recommended > Multipette® M4 > Multipette® E3(x)				
	Advanced surface robustness: PTFE in surfaces strengthens cleaning and decontamination properties.	> Eppendorf Reference® 2 family > Eppendorf Research® plus family	*Surface does not contain PTFE*				
	Smooth surface: Surface without interrupted surfaces or recesses enable easy and effective wipe disinfection.	> Eppendorf Reference® 2 family	*not applicable*				
Chemical resistance	**Robust chemical resistance**	> Eppendorf Reference® 2 family (1), [6] > Eppendorf Research® plus family (1), [7] > All lower parts of Eppendorf Xplorer® family	> Multipette® M4 [8]				
	Option: Advanced chemical resistance	Special lower part available with resistance to highly aggressive chemicals (e.g. TFA) > Eppendorf Reference® 2 variants (2) > Eppendorf Research® plus variants (2)	*not applicable*				
Leachables	**Certified absence of additives:** Plasticizer, biocides, slip agents cannot interfere with biological analyses.	> epT.I.P.S.® > ep Dualfilter T.I.P.S.® > ep Dualfilter T.I.P.S.® SealMax®	> Combitips® advanced				

> Light labelling: applicable, dark labelling: recommended
> Family includes all pipette variants (fixed and variable volumes, single- and multi-channel, Move It®)

(1) Variants > 20 µL without metal pistons, except 16- and 24-channel pipettes
(2) Available for Reference 2 and Research plus single-channel pipettes: 1,000 µL (color code: blue); 5 mL (color code: violet); 10 mL (color code: turquoise); available for Reference 2 single-channel pipettes only: 2.5 mL; 2 mL, fixed (color code for both: red).

References

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About Eppendorf

Since 1945, the Eppendorf brand has been synonymous with customer-oriented processes and innovative products, such as laboratory devices and consumables for liquid handling, cell handling and sample handling. Today, Eppendorf and its more than 5,000 employees serve as experts and advisors, using their unique knowledge and experience to support laboratories and research institutions around the world. The foundation of the company's expertise is its focus on its customers. Eppendorf's exchange of ideas with its customers results in comprehensive solutions that in turn become industry standards. Eppendorf will continue on this path in the future, true to the standard set by the company's founders: that of sustainably improving people's living conditions.

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