USERGUIDE No. 24

Cleaning and Inspection of Pipettes

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Abstract

Modern quality management in the laboratory calls for regular cleaning and inspection of liquid handling systems. Liquid handling systems need to be cleaned, decontaminated and inspected at varying intervals, depending on their usage. This article gives an overview of how to avoid contamination, presents a list of possible contaminants, and explains suitable cleaning and decontamination methods. The recommendations are relevant both for normal laboratory routines and for sending devices to a calibration lab.

Sources of contamination and contamination prevention

There are three distinct channels of contamination:
> From the sample to the pipette
> From the pipette to the sample
> From sample to sample (carryover)

The first form of contamination occurs when a liquid or its aerosols enter the pipette cone. In most cases, liquid enters the cone as a result of handling errors. For example, the user may attach a pipette tip that is too small for the set volume, or the user may press the operating button too quickly. In contrast, contamination from the pipette to the sample usually involves aerosols. Aerosols are movable matter, such as dust or tiny liquid drops, that can carry contaminants like nucleic acids. Aerosols may form whenever liquids are moved. Consequently, pipetting creates aerosols. Whether contamination occurs is dependent on the ultimate destination of these floating aerosols. It is vital to prevent these aerosols from trespassing into the pipette's cone.

Once an aerosol particle has entered the pipette cone, it may either attach to the inner surface or remain in the air cushion. The air movement involved in subsequent pipetting steps may push it down into the pipette tip and thus into the liquid within the tip, contaminating the next sample.
Filter tips prevent aerosols from entering the pipette's cone and piston/cylinder system (see Fig. 1). When filter tips are used, aerosols remain within the tip and are thus discarded along with the tip. It is important to use a filter tip that is proven to have a high filter efficiency.
As a second option, positive displacement systems methodically exclude the first two forms of contamination, as their tips feature a piston with an integrated leakage seal (see Fig. 1).
Aside from using special tips or systems, users can decrease aerosol creation in general by pipetting slowly.

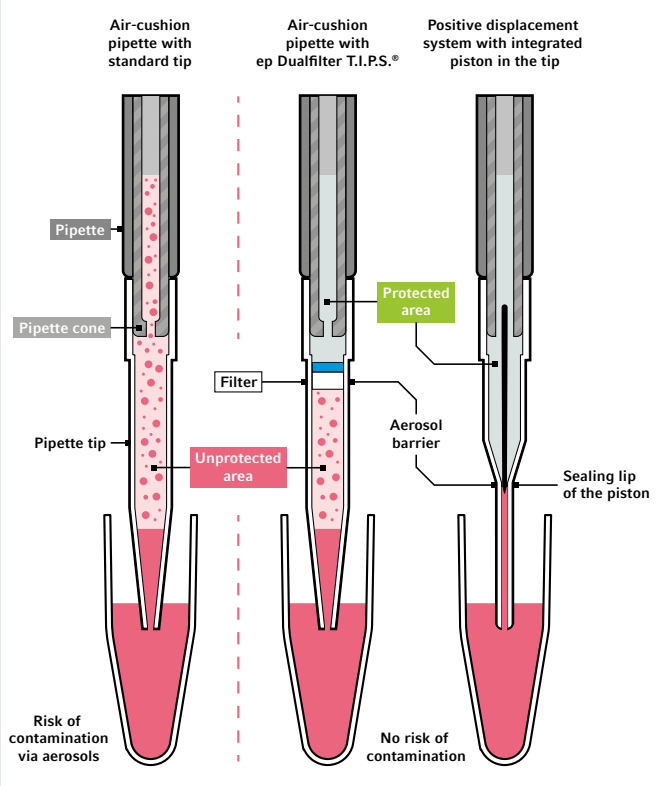

Figure 1: Filtered pipette tips with proven efficiency, such as the ep Dualfilter T.I.P.S.®, and positive displacement systems, such as the Multipette®/Combitips® advanced system, reliably prevent internal contamination of liquid handling instruments.

When not in use, storing air cushion pipettes in an upright position, ideally in a pipette carousel, makes it unlikely that aerosols will float into the cone.
Sample-to-sample contamination is the result of reusing pipette tips. This inadvisable practice causes sample contents to be carried over. For this reason, the tip needs to be changed following each pipetting. Dispensing quickly against a wall may also cause carryover, as it creates splashes that may, for example, hit the well of a plate and contaminate the next sample. Again, slow pipetting is preferable.

Decontamination and cleaning

As mentioned above, positive displacement instruments prevent contamination of the instrument's inner parts by their functional principle. In contrast, air cushion pipettes may require a more extensive cleaning regime, as the air cushion may transport aerosols into the lower part of the instrument. This chapter presents an overview of decontamination procedures.

Decontamination by reagents

Table 1 lists recommendations for handling contamination involving different kinds of liquids and other contaminants, with a special focus on air cushion pipettes. For more specific information about cleaning and decontamination, please refer to the operating manual of the respective liquid handling system. When using decontaminating reagents, please check the chemical resistance of the pipettes before starting the decontamination process.

External contamination can be removed by wiping off the instrument with a soap solution, isopropanol or suitable disinfectants/decontaminating reagents. When using aggressive reagents, please check the chemical resistance of the instrument's materials. For most liquid handling systems, a chemical resistance table is available on the Eppendorf website. The table lists the chemical resistance of specific instruments to the reagents most often applied in laboratories.
After decontamination, the reagent should be wiped off using a cloth soaked in distilled water. Wiping off the active molecules after decontamination is important for the longevity of the material's surface.

Internal contamination is more complicated. Liquids that have been accidentally absorbed should not be allowed to dry. The respective parts (e.g. cone, piston/cylinder) must be cleaned immediately, and if needed, the piston-cylinder system should be lubricated. Regular decontamination using reagents can be performed by immersing the respective parts into the decontaminating reagent. Afterward, the parts should be rinsed well with distilled water to wash off the active molecules, then left to dry. If needed, lubrication of the piston-cylinder system should follow. The respective instrument operating manuals give comprehensive, step-by-step instructions regarding how to disassemble, clean, lubricate and reassemble the instruments.

If **volatile organic liquids** are frequently used, the otherwise maintenance-free seal may swell up, causing the pipette to become stiff. In this case, the pipette should be left under the hood overnight to air out. In addition, inspection intervals should be shortened, and more frequent exchange of the piston seal should be scheduled.

When pipetting **saturated solutions**, the aerosol's precipitate inside the pipette may produce crystals that can destroy the seal. To avoid this, regular cleaning and greasing of the piston-cylinder system is recommended, as is regular inspection of the piston seal.

Table 1: Decontamination and cleaning

Substance classification	Handling, special features	Decontamination and cleaning
Aqueous solutions, detergents, buffers	Some of these liquids create foam; in this case the use of filter tips or a positive displacement instrument is recommended.	Rinse lower part with distilled water. Allow to dry at maximum 60 °C in dryer compartment. Lubricate piston-cylinder system as described in the operating manual.
Inorganic acids	Note the chemical resistance of the liquid handling system. Wipe away splashes immediately. Filter tips do not prevent vapors to enter the cone. Prefer a positive displacement instrument.	Vapors from acids may enter the lower part of air-cushion pipettes and affect the performance of the pipette. Clean as described above for "aqueous solutions".
Alkalis	Note the chemical resistance of the liquid handling system. Wipe away splashes immediately. Vapors may occur. Filter tips do not prevent vapors from entering the cone. Prefer a positive displacement instrument.	With some alkalis, vapor may enter the lower part of air-cushion pipettes and affect the performance of the pipette. Clean as described above for "aqueous solutions".
Potentially infectious liquids	To avoid contaminations, filter tips should be used or a positive displacement system applied.	Autoclave at 121 °C, 1 bar overpressure, 20 min. Or immerse the lower parts in disinfectants. Afterwards rinse well with distilled water and allow to dry at maximum 60 °C in a dryer compartment. Lubricate the piston-cylinder system if needed.
Cell cultures	To guarantee sterility, apply filter tips with respective purity grade.	Proceed as described above for "potentially infectious liquids".
Organic solvents	Note the chemical compatibility of the liquid handling system. Higher evaporation pressures will lead to dripping. Prewet thoroughly or, better, apply a positive displacement system.	Give time to evaporate from the piston seal. Additionally, immerse the contaminated parts in detergent, rinse well with distilled water and dry at maximum 60 °C in a dryer compartment. Lubricate the piston-cylinder system if needed.
Proteins	To avoid contaminations, filter tips should be used or a positive displacement system applied.	Open the pipette. Rinse with or immerse in detergent. Afterwards rinse / wipe off with distilled water and let dry at maximum 60 °C in a dryer compartment. Lubricate the piston-cylinder system if needed.
Nucleic acids	To avoid contaminations, filter tips should be used or a positive displacement system applied.	1) Use commercial decontaminating reagent if materials are chemically resistant. Apply as recommended by manufacturer. Rinse with distilled water and let dry at maximum 60 °C in a dryer compartment. Lubricate the piston-cylinder system if needed. 2) Decontaminate by sodium hypochlorite (max. 4%), rinse well with distilled water and dry at maximum 60 °C in a dryer compartment. Lubricate the piston-cylinder system.
Radioactive solutions	To avoid contaminations, filter tips should be used. A positive displacement system gives maximum security when handling this liquid type.	Open the pipette and place contaminated parts into a complex solution or a special cleaning solution. Rinse well with distilled water and dry at maximum 60 °C in a dryer compartment. Lubricate the piston-cylinder system if needed.

Autoclaving

The autoclaving of air cushion pipettes and pipette tips is generally performed at 121 °C with an overpressure of 1 bar (100 kPa) for a period of 20 minutes. Filter tips and dispenser tips should not be autoclaved; instead, they should be purchased in the required purity grade. Eppendorf mechanical pipettes are fully autoclavable. Due to the electronic components inside Eppendorf electronic pipettes, only the lower part of each pipette can be autoclaved. Aside from removing the lower part, no further disassembly is required for autoclaving. Multipette® pipettes are generally not autoclavable due to their electronic components.
After autoclaving, the autoclaved pipette or lower part of the pipette must be allowed to dry and cool to room temperature. With Eppendorf pipettes, it is not necessary to lubricate the piston-cylinder system after autoclaving.

Ultra-violet light

Eppendorf pipettes and other liquid handlers are resistant to UV irradiation when certain criteria are met. A 30-watt low-pressure mercury-vapor lamp with a characteristic wavelength of 254 nm is required. The optimum distance between the lamp and the pipette is approximately 60 cm. Smaller distances raise the level of irradiation per cm^2 unfavorably.

Decontamination before shipment

Prior to repairing, calibrating, or performing maintenance on a liquid handling system, the system must be decontaminated. Laboratory instruments that have not been decontaminated are not allowed to enter public spaces. The decontamination requirements vary between laboratories and across applications depending on the contaminants involved and their hazardous nature. Table 2 lists the decontamination steps that need to be performed before delivering a liquid handling system to a calibration lab. After decontamination, a decontamination form must be filled out and signed. This form must be attached to the shipment. Deliveries without a decontamination form will not be processed by the calibration lab; instead, they will be left in a designated area until the decontamination status has been clarified with the customer. The form can be downloaded from the Eppendorf website [1] or obtained from a local calibration service provider.

Table 2: Decontamination steps needed before shipment to a calibration lab

Type of contamination	Decontamination methods
Biological contamination **(BSL 1)** Bacteria, fungi, viruses allowed in BSL 1 laboratories Biological contamination **(BSL 1)** Bacteria, fungi, viruses with sensitizing, toxic or other harmful health effects Biological contamination **(BSL 2)** Bacteria, fungi, viruses allowed in BSL 2 laboratories	**1) Mandatory** cleaning: Clean surfaces and decrease the load of microorganisms 2) Recommended decontamination with suitable reagents: Microorganisms on the surface killed, inactivated or made harmless 3) Recommended disinfection: Ensure that materials or products pose no risk of infection (if applicable) 4) Recommended sterilization: Destruction of all reproductive microorganisms (if applicable) **Note:** Eppendorf offers a decontamination form which is to be filled out.
Biological contamination **(BSL 3**) Bacteria, fungi, viruses allowed in BSL 3 laboratories Biological contamination **(BSL 4)** Bacteria, fungi, viruses allowed in BSL 4 laboratories	1) **Mandatory** cleaning: Clean surfaces and decrease the load of microorganisms 2) **Mandatory** decontamination with suitable reagents: Microorganisms on the surface killed, inactivate or made harmless 3) **Mandatory** disinfection: Ensure that materials or products pose no risk of infection (if applicable) 4) **Mandatory** sterilization: Destruction of all reproductive microorganisms (if applicable) **Note:** Users must prove that these procedures have been applied or that the respective contamination risks can be excluded. Eppendorf offers a decontamination form for this purpose which is to be filled out.
Chemical contamination: Organic contamination including CMR substances Chemical contamination: Anorganic contamination including CMR substances	1) **Mandatory** cleaning: Clean surfaces and decrease load of contaminants
Radioactive contamination: Short and long lived low intermediate residues, high-level and transuranic residues	1) **Mandatory** cleaning: Clean surfaces and reduce the load of contaminants **Note:** Users must prove that all radioactive contaminants have been efficiently removed or that a contamination risk can be excluded. Eppendorf offers a decontamination form for this purpose which is to be filled out.

Legend: BSL1/2/3/4 specifies the biosafety level of a laboratory. CMR substances are carcinogenic, mutagenic or reprotoxic substances.

Regular inspection of pipettes

It is of foremost importance that samples and reagents are dispensed correctly and with precision. To ensure reliable results, it is necessary to check liquid handling systems at regular intervals to confirm they are functioning properly. Guidelines stipulate how often pipettes and dispensers should be inspected as well as which tools should be used for inspection.

The following factors determine how often inspections should be performed [2]:
> Frequency of use
> Number of users of the liquid handling system
> Aggressiveness of the liquids dispensed
> Acceptable error limits as defined by the user
> Laboratory regulations

Aside from these recommendations, ISO 8655 requires a test for error limit compliance (calibration) at least once a year [2].

Taking the stated factors into account, Table 3 shows five distinct types of inspection:

Table 3: Brief overview of liquid handling system tests and their purposes

Test	Purpose
Functional test	Check for broken parts, visible contaminations, etc.
Leak test	Check for airtightness of the tip fit and piston seal; note that this test does not determine the performance of the liquid handling system.
Volumetric check	Fast performance test yielding rule-of-thumb information about system performance. This is a two-fold determination of dispensed volumes. Test to be performed, for example, when starting a new batch of alternative pipette tips.
Quick check	Four-fold determination of dispensed volumes. This is a rough check for random and systematic errors. Meaningful as an intermediate check between calibrations.
Calibration	Ten-fold determination of dispensed volumes. Reliable determination of random and systematic errors.

Further information about these tests and how to perform them is provided by the Eppendorf SOP for manual liquid handling systems [3]. The SOP also contains a detailed description of how to perform a calibration.

Literature

[1] epServices Decontamination Certificate. www.eppendorf.com
[2] ISO 8655-1:2002: Piston-operated volumetric apparatus. Part 1: Terminology. www.iso.org

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