Userguide

Liquid Handling 6 | No 024

Cleaning and inspection of pipettes

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Abstract

Modern quality management in the laboratory calls for the regular cleaning and inspection of dispensing systems. How often a pipette needs to be cleaned and inspected depends on actual practice, i.e. frequency of usage, number of users of the device, aggressiveness of liquids to be dispensed and, also, the acceptable error limits that have been defined by the user.

Sources and prevention of contamination

A distinction is made between three possible sources of contamination:

- From the pipette to the sample
- From the sample to the pipette
- From sample to sample, also known as carry over.

The first source of contamination is via a contaminated pipette tip or pipette. The contamination of samples can be prevented by using sterile pipette tips and cleaning or autoclaving the pipette. The second type of contamination occurs if the sample or its aerosols enter the pipette. To prevent this, the pipette should be held vertically when aspirating the liquid. It is also recommended to immediately eject the pipette tip after usage to prevent vapors from entering the pipette. Furthermore, the pipette should be stored suspended in a dedicated pipette stand.
In addition, the pushbutton should always be slowly moved up on aspiration. The most effective protection from contamination for pipettes is the use of filter tips. They prevent aerosols from entering the pipette and, thus, contamination of the pipette. The use of positive displacement pipettes whose tips feature a piston with an integrated leakage seal also excludes contamination of the pipette (Fig. 1A). The third type of contamination occurs during the dispensing of samples. Carry over takes place when parts of sample A adhere to the inside surface of the pipette tip in droplet form. These are then mixed with sample B, thus producing a false test result (Fig. 1B). To prevent sample-to-sample contamination, the pipette tip should be replaced after dispensing of each sample.

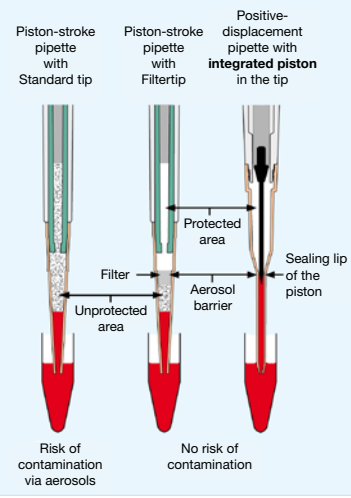

Fig. 1A: Preventing contamination of piston-stroke pipettes

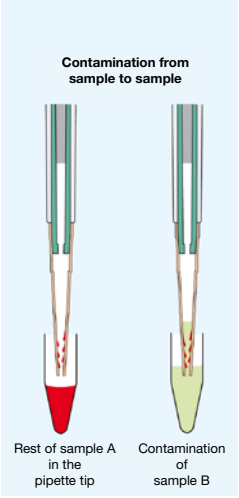

Fig. 1B: Contamination from sample to sample (carry over)

Decontamination and cleaning

External contamination can be removed with soap solution or isopropanol. The individual parts should then be rinsed with distilled water and dried. Accidentally absorbed liquids should not be allowed to dry; the piston must be cleaned, and a small amount of silicone grease must then be applied (Tab. 1).

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

Tab. 1: Decontamination and cleaning of air-cushion pipette

If readily volatile organic reagents are frequently used, the normally maintenance-free seal may swell up, causing the pipette to become stiff. In this case the lower section of the pipette should be unscrewed to allow the airing of the seal overnight. When pipetting saturated solutions, crystals may be produced from the aerosol precipitate inside the pipette which can destroy the seal. To avoid this, regular cleaning and regreasing of the piston as well as regular inspection of the piston seal is recommended.

Decontamination and cleaning

Autoclaving

The piston-stroke pipettes used today can either be fully autoclaved, or just the parts that have been contaminated through improper usage can undergo autoclaving. This removes any residual doubts on the part of the user regarding sterility, thus opening up new fields of application for these instruments. 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 min. Filter tips should not be autoclaved but rather, sterile products should be used if needed.
Before autoclaving fully autoclavable pipettes, they should be opened slightly in the middle by two turns to allow the vapor to enter more easily. After autoclaving, the pipettes or autoclaved parts must be allowed to dry and cool down to room temperature. Pipettes should only be screwed back together once they have cooled right down as otherwise plastic parts could become stretched and damaged. With Eppendorf pipettes, it is not necessary to regrease pipette pistons after autoclaving.

Decontamination with ultraviolet light

The UV-resistance of the plastics used in a pipette is of key importance for a wide range of applications.
UV-resistant pipettes can be left in inoculation rooms or benches without risk as the ultraviolet light used to disinfect workstations does not have any adverse effect on the pipette material and thus on the function of the pipette. The following criteria should be taken into account when using ultraviolet light for decontamination: a 30-watt low-pressure mercury-vapor lamp with a characteristic wavelength of 254 nm is required. The optimum distance between the lamp and pipette is approx. 60 cm.

Regular inspection of pipette condition

The precise and correct dispensing of samples and reagents is of prime importance for both research and diagnostics applications. To ensure reliable results it is necessary to check the dispensing devices used for this purpose for proper function at regular intervals. Guidelines stipulate the regular control of pipettes and dispensers as well as the tools used for inspection.

Leak test

To check for leaks, the nominal volume of the pipette is aspirated into the pipette tip (distilled degassed water) while the pipette is held vertically. The pipette, pipette tip and test liquid should all have the same temperature. If after 1 min no distinct drop has formed on the tip, the pipette does not leak for volumes up to 20 µl the tip should always be pre-wetted.

Function test

A visual check for leaks, broken parts, air bubbles and contamination should be carried out on a daily basis.

Volumetric check

Single or double determination of the volumes required for each pipette type should be carried out if any relevant changes to the device have occurred (e.g. using a new batch of pipette tips or after replacing volume-determining parts).

Quick check

Four-fold determination of the volumes required for each pipette type should be carried out on a monthly basis as a rough check for random and systematic measurement deviation.

Calibration

Ten-fold determination of the volumes required for each pipette type should be carried out on a quarterly basis to determine random and systematic measurement deviation.

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