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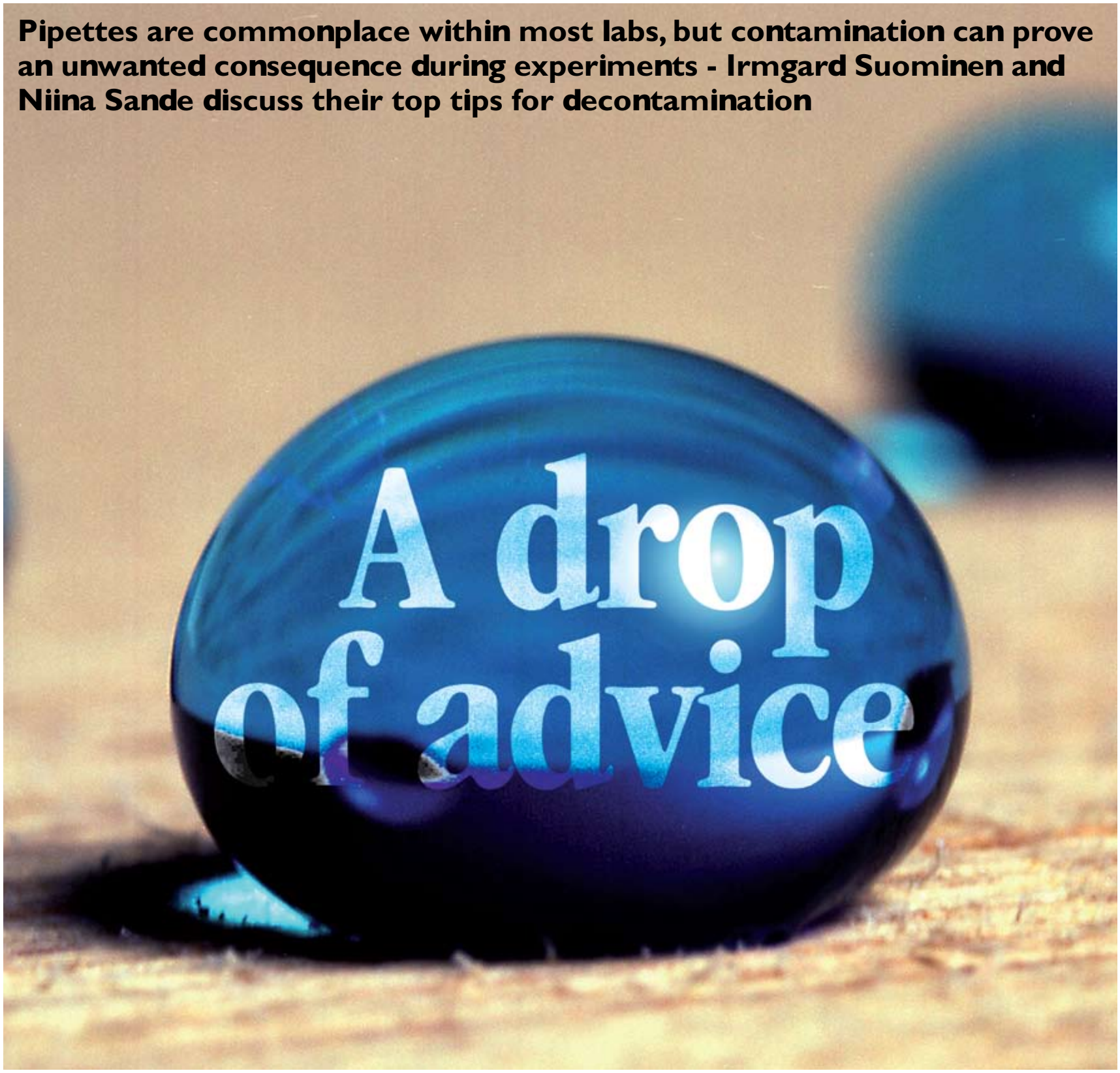
Pipettes are commonplace within most labs, but contamination can prove an unwanted consequence during experiments - Irmgard Suominen and Niina Sande discuss their top tips for decontamination



AS a regularly used piece of equipment, pipettes are commonplace within most laboratories. With uses in a wide range of applications, these tools can accidentally come into direct contact with a large number of different liquids. As a result, contamination is an issue of great importance, especially in highly sensitive applications such as PCR, where minute quantities of foreign particles can have detrimental effects on the resulting data.

Disposable pipette tips are used to transfer liquid and prevent any aspired liquids from coming into direct contact with the pipette, however splashes and aerosols from some liquids can still cause contamination of the tool itself. It is therefore recommended that cleaning and disinfection processes are established and routinely carried-out as part of overall good laboratory practice (GLP). As well as preventing cross-contamination, such practices help maintain functionality and extend the life-span of the pipette. Cleaning should also be performed if contamination is known, or suspected to have occurred. This is especially important if the pipetted liquid is hazardous, to ensure safety for the user as well as the surrounding environment.

As part of GLP, it is advised that each laboratory creates its own practices, including the completion of a risk assessment to determine any potential hazards related to each process or procedure. This will include details on the classification of any hazardous materials, such as microorganisms and radiolabels. Information on dangers to the surrounding environment, animals and humans, as well as how to avoid problems should also be included. As a result, the necessary precautions can be implemented before the experimental protocol is carried out, and are therefore very important to establish correctly.

There are a number of international (ASTM, ISO, IAEA) as well as local (FDA, EPA) statements and guidelines in place to ensure safety standards are met in respect to contamination. Guidelines for accurate decontamination in different situations have also been issued by the World Health Organisation (WHO)[1] for laboratory biosafety, and by the Healthcare Infection Control Practices Advisory Committee (HICPAC)[2] for cleaning, disinfection and sterilisation within the healthcare environment.

When aspirating and dispensing a liquid, aerosols and/or splashes have the potential to penetrate the inside of the pipette. >>

Feature: **Liquid Handling**

"As a commonly used laboratory tool, scientists need to be aware of the potential issues of contamination that surround the regular use of pipettes"

<< When pipetting a subsequent sample, the liquid can become contaminated with traces of the previous solution. While the occurrence of this cross-contamination is most critical in sensitive analyses, it can be detrimental to the resulting data of any experiment, or introduce contaminating pathogens to cultures.

The most efficient ways to prevent cross-contamination are through the use of a good pipetting technique, and appropriate pipette tips. For example, filtered pipette tips are recommended for use in molecular biology, cell culture, radioactive work and when using potentially infectious samples. The filter tips not only protect the sample from contamination, but also the pipette itself. When dosing corrosive or highly volatile chemicals, the filter tip protects the pipette cone from exposure to these reagents and any aerosols. Furthermore, it is recommended that a dedicated set of pipettes are set aside for the most sensitive applications, or where the risk of contamination is high. For example, the pipettes that are used for dosing amplified DNA should not then be used for the pipetting of a PCR reaction, or those used for handling radiolabels should be reserved solely for this.

In compliance with GLP, users should clean their pipettes on a regular basis, whether that be monthly, quarterly, or biannually. This is often coordinated with the calibration process. However, cleaning is also recommended at any point in time where contamination is suspected, to ensure an effective performance. In addition, pipettes should be checked on a daily basis for any dust and dirt on the surface, with particular attention to the inner and outer surfaces of the cone tip. If there is any visible contamination inside the cone tip, then the lower part of the pipette must be disassembled and thoroughly cleaned. The cleaning requirements for each pipette will differ, depending on its use and the liquid type. As a result, the chemical compatibility of the pipette must also be carefully checked.

Aqueous solutions and buffers: Dilute buffers with a neutral pH are not harmful to the pipette, however if any salt crystals are visible then the pipette must be opened and the contaminated parts thoroughly rinsed with distilled water and left to dry. The piston must be cleaned as standard with 70% ethanol and lubricated (as per the manufacturer's suggestions).

Acids and alkalis: The plastics used in the construction of most commercially available pipettes are highly resistant to both acids and alkalis. However, aerosols from these reagents can enter the lower parts of the pipette, affecting its performance. For example, aerosols from concentrated hydrochloric acid may condense on piston surfaces and cause corrosion. It is therefore advisable to frequently clean the lower part of the device with distilled water.

Organic solvents: Alcohols are generally well endured; however the chemical compatibility of other solvents can be problematic. After use with an organic solvent, any contaminated parts should be immersed in a detergent solution. All parts should be rinsed well with distilled water and left to dry and the piston cleaned and lubricated in the standard way.

Radioactive solutions: If contamination with radioactive material is suspected, the pipette must be opened and the contaminated parts placed in a large amount of detergent solution. The parts must be

Feature: Liquid Handling

thoroughly rinsed with distilled water and allowed to dry. The piston then needs to be cleaned and lubricated in accordance with the standard protocol. Decontamination of radioactive materials must always be followed with a confirmation that the radiation has been reduced to an acceptable level. All cleaning materials used are classed as radioactive waste and must be disposed of in accordance with effective regulations.

Proteins: Protein contamination can decrease or inactivate the effectiveness of some disinfectants. As such, it is strongly recommended that cleaning of all surfaces takes place before the disinfection. The pipette must be opened and the contaminated parts immersed in a detergent solution. The parts must be thoroughly rinsed with distilled water and allowed to dry before reassembly. Standard procedures must be used to clean and lubricate the piston.

Nucleic acids: DNA can be eliminated by immersing the pipette parts in 3 – 10% (w/v) sodium hypochlorite for at least 15 minutes[3,4]. This must be followed with a thorough rinse in distilled water and standard cleaning protocols carried-out on the piston. Also, commercially available wipes or solutions can be used to eliminate DNA from the pipette surface. After treatment, the parts must be wiped or rinsed according to the package instructions. One additional way in which to reduce DNA contamination is with the use of UV light; however this is not sufficient to completely eliminate the presence of DNA5. RNA is less problematic since it degrades rapidly and is sensitive to ubiquitous RNases.

Nucleases: RNase can be removed by cleaning the pipette with a detergent solution, followed by thorough rinsing with distilled water and 95% ethanol, which is then left to dry. The pipette parts are subsequently soaked in a 3% hydrogen peroxide solution for 10 minutes, and are then rinsed with DEPC-treated water[6]. Standard cleaning procedures of the piston must be followed. Alternatively, commercially available wipes or solutions can be used to easily remove RNase and DNase. After treatment, the parts must be wiped or rinsed according to the instructions on the package and allowed to dry. DNases can also be effectively destroyed by autoclaving[7].

Disinfection and sterilisation methods can be grouped into two categories: one for materials that tolerate heat; and one for materials that are heat intolerant. The presence of dirt, soil and organic matter such as blood or serum, can shield microorganisms and interfere with the killing action of decontaminants and sterilisation procedures. Pre-cleaning is therefore an essential step to achieve proper disinfection or sterilisation. Many germicidal products claim activity only on pre-cleaned items, however the process must be carried out with care so as not to come into contact with any infectious agents between the pre-clean and disinfection steps. Other factors, including temperature, relative humidity, concentration and contact time can also affect the success of disinfectants.

Heat-tolerant objects are usually best sterilised via autoclaving (steam sterilisation) and dry heat sterilisation. Autoclaving is the simplest and most frequently used form of cleaning, since heat is the most common physical agent used for the decontamination of pathogens. Dry heat sterilisation is completely non-corrosive, and is used to process laboratory ware which can withstand temperatures in excess of 160 °C for two to four hours. Autoclaving involves saturated steam under pressure, and for most purposes the following cycles will ensure sterilisation of correctly loaded autoclaves[1,2]:

1. 15 min holding time at 121°C
2. 25 min holding time at 115°C

When autoclaving, always follow the manufacturers' guidelines and pre-clean for the best possible results.

For materials that are not heat-tolerant, chemical disinfection or sterilisation, gaseous decontamination (such as ethyl oxide) or irradiation (UV, gamma or e-beam) can be used. Sterilisation by gamma or e-beam irradiation is an industrial process and particularly suited for the sterilisation of large batches of product. Chemical disinfectants or sterilants are used for the decontamination of surfaces and equipment if autoclaving is not possible or practical. The choice of disinfectant is dependent upon the microorganisms of concern, as well as the chemical compatibility of the materials. Many types of chemical can be used to disinfect, and there is an ever increasing number and variety of products available. The germicidal activity of many chemicals is faster and more effective at higher temperatures, but these temperatures can also accelerate their evaporation and degrade them. Many of these products are hazardous to humans and must therefore be stored, handled, used and disposed of with care. For personal safety, gloves and goggles are recommended while preparing any germicide dilutions. The correct use of such products contributes to the production of a safe working environment, while reducing the risk from infectious agents. As far as possible, the number of germicide chemicals used should be kept to a minimum, for economic reasons, as well as to limit environmental pollution. Commonly used chemical detergents include: chlorine, iodine, phenolic compounds, and formaldehyde[1]. Note - disinfection is mandatory before a pipette is sent for service to protect the service personnel from potentially hazardous subjects.

As a commonly used laboratory tool, scientists need to be aware of the potential issues of contamination that surround the regular use of pipettes. Sample contamination can be detrimental to resulting data, whatever the area of research. Minimising the risks of contamination is therefore of extreme importance, which is predominantly addressed through the use of good pipetting techniques and the correct pipette tips. However, in addition, there is a number of different cleaning and decontamination steps which can be put into place to further alleviate these associated risks. These should be performed on a regular basis in conjunction with calibration, but must also be used when contamination is suspected. Furthermore, assigning a dedicated set of pipettes to highly sensitive applications removes the potential occurrence of cross-contamination, which could be disastrous. Decontamination methods need to be tailored to the type of pipette as well as the type of liquid being used. For example, an electronic pipette cannot be autoclaved and DNases require different decontamination methods to buffers. As a result, knowing which decontamination method is best suited to your needs could have a significant impact on experimental integrity. LN

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CONTACT

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