Contamination-pipetting: relative efficiency of filter tips compared to Microman® positive displacement pipette

Filter tips are generally accepted as the solution for preventing cross-contamination of samples during pipetting tasks in applications such as bacteriological work, PCR and radioimmunoassays. Fundamentals of aerosol behavior demonstrate that filter tips cannot achieve 100% protection over the full range of particle sizes. When contamination issues are of the utmost importance, the Microman positive displacement pipette provides an effective alternative to filter tips.

PCR provides an extremely sensitive means of amplifying small quantities of DNA. The most important consideration in PCR is avoiding contamination. If the test sample has even the smallest contamination of DNA from the target, the reaction could amplify this DNA and report a false positive identification. For example, if a technician in a crime lab performs PCR on a blood sample, cross-contamination between samples could result in an erroneous incrimination, even if the technician changes pipette tips between samples. A few blood cells could volatilize in the pipette shaft, stick to the plastic of the pipette, and then get ejected into the next test sample. Modern laboratories have taken account of this fact and are devoting tremendous efforts to avoid this problem through the use of filter tips. But can you be 100% confident in the efficiency of your filter tips when a single aerosol drop of PCR product may contain thousands of strands of DNA that can easily contaminate reagents?

The Microman positive-displacement pipette ensures complete protection against cross-contamination. It uses a mechanism that isolates the aspirated sample from the body of the pipette, eliminating the air space in which aerosols can form by resorting to disposable capillary and piston tips (**Fig. 1**). The wiping action of the piston against the capillary wall ensures accurate dispensing of even the most viscous sample and avoids any carryover.

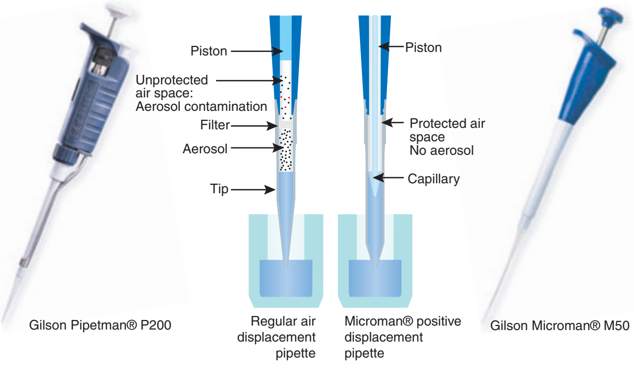

Figure 1 | Two pipetting concepts: air displacement using standard filter tips, and positive displacement as applied in the Microman pipette.

What is an aerosol?

An aerosol can be defined as a dispersion of solid or liquid particles suspended in gas. The size of aerosol particles is usually expressed as the radius of the particle, assuming a spherical shape (**Fig. 2**). They are mechanically produced by many laboratory activities, such as routine air-displacement pipetting, and are a considerable cause of contamination when working with infectious, toxic, corrosive or radioactive agents.

Tip manufacturers have developed filter tips as a solution to address this issue. These tips have a porous filter positioned inside the tip. During pipetting, air flows through the filter, which captures aerosols, preventing contact with the pipette's shaft and, subsequently, sample carryover.

How a filter works

Understanding how aerosols behave as they pass through a filter is important because the mechanism by which the particles are trapped varies with the particle size[1,2].

Depending on an aerosol's diameter, three mechanisms can explain aerosol interception[3] (**Fig. 3**). (i) Small (aerosol diameter below 0.3 μm) aerosols are so light that the collision between the small particles and air molecules affects their trajectory. The aerosols then diffuse out of the air-flow line and come in contact with the filter fibers.

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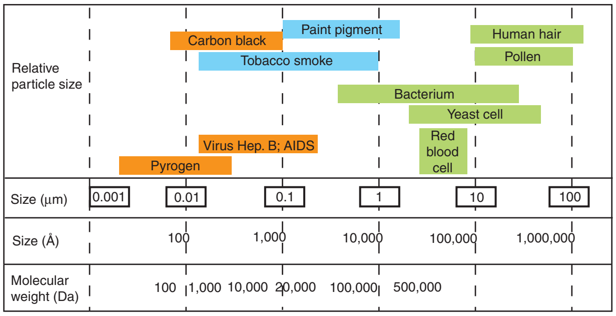

Figure 2 | Aerosol content size ranges.

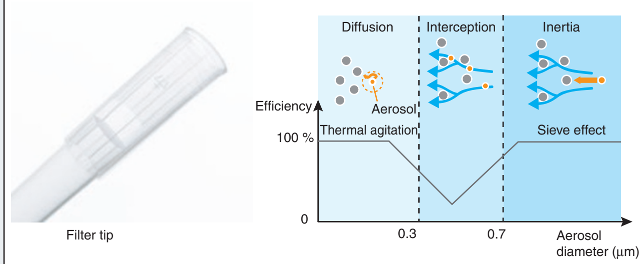

Figure 3 | Filtration mechanisms. Three mechanisms of particle collection depend on the aerosol content size. The boundary area between the three mechanisms is not strictly defined and two or even three regimes can coexist for a given particle diameter. Blue lines, air flow lines; gray circles, filter fibers; orange circles, aerosol.

(ii) Medium-size particles (aerosol diameter of 0.3–0.7 µm) are more likely to pass through the filter, but they can be intercepted as their diameter is not negligible compared to the diameter of the fibers. (iii) Large (aerosol diameter above 0.7 µm) particles exhibit high mass and inertia, and therefore cannot follow the air flow line around the fibers; they go straight ahead and are intercepted by the fibers.

The real efficiency of the filter tips against carryover contamination

We performed an evaluation of four different filter-tip brands of comparable quality over a complete aerosol size range of 0.05, 0.1, 0.3, 0.5 and 1 µm, and found that these particles behave as the theory of filtration summarized above would predict. We observed a minimum of filtering efficiency as low as 85% between the areas where diffusion and interception mechanisms intervene (0.3–0.7µm), an area that corresponds to the size of viruses and bacteria (data not shown).

Nowadays, more and more laboratories face the necessity of adopting good laboratory practices. Stringent conditions are required for DNA applications, and to avoid biological or radioactive contamination, special areas dedicated to DNA analysis, sterile tools, clean gloves and dedicated pipetting systems are highly recommended, if not mandatory. The Gilson Microman was designed for use in such situations, to work with disposable capillary and piston tips that create a shield that prevents the formation of aerosols and subsequent contamination of samples by carryover. The capillary piston technology avoids direct contact of the sample with the pipette shaft to ensure complete protection against contamination (additional information is available online; http://www.gilson.com/PDFs/pdfid238.pdf). Ultimately, the ejection of the disposable capillary and piston protects the users from potential hazards; changing capillary piston tips is all it takes between each assay to provide an absolute protection.

Conclusion

Preserving sample integrity is paramount to achieving accurate results in biological research, and pipette filter tips have been specifically designed for that purpose. Regardless of the source of potential aerosol contamination, filter tips cannot guarantee 100% protection against carryover contamination.

In environments in which DNA probes are being used and the results of the tests must be reliable, including forensic science, clinical diagnostic analyses or genetic disease testing, we recommend using Gilson Microman pipettes. They are the ideal complement to the Gilson Pipetman and are recommended for critical protocols when unquestionable results need to be provided.

1. Lee, K.W. & Liu, B.H.Y. Experimental study of aerosol filtration by fibrous filters. *Aerosol Sci. Technol.* **1**, 35–46 (1982).
2. Lee, K.W. & Liu, B.H.Y. Theoretical study of aerosol filtration by fibrous filters. *Aerosol Sci. Technol.* **1**, 147–162 (1982).
3. Renoux, A. & Boulaud, D. The aerosols: physics and metrology (Lavoisier Technique et Documentation, Paris, 1998).