



PRODUCT FEATURE

Pipettor Tip Cone Filters Minimise the Risk of Contamination

Mannonen, S., Ph.D.



Bacteria and fungi are the two most common contaminants observed in cell cultures. There are thousands of species of bacteria, and they can live on or in almost any material and environment from soil to water and air – even in us. Bacteria consist of a single cell and can live in temperatures above boiling point and in cold that would freeze blood. Bacteria sometimes form protective spores, which have hard protective coating. With this coating, spores are able to survive for weeks, even years, through drought, heat and even radiation. Fungal spores are lightweight and present everywhere in the environment. If a fungal spore comes in contact with cell culture media, contamination of the cell culture is in practice inevitable.

Membrane filters have been used widely for decades to sterilize liquids used in cell culture. Filters with a 0.45 µm pore size have been recognized as the standard for growth of micro organisms and are used to recover bacteria and other micro organisms from many samples and environments.

In 1987, the FDA "Guidelines on sterile drug products produced by aseptic processing" (1) defined 0.2/0.22 µm filter as a standard sterilizing filter. Often, liquids and the environment are carefully checked for sterility, and the user is well protected by appropriate workstations, seats, clothing and masks, but the pipettor is forgotten. However, pipetting with air displacement pipettors is one of the most common laboratory tasks and includes a number of potential contamination risks.

This contamination can occur through handling of infectious or toxic agents, such as bacteria, and in situations where the sample or specimen is at risk through contamination from other samples or environmental factors. In bacteriological work, Pasteur pipettes or serological pipettes, with a piece of cotton as a filter in the upper end have traditionally been used together with pipetting aids featuring a filter of 0.45 or 0.2 µm (Figure 1).

However, modern air displacement pipettors, especially electronic pipettors with disposable tips enable fast, more accurate and precise pipetting and dispensing (Figure 2), but contamination of the pipettors may increase both the possibility of unreliable results and health risks in laboratory work. Scientific literature on the contamination of modern air displacement pipettors hardly exists.



Figure 1. Pipetting aids together with serological pipettes are commonly used in bacteriological work.



Figure 2. Electronic pipettors can be used effectively and accurately in dispensing to various cell culture plates.



Figure 3. Biohit filtered tip cones prevent contamination of the pipettor and carryover to next sample.

PREVENTING CONTAMINATION

Good laboratory practice – pipetting slowly and carefully minimises aerosol formation and foaming which is important to avoid contamination of the pipettor and the subsequent sample. This may not be sufficient and aerosol-barriers, such as filters, between the sample and the pipettor have been introduced. Tip manufacturers have developed aerosol-barrier tips, which are useful in contamination sensitive work, such as bacteriological work, PCR, and RIA. These tips have a porous filter positioned inside the tip. During pipetting air flows through the filter, aimed to reduce the flow of aerosols or liquid into the pipettor barrel and subsequently to the next sample (carryover contamination). The material and the features of the filter tips on the markets vary. The small pore size of some filters reduces pipetting speed due to slow air flow through the filter. On the other hand, the pore size in many filters is large enough to let small molecules (100 nm) and aerosols pass through easily (2). In other words, these filters mounted in the tips have low blocking efficiency. Moreover, using filter tips instead of standard tips is rather expensive.

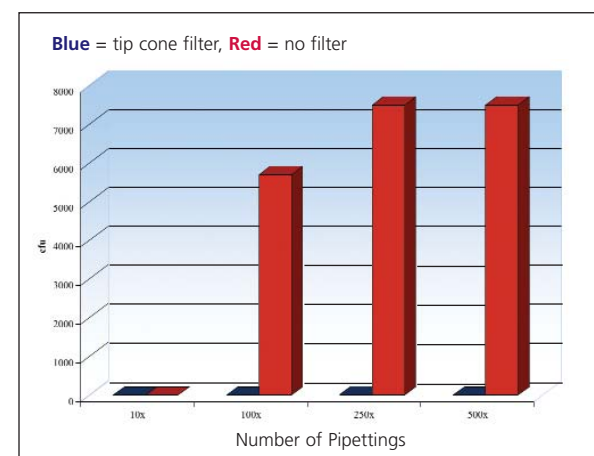
An alternative to the filtered tips is provided with pipettor that features a tip cone design that is adapted for the placement of a protective filter (Figure 3). Two types of filters are available for Biohit pipettors: Biohit Standard Safe-Cone filter and the Biohit Safe-Cone Plus filter. The Biohit standard and Plus filters have a different barrier capacity in a situation simulating overdraw of liquid. The standard filter allows the liquid to penetrate the filter if drawn through whereas no liquid can penetrate through the Plus filter.

ASEPTIC PIPETTING LIFETIME OF A PIPETTOR

The aseptic pipetting lifetime of air displacement pipettors during serial pipetting has been investigated by Kolari et al. (3). A liquid culture of *Micrococcus luteus* bacteria was repeatedly pipetted with standard tips, filter tips, and with or without a Biohit Safe-Cone filter placed in the pipettor tip cone. Asepticity of the interior of the pipettor barrel was maintained for less than 50 pipettings, when no filter was used (Table I). With 100 pipetting in most cases a heavy contamination of both mechanical and electronic pipettor barrels was observed even if good laboratory practice – pipetting slowly and carefully to minimise aerosol formation and foaming – was followed. Sometimes contamination was detected in as few as 10 pipettings. The same was true with radioactive solution and plasmid DNA.

When filter tips (Biohit Plc., Finland) were used, no contamination of the pipettor barrel was detected even after 550 pipettings. But -the same was true with the Plus filter attached to the tip cone. The comparison of contamination in the pipettor barrel using the Safe-Cone Plus filter in the tip cone and pipetting without filter (standard tips) is shown in Table I. Also, the Standard filter protected the pipettor quite well. Only in 18% of the pipettings the barrel was slightly contaminated. This is due to the different barrier capacity of the Standard and the Plus filter. However, if the standard filter would be changed more often, contamination can be eliminated. The recommendation is to change the Plus filter once per 500 pipettings and the Standard filter once per 50-100 pipettings in bacterial work. The same applies to radioactive and DNA work.

Table I. Contamination of the pipettor barrel during repeated pipetting of *M. luteus* bacterial culture with no filter and the Biohit Safe Cone Plus filter on pipettor tip cone. Sterile tips were replaced at intervals of 10 pipettings. As seen after 100 pipettings heavy contamination can be detected in the pipettor barrel if no filter is used.



CONTAMINATION AND CORROSION OF THE PIPETTOR PISTON

Pipetting even commonly used laboratory liquids can be enough to contaminate the pipettor tip cone and the pipettor barrel extremely easily, not to mention pipetting infectious agents, blood (Figure 4), radioactive solutions or DNA. If the pipettor is not protected with a tip cone filter, the metallic piston may be contaminated or corroded quickly. Contamination as well as corrosion certainly has big effect on accuracy and function of the pipettor. However, both Biohit Safe-Cone Standard and Plus filters protect the pipettor piston from dirt, contamination as well as from corrosion, which are typical reasons to send a pipettor for service (Figure 4).

CARRYOVER CONTAMINATION

The presence of contaminating bacteria inside the pipettor barrel, at the pipettor tip cone or the filter may lead to carryover contamination. If deliberate over-pipetting is done, a pipettor without any filter works as a continuous source of contamination for at least 50 pipettings (3). The same is true for the Standard filter. When a Biohit Safe-Cone filter was placed in the tip cone, no carryover was observed within 2620 pipettings (Table I). With DNA samples carryover was not observed with either filter (Table II). This data proves that a tip cone filter replaced at intervals of 50 - 250 (Safe-Cone Standard filter) or 500 (Safe-Cone Plus filter) pipettings will protect the pipettor barrel from contamination and the samples from carryover. Nevertheless, in case of over-pipetting, the filter should be changed immediately and the pipettor tip cone decontaminated.

RECOMMENDATIONS

If no protection (filter tips or filter in the pipettor tip cone) is used in pipetting, the pipettor is contaminated easily. Even when working with buffers or aqueous solutions, small amount of liquid gets easily inside the pipettor tip cone and the tip cone becomes contaminated and eventually the piston gets either jammed or corroded.

The filters protect the internal mechanism of the pipettors effectively but they need to be changed regularly. Filter replacement intervals depend completely on the application and the sample. However, according to the studies above, the filter should be changed daily (50-250 pipettings) and always in case of over-aspiration. To ensure the safety of the user, forceps or automatic filter ejection (Biohit mLINE) (Figure 5) should be used to avoid touching the dirty filters by hand. In addition, the tip cone should be cleaned regularly with ethanol or decontamination liquid, such as Biohit Proline Biocontrol. It is recommended to use standard filters for general applications and the Biohit Safe Cone Plus filter for more demanding applications such as cell culture, bacterial and virological work and molecular biology. Standard filters can be used also for this type of work, but they need to be changed more frequently.

REFERENCES

1. FDA, "Guidelines on sterile drug products produced by aseptic processing". Centre for Drugs and Biologics, Rockville. MD (1987).
2. Riebold K. Comparative investigation on the blocking efficiency of filter tips. G.I.T. Lab. Journal 3, 25-26, 2005
3. Kolari, M., Mannonen, S., Takala, T., Saris, P., Suovaniemi, O. and Salkinoja-Salonen, M. The effect of filters on aseptic pipetting lifetime of mechanical and electronic pipettors and carryover during pipetting. Lett. Appl. Microbiol. 29, 123-129, 1999.
4. Lambert, N.G., Jétte, L., Caprioli, T. and Kasatiya, S. Risk of contamination by pipetting bacterial culture broth with Pasteur pipettes. French. Ann. Microbiol. (Inst. Pasteur), 130A, 351-354, 1979.

AUTHOR DETAILS

S. Mannonen is Vice President, Sales and Marketing, Biohit Plc., Helsinki, Finland

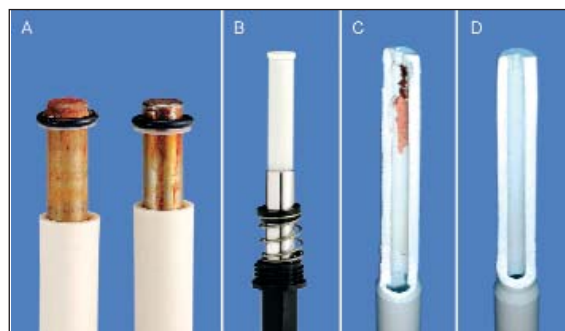


Figure 4. If the pipettor is not protected with a tip cone filter, the tip cone may be contaminated easily and the metallic piston corroded quickly:

- A. Badly corroded and contaminated pistons taken from a commonly used brand of pipettor sent for service.
 B. Biohit pipettor tip cone used with Biohit Safe Cone filter.
 C. Tip cone taken from a commonly used brand of pipettor after over-aspiration of blood sample.
 D. Clean tip cone.

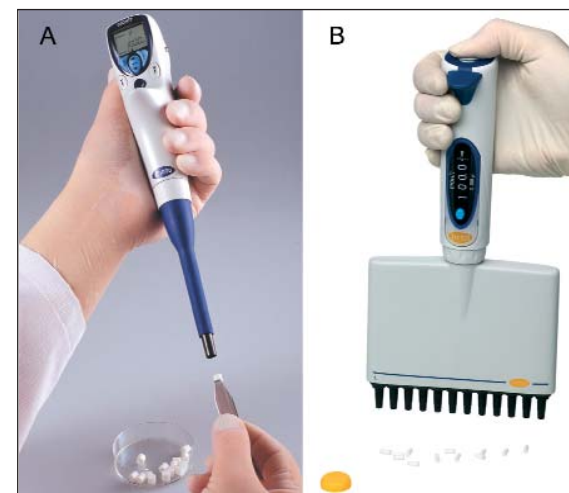


Figure 5. The tip cone filter can be changed easily and safely with the use of forceps supplied with every Biohit pipettor (a). Biohit mLINE features tip cone filter ejection mechanism (b)

Table II. Both the Biohit Standard and the Biohit Safe Cone Plus filter prevent from DNA contamination. However, after 100 pipettings contamination of the pipettor barrel starts to show if no filter is used and after 500 pipettings contamination is already severe.

DNA (50µl plasmid DNA, 120 µg/ml) contamination in pipettor barrel			
No. of pipettings	No filter	Biohit Standard filter	Biohit Plus filter
50	-	-	-
100	+	-	-
250	++	-	-
500	+++	-	-

CONCLUSIONS

Controlling contamination means above all eliminating aerosols and spillage in liquid handling. Traditionally in contamination sensitive work, such as PCR this has been done using either positive displacement tips or aerosol-barrier tips. In bacteriological work, Pasteur pipettes or serological pipettes, with a piece of cotton as a filter in the upper end have been used.

Firstly, positive displacement tips are rather expensive. Secondly, the material and the features of the filter tips on the markets vary. The small pore size of some filters reduces pipetting speed due to slow airflow through the filter. On the other hand, the pore size in some filters is large enough to let small molecules and aerosols pass through easily. Third, the insufficient performance of the cotton plugs in Pasteur pipettes has been shown by Lambert et al. (4), where bacterial aerosols formed in just two pumpings contaminated the upper end of the cotton plugged Pasteur pipettes in 4% of the tests.

An economic but effective way to protect both the pipettor and the sample from contamination is to use filter in the tip cone of the pipettor. Not just any filter, but filters with documented data. Biohit offers two types of tested & validated filters, the Biohit Safe-Cone standard and the Plus filter, to be used depending on application. By using these protective filters, the most modern, time-saving, accurate and precise tools, such as Biohit electronic pipettors, can be used in contamination sensitive work. Cotton plugs, Pasteur pipettes and rubber bulbs are history.

WISH YOU WERE HERE?

The place to be seen...

Put your press releases in front of over 24,000 readers.

PR@INTLABMATE.COM

or contact our sales team on +44 (0) 1727 855 574