

Mycoplasma Protection in Eppendorf Cell Culture Flasks with Advanced Filter Technology

Jessica Wagener¹, Ute Kowanz²

¹Eppendorf AG, Hamburg, Germany

²Eppendorf Polymere GmbH, Oldenburg, Germany

Executive Summary

Are mycoplasmas able to pass through the filter in the screw cap of a T-flask? Eppendorf assigned one of Europe's leading providers of non-medical laboratory analysis (SGS Institut Fresenius) to test the filter screw caps of different cell culture flasks. A challenge test was developed for simulating everyday lab routine using *Mycoplasma orale*, one of the most common mycoplasma species contaminating cell culture labs worldwide [1].



Introduction

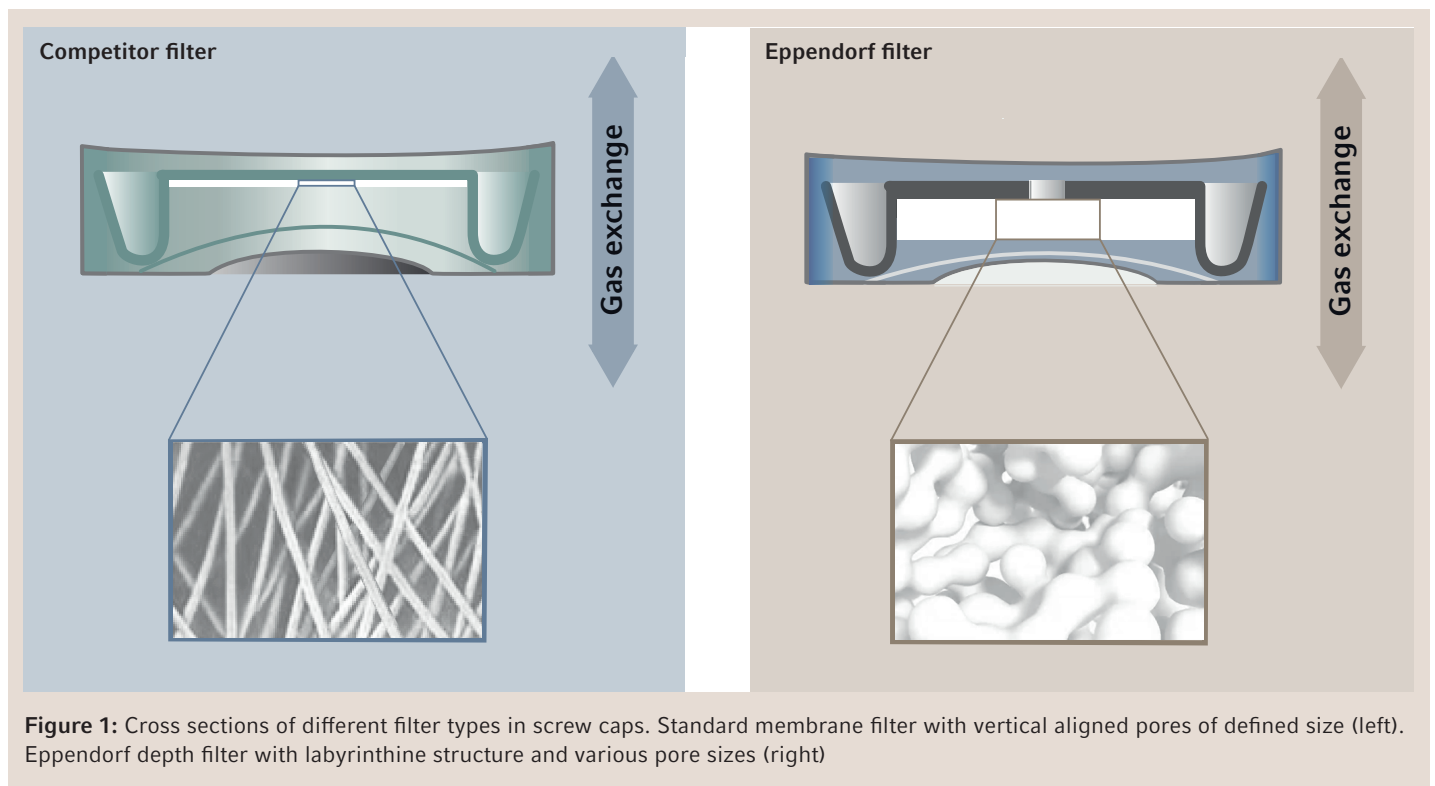
Mycoplasmas are common contaminants in eukaryotic cell cultures. Unlike other bacterial contaminations, mycoplasmas do not cause a visual change of the culture media like turbidity or color change. Thus, they can often go undetected causing cellular effects such as alteration of cellular metabolism, gene expression profiles etc. leading to irreproducible experimental results [2].

Mycoplasmas are the smallest type of bacteria at only 0.1-0.3 µm in size. Their lack of a rigid cell wall results in a certain elasticity and variable morphology. This allows mycoplasmas to penetrate filter membranes with a pore size of 0.2 µm. Whereas this fact is already widely known with regards to liquid filtration, it is disregarded when it comes to filters in the screw cap of T-flasks.

The most common source of a mycoplasma contamination in cell culture laboratories is a cross-contamination from a previously infected culture that was used in the same

culture room. Mycoplasmas can easily spread throughout the complete lab when they go undetected. Regular testing of the cultured cells (e.g. by PCR) and immediate disposal of infected cultures is crucial. Besides applying standards in aseptic techniques for all people sharing the lab, and regular cleaning of equipment like water baths and CO₂ incubators, the choice of the right culture vessel can help to reduce mycoplasma contaminations. Whenever possible a T-flask with a tight filter screw cap should be preferred over plates and dishes with their loose lids. But does the type of filter in the screw cap make any difference?

The screw caps of cell culture flasks usually contain a thin membrane filter with vertically aligned pores of 0.2 µm size. In contrast, the Eppendorf T-flasks are equipped with caps containing a depth filter, which is thicker and has a labyrinthine structure with various pore sizes and shapes (Figure 1).



Imagine a busy day in the culture lab. You take the T-flasks out of the CO₂ incubator and carry them to the microscope to check the status of your cells. Luckily, you don't see any black particles moving between the cells or any other obvious sign of microbial contamination like turbid and yellow culture medium. The cells look healthy and are ready for splitting. You take the T-flask to the safety cabinet and begin working with the cells. It happens very randomly that the medium is slewed against the filter cap from the inside while carrying the flask around the lab. Or while you reposition the flasks inside the incubator to find the right one or make space for other vessels.

On behalf of Eppendorf, the SGS Institut Fresenius tested the potential of different filter types to hold back mycoplasma. Cell culture flasks from different suppliers were filled with mycoplasma containing medium. The medium

was manually slewed against the inside of the flask cap as can happen accidentally during transportation of the flasks or when they are moved within the incubator. Samples were taken with swabs from the outer filter surface. These samples were then used for inoculation of liquid mycoplasma media and subsequent cultivation on agar plates. While gas exchange through the filter is not impacted, the Eppendorf filter cap is able to hold back mycoplasma and thus prevents spreading an infection throughout the lab. In contrast to the standard membrane filters with defined pore size used by other suppliers of T-flasks, the Eppendorf filter caps cannot be penetrated by mycoplasma. The labyrinth-like structure of the thick filter pad with pores of various sizes and shapes forms a reliable barrier for mycoplasma (Figure 2).

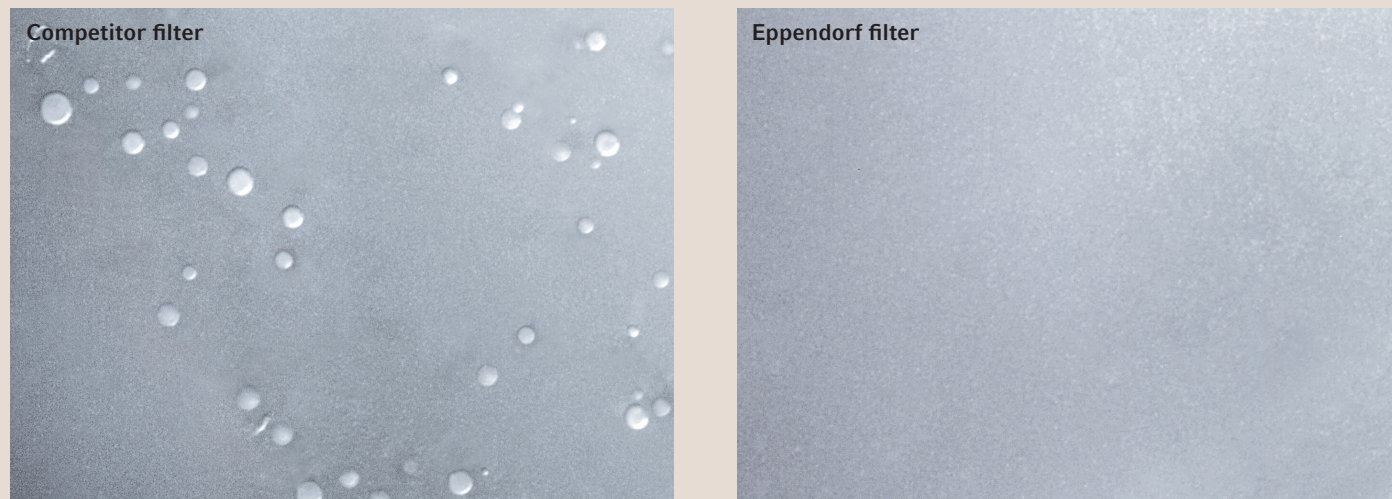


Figure 2: *Mycoplasma orale* colony growth on agar plates. Swabs taken from a competitor membrane filter (left) and from the Eppendorf filter (right).

Summary

These results clearly indicate that a 0.2 µm filter membrane is not a sufficient barrier for mycoplasma when contaminated culture media comes into contact with the filter. The Eppendorf filter cap is certified to be mycoplasma retentive by one of Europe's leading providers of non-medical laboratory analysis [3].

[1] Uphoff and Drexler (2003) Cell Culture Mycoplasmas, www.dmsz.de

[2] Drexler HG, Uphoff CC, Cytotechnology 2002; 39(2):75-90

[3] SGS Institute Fresenius, Certificate of analysis (2019), Eppendorf Cell Culture Flasks with filter cap, www.eppendorf.com/certificates

About Eppendorf

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