Invisible Threats:

Mycoplasma Aerosol Formation in Pipetting

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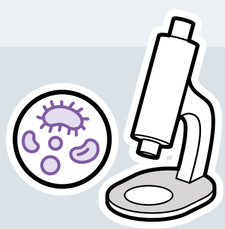
? How does pipetting affect mycoplasma contamination spread in cell cultures?

1 Mycoplasma contamination affects results

Fig. 1.1 Air-cushion pipettes used in cell culture assays

Mycoplasma contamination is a common, often unnoticed issue in (stem) cell cultures that can significantly affect cell physiology, metabolism, and genetic stability, thereby undermining the reliability and reproducibility of experimental results [1, 2].

This study examined whether simple standard pipetting of mycoplasma contaminated samples leads to aerosol formation and consequently to contaminated pipettes, and how to prevent such events.



Most difficult contamination to detect and eliminate

Estimated to affect over 10 % of cultures

Sources: animal sera, media, and improper septic technique

Fig. 1.2 Mycoplasma in cell cultures [1, 2]

2 Mixing of mycoplasma samples leads to aerosol formation and subsequently contaminated pipettes

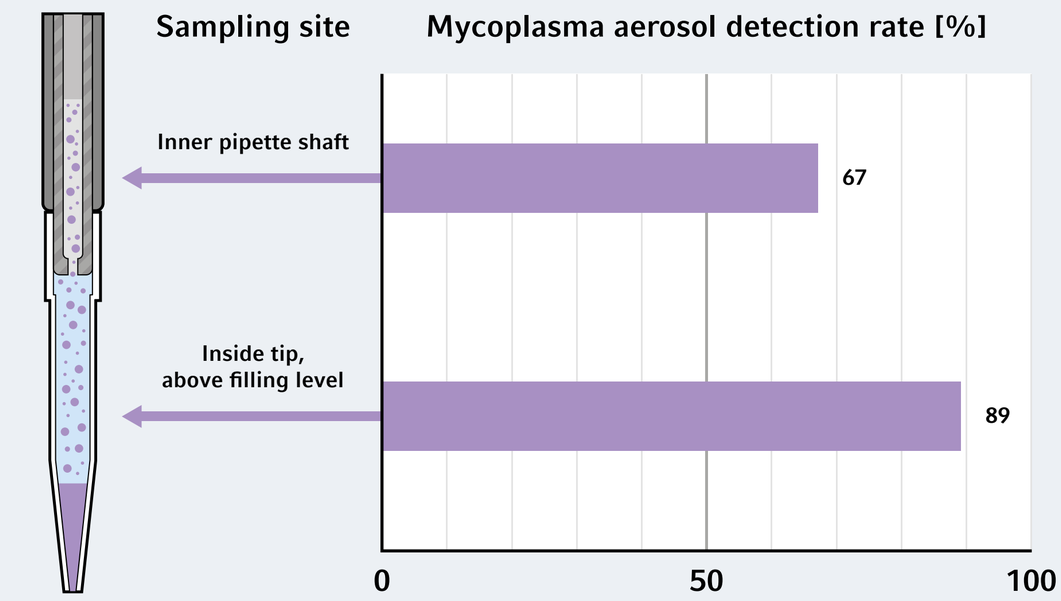


Fig. 2 Aerosol detection during standard pipetting. 20 × mixing 600 µL *A. laidlawii* culture, Eppendorf Xplorer® plus electronic pipette at speed 8, N = 9

A sample of a mycoplasma culture was mixed 20 times with an electronic air-cushion pipette. The air space above the filling level within the tip and the inner pipette shaft were sampled afterwards. At both sampling sites, viable mycoplasma cells were found, demonstrating aerosol-based spread from pipetting [3]. Two thirds of pipette shafts were contaminated [4].

3 Mycoplasma contamination remains within pipette for at least six weeks

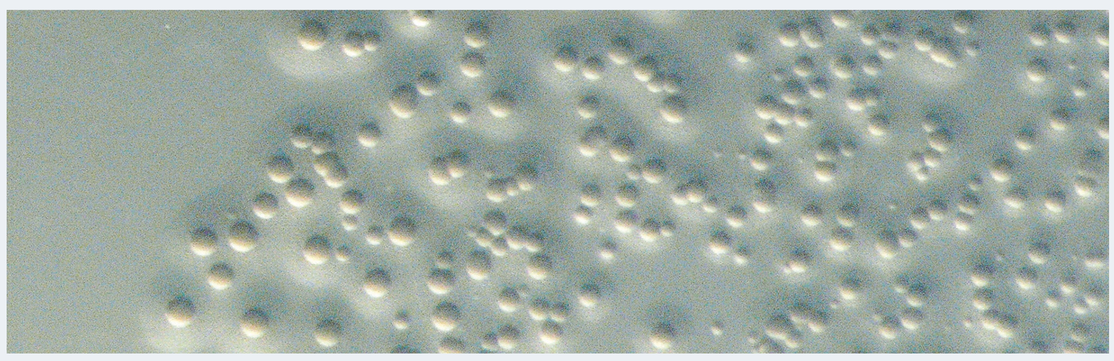


Fig. 3.1 *A. laidlawii* mycoplasma found in pipettes after mixing experiments

To decontaminate the used pipettes, the lower parts were wiped with 70 % isopropyl alcohol (IPA). After six weeks, two pipettes remained contaminated. Subsequent soaking in IPA did not decontaminate. Only autoclaving led to decontamination of the pipette [4], as described previously [1].

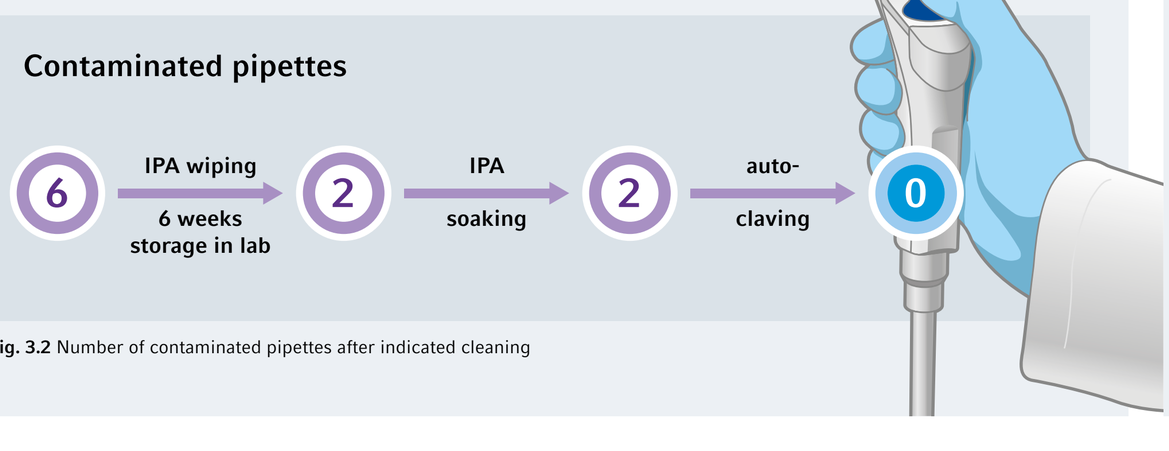


Fig. 3.2 Number of contaminated pipettes after indicated cleaning

4 ep Dualfilter T.I.P.S.® pipette filter tips retain mycoplasma aerosols and protect pipette contamination

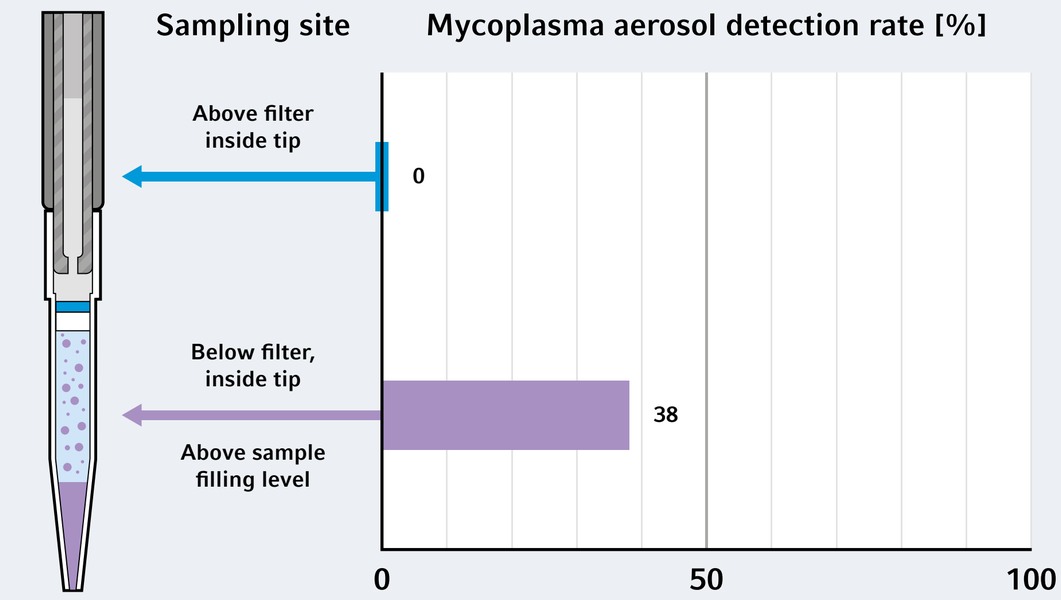
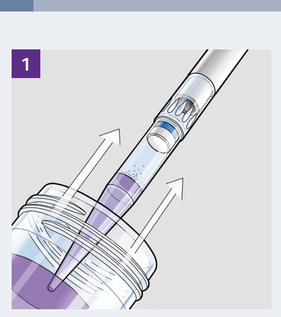


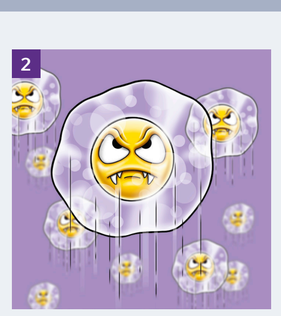
Fig. 4 Aerosol detection during pipetting with filter tips. 20 × mixing 600 µL *A. laidlawii* culture, ep Dualfilter T.I.P.S.® filter tip, Eppendorf Xplorer® plus electronic pipette at speed 8, N = 21

Repeating the experiment with a pipette filter tip revealed that the filter successfully retained all mycoplasma aerosols within the tip and efficiently protected the pipette from contamination. No viable mycoplasma cells were found within the pipette shaft.
Filter tips are thus essential to prevent mycoplasma spread across cultures [4]. Equal results were found for bio-aerosols formed from pipetting other bacteria, virus or DNA samples: they form over the sample but are blocked by the filter [4].

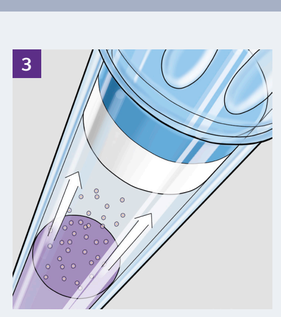
! Read Application Note 490: Bio-Aerosol formation during pipetting



Sample mixing via pipetting

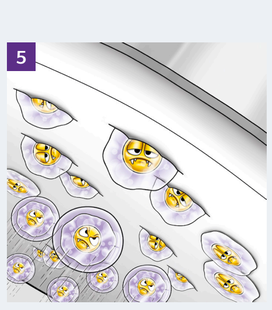


Mycoplasma in sample



Aerosol formation

Filter in place



Aerosol hits filter

Efficient aerosol blockage

Pipette protected from bio-aerosols

Read full research here

Literature.
[1] Nikfarjam & Farzaneh, Cell J. (2012); 13(4):203-212
[2] Gedye *et al.* Stem Cell Rev Rep (2016); 12(1).
[3] Masters *et al.* Br J Cancer (2000); 82(9): 1495-1509
[4] Schmidt *et al.*, Application Note 490 Eppendorf SE (2025)

Methods.
Acheloplasma laidlawii (10^9 CFU mL^{-1}) was mixed 20 times (Eppendorf Xplorer® plus, speed 8) and indicated sampling sites were sampled with a sterile swab. Swabs were transferred to 5 mL liquid Medium (Pharm. Eur. 2.6.7), incubated at 36 °C for 14 days, with subculture on solid medium after 7 days. Agar plates were incubated in 95 % N_2/5 % CO_2 at 36 °C for 7 days. Aerosol detection was positive if the liquid culture changed color; phenotype was confirmed microscopically.

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