Simplifying Progress

How to Prevent Mycoplasma Contamination and Spread in Your Cell Culture Laboratory

Sandra Söderholm[1*] and Emilia Varhimo[2]

[1] Lab Essentials Applications Development, Sartorius Biohit Liquid Handling Oy, Helsinki, Finland
[2] Pipetting and Dispensing, Sartorius Biohit Liquid Handling Oy, Helsinki, Finland

Correspondence | Email: LHinfo.Finland1@Sartorius.com

Introduction

Mycoplasma is a common bacterial contaminator in cell culture laboratories. In one study, researchers identified mycoplasma contamination in 11% of 10,000 cell lines that they tested (Olarerin-George *et al.* 2015). Moreover, mycoplasmas have been shown to even contaminate liquid nitrogen storage tanks where cell stocks are stored (Bajerski *et al.* 2020).

Mycoplasmas are resistant to commonly used antibiotics, and cannot be detected under the light microscope, because they lack a cell wall and are extremely small – only 0.2-0.4 µm in diameter. This increases the risk of failing to detect mycoplasma contamination in the laboratory. Mycoplasma contamination has been shown to induce cellular changes, e.g. susceptibility to drugs. Therefore, any results obtained from mycoplasma-contaminated tissue cultures potentially render the data invalid (Kim et al., 2015; Gedye *et al.*, 2016).

Pipettes are the most frequently used tools in the laboratory and therefore prone to contamination. Contaminated pipettes can cross-contaminate samples and cell cultures. Regular cleaning of pipettes is absolutely essential for contamination control. Some pipettes are fully autoclavable or have parts that can be autoclaved. Wrong pipetting technique can also cause cross-contaminations. Therefore, an ergonomic pipette and the right pipetting technique are absolutely essential to prevent mycoplasma contamination and to ensure clean samples and successful experiments.

In this study, we demonstrate that autoclaving the pipette is the most efficient method to remove mycoplasma from pipettes. We also show that the pipette and pipetting technique have a great impact on cross-contamination

1. Experimental setup

1.1 Surface Contamination and Decontamination of Mechanical Pipettes

Five to seven spots on mechanical pipettes were contaminated with *A. laidlawii* (ATCC 23206) liquid culture, 2.0-3.7x10^5 colony-forming units (CFUs) per spot (Figure 1). One Tacta pipette was not inoculated and served as negative control (Table 1). The pipettes were incubated in a closed laminar flow cabinet for 24 h at room temperature. The autoclave-resistant pipettes and parts were sterilized at 121 °C for 15 min. The non-autoclavable pipettes and parts were cleaned by wiping surfaces, seams, and grooves with 70% ethanol.

Samples were taken from each spot on the pipettes with cotton swabs soaked in 0.9% sterile saline solution. After sampling, the swabs were placed back into the container with saline solution (1.5 mL) and vortexed for 30 sec, after which the swabs were discarded.

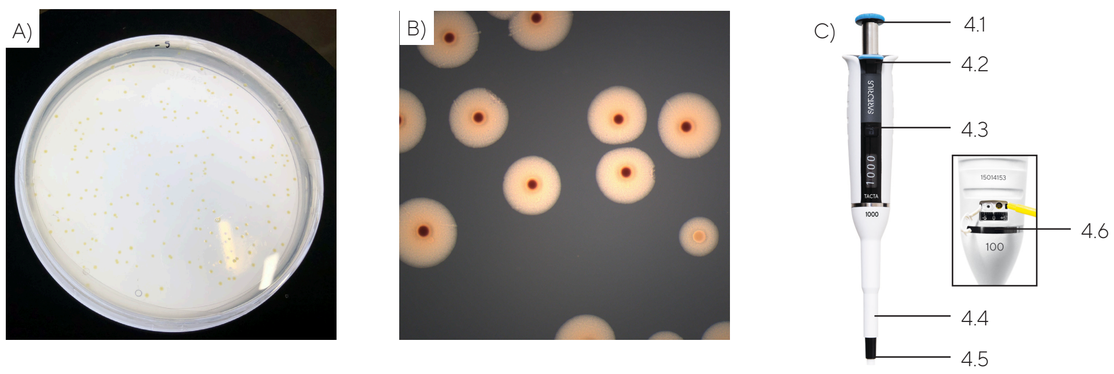

Figure 1: (A) Mycoplasma (*A. laidlawii*) colonies on Luria broth agar plates incubated for 72 h at 36°C. (B) *A. laidlawii* colonies after 5 days (10x magnification). (C) The pipettes were contaminated with *A. laidlawii* on five to seven different spots. In this picture the spots are indicated for the Sartorius Tacta.

Pipette ID	Pipette Type	Number of Contaminated Spots	Decontamination Procedure
1.	Pipette with autoclavable lower parts	7	70% ethanol and autoclave
2.	Non-autoclavable pipette	6	70% ethanol
3.	Non-autoclavable pipette	5	70% ethanol
4.	Sartorius Tacta (0.1-3 µl)	6	Autoclave
5.	Non-autoclavable pipette	6	70% ethanol
6.	Fully autoclavable pipette	5	Autoclave
7. Positive control	Sartorius Tacta (0.5-10 µl)	6	No decontamination
8. Negative control	Sartorius Tacta (20-200 µl)	0	Autoclave

Table 1: Mechanical pipettes included in the surface contamination and decontamination study and their decontamination procedure.

1.1.1 Detecting Live Mycoplasma

Agar plates (MS5, Mycoplasma Experience) were inoculated with 200 µl of sample. The plates were sealed with parafilm and incubated at 36°C for 72 h. The CFUs were counted and the total CFUs in the original samples were determined.

1.1.2 Detecting Mycoplasma DNA

Total DNA was extracted from 200 µl of sample with the Sartorius Microsart® AMP Extraction Kit (Cat No. SMB95-2003). The procedure for low complexity aqueous samples was applied, adding 80 µl Microsart® AMP Coating Buffer (Cat No. SMB95-2002) to the sample before extraction procedure. The eluate (possibly containing mycoplasma DNA) was analyzed with RT-qPCR applying Sartorius Microsart® ATMP Mycoplasma kit (Cat No. SMB95-1003) and the LightCycler 480 Real-Time PCR Instrument (Roche).

1.2 Cross-contamination Test for Mechanical Pipettes and Bad Pipetting Technique

One milliliter of *A. laidlawii* culture (1.9x10^8 CFU/ml) was pipetted in total 25 aspiration-and-dispensing steps according to three different pipetting conditions. The tip was changed after every 5th step. After completion, 1 mL of sterile PBS was pipetted under the same pipetting condition. Sartorius Tacta 100-1000 µl pipette and two other manufacturers' mechanical 1000 µl pipettes (Pipette A and B) were tested (Figure 4). Agar plates were inoculated with the PBS sample and CFUs were determined after 72 h of incubation at 36°C.

2. Results – Autoclaving Eliminates Mycoplasma

In comparison to mechanical non-autoclavable pipettes, the fully autoclavable Tacta pipettes can be 100% decontaminated of mycoplasma. Autoclaving is the only way to ensure that the surface of your pipette is fully decontaminated of infectious mycoplasma (Figure 2).

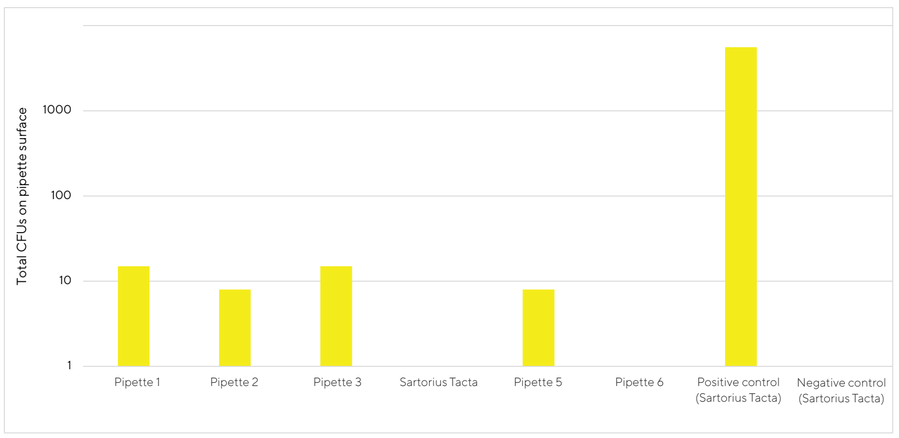

Figure 2: Autoclaving ensures complete decontamination of mycoplasma. The surface of the pipettes (except negative control) were contaminated with mycoplasma. Only autoclaving (Sartorius Tacta and Pipette 6) completely eliminated mycoplasma. After 24 h, ca. 0.5% of the total inoculated number of mycoplasma could still be recovered when no decontamination procedure was applied (Positive control).

3. Results- Autoclaving or Ethanol Do Not Remove Mycoplasma DNA

Neither autoclaving nor 70% ethanol removes mycoplasma DNA - samples taken from every pipette, except the pipette that was not contaminated, were positive for mycoplasma when analyzed with RT-qPCR (Figure 3).

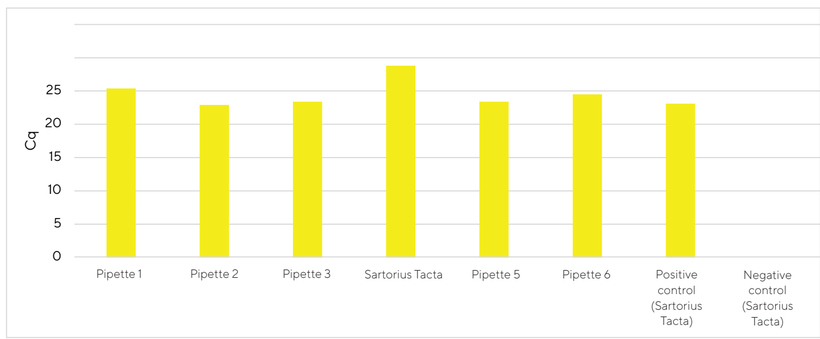

Figure 3: Mycoplasma DNA is not removed by autoclaving or 70% ethanol. The DNA extracted from the samples taken from the surface of the pipettes were analyzed with RT-qPCR with the the Microsart® ATMP Mycoplasma kit. The average quantification cycle (Cq) values for are shown. All the pipettes, except for the negatve control, were positive for mycoplasma DNA.

4. Results - Pipette and Pipetting Technique Matter

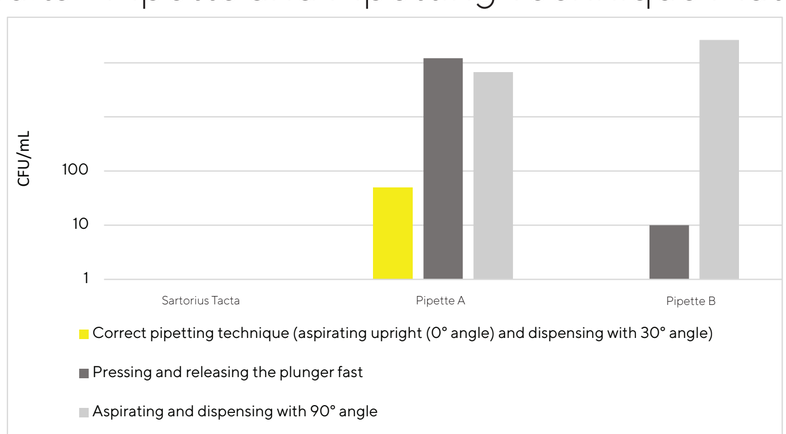

Figure 4: Bad pipetting technique, in particular in combination with mechanical pipettes with stiff plungers, increases the risk for cross-contamination. Sartorius Tacta pipettes have soft plungers, preventing retraction of contaminating droplets into the pipette. In comparison to the two pipettes from other manufacturers, cross-contamination was not observed with the 100-1000 µl Tacta pipette.

5. Conclusion

Pipettes are potential sources of mycoplasma contamination, as mycoplasmas can survive for at least 24 h on their surface. Autoclaving eliminates infectious mycoplasma. Sartorius Tacta pipettes can be completely autoclaved, significantly reducing the risk of contaminating your cell cultures. However, for removing mycoplasma DNA from the pipette we suggest sodium hypochlorite solution, DNA AWAY™, or PCR Clean™ Wipes (Minerva Biolabs). Additionally, correct pipetting technique and pipettes with soft plungers such as Sartorius Tacta are essential for preventing contamination and spread of mycoplasma.

Tips and tricks to prevent mycoplasma contamination in your laboratory:

- Wear protective clothes and gloves
- Frequently clean the laminar flow cabinet and surfaces where you work with cells
- Set up a regular cleaning-schedule for your pipettes
- Use pipettes in one single laboratory – do not move them around
- Add Safe-Cones to the tip cones of your pipettes if you are not using filter tips
- Use Safetyspace™ Filter tips
- Use Sartorius Tacta ergonomic pipettes with soft plunger and tip ejector, avoiding splashes and retraction of droplets into the pipette while pipetting
- Aspirate with the pipette upright (0° angle) and dispense with 30° angle
- Test your cell cultures and cell lines on a regular basis – use Sartorius Microsart® Mycoplasma PCR kits to detect any traces of mycoplasmas in your cell cultures

References

Bajerski et al. Applied Microbiology and Biotechnology (2020); 104.
Gedye et al. Stem Cell Rev Rep (2016); 12(1).
Kim et al. Biomaterials Research (2015); 19(6).
Olarerin-George AO & Hogenesch JB. Nucl Acid Res (2015); 43(5).