

Standard Operating Procedure: 256a

Title: How To Take a Mycoplasma Sample

Issue Date: 9/6/07

Effective Date: 9/6/07

SOP reviewed by: _____ **Date Signed:** _____
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Revision #: 2 **Revision Date(s):** 9/27/13

1.0 Purpose and Applicability

To collect a mycoplasma sample for testing. Mycoplasma-infected cells tend to change morphology and tend not to grow *in vivo*. Mycoplasma can cause changes in cell growth characteristics, inhibition of cell metabolism, disruption of nucleic acid synthesis, chromosomal aberrations, change in cell membrane antigenicity, and can alter transfection rates and virus susceptibility.

2.0 Definitions

Mycoplasma spp.: a group of BACTERIA, some of whose members cause disease in animals and humans. They penetrate and infect individual cells.

3.0 References

SOP# 729 Cleaning The Laminar Flow Hood

4.0 Responsibilities

- Research Specialist Principal
- Research Specialist Senior
- Research Specialist
- Research Technician

5.0 Supplies

- 5, 10, 25, 50 ml Pipets
- 70% EtOH

10% Bleach
Gloves
Kimwipes
Beaker with 100% Bleach
Biohazard Waste

6.0 Equipment

CO₂ Water Jacketed Incubator Maintained at appropriate cell culture environment depending on cells
Pipet Aid
Laminar Flow Hood

7.0 Procedure

1. Put on gloves, clean the hood (SOP# 729 Cleaning The Laminar Flow Hood),.
2. Remove flasks that will be tested for mycoplasma from incubator, view media color and cell confluency macroscopically.
3. Put the flask into the hood. Remove cap and place behind flask4.
Mycoplasma samples are taken BEFORE the regular feeding of that particular cell line. Get a sterile 5 mL pipette, unwrap it being sure not to touch the pipette with anything, and, for an adherent cell line, collect **2 mLs only** of conditioned media that has been on the cells and in the incubator for **at least 3 days**.
5. For a suspension cell line, stand the flask up so the neck of the flask is perpendicular to the hood surface for about 3 minutes. Collect 2 mLs of conditioned media that has been on the cells and in the incubator for **at least 3 days** media for the mycoplasma sample from the surface of the cell/media solution.
7. Preferably take a mycoplasma sample from a cell line that has been split at least once from time of thawing and is at least 60% confluent with media on the cells for at least 3 days.
8. Place the sample into a **15 mL** plastic tube and cap it. It is important to put the sample into only this type of tube for centrifuging and to eliminate the need for transferring the sample, which could cause contamination. Refrigerate the sample for up to one week until it is taken to the EMSS for testing (sample is only good for 1 week). Fill out appropriate paperwork when taking mycoplasma samples to the EMSS.

8.0 Records

Cell Culture Record Book
EMSS Database

9.0 History

Changed SOP since Kathy Brown doesn't work for the EMSS any longer.