

## TITLE: Mycoplasma Testing-Sample Preparation/Sample Submission

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### Background:

Mycoplasmas are wall-less microorganisms belonging to a genera within the class *Mollicutes*. These flexible organisms are as small as 0.2-0.3  $\mu\text{m}$  and can achieve high densities in cell cultures, (10<sup>7</sup>-10<sup>8</sup> organisms/ml). Additionally, they can be found attached to the cell membranes of the cultured cells. Unlike bacteria and fungi, mycoplasma can not easily be detected. No changes in pH or turbidity of the cell culture media is observed with mycoplasma contamination. The only way to know that your cell cultures are contaminated is to routinely test them.

Tests fall into two categories: direct culture with media and indirect tests measuring specific biochemical markers or other characteristics associated with mycoplasmas.

The Cell Center will have your cells tested using an indirect method called DNA fluorochrome staining. The cells are fixed on a slide treated with a special coating (Cellshipper slide) and sent to Bionique Testing Laboratories, Inc. for DNA fluorochrome staining. Results are reported within 48 hours. Appropriate positive and negative controls are processed with each assay and the test is 95% effective in detecting mycoplasma.

DNA from all mycoplasma species can be detected. Bacteria, fungi, and large viruses (such as smallpox) can lead to a questionable result.

This test is able to detect levels as low as 10,000-100,000 mycoplasma colony forming units per ml of culture media.

### Sample Preparation for Mycoplasma Testing

Grow cells in antibiotic-free media for 7 days. Do not change the medium or dilute with fresh medium during the last 24-72 hours before testing. Cells should be 50-80% confluent.

**Testing adherent cells:** remove most, but all, of the used medium. Leave approx. 3 ml in a T25 flask or 5 ml in a T75 flask. Use a cell scraper to dissociated cells from the substrate. Break up clumps by vigorously pipetting. Pipet 3 or 5 ml cell suspension into a 6 ml test tube.

**Testing suspension cells:** Pipet 2 ml of a heavy cell suspension to a 6 ml test tube.

### Submitting Sample to the Cell Center

Bring sample(s) to **Blalock 1017B** on **Tuesdays 10 am**. Samples received after 10:15 am will not be processed.

**Important Note:** Samples grown in antibiotics or given fresh medium prior to testing may have artificially low mycoplasma levels and may give a false negative result. Likewise, trypsinizing adherent cells may remove some mycoplasma from the cell membrane of the cell line. Conversely, samples that are contaminated with bacteria, fungi, or large viruses will give a questionable result because the test stains all prokaryotic cells.

The test can detect **all** species of Mycoplasma and Acholeplasma. There are over 200 species of mycoplasma.

Reference: Cell Shipper instructions, Bionique Testing Laboratories, Inc., Saranac Lake, NY.

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