Center For Inherited Disorders of Energy Metabolism
University Hospitals Case Medical Campus

CELL CULTURE MYCOPLASMA TESTING INFORMATION

General Mycoplasmal Biology:

Mycoplasmas are wall-less bacteria which can be as small as 0.2 – 0.3 μm and can achieve very high densities in cell cultures, (10^7 – 10^8 organisms/mL), without discernable pH changes or turbidity. There are currently more than 120 species in 6 genera, many of which are pathogenic. The vast majority of cell culture contaminants belong to only 6 species of human, bovine or porcine origin.
These very common contaminants are ***Mycoplasma hyorhinis***, ***Mycoplasma arginini***, ***Mycoplasma salivarium***, ***Mycoplasma orale***, ***Mycoplasma fermentans***, and ***Acholeplasma laidlawii***.

Causes of High Rates of Mycoplasma Contamination:

The high mycoplasmal contamination rate of cell cultures is primarily due to four factors:

1. The absence of visible signs of mycoplasmal contamination including changes in turbidity, cytopathic effect and pH, even in heavily contaminated cultures, leads to a false sense of security. Once present in the laboratory, mycoplasmally infected cultures often cross-contaminate the other cell lines being used.
2. The continuous use of antibiotics which are not usually mycoplasmacidal in long-term cell cultures can mask poor aseptic technique. This leads to accidental introduction of mycoplasmas along with other microorganisms.
3. The lack of simple, easy-to-use and reliable detection methods in the past has resulted in many cell lines being untested. Because of this, many cell lines brought into a lab from outside sources although appearing normal and healthy, are in fact already infected with mycoplasma.
4. Because of their small size and lack of cell wall, mycoplasmas occasionally penetrate the filters used to "sterilize" cell culture media and sera, resulting in low levels of these organisms being accidentally introduced into cultures during routine feeding.

Detection of Mycoplasma Contamination:

Unlike contamination caused by most bacteria, yeast or fungi, mycoplasmas typically do not alter the turbidity or pH of cell culture media. Therefore, they can only be detected through tests that fall into two basic categories:

1. Direct culture with media
2. Indirect tests measuring specific biochemical markers or other characteristics associated with mycoplasmas.

Direct culture requires the use of one or more complex nutritionally enriched mycoplasmal media and carefully controlled environmental conditions. Even then, some mycoplasmal strains are difficult to grow in culture without cells. Properly done, with appropriate positive and negative controls, direct culture testing offers the greatest security, but is rather slow, usually requiring up to 28 days for completion.

There are many indirect tests of varying sensitivity and convenience, including DNA fluorochrome staining, DNA probes, PCR, ELISA, autoradiography, immunofluorescence and specific biochemical assays. While faster than direct culture methods, indirect tests are not yet as sensitive and usually require higher levels of contamination (i.e. 10^4+ organisms/mL) for detection. Indirect tests offer two advantages over direct culture methods: first, they can detect so-called "non-cultivable" mycoplasmal strains that direct culture may miss; second, they are faster, usually taking only 1-5 days to complete.

The CIDEM Lab Uses:

CELLshipper Cat # M-100
Bionique Testing Laboratories, Inc.
RR #1, Box 2, Bloomingdale Rd.
Saranac Lake, NY 12983
Phone: (518) 891-2356 Fax: (518) 891-5753

REFERENCE:
Cell Culture User's Guide to Mycoplasma Detection and Control

Note: All cells sent to CIDEM must have a copy of mycoplasma test results sent with the cells. CIDEM will not accept cells that have not been tested for the most common mycoplasma contaminants listed above. General clinical analysis that tests for Ureaplasma urealyticum and Mycoplasma hominis is not acceptable. Please note on your result sheet the test method that was used.