13: Quality Control In Cell Culture: Testing for Mycoplasma Contamination

Performance of virology laboratories depends largely on continuous uninterrupted supply of healthy cell cultures for virus work. Mycoplasma contamination of cell lines presents as a unique problem in the cell culture laboratory. Use of excess of antibiotics may mask poor aseptic techniques leading to introduction of mycoplasma in cell cultures. Mycoplasma contamination does not show visible signs such as turbidity of the medium, cytopathic effect or pH changes. Yet it may deplete arginine in media, alter nucleic acid synthesis and RNA profiles and growth rate of the cell line.

As part of internal quality control programme of the laboratory monthly testing of all cell lines for mycoplasma was introduced. Mycoplasmas were detected by staining the cells with DNA specific fluorescent dye, 4’ 6 diamidino-2phenylindole (DAPI). In mycoplasma free cultures only the fluorescent cell nuclei can be seen. Infected cultures show typical starry sky appearance due to mycoplasma cells (Figure 18).

Figure 18. Mycoplasma detection by DAPI staining.

Mycoplasma +ve cells, Cytoplasmic blue fluorescence of Mycoplasma.

We have now extended mycoplasma-testing facility to Polio Network Laboratories in Southeast Asia region. Laboratory personnel of these laboratories were trained during the annual workshops conducted at ERC. All laboratories submit cultures for testing for presence of mycoplasma on a quarterly basis. The results are made available in 3 days. Initially, Mycoplasma contamination was observed frequently in some laboratories.
Monitoring cell lines for mycoplasma has drastically reduced the rate of contamination in all laboratories indicative of improvement in good laboratory practices.

14. Research papers


