Development and application of a protocol for detection, prevention and elimination of Mycoplasma contamination in cell cultures

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Mycoplasma contamination in cell culture is an extremely serious setback for the cell-culturist. It was first time discovered by Robinson et al., in 1956. The latest surveys indicate about 15-80% of all cultures might be contaminated with mycoplasma from outside India. However, till date such survey reports are not available from India. Experiments using mycoplasma contaminated cultures yield unreliable and non-reproducible results and unsafe biological products.

There were total 77 cell cultures were collected from different cell culture laboratories situated in Delhi and subjected to Immunofluorescence Assay (IFA), Hoechst staining and PCR for detection of mycoplasma contamination. Out of 77 cell cultures, 18 cultures were uniformly positive for mycoplasma contamination by both IFA & Hoechst staining. Conversely, 64 cultures (83.1%) out of 77 were positive and 13 cultures were negative by PCR. There were 46 false positive cell cultures that showed clear mycoplasma negative status by Hoechst and IFA were found mycoplasma positive by nested-PCR kit and interestingly there was one false negative result that very apparently showed positive result by Hoechst and as well as IFA tests. Adobe Photoshop (version 7.0) was used to find out the low-level mycoplasma contamination on IFA preparations.

This study reveals that out of 21 cell culture laboratories only 6 (28.6%) were having mycoplasma positive cultures. Ciprofloxacin and gentamycin were able to prevent mycoplasma contamination in cell cultures as compared to penicillin-streptomycin combination. Number of mycoplasma positive cell cultures significantly more, which were handled by technicians as compared to researchers. Suspension cell lines were more prone to get mycoplasma infection as compared to adherent cell lines but there was no significant association between the incidence of mycoplasma positivity of primary and continuous cell cultures. For preventing the mycoplasma contamination point of view, use of 0.1 μm pore sized filter membrane was more significant than 0.22 μm and 0.45 μm pore sized filter membrane for culture media sterilization. The use of type I or type II laminar airflow workbenches and implementation of unwarranted fumigation in cell culture laboratories would not help contain an already existing outbreak of contamination. The granularity in the cytoplasm of cultured cells is an indication of mycoplasma contamination.

Higher doses of ciprofloxacin, gentamycin and azithromycin were able to reduce the mycoplasma contamination level significantly but simultaneously these doses were toxic to cell line and lower doses of these anti-mycoplasma agents were ineffective to trim down the contamination level. Mycokill AB and Mynox were ineffective to eliminate the contamination and they were toxic to the cells.

In vitro cultured human keratinocytes and 3T3 cells were found mycoplasma negative by IFA, Hoechst staining and PCR. *In vitro* cultured human epidermis was subjected to light & electron microscopy and immunohistochemistry for its structural and functional confirmation. During the keratinocytes culture experiments, all preventive measures were undertaken to prevent mycoplasma contamination.