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Catalog ID: M-250 Multi-media Direct Culture and DNA Fluorochrome Test

The purpose of this test is to determine whether or not mycoplasmal contaminants are present in a cell culture sample, be it a primary culture, hybridoma or continuous cell line.

Cells should be cultured at least once, preferably 3 passages without antibiotics &/or selective agents to increase sensitivity. This procedure combines an indirect DNA fluorochrome assay (see M-150) to detect non-cultivable mycoplasmas with a direct culture assay utilizing three mycoplasmal media formulations. The DNA fluorochrome procedure is a non-selective indirect DNA fluorochrome assay which involves the inoculation of a 1.0 ml sample into a mycoplasmal free indicator cell culture (VERO), incubation for 3 to 5 days, then performance of a DNA fluorochrome (Hoechst) staining assay on a slide prepared from the co-incubated sample/Vero cell culture (see M-100 CellShipper®). This assay is designed to enhance the level of sensitivity by reducing background from genetically unstable cell lines (e.g. hybridomas, etc.) and amplifying the titer of mycoplasmal contaminants. Appropriate positive ($<10^3$ CFU/ml) and negative controls are processed with each sample.

The direct culture procedure utilizes three mycoplasmal media formulations: Fortified Commercial (FC), Modified Hayflick (MH), and Heart infusion (HI) broth and agar formulations. A 0.5 ml sample is inoculated into 6 ml of each broth (FC, MH, & HI). A 0.1 ml sample is inoculated onto duplicate plates for each agar formulation. Each broth is subcultured onto like agar plates on Day 7 post setup. The agar plates are incubated aerobically and microaerophilically and examined microscopically at 7 day intervals. Appropriate positive (< 100 CFU) and negative controls are processed with each sample. The theoretical sensitivity is approximately 50 CFU. The accuracy of the combined test is approximately 99%. Total testing time is 28 days.