# Mycoplasma – A Cells Worst Enemy

**Pippa Bracegirdle, Clare Wilson and Bryan Bolton** *European Collection of Cell Cultures, Health Protection Agency, Porton Down, Wiltshire, UK*

## Answer from front page

A scanning electron micrograph of an infected cell, showing the cell membrane completely covered with Mycoplasma.

## Are your cell lines free from Mycoplasma contamination?

Consider the effects Mycoplasma contamination has on the properties and functions of a cell line and how this might affect your research:

- Affect uptake across cell membranes
- Interfere with membrane receptor function
- Cause morphological change
- Influence amino acid and nucleic acid metabolism
- Induce cell transformation

In addition to the effects mycoplasmas might have on an individual cell line, the introduction of a contaminated culture can devastate a cell culture facility due to it's ability to spread rapidly through all cell cultures, causing an outbreak situation.

## The solution

The best way to avoid the introduction of Mycoplasma is to obtain your cell lines from a recognised culture collection such as ECACC. However, it is still necessary to carry out regular testing of cell lines in routine culture and at the time of cell banking, so that any contamination can be quickly identified and removed from the facility. ECACC provides a Mycoplasma testing service, which is used by many of our customers. Three Mycoplasma detection methods are currently routinely employed at ECACC, each having particular strengths and weaknesses (see Table 1). Reliance on a single detection method for anything other than screening purposes is not advisable, and if a cell line has not been tested for some time ECACC recommends testing by all three methods.

| Method | Sensitivity | Species range |
|---|---|---|
| PCR | Low/Medium | Uncertain |
| Indirect DNA Stain | Medium | All |
| Culture Isolation | High | Majority |

| Method | Speed | US FDA Approval |
|---|---|---|
| PCR | 1 day | No |
| Indirect DNA Stain | 2-3 days | Yes |
| Culture Isolation | 3-4 weeks | Yes |

**Table 1: Mycoplasma detection methods.**

# Testing for Mycoplasma

The protocols outlined opposite are routinely used by ECACC for testing all manufactured cell banks. A more detailed version of these protocols is provided in the popular ECACC and Sigma-Aldrich joint publication "Fundamental Techniques in Cell Cultures – A laboratory handbook'. For a free copy of this publication complete the reply card or visit **www.ecacc.org.uk.**

## Detection of Mycoplasma by Indirect DNA Stain (Hoechst 33258)

DNA staining methods such as Hoechst stain, are suitable for the detection of Mycoplasma in both cell cultures and cell culture reagents and can give results within 24 hours (Figure 1). However, direct staining is relatively insensitive, with a detection limit of $10^6$ colony forming units (CFU) $ml^{-1}$. Co-culturing the test sample with an indicator cell line such as Vero (ECACC Product No. 84113001-1v1) can improve the sensitivity to $10^4$ CFU $ml^{-1}$ by increasing the available surface area upon which mycoplasmas can adhere.

## Detection of Mycoplasma by Culture

Detection of mycoplasmas using both direct culture and an enrichment step, is regarded as the reference method, with a theoretical detection level of 10 CFU $ml^{-1}$ (Figure 2). This method is suitable for the detection of mycoplasmas in both cell cultures and reagents, with results available within four weeks. However, it is worth noting that certain strains of *Mycoplasma hyorhinis* can not be cultured *in vitro*. *Mycoplasma sp.* Colonies have a characteristic "fried egg" appearance.

**Figure 1: Detection of Mycoplasma by Indirect DNA Stain**

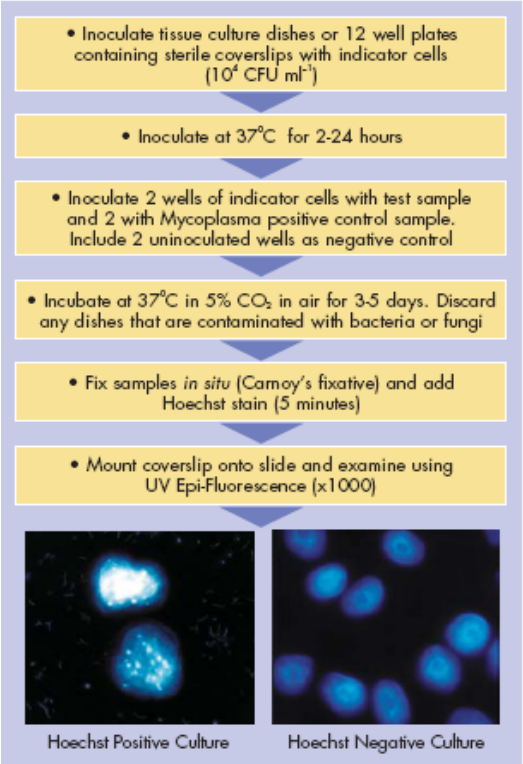
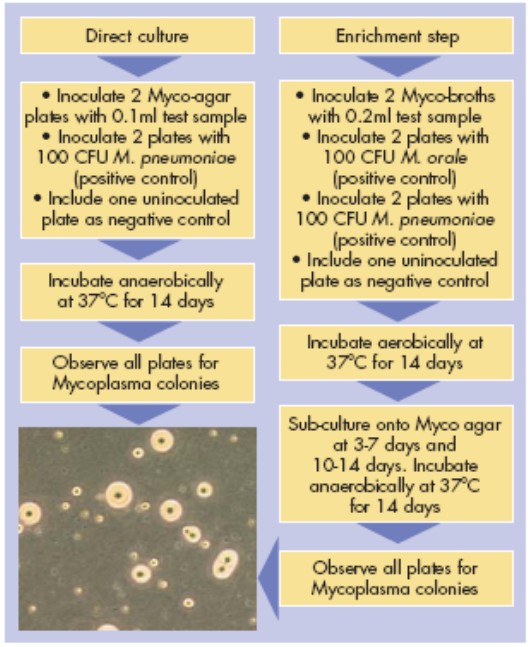



**Figure 2: Detection of Mycoplasma by Cell Culture Isolation**




## Important Notes for both Methods

1. These test procedures should be carried out in a microbiological laboratory away from the cell culture laboratory.
2. *M. pneumoniae* is a potential pathogen and must be handled in class II microbiological safety cabinet operating to ACDP Category 2 Conditions.
3. Hoechst stain is toxic and should be handled and discarded with care.

## Ordering Information

| Description | Price £ |
|---|---|
| Indirect DNA Stain | 110* |
| Culture Isolation | 150* |
| PCR | 60* |
| All three tests together | 260 |

*A sample preparation fee of £90 per sample is charged if samples are received frozen or require passaging without antibiotics.

Health Protection Agency

**ECACC is a Health Protection Agency Culture Collection**