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

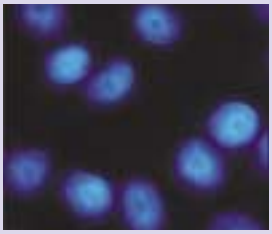
Detection of mycoplasmas using both direct culture and an enrichment step, is regarded as the reference method, with a theoretical detection level of 10 CFU ml^{-1} (Figure 2). This method is suitable for the detection of mycoplasmas in both cell cultures and reagents, with results available within four 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.

ECACC Service

Figure 1: Detection of Mycoplasma by Indirect DNA Stain

- Inoculate tissue culture dishes or 12 well plates containing sterile coverslips with indicator cells (10^4 CFU ml^{-1})
- Inoculate at 37°C for 2-24 hours
- Inoculate 2 wells of indicator cells with test sample and 2 with Mycoplasma positive control sample. Include 2 uninoculated wells as negative control
- Incubate at 37°C in 5% CO_2 in air for 3-5 days. Discard any dishes that are contaminated with bacteria or fungi
- Fix samples *in situ* (Carnoy's fixative) and add Hoechst stain (5 minutes)
- Mount coverslip onto slide and examine using UV Epi-Fluorescence (x1000)

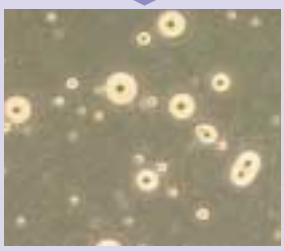
Hoechst Positive Culture



Hoechst Negative Culture

Figure 2: Detection of Mycoplasma by Cell Culture Isolation

Direct culture	Enrichment step
• Inoculate 2 Myco-agar plates with 0.1ml test sample • Inoculate 2 plates with 100 CFU *M. pneumoniae* (positive control) • Include one uninoculated plate as negative control	• Inoculate 2 Myco-broths with 0.2ml test sample • Inoculate 2 plates with 100 CFU *M. orale* (positive control) • Inoculate 2 plates with 100 CFU *M. pneumoniae* (positive control) • Include one uninoculated plate as negative control
Incubate anaerobically at 37°C for 14 days	Incubate aerobically at 37°C for 14 days
Observe all plates for Mycoplasma colonies	Sub-culture onto Myco agar at 3-7 days and 10-14 days. Incubate anaerobically at 37°C for 14 days
	Observe all plates for Mycoplasma colonies



Important Notes for both Methods

1. These test procedures should be carried out in a micro-biological 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.

To make use of this service visit the ECACC website for details on how to send samples for testing or contact our technical support co-ordinator on **+44 1980 612684**

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.

Information Request 201

Cell Culture Training Courses

Learn how to maintain your cell lines free from contamination by attending ECACC's long established training courses. Call today to book your place and gain valuable in depth practical and theoretical knowledge from ECACC's cell culture experts. The ECACC Cell Culture training courses are designed to accommodate the newcomer to cell culture (Level 1) through to the experienced cell biologist (Level 2).

Level I (2004)
13 - 15 January
11 - 13 May
9 - 11 November

Level II (2004)
2 - 5 March
5 - 8 October

Contact our Business Administrator and Training Coordinator:
lisa.reynolds@hpa.org.uk