Mycoplasma detection by culture

Generalities

Detection of mycoplasma by culture is the reference method of detection and has a theoretical level of detection of 1 colony-forming unit (cfu). However, there are some strains of mycoplasma that are **non-cultivable** (certain strains of *Mycoplasma hyorhinis*) and need DNA-based methods to be detected. The current method is suitable for the detection of mycoplasma in both cell cultures and cell culture reagents and results are obtained within 4 weeks. Mycoplasma colonies observed on agar plates have a 'fried egg' appearance (see **figure 1**).

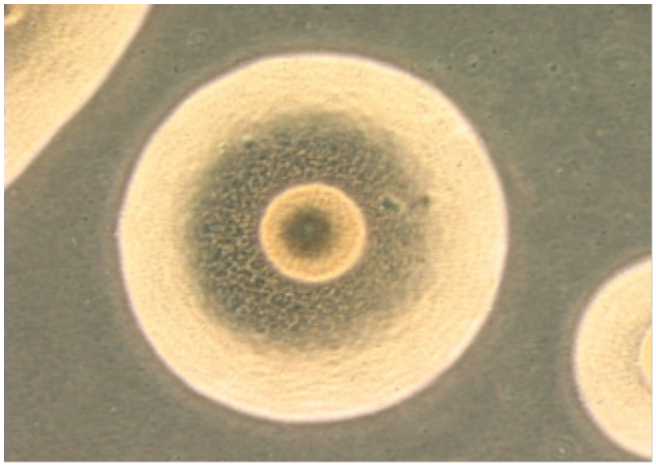

Figure 1. Colony of Acholeplasma laidlawii showing typical "fried egg" morphology.

Materials

- 70% (v/v) isopropanol in sterile water
- Mycoplasma plates (in 5cm petri dishes)
- Mycoplasma horse serum broths (in 1.8ml aliquots)
- *M. orale* (ATCC #23714, from $185.00)
- *M. pneumoniae* (ATCC #15531, from $185.00)

Equipment

- Personal protective equipment (gloves, laboratory coat, safety visor)
- Water bath set to 37°C
- Microbiological safety cabinet at containment level II
- Incubator set at 37°C
- Anaerobic jar system

Sample Preparation

- Cell should be cultured in antibiotic-free media for at least three passages prior to the preparation of the test sample.
- Prepare cell suspension at 1 to $2x10^6$ cells/mL.
- Induce cell lysis either by sonication or using Triton x100 (see p. 33).
- Samples can be stored at ≤ - 60°C.

Procedure

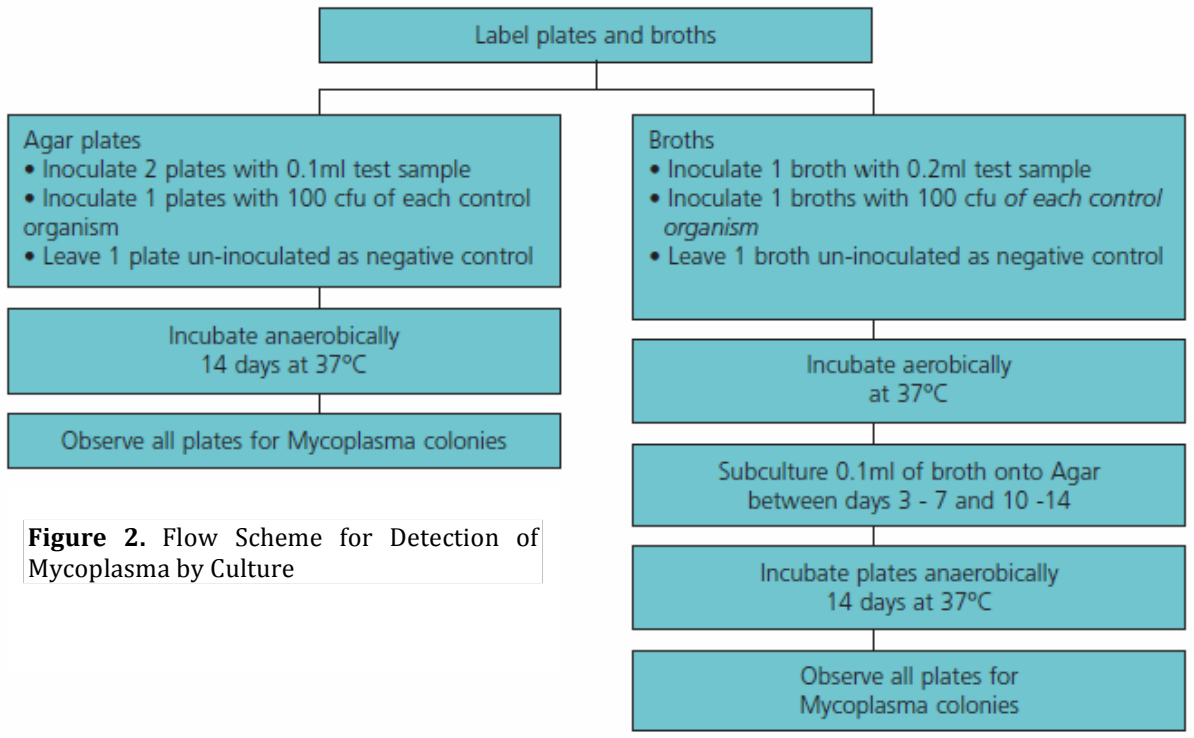

Figure 2. Flow Scheme for Detection of Mycoplasma by Culture

1. Inoculate 2 agar plates with 0.1 ml of test sample.
2. Inoculate an agar plate with 100 cfu of each control organism (M. orale and M. pneumonia).
3. Leave 1 agar plate un-inoculated as a negative control.
4. Inoculate 1 broth with 0.2 ml of test sample.
5. Inoculate a broth with 100 cfu of each control organism.
6. Leave 1 broth un-inoculated as a negative control.
7. Incubate agar plates anaerobically for 14 days at 37°C.
8. Incubate broths aerobically for 14 days at 37°C.
9. Between days 3 - 7 and 10 - 14 of incubation, subculture 0.1 ml of test broth onto an agar plate and incubate plate anaerobically as above.
10. Observe agar plates after 14 days incubation at x400 magnification for the presence of mycoplasma colonies (see **Figure 1**).

Results

- Criteria for a Valid Result:

- All positive control agar plates and broths show evidence of mycoplasma by typical colony formation on agar plates and usually a colour change in broths.
- All negative control agar plates and broths show no evidence of mycoplasma.

- Criteria for a Positive Result
  Test agar plates infected with mycoplasma show typical colony formation.

- Criteria for a Negative Result
  The test agar plates show no evidence of mycoplasma.

Notes

1. *Mycoplasma colonies* have a typical colony formation commonly described as "fried egg" due to the opaque granular central zone of growth penetrating the agar surrounded by a flat translucent peripheral zone on the surface.
2. *Mycoplasma pneumoniae* is a potential pathogen and must be handled in a class 2 microbiological safety cabinet operating to ACDP Category 2 Conditions.
3. This test procedure should be carried out in a microbiology laboratory away from the cell culture laboratory.
4. It is recommended that samples be tested for mycoplasma using at least two detection methods (e.g. indirect DNA stain and culture isolation) for a more reliable result. This is due to the varying detection sensitivities of the methods for different species of mycoplasma.