

No. 4: Mycoplasma - horror of all cell cultures



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Mycoplasma infection is the ultimate horror vision, not only for Susi and other suspension cells but also for experienced laboratory personnel. Adi, our adhesion cell representative and a fairly solid type who doesn't tend to become hysterical about such things, also gets a shock when mycoplasma is mentioned.

Mycoplasma is not just a horror vision for Susi; the problem has been known for some 50 years and in the meantime it is estimated that between 30 and 45% of all cell cultures are either infected with mycoplasma or have been infected at some time. Mycoplasma, in spite of the name, is not myxomycetes but rather prokaryotic micro-organisms of an ancient development type. In contrast to many other bacteria, they have no cell wall and cause disease in both animals and humans.

Adi doesn't believe in ghosts. As far as he is concerned, mycoplasma is not anything unnatural, simply a source of danger. They endanger the vitality of cultivated cells, prevent their morphological progress and influence their metabolism. For this reason, working with cells infected with mycoplasma becomes a game of roulette. In Susi's opinion, the matter is almost unnatural as this particular danger is everywhere. Contamination with mycoplasma can be brought about by sera, humans or by already infected cell cultures. In Adi's opinion, one should react immediately to the first warning signals. Mycoplasma infections often cause morphological changes in cells or the appearance of black particles in the medium. However, these parameters are unreliable and can not always be adequately controlled. It is thus better to use the more professional methods of detection. Adi is always surprised to hear that there are more detection methods than pathogens. 95% of all mycoplasma infections are brought about by only 4 pathogens. For these, there are at least 5 methods of detection:

1. Culture Method

In this method, mycoplasma is cultivated in special nutrient solutions using the necessary aerobic and anaerobic culture techniques. This method is not only time-consuming but, unfortunately, also unreliable in Adi's opinion as not all mycoplasma species can be cultivated in this way.

2. Electron Microscopy

This method requires a lot of time and expensive apparatus and thus can not always be used.

3. Fluorescence Detection

The principle of this method is that the DAPI or bis-benzimide recognise and specifically bind double-stranded DNA. Single-stranded DNA is not detected. A culture infected with mycoplasma can be recognised by the fact that, apart from a clearly defined core fluorescence of the culture cells, the cytoplasm or the outer area of the cells is dyed. Contamination can be recognised by uniform mottled fluorescence. However, one must be careful in interpreting the situation: If the fluorescence dye has not been properly washed out, the whole cell will exhibit fluorescence.

4. Enzyme immunodetection

The most sensitive detection method in use up to now is the enzyme immunoassay. This is an immunoassay based on polyclonal antibodies against the four principle pathogens. The sensitivity of the enzyme test is 10⁴ to 10⁶ KBE/ml* depending on the strain used.

* Germ-forming units

5. Biochemical detection methods

The detection method using 6-methyl-purine-deoxyriboside (6-MPDR) has established itself as the most important routine method and requires little in the way of apparatus. This test is based on the basic differences in the enzyme composition of mycoplasma and some prokaryotes and mammalian cell cultures. For example, the enzyme adenosine phosphorylase occurs in negligibly small amounts only, if at all, in mammalian tissue, whilst significant amounts occur in mycoplasma and other microorganisms. This enzyme converts the synthetic nucleotide 6-methylpurine-deoxyriboside into 6-methylpurine and 6-methylpurine-riboside, both of which are toxic to cells. These two antimetabolites can kill the test cells necessary for this test (3T6 mouse fibroblasts) at a concentration of only $< 1 \mu\text{mol/litre}$. The test is carried out as follows:

Using a micrititre plate, 5×10^3 3T6 cells are placed in each well containing $150 \mu\text{l}$ DME. Once the cells have adhered, the supernatant ($20 \mu\text{l}$) is pipetted into three wells. 24 hours later, 6 MPDR is added to 2 of these wells, one $5 \mu\text{l}$ and one $2.5 \mu\text{l}$. The third well serves as a control as to whether the test supernatant is already toxic for the 3T6 cells. The medium is also checked, i.e. one mole Mycotect in PBS is added to a DME sample in the specified concentration. The test cells are incubated for 3 days at 37°C and subsequently dyed using crystal violet. Intact 3T6 cells are dyed completely violet. In those wells containing supernatant infected with mycoplasma, the test cells die and there is no dyeing effect.

Comments: False positive results can occur if cells are contaminated with *Bacillus subtilis*, *leishmaniae*, *trypanosomas* and *schistosomas* as these contain adenosine phosphorylase and thus convert 6 MPDR into its toxic components.

Literature References

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