

No 5: Mycoplasma infection - therapy is possible



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After going through the shock of a mycoplasma infection of the cell culture, Susi and Adi report in their capacity as "health consultants" on successfully introduced therapy for saving valuable cell cultures.

Whilst Susi sees mycoplasma infections as horror visions, Adi is more practical. In his opinion, the slow growth rates of mycoplasma provide a better chance of combatting them as is the case with bacterial and fungal infections.

Adi of course is referring to the use of specific antibiotics against mycoplasma and even Susi, who is usually against the constant use of chemicals in media, has to admit that this procedure can be effective in the fight against mycoplasma infection. Two procedures have proved of value in recent years, according to Adi:

In the first instance, the alternating method using minocyclin and tiamulin and on the other hand the continuous use of ciproflaxin over a period of 14 days. In the first method, apart from the usual additives such as FCS, either 5 µg/ml minocyclin or 10 µg/ml tiamulin are added (sequence unimportant). Other antibiotics should not be used during the period of treatment.

Inoculate the cells in a concentration of 10⁵ cells per T-bottle and treat for 4 days with minocyclin or tiamulin. Change the medium and subsequently treat the cells with the second antibiotic for three days. This alternating method of treatment should be continued for one or, better, two weeks. Subsequently, method number 4 as described should be used to identify whether mycoplasma has accumulated in the cell cultures. As a rule, this is no longer the case and the cells can be cultured and aliquotted for storage.

Apart from this alternating method, the use of ciproflaxin (Ciprobay, Bayer) in a concentration of 10 µg/ml has proven of value. This treatment should be carried out over a period of 14 days.

In Susi's opinion as a suspension cell, co-cultivation with macrophages is the method of choice. In this case, primary peritoneal macrophages are obtained from the mouse and pipetted onto a hydrophobic Petri dish (e.g. Petriperm, Heraeus). The layer of macrophages must be uniform and must cover the whole of the area. The cells to be treated are then pipetted directly onto the surface of the macrophages and cultivated for one to three days. Both adherence cells (such as for example infected fibroblasts) and myeloma or hybridoma cells cannot adhere to a complete layer of macrophages and hence remain in suspension during the period of incubation.

The macrophage layer digests the mycoplasma adhering to the cell surfaces or to the cell membrane via phagocytosis and after one to three days it can be relatively easily pipetted off and placed in a new culture vessel. Strict adhering cells are better left for only one day on the macrophage layer whilst myeloma cells may be left for three days. This treatment with macrophages should be supported with antibiotic treatment so that the whole represents a relatively reliable system of treatment.

Although Adi is sometimes a little bit bashful on questions of costs, these questions have to be asked. In the case of less exclusive cells that may be infected, these are normally better replaced by new ones which can be obtained from cell banks. In the case of the more precious cell lines, the therapy methods described above can be used to clone the cells and, subject to adequate testing, they can be frozen.

In any event, strict quarantine conditions should be maintained with all cells infected by mycoplasma. Susi's motto is always "prevention is better than cure"; this principle can prevent infection spreading to other cell lines.

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