Troubleshooting & FAQ

Mycoplasma

The recent interest in mycoplasma diagnostics has resulted in the development of new PCR-based methods for detecting mycoplasma contamination. In the following, the general mycoplasmal biology and the features of the Minerva Biolabs mycoplasma detection system Venor®*GeM* are discussed. The technical basis and utility of this system are highlighted and explained.

What are mycoplasmas?

"Mycoplasma" is used to describe members of the genus *Mycoplasma*, in the class *Mollicutes*, representing a group of minute, wall-less bacteria comprising over 120 species. They are the smallest self-replicating prokaryotic organisms. As a consequence of their small genome (600-1700 kb depending on the species), mycoplasma lack many biosynthetic pathways and are dependent on nutrients obtained from their environment. Their small size (~ 0.15 µm) allows them to pass through common 0.22- or 0.45-µm sterilization filters and they are resistant to antibiotics such as penicillin and streptomycin.

Very common contaminants are: *Mycoplasma hyorhinis, Mycoplasma arginini, Mycoplasma salivarium, Mycoplasma orale, Mycoplasma fermentans,*

Acholephasma laidlawii, Mycoplasma pneumoniae, Ureaplasma urealyticum.

What is the problem with mycoplasma contaminations?

Mycoplasma are parasites and many are pathogens that infect a variety of animal hosts. In humans, mycoplasma are mostly surface parasites, colonizing the epithelial lining of the respiratory and urogenital tracts.

Mycoplasma are common and serious contaminants found in cell cultures. Mycoplasma contamination is widespread in laboratories, depending on the cell lines and quality management systems used. Several studies (including our own data) support an incidence of ~15-85%. A cell culture infection may persist for an extended amount of time without apparent cell damage, but can affect nearly every parameter of cell metabolism including altering the cells phenotypical characteristics and normal growth. As a result of this widespread problem, bio production and research is often unknowingly done using mycoplasmal contaminated cell cultures. The validity and significance of research and the safety of the biologicals produced from contaminated cell cultures must be questioned.

Can trypsin be a possible source of contamination in cell cultures?

Trypsin Trypsin is derived from swine sources and believed to be a source of *Mycoplasma hyorhinis* contamination. However, *Mycoplasma hyorhinis* is lysed at room temperatures by trypsin within minutes, thus it presents no source of contamination.

Can mycoplasma contamination be observed with the naked eye?

No, mycoplasma can only be observed through electron microscopy. For highly sensitive detection of mycoplasma contamination, we recommend the Venor® *GeM* Mycoplasma Detection Kit.

How can mycoplasmas be detected?

It is important to note that mycoplasma infection is usually undetectable by microscope. A number of techniques - direct and indirect tests - have been developed for detecting mycoplasma contamination in cell cultures.

Direct culture requires the use of one or more complex nutritionally enriched mycoplasmal media and carefully controlled environmental conditions and can offer the highest sensitivity for vital mycoplasma. The method is rather slow and usually requires a month for completion. There are various indirect tests of varying sensitivity and convenience, including DNA fluorescence, RNA hybridization, immunofluorescence, electron microscopy, ELISA, DNA probes, biochemical assay. Indirect tests offer two advantages over direct culture methods: first, they can detect so-called "non-cultivable" mycoplasmal strains that direct culture may miss; second, they are faster, usually taking only a few hours to complete. In the past, two independent methods were generally used to insure that cultures were mycoplasma contamination free.

Recently, the PCR technology was adapted for mycoplasma detection and has proven to be a powerful and reliable tool for mycoplasma contamination control.

What is the Venor®*GeM* system?

Minerva Biolabs offers Venor®*GeM*, that detects nearly all mycoplasma species commonly appearing as contaminants in cell cultures, such as *M. orale, M. hyorhinis*, and *A. laidlawii*. Venor®*GeM* is based on a PCR technology that uses the conserved 16S RNA coding region, specific to mycoplasmas, as a template.

What are the advantages of Minerva Biolabs' detection system?

Minerva Biolabs offers Venor®*GeM* for conventional and real-time applications. The Venor®*GeM* system facilitates the detection of mycoplasma species with an easy-to-read, positive or negative PCR test. The coding region used as a template is highly specific to discriminate from other bacterial contaminates. Positive tests produce a single DNA fragment (~ 270 bp) that is easily identified by agarose gel electrophoresis. With the qPCR system a quantification of the mycoplasma load is possible. The test is highly sensitive and allows detection of 1.5 copies/µl. Venor®*GeM* is validated and due to the internal and the positive control very reliable. The controls minimize operator-dependent variability of the results and optimize the reproducibility. The samples are quick and easy prepared by boiling extraction.

When should I use the Venor®*GeM*?

The Venor®*GeM* System is designed for the detection of mycoplasmas in cell cultures and virus stocks as well as many other biological samples. Cell samples

may be derived from either cultured cells or tissue extracts.

What sample size is required?

The Venor®*GeM* System requires as little as 100 µl of cell culture supernatant for a boiling extraction, of which 2 µl is used in the mastermix preparation. The cells should be cultivated to 90 % confluence to guarantee a maximal density of mycoplasmas in the sample volume but to avoid any inhibition due to the sample.

What type of detection equipment is required?

Only a PCR thermocycler (and in case of a conventional PCR a gel electrophoresis system) is required for mycoplasma detection, plus pipets, pipet tips, sterile reaction tubes and RNA- and DNA-free water.

What controls should be performed?

It is highly recommended that both positive and negative control reactions be performed for each test series. These controls are to insure assay conditions, as well as biological positive controls. A negative control reaction, in which the sample volume is replaced by sterile water, should also be performed. The internal control should by processed with all reactions to avoid false-negative results caused by PCR inhibition.

Do I need fixative agents for sample preparation?

Venor®*GeM* eliminates the use of hazardous chemicals found in most standard fixatives used in cell culture technique (e.g., formaldehyde) that also interfere with the polymerase reaction. Samples are prepared and stored after a simple heat inactivation of five minutes at 95°C, which is sufficient for mycoplasma DNA extraction and gives the best results in testing.

Can the processed samples be stored for later analysis?

After heat treating the cell culture supernatant, samples can be stored for several days at 4°C prior to analysis. For long-term storage, it is recommended that samples be stored at -20°C either in their native state or after heat inactivated.

How long does it take to perform the assay?

Detection of mycoplasma DNA using the Venor®*GeM* system requires approximately 2-3 hours for completion. This accounts for sample preparation (10 minutes), the reaction mixture set-up (15 minutes), the cycling step (approx. 2 hours depending on the PCR cycler), and finally the gel electrophoresis (20 minutes). In comparison, a qPCR application requires only 1 hour for completion.

What does Minerva Biolabs recommend?

Mycoplasma should be monitored at regular intervals. It is highly recommended to t est cultures once a month, to test newly arrived cultures immediately, and to test cultures prior to freezing. Suspect cultures should be tested immediately.

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