NEWS on diagnostics

2024 Volume 2

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Welcome to the second issue of News on Diagnostics for 2024. Cultivating healthy cell growth means depending on a quality mix of media, sera and reagents. That's why we offer an unparalleled line of cell culture solutions. From classical and specialty media to foetal bovine serum, supplements and reagents, our offering is extensive and comprehensive — exactly what you would expect from a leader in R&D and manufacturing for diagnostics.

Cell Culture

Cell culture has become one of the most fundamental techniques for modelling biological systems and is of increasing importance in the biotechnology and pharmaceutical sectors as well as an essential process in life science research labs. Though this technique is highly accessible, successful propagation of cells for stock expansion or modelling experiments can be plagued by contamination or other conditions that negatively impact cell viability.

Types of Contamination

Microbial Contamination; Bacterial, Fungal or Yeast

Maintaining a sterile tissue culture environment is essential for successful cell culture work. Rapid-onset turbidity and colour change of the culture medium, particularly when supplemented with a non-toxic pH indicator like phenol red, can indicate the presence of bacterial, fungal, or yeast contamination. Daily microscopic observation of cultures is crucial for the early detection of microbial contamination. This allows for prompt action to protect neighbouring cultures and the overall sterile environment, including removing contaminated cultures as soon as signs become apparent.

Furthermore, specific testing for bacteria and fungi should be part of a routine and regular quality control screening procedure to ensure the integrity of cell cultures. This proactive approach to detection and removal of contamination helps to maintain the quality and reliability of cell culture experiments.

Tips for reducing Microbial Contamination:

- Work within the biological safety cabinet with proper air flow thus maintaining a sterile environment
- Disposable plastic pipettes—also called serological pipettes, available in capacities from 1-100 mL—are indispensable tools for cell culture.
- Use of disinfectants such as sodium hypochlorite/ other bleach, or alcohol must be used with care and safety of lab personnel in mind.

Viral Contamination

Viruses represent a particularly challenging cell culture contaminant due to the difficulty of detecting them using standard microscopy methods, which are generally impractical for most research laboratories. They can originate from the patient or host animal cell source, and certain cell lines of biotechnological significance have been found to contain endogenous retroviruses.

The small size of viruses makes them exceptionally challenging to remove from media, sera, and other solutions of biological origin.

In addition to their potential impact on cultured cells, the use of virally infected cell cultures raises significant health concerns for laboratory personnel. Special safety precautions must be taken when working with tissues or cells from humans or other primates to avoid the potential transmission of viral infections, such as HIV, hepatitis B, Epstein-Barr, and simian herpes B virus, from the cell cultures to laboratory personnel.

Best practices for reducing the incidence of viral contamination of cell cultures:

- Limit the number of biological sources (suppliers, animals) from which cells are extracted.
- Select animals/cells which are less virus-susceptible.
- Source cells from repositories that perform virus testing and provide certification of virus-free cell lines.

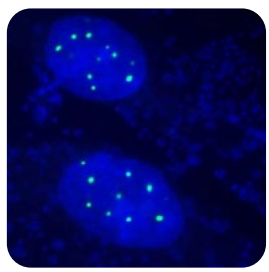
Chemical Contamination

Chemical contamination in cell culture refers to non-living contaminants that can originate from various sources such as reagents, water used in media or buffers, equipment, and supplies. Examples of chemical contaminants include free radicals, metal ions, disinfectant, or detergent residues, and even endotoxins that may persist after upstream bacterial contaminants are no longer present. These contaminants can have detrimental effects on cell culture and may impact experimental outcomes. Therefore, it is important to maintain high standards of quality control and ensure that all reagents, water, and equipment used in cell culture are free from chemical contaminants.

Tips for preventing chemical contamination:

- Always use laboratory-grade water for preparing buffers and solutions, and resuspending lyophilized reagents.
- Any reusable labware must be rinsed exhaustively and air-dried—autoclaving will have no effect on detergent residue.
- Obtain media, supplements, and serum (FBS, FCS) exclusively from suppliers that provide endotoxin testing certification.

Mycoplasma Contamination



Mycoplasma, as a genus of bacteria lacking cell walls, present unique challenges in cell culture environments. Their lack of susceptibility to antibiotics that limit bacterial growth by inhibiting cell wall formation makes them particularly resilient. Additionally, their flexible morphology and small cell size in the range of 0.15 to 0.3 µm enable them to potentially escape

standard cell culture filtration approaches that typically use filters with 0.22 µm pores.

One of the most concerning aspects of mycoplasma contamination is their inconspicuous nature. Unlike most other bacterial contaminants, mycoplasma is not readily apparent by casual inspection and are difficult to detect by light microscopy due to their notably small size and unique morphology. Even at high titres, reaching 108 organisms/mL, mycoplasma may not cause turbidity of the medium.

Infections by mycoplasma do not typically result in the immediate death of the mammalian cells they infect. However, they can significantly impact cell cultures by altering cellular metabolism, causing chromosomal aberrations, slowing cell growth, and interfering with cell attachment. As a result, mycoplasma contamination is likely to substantially influence the outcomes of experiments using affected cell lines.

Given these implications, it's crucial to maintain rigorous mycoplasma testing and implement preventive measures to ensure the integrity of cell cultures and the reliability of experimental results.

Common Sources of mycoplasma & how to control them.

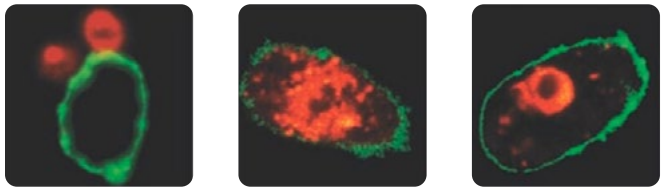
The causes of mycoplasma contamination in cell culture are diverse and can originate from various sources that are challenging to trace. These sources include:

- Laboratory Personnel
- Cross-Contamination and Poor Lab Techniques
- Aerosols
- Media Contamination

Mycoplasmas present a unique challenge as they can remain viable in a dry state for extended periods and rapidly proliferate upon contact with a nutrient source. Once introduced into the laboratory environment, mycoplasmas are difficult to completely eradicate. While it is possible to suppress their growth, complete eradication is challenging.

There are three effective detection methods:

Method	Pros	Cons
Mycoplasma culture	Definitive method	Can take over 4 weeks to obtain results
DNA staining methods	Quick & simple	Not definitive
PCR	Sensitive & rapid results	Risk of false positives/ contamination carry over during pipetting



LookOut® Mycoplasma PCR Detection Kits are exceptionally sensitive, with a detection limit of as little as 2 genomes per µL of sample. Our newest one-step PCR kit is designed to reduce pipetting to a minimum, as the PCR mix includes all reagents required for the reaction: primers, nucleotides, polymerase and the internal amplification control, provided in a ready-to-use lyophilised reaction mix.

Mycoplasma Elimination

The eradication of mycoplasma contamination in cell cultures is a crucial step to ensure reliable research results.

Antibiotics like penicillin and streptomycin are ineffective against mycoplasma due to their unique cell wall structure.

Good practices, such as aseptic techniques, quarantining new cell lines, and proper cell banking, help prevent contamination.

The process takes four to six weeks:

- **Pre-treatment:** After thawing for mycoplasma detection assays.
- **Treatment:** Cells are treated for one to three weeks, depending on the antibiotic used.
- **Post-treatment:** At least two weeks to ensure complete eradication.

Antibiotics for Mycoplasma Eradication

Specific antibiotics effectively kill mycoplasma.

Tetracyclines, macrolides, and quinolones are used at relatively low concentrations.

These classes of antibiotics target mycoplasma and are more effective than standard antibiotics.

The LookOut® Mycoplasma Elimination Kit has been developed to quickly and efficiently eliminate mycoplasma contamination from cell cultures.

The kit is comprised of a combination of biological agents that reliably and completely eliminate mycoplasma contamination. The initial treatment of this eradication procedure is adequate for mycoplasma elimination in most applications. The second step suppresses and inactivates any remaining mycoplasma using a follow-up antibiotic treatment.

Find out more information, including how to order a kit **here**.