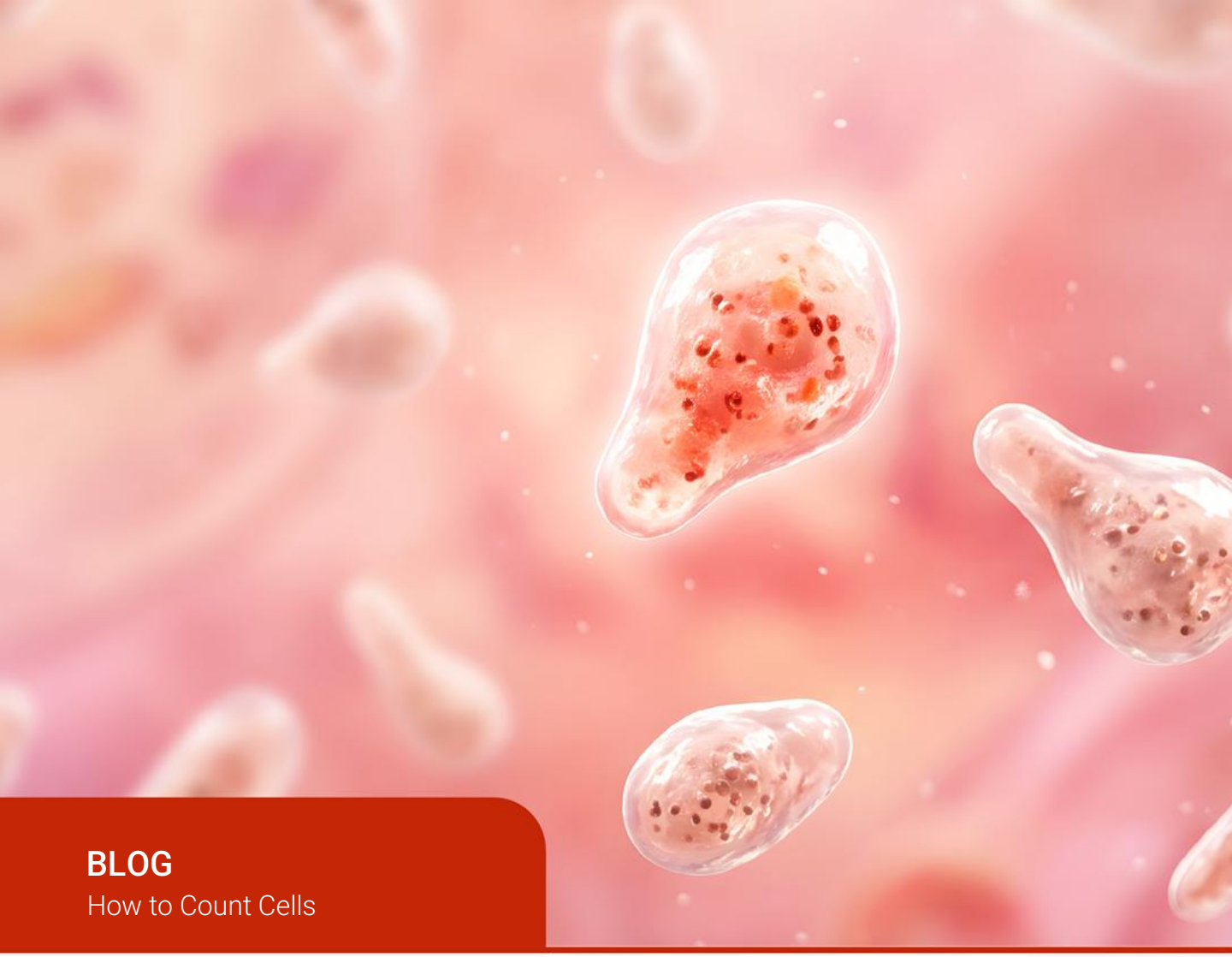

BLOG
How to Count Cells

Mycoplasma Contamination : The Silent Threat in Your Lab

- If your cells look healthy but your experiments keep failing, this might be why

logos biosystems
by ALIGNED GENETICS

Introduction : Why Are Cell Counting Results Inconsistent?

When reproducibility suddenly breaks down, growth rates drop without explanation, or viability readings come back low despite cells looking perfectly fine — most researchers instinctively reach for the usual suspects: the medium, the serum, the protocol. But the real cause is often somewhere else entirely.
Mycoplasma. Because it does not kill cells, contamination is easy to miss — and by the time it is finally detected, months of accumulated data may already be compromised. Across the published literature, estimates suggest that approximately 15–35% of cell lines in culture laboratories worldwide carry Mycoplasma contamination.
It is one of the most common yet most underappreciated sources of error in cell biology research.

Why Is It So Hard to Detect?

Mycoplasma are bacteria without a cell wall. That single characteristic is what makes them so difficult to catch and eliminate.

They are small enough to pass through a 0.2 µm sterilization filter. Filter sterilization does not remove Mycoplasma from media.
They are invisible under a standard light microscope. Phase contrast microscopy cannot detect them either, so there is no way to spot contamination by eye.
Common antibiotics do not work. Beta-lactams such as penicillin and ampicillin target cell wall synthesis — a mechanism that is irrelevant to organisms that have no cell wall.
Cells do not die. Contaminated cultures continue to grow and look normal. This is precisely why contamination goes unnoticed for so long, quietly corrupting data the whole time.

The species most commonly responsible for contamination in cell culture are M. orale (originating from the human oral cavity), M. hyorhinis (from porcine serum), and M. arginini (transmitted via serum or human contact). Of these, M. orale is most often traced back to the researcher working at the bench.

Where Does It Come From? — Main Routes of Contamination

Mycoplasma contamination does not appear overnight. It enters gradually over time and only becomes detectable once it reaches a sufficient threshold. Understanding the routes is the first step toward preventing it.

Source	Typical Scenario	Risk Level
Laboratory personnel	Oral Mycoplasma transmitted during culture work without a mask	⚠ ⚠ ⚠ Very high
Cell line sharing	Received cell lines already contaminated at source institution	⚠ ⚠ ⚠ Very high
Serum (FBS)	Fetal bovine serum containing M. hyorhinis or other species	⚠ ⚠ High
Trypsin	Porcine pancreas-derived trypsin acting as a contamination vector	⚠ ⚠High
Shared equipment	CO2 incubators, pipettes, media bottles shared between u sers	⚠ Medium
Cross-contamination	Contaminated and clean cells co-housed in the same incubator	⚠ Medium

Two scenarios account for the majority of cases. The first is working at the biosafety cabinet without a mask. Oral droplets released during talking, coughing, or sneezing can carry Mycoplasma directly into open culture vessels. The second is putting a new cell line into use without testing it first. Even from a reputable source, contamination can arise during shipping and handling. Test before use — every time.

Why Does It Matter? — The Silent Corruption of Experimental Data

Mycoplasma does not kill cells. But it interferes with nearly every experimental parameter you care about.

Growth kinetics shift. Mycoplasma competes for nutrients, which can slow cell proliferation — but in some cases it induces cytokine secretion that drives abnormally rapid growth. Either way, your growth curves and doubling time data become unreliable.

Viability measurements become untrustworthy. Mycoplasma disrupts cellular metabolism and membrane function, distorting viability assay results. The percentage readout on your cell counter may not accurately reflect the true health of the culture.

Downstream experiments are affected across the board. Transfection efficiency drops. Mycoplasma-derived RNA contaminates RNA-seq and PCR results. Drug screening assays yield inconsistent IC50 values and aberrant dose-response curves.

In GMP-based cell therapy manufacturing, a confirmed Mycoplasma positive can mean batch rejection or full process shutdown — with direct consequences for product timelines and regulatory filings.

Taken together, Mycoplasma contamination is one of the most reliable ways to compromise experimental reproducibility, which is why routine monitoring is not optional.

> ⚠ **The core problem**
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> Mycoplasma is dangerous not because it kills cells, but because it keeps cells alive while quietly corrupting data — often for months before anyone notices. The later the detection, the greater the damage.

How to Check — Detection Methods Compared

There are five main approaches to Mycoplasma detection. For most research labs, PCR is the standard.

Method	Sensitivity	Turnaround	Cost	Notes
PCR-based	★★★★★	Hours	Medium	Lab standard. Most widely used
Fluorescent staining (Hoechst)	★★☆☆☆	1–2 days	Low	Screening only; not for confirmation
Culture method	★★★★☆	2–4 weeks	High	Required for GMP / regulatory compliance
ELISA / kit-based	★★★☆☆	Hours	Medium	Easy to use without specialized equipment
Next-gen sequencing (NGS)	★★★★★	Days	Very high	Species-level identification; research use

PCR — Why It's the Standard

PCR is fast, sensitive, and easy to implement with a commercial kit. By targeting the Mycoplasma 16S rRNA gene with universal primers, a single run covers the major contaminating species. One thing to watch for is DNA quality: inhibitors in culture supernatant can affect results, so using a kit with an internal positive control is strongly recommended.

Fluorescent Staining (Hoechst 33258) — For Screening Only

With Hoechst 33258 or DAPI staining, small punctate fluorescent signals may be visible in the cytoplasm surrounding the nucleus — an indicator that Mycoplasma may be present. However, sensitivity is low and low-level contamination is easily missed. This method is not suitable for definitive diagnosis; use it only as a preliminary screen alongside PCR.

Culture Method — May Be Required in GMP Settings

This classical approach involves culturing for up to four weeks and offers high sensitivity and specificity, but requires time and specialized expertise. Regulatory agencies may require this method for cell therapy and other GMP products.

When to Test — Timing Matters

In contamination detection, timing is everything. Without a routine testing schedule, you will almost certainly find out too late.

Test in these situations — without exception

- Upon receiving any new cell line — regardless of source, test before use.
- Routine scheduled testing — at least monthly is generally recommended; more frequently for high-priority or heavily used lines.
- When something seems off — unexplained growth slowdown, viability drop, or morphological changes: test immediately.
- Before sharing cells with another lab — contaminated cells passed to colleagues spread the problem.
- Long-passaged cell lines — established lines in long-term use still need periodic testing.

Prevention Is Far Better Than Treatment

Dealing with an active contamination is far more costly than preventing one. Four core principles cover most of the risk.

Wear a mask — non-negotiable

Oral Mycoplasma is the single most common route of contamination. Always wear a mask during cell culture work and minimize unnecessary talking in front of the biosafety cabinet. It sounds simple, and it is — but it is also the most effective single preventive measure.

Quarantine new cell lines until tested

Any cell line received from outside the lab should be kept in a dedicated quarantine incubator until confirmed Mycoplasma-negative. A supplier's 'Mycoplasma-free' certification is not a substitute for your own testing — contamination can occur during transit, and conditions may have changed since the supplier's last test.

Use certified reagents

Use FBS lots that carry Mycoplasma-negative certification. For trypsin, consider recombinant or certified alternatives given the contamination risk associated with porcine pancreas-derived products. Never share media bottles between cell lines.

Maintain a frozen stock — your insurance policy

Banking early-passage cells that have been confirmed Mycoplasma-negative is both a prevention strategy and a contingency plan. If contamination does occur, replacing from a clean frozen stock is faster and more reliable than antibiotic treatment. Log passage number and test history so you can trace back to a specific time point if needed.

Penicillin/Streptomycin Won't Help — Elimination Antibiotics

This is one of the most common misconceptions in cell culture. Penicillin has no activity against Mycoplasma, as it targets cell wall synthesis and Mycoplasma have no cell wall. Streptomycin likewise has only limited effectiveness for Mycoplasma prevention or elimination. A different class of antibiotic is required.

Antibiotic	Mechanism of Action	Considerations
Ciprofloxacin	DNA gyrase inhibition (cell wall-independent)	Potential cytotoxicity; avoid prolonged use
Doxycycline	30S ribosomal inhibition; blocks protein synthesis	Resistance may develop with repeated use
Azithromycin	50S ribosomal inhibition	Some Mycoplasma species show resistance
Commercial kits (e.g., BM-Cyclin)	Alternating dual-antibiotic regimen	Follow kit protocol strictly

> ⚠⚠ **The limits of antibiotic treatment**
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> Antibiotic treatment does not guarantee complete eradication. PCR confirmation of a negative result is required after treatment. For important cell lines, replacing from a clean frozen stock is a more reliable solution.

If You Get a Positive Result — Step-by-Step Response

Knowing the response sequence in advance makes a positive result much less disruptive.

Isolate immediately. Separate contaminated cells from all other cultures and stop using the shared incubator.

Assess the scope. Test all other cell lines that shared the same incubator or media bottles.

Replace or treat. If a clean frozen stock exists, replacement is the preferred option. If not, attempt antibiotic treatment — and confirm negative by PCR afterward.

Decontaminate the incubator. Clean all interior surfaces thoroughly with 70% ethanol or a dedicated decontamination agent.

Identify the source. Determine how contamination entered and update your SOPs accordingly.

Re-test after recovery. Confirm Mycoplasma-negative by PCR at least two weeks after completing antibiotic treatment.

Closing Thoughts

Mycoplasma is dangerous precisely because it does not kill cells. The typical pattern is months of silent data corruption before anyone realizes something is wrong.
Prevention beats remediation, and routine testing beats both. Building monthly PCR testing into your lab schedule, committing to masks and cell line quarantine, and keeping clean frozen stocks on hand — these three habits are the most practical defense against Mycoplasma contamination.

While automated cell counters cannot detect Mycoplasma directly, they are valuable for monitoring the changes in cell count and viability that contamination can cause.

Logos Biosystems — Cell Counting Solutions for Mycoplasma Monitoring

LUNA-FX7™ – Fluorescence + brightfield automated cell counter. Quickly confirm cell count and viability when contamination is suspected

LUNA-III™ – Simple, accurate brightfield automated cell counter

CELENA® X – High-Content Imaging System for monitoring morphological changes and analyzing 3D structures

Learn more: www.logosbio.com

Frequently Asked Questions (FAQ)

Q1. Do I have to discard all data generated from a contaminated culture?

A. Not necessarily. If you can estimate when contamination began and have a confirmed negative test result predating that point, earlier data may still be usable. Data generated after the estimated onset of contamination should be interpreted cautiously — reproducibility cannot be assumed.

Q2. I use Penicillin/Streptomycin — am I covered?

A. No. Penicillin targets cell wall synthesis, and Mycoplasma have no cell wall. Streptomycin has very limited efficacy against Mycoplasma as well. Routine antibiotics in your culture medium do not protect against Mycoplasma. Prevention requires aseptic technique and regular testing.

Q3. Doesn't filtering media through a 0.2 µm filter eliminate Mycoplasma?

A. No. Mycoplasma range from 0.1 to 0.8 µm in size, and some species pass through a 0.2 µm filter. Filter sterilization should not be assumed to remove Mycoplasma from media.

Q4. The supplier says the cell line is Mycoplasma-free. Do I still need to test?

A. Yes. Contamination can occur during shipping and handling after the supplier's test was performed, and conditions may change over time. Every incoming cell line should be tested upon arrival, regardless of documentation.

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