

# Troubleshooting Guide for Cell Culture Contamination

CORNING

Common cell culture contaminants, their sources, detection, and prevention

Type	Source	Detection	Prevention
<b>Bacteria</b> (Gram +, Gram -)	<ul style="list-style-type: none"> <li>• Lab personnel</li> <li>• Unfiltered air</li> <li>• Humidified incubators</li> <li>• Purified water</li> <li>• Insects</li> <li>• Plants</li> <li>• Contaminated cell stock</li> <li>• Media</li> <li>• Equipment (Biosafety cabinets)</li> </ul>	<ul style="list-style-type: none"> <li>• Microbial culture</li> <li>• Gram's stain test</li> <li>• Visual turbidity</li> <li>• pH becomes acidic</li> </ul>	<ul style="list-style-type: none"> <li>• Aseptic technique</li> <li>• Antibiotics</li> <li>• Filtration (&lt;0.22 μm)</li> <li>• Use of sterile products</li> <li>• Disinfection of CO<sub>2</sub> incubators</li> <li>• Daily cleaning of hood space with 70% alcohol, as well as monthly cleaning with 10% bleach or equivalent products</li> <li>• Daily, or at minimum weekly, emptying of used media traps</li> <li>• Storing cell line stocks in vapor phase of liquid nitrogen (LN<sub>2</sub>), not in liquid phase</li> </ul>
<b>Yeast</b>	<ul style="list-style-type: none"> <li>• Humidified incubators</li> <li>• Lab personnel</li> <li>• Unfiltered air</li> <li>• Contaminated cell stock</li> <li>• Equipment (Biosafety cabinets)</li> </ul>	<ul style="list-style-type: none"> <li>• Microbial culture</li> <li>• Visual turbidity</li> <li>• Odor</li> </ul>	<ul style="list-style-type: none"> <li>• Aseptic technique</li> <li>• Antimycotics</li> <li>• Filtration (&lt;0.5 μm)</li> <li>• Use of sterile products</li> <li>• Disinfection of CO<sub>2</sub> incubators</li> <li>• Daily cleaning of hood space with 70% alcohol, as well as monthly cleaning with 10% bleach or equivalent products</li> <li>• Daily, or at minimum weekly, emptying of used media traps</li> <li>• Storing cell line stocks in vapor phase of LN<sub>2</sub>, not in liquid phase</li> </ul>
<b>Fungus</b>	<ul style="list-style-type: none"> <li>• Fruit</li> <li>• Cellulose products (cardboard)</li> <li>• Plants</li> <li>• Unfiltered air</li> <li>• Lab personnel</li> <li>• Contaminated cell stock</li> <li>• Equipment (Biosafety cabinets)</li> </ul>	<ul style="list-style-type: none"> <li>• Microbial culture</li> <li>• Visual particulates, visual mycelia</li> </ul>	<ul style="list-style-type: none"> <li>• Aseptic technique</li> <li>• Antimycotics</li> <li>• Filtration (&lt;0.5 μm)</li> <li>• Use of sterile products</li> <li>• Daily cleaning of hood space with 70% alcohol, as well as monthly cleaning with 10% bleach or equivalent products</li> <li>• Daily, or at minimum weekly, emptying of used media traps</li> <li>• Storing cell line stocks in vapor phase of LN<sub>2</sub>, not in liquid phase</li> </ul>
<b>Virus</b>	<ul style="list-style-type: none"> <li>• Original tissues</li> <li>• Serum</li> <li>• Cross-contamination</li> <li>• Lab personnel</li> <li>• Equipment (Biosafety cabinets)</li> </ul>	<ul style="list-style-type: none"> <li>• Co-cultivation</li> <li>• PCR</li> <li>• Electron microscopy</li> <li>• <i>In vivo</i> testing</li> <li>• Assays</li> </ul>	<ul style="list-style-type: none"> <li>• Aseptic technique</li> <li>• Ultrafiltration</li> <li>• Chemical treatment</li> <li>• Gamma-irradiated serum</li> <li>• Use animal-free products</li> <li>• Storing cell line stocks in vapor phase of LN<sub>2</sub>, not in liquid phase</li> </ul>
<b>Endotoxin</b>	<ul style="list-style-type: none"> <li>• Serum</li> <li>• Bacterial contamination</li> <li>• Contaminated or improperly maintained water supply</li> <li>• Equipment (Biosafety cabinets)</li> </ul>	<ul style="list-style-type: none"> <li>• Limulus amoebocyte lysate (LAL) assay</li> </ul>	<ul style="list-style-type: none"> <li>• Aseptic technique</li> <li>• Ultrafiltration (&lt;5,000 daltons)</li> <li>• Endotoxin-specific affinity or size exclusion chromatography</li> <li>• Use animal-free products</li> </ul>
<b>Mycoplasma</b>	<ul style="list-style-type: none"> <li>• Contaminated cell lines</li> <li>• Serum</li> <li>• Media</li> <li>• Lab personnel</li> <li>• Equipment (Biosafety cabinets)</li> </ul>	<ul style="list-style-type: none"> <li>• Hoechst stain</li> <li>• Microbial culture</li> <li>• Specialized kits</li> <li>• PCR</li> </ul>	<ul style="list-style-type: none"> <li>• Aseptic technique</li> <li>• Antibiotics</li> <li>• Ultrafiltration (&lt;0.04 μm)</li> <li>• Use of sterile products</li> <li>• Use animal-free products</li> <li>• Storing cell line stocks in vapor phase of LN<sub>2</sub>, not in liquid phase</li> </ul>
<b>Cellular</b>	<ul style="list-style-type: none"> <li>• Cross-contamination of cultures</li> <li>• Cross-use or sharing of media from a different cell line</li> <li>• Equipment (Biosafety cabinets)</li> </ul>	<ul style="list-style-type: none"> <li>• Cell authentication to determine identity and species of cells</li> </ul>	<ul style="list-style-type: none"> <li>• Work with only one cell line at a time</li> <li>• Thoroughly clean before and after introducing a new cell line into the laminar flow hood</li> <li>• Daily cleaning of hood space with 70% alcohol, as well as monthly cleaning with 10% bleach or equivalent products</li> <li>• Storing cell line stocks in vapor phase of LN<sub>2</sub>, not in liquid phase</li> </ul>

**NOTE:** When applicable, resterilize media using a Corning® syringe filter or bottle-top filter.

## Corning® media products used for the prevention of contamination

### Sterile Media and Reagents

Cat. No.	Description	Size	Qty/Pk
25-950-CQC	Dimethyl Sulfoxide (DMSO)	250 mL	1
35-070-CV	Fetal Bovine Serum (FBS), premium, US Origin (gamma irradiated)	500 mL	1

### Antibiotics and Antimycotics

Cat. No.	Description	Size	Qty/Pk
30-001-CI	Pen-Strep solution, 50X	100 mL	6
30-002-CI	Pen-Strep solution, 100X	100 mL	6
30-003-CF	Amphotericin B, liquid	50 mL	6
30-004-CI	Antibiotic-Antimycotic solution	100 mL	6
30-005-CR	Gentamicin Sulfate, liquid	10 mL	10
30-006-CF	Kanamycin Sulfate, liquid	50 mL	6
30-009-CI	Pen-Strep-L-Glutamine, 100X	100 mL	6
61-238-RH	Ampicillin, Sodium Salt, powder	10 g	1
61-238-RM	Ampicillin, Sodium Salt, powder	100 g	1
61-239-RI	Chloramphenicol, powder	25 g	1
61-277-RF	Ciprofloxacin Hydrochloride, powder	1 g	1
61-277-RG	Ciprofloxacin Hydrochloride, powder	5 g	1

For more specific information on claims, visit [www.corning.com/certificates](http://www.corning.com/certificates).

**Warranty/Disclaimer:** Unless otherwise specified, all products are for research use only. Not intended for use in diagnostic or therapeutic procedures. Not for use in humans. Corning Life Sciences makes no claims regarding the performance of these products for clinical or diagnostic applications.

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