# Troubleshooting Guide for Cell Culture Contamination

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



Туре	Source	Detection	Prevention
Bacteria	Lab personnel     Unfiltered air	Microbial culture     Cram's stain tost	Aseptic technique
(Gram +,		Gram's stain test     Visual turbidity	• Antibiotics
Gram -)	Humidified incubators     Durified water	Visual turbidity	• Filtration (<0.22 μm)
	<ul><li>Purified water</li><li>Insects</li></ul>	• pH becomes acidic	Use of sterile products     Disinfection of CO incubators
	• Plants		<ul> <li>Disinfection of CO<sub>2</sub> incubators</li> <li>Daily cleaning of hood space with 70% alcohol, as well as</li> </ul>
	Contaminated cell stock		monthly cleaning with 10% bleach or equivalent products
	Media		Daily, or at minimum weekly, emptying of used media trap
	• Equipment (Biosafety cabinets)		• Storing cell line stocks in vapor phase of liquid nitrogen (LN <sub>2</sub> ),
	Equipment (Biosurety cubinets)		not in liquid phase
Yeast	Humidified incubators	Microbial culture	Aseptic technique
	• Lab personnel	Visual turbidity	• Antimycotics
	Unfiltered air	• Odor	• Filtration (<0.5 μm)
	Contaminated cell stock		• Use of sterile products
	<ul> <li>Equipment (Biosafety cabinets)</li> </ul>		• Disinfection of CO <sub>2</sub> incubators
			Daily cleaning of hood space with 70% alcohol, as well as
			monthly cleaning with 10% bleach or equivalent products
			Daily, or at minimum weekly, emptying of used media trap     Staying call line stacks in vapor place of LNL not in liquid place.
			• Storing cell line stocks in vapor phase of LN <sub>2</sub> , not in liquid pha
Fungus	• Fruit	Microbial culture	Aseptic technique
	Cellulose products (cardboard)	<ul> <li>Visual particulates, visual mycelia</li> </ul>	• Antimycotics
	• Plants		• Filtration (<0.5 μm)
	Unfiltered air		<ul><li>Use of sterile products</li><li>Daily cleaning of hood space with 70% alcohol, as well as</li></ul>
	<ul><li>Lab personnel</li><li>Contaminated cell stock</li></ul>		monthly cleaning with 10% bleach or equivalent products
	• Equipment (Biosafety cabinets)		Daily, or at minimum weekly, emptying of used media trap
	Equipment (biosaicty cabinets)		• Storing cell line stocks in vapor phase of LN <sub>2</sub> , not in liquid pha
Virus	Original tissues	Co-cultivation	Aseptic technique
	• Serum	• PCR	• Ultrafiltration
	Cross-contamination	• Electron microscopy	Chemical treatment
	• Lab personnel	• In vivo testing	Gamma-irradiated serum
	<ul> <li>Equipment (Biosafety cabinets)</li> </ul>	• Assays	Use animal-free products  Charing a sell line at a leading and a sell line at a leading at a leading and a sell line at a leading at
F 1 4 1			• Storing cell line stocks in vapor phase of LN <sub>2</sub> , not in liquid pha
Endotoxin	• Serum	Limulus amoebocyte lysate (LAL)	Aseptic technique
	Bacterial contamination	assay	• Ultrafiltration (<5,000 daltons)
	<ul> <li>Contaminated or improperly maintained water supply</li> </ul>		<ul> <li>Endotoxin-specific affinity or size exclusion chromatograph</li> <li>Use animal-free products</li> </ul>
	• Equipment (Biosafety cabinets)		• Ose animal-free products
Mycoplasma	Contaminated cell lines	Hoechst stain	Aseptic technique
	• Serum	Microbial culture	• Antibiotics
	• Media	Specialized kits	• Ultrafiltration (<0.04 μm)
	• Lab personnel	• PCR	Use of sterile products     Use a primal free products
	<ul> <li>Equipment (Biosafety cabinets)</li> </ul>		<ul><li>Use animal-free products</li><li>Storing cell line stocks in vapor phase of LN,, not in liquid pha</li></ul>
Cellular	Cross-contamination of cultures	Cell authentication to determine	Work with only one cell line at a time
	<ul> <li>Cross-use or sharing of media</li> </ul>	identity and species of cells	• Thoroughly clean before and after introducing a new cell li
	from a different cell line		into the laminar flow hood
	<ul> <li>Equipment (Biosafety cabinets)</li> </ul>		<ul> <li>Daily cleaning of hood space with 70% alcohol, as well as</li> </ul>
			monthly cleaning with 10% bleach or equivalent products

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

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### 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.

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