Troubleshooting: Cell Culture

There are several common problems encountered when culturing cells. Problems in primary cultures may have different causes than the same problem in established cell lines.

The table below addresses some of the common problems encountered when culturing cells, along with their possible causes and suggested solutions.

Problem	Possible Cause	Suggested Solution
Rapid pH shift in medium	Incorrect carbon dioxide (CO_2) tension	Increase or decrease percentage of CO_2 in the incubator based on concentration of sodium bicarbonate in medium. For sodium bicarbonate concentrations of 2.0 to 3.7 g/L, use CO_2 amounts of 5% to 10%, respectively.
		Switch to CO_2-Independent Medium.
	Overly tight caps on tissue culture flasks	Loosen caps one-quarter turn.
	Insufficient bicarbonate buffering	Add HEPES buffer to a final concentration of 10 to 25 mM.
	Incorrect salts in medium	Use an Earle's salts-based medium in a CO_2 environment and a Hanks' salts-based medium in atmospheric conditions.
	Bacterial, yeast, or fungal contamination	Discard culture and medium.
		Try to decontaminate culture. (See *Decontaminating Cultures with Antibiotics and Antimycotics*.)
Precipitate in medium, no change in pH	Residual phosphate left over from detergent washing, which may precipitate powdered medium components	Rinse glassware in deionized, distilled water several times, then sterilize.
	Frozen medium	Warm medium to 37°C and swirl to dissolve. If precipitate remains, discard medium.
Precipitate in medium, change in pH	Bacterial or fungal contamination	Discard medium.
		Try to decontaminate culture. (See *Decontaminating Cultures with Antibiotics and Antimycotics*.)
Cells not adhering to culture vessel	Overly trypsinized cells	Trypsinize for a shorter time, or use less trypsin. (See *Dissociation of Cells from Culture Vessels*.)
	Mycoplasma contamination	Segregate culture and test for mycoplasma infection. Clean hood and incubator. If culture is contaminated, discard.
	No attachment factors in medium	For serum-free formulations, be sure they contain attachment factors.
Decreased growth of culture	Change in medium or serum	Compare media formulations for differences in glucose, amino acids, and other components.
		Compare the old lot of serum with the new lot in a growth experiment.
		Increase initial cell inoculum.
		Adapt cells sequentially to new medium.

Problem	Possible Cause	Suggested Solution
Decreased growth of culture (continued)	Depletion, absence, or breakdown of essential growth-promoting components such as glutamine or growth factors	Remove medium and add fresh medium.
		Supplement medium with growth-promoting components.
		Substitute GLUTAMAX I or II for glutamine in the medium.
	Low-level bacterial or fungal contamination	Grow culture without antibiotics. If contaminated, discard culture.
		Try to decontaminate. (See *Decontaminating Cultures with Antibiotics and Antimycotics*.)
	Improper storage of reagents	Store sera at -5°C to -20°C. Store media at 2°C to 8°C. Store complete media at 2°C to 8°C and use within recommended shelf life.
		Minimize exposure of sera and media to light.
	Excessively low initial cell inoculum	Increase viable cell inoculum.
	Senescence of finite culture	Discard culture; obtain new cell stock.
	Mycoplasma contamination	Segregate culture and test for mycoplasma infection. Clean hood and incubator. If culture is contaminated, discard.
Death of culture	No CO_2 in the incubator	Monitor rate of CO_2 use in incubators to determine when to change tanks. Check line connections frequently for leaks. Avoid opening and closing incubator doors.
	Temperature fluctuations in the incubator	Monitor temperature of incubator.
	Use of FUNGIZONE® at toxic concentrations	Use less FUNGIZONE. (See *Use of Antibiotics and Antimycotics*, for appropriate concentrations.)
	Cell damage during thawing or cryopreservation	Obtain another aliquot of cells. (See *Cryopreservation of Mammalian Cells.*)
	Incorrect osmotic pressure in medium	Check osmolality of complete medium. Note: Most mammalian cells can tolerate an osmolality of 260 to 350 mOsm/kg. Additions of reagents such as HEPES and drugs may affect osmolality.
	Buildup of toxic metabolites in the medium	Remove medium and add fresh medium.
Suspension cells clumping together	Presence of calcium and magnesium ions	Wash cells in a balanced salt solution without calcium and magnesium. Gently pipette cells to obtain a single cell suspension.
	Mycoplasma contamination	Segregate culture and test for mycoplasma infection. Clean hood and incubator. If culture is contaminated, discard.
	Cell lysis and release of DNA resulting from overdigestion with proteolytic enzymes	Treat cells with DNase I.
Primary cell culture is contaminated	Contamination of primary tissue carried over to culture	Wash tissue pieces several times in a balanced salt solution containing a higher concentration of antibiotics and antimycotics before starting culture.