



Best practices in cell culture: an overview

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Abstract This overview describes a series of articles to provide an unmet need for information on best practices in animal cell culture. The target audience primarily consists of entry-level scientists with minimal experience in cell culture. It also include scientists, journalists, and educators with some experience in cell culture, but in need of a refresher in best practices. The articles will be published in this journal over a six-month period and will emphasize best practices in: (1) media selection; (2) use and evaluation of animal serum as a component of cell culture medium; (3) receipt of new cells into the laboratory; (4) naming cell lines; (5) authenticating cell line identity; (6) detecting and mitigating risk of cell culture contamination; (7) cryopreservation and thawing of cells; and (8) storing and shipping viable cells.

Keywords Best practices in cell culture · Overview

Introduction

Propagation and culturing of animal cells for various cell-based assays are fundamental to biomedical and pre-clinical research. However, the rapid growth in such areas as gene therapy, genomics, and proteomics has resulted in a remarkable increase in cell culture activities with little regard for the impact of poor tissue culture practices on the experimental outcome. Consequently, this lack of adherence to best tissue culture practices has been costly (Freedman et al., 2015a; Freedman et al., 2015b) due to erroneous data and irreproducible results (Freedman et al., 2015a; Freedman et al., 2015b; Reid, 2011).

This overview describes a series of articles to provide an unmet need for information on best practices in animal cell culture. The target audience primarily consists of entry-level scientists with minimal experience in cell culture. It also include scientists, journalists, and educators with some experience in cell culture, but in need of a refresher in best practices. The articles will be published in this journal over a six-month period and will emphasize best practices in: (Barallon et al., 2010) media selection; (Baust, 2007) use and evaluation of animal serum as a component of cell culture medium; (Baust et al., 2009) receipt of new cells into the laboratory; (Capes-Davis et al., 2010) naming cell lines; (Dirks et al., 2015) authenticating cell line identity; (Freedman et al., 2015a) detecting and mitigating risk of cell culture contamination; (Freedman et al., 2015b) cryopreservation and thawing of cells; (Fuller, 2003) storing and shipping viable cells. Below is a list of the articles provided in Table 1.

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Table 1. List of best practices articles

	Title
1	Best Practices in Cell Culture:An Overview
2	Best Practices for Media Selection for Mammalian Cells
3	Best Practices for use and evaluation of animal serum as a component of cell culture medium
4	Best Practices for Naming, Receiving and Managing Cells in Culture
5	Best practices for authenticating cell lines
6	Best practices for detecting and mitigating the risk of experiencing cell culture contaminants
7	Best Practices for Cryopreserving, Thawing, Recovering and Assessing Cells
8	Best Practices for Storing and Shipping Frozen Cells

Best practices of media selection for mammalian cells

The cell culture medium is a complex mixture of nutrients and growth factors that along with the physical environment can either enable or destroy your cell culture experiment or biologicals production run. Nutritional requirements differ with different cell types and functions, as do optimal pH and osmolality. As cell growth proceeds from initial seeding to confluence or maximal cell density, different cells will utilize amino acids and other components at different rates. By controlling for ammonia, free radicals, heavy metal toxicity, pH shifts, fluctuations in osmolality, nutrient depletion, and chemical and biological contaminants, you will optimize the chances of success.

Best practices for the use and evaluation of animal serum as a component of cell culture medium

Animal serum is a common additive for cell culture medium and is often required at 5 to 10% (v/v) for the attachment and growth of primary and continuous anchorage-dependent (monolayer) cultures. The use of animal serum in cell culture medium confers several advantages but also some risks. This article will discuss the use of animal serum as a component of cell culture medium. The best practices associated with the sourcing, storage, thawing, testing, and mitigation of risk associated with the use of animal sera are among the topics to be described.

Best practices for naming, receiving and managing cells in culture

One of the first considerations in the management of a cell line is the receipt of the cell line into the laboratory. It is necessary to have a well-trained practitioner in best practices in cell culture who has experience in the preparation of cell banks, microscopic observation of cells in culture, growth optimization, cell count, cell subcultivation, as well as detailed protocols on how to expand and store cells. Indeed, the practitioner

should ensure that the appropriate certified facilities, equipment, validated supplies, and reagents are in place.

When establishing a new cell line from a donor tissue, it is very important to consider the name or designation to be used for the cell line early in the process. Often the cell line name on the donor tissue vial in storage is not the name used in succeeding cell line progenies, notebooks or publications; later, this information becomes confusing due to lack of traceability or insufficient documentation. Designation on the vial should be traceable, not only to the cell line name, but also to the historical information including the tissue of origin, clinical information pertaining to the tissue, passage number, population doubling level, viable cell number per vial, cryopreservation medium (including percent of cryoprotectant), and date of manufacturing.

Best practices for authenticating cell lines

Over the years, numerous cell lines have been shown to be misidentified due, in part, to poor tissue culture technique and inadequate identity authentication practices (Capes-Davis et al., 2010; Dirks WG et al., 2010). Technological advances have given rise to improved capabilities to determine the identity of cell lines, both at the intraspecies (donor) level and interspecies level. Cell line identification now requires a comprehensive strategy that employs several complementary technologies such as STR profiling for human cells (Barallon et al., 2010; Reid et al., 2013), and CO1 barcoding for non-human animal cells. The validity of conclusions drawn from research data is dependent on consistent and unequivocal verification of cell line identity. An overview of the current technologies used to identify mammalian cells will be presented in this article.

Best practices for detecting and mitigating the risk of experiencing cell culture contaminants

The types of cell contaminants that might be experienced includes cross-contaminating cells, microbial (bacterial, fungal,

mycobacterial, mycoplasma, and viruses), and chemical contaminants (e.g., free radicals, endotoxins, heavy metals, and detergents). Cross-contamination is detected through incorrect identity assignment (STR profiling for human cells, and CO1 bar coding for animal cells) and through multiplex PCR. Microbial contamination of cell cultures continues to be problematic, especially for the most insidious bacteria—mycoplasma and mycobacteria. Other bacterial and fungal contaminations are more easily detected as these rapidly destroy the cell culture. The detection of viruses remains challenging, as specialized test methods are required, especially if there is no overt cytopathic effects on the cells. The impacts of chemical contaminants such as Gram-negative bacterial endotoxin, residual detergents, free-radicals, heavy metals, osmolality, and pH changes and others to include residues of disinfectants, antibiotics, volatilized fixatives, impurities in gases, will be discussed. Best practices for mitigating the risk of experiencing cellular, microbial, and chemical contamination are also discussed.

Best practices for cryopreserving, thawing, recovering and assessing cells

Long-term storage of cell stocks insures that cells are available for use whenever needed. Cryopreservation of cells is the method of choice for preservation cell stocks. There are several factors to consider when establishing a protocol for freezing cells. These parameters may include cell concentration, cryoprotectant choice and concentration, and volume for storage, among others. This article will provide guidance and insight into developing robust and successful protocols for preserving cells that will preserve cell stocks and provide optimal cell yield and viability. It is important to note, that as with freezing, the thawing process may critically impact the viability and downstream utility of cultured cells. The important aspects include: air-time, thaw temperature/rate, final sample temperature, thaw time, mixing consistency, and dilution process. Other issues include aseptic technique, consistency, controllability, documentation, and cleanliness.

Sample quality prior to freezing greatly impacts post-thaw outcome (Snyder et al., 2004; Baust et al., 2009;). Cell samples which have been stressed in culture (nutrient starved, over confluent, high passage, etc.) prior to freezing respond differently to the cryopreservation process compared to non-stressed (healthy) cells, and often result in decreased sample quality post-thaw (yield, viability and function) (Fuller, 2003; Baust, 2007; Baust et al., 2009). It is important to recognize that optimization of each step of the handling process can improve pre-freeze sample quality and therefore post-thaw outcome.

Best practices in storing and shipping frozen cells

Low-temperature storage is the best means of preserving biological materials; however, the best preservation methods cannot compensate for poor quality starting material. Freezing and cryogenic storage can exert selection pressure on cells and a population of cells to be preserved must be in the best possible physiological state to ensure optimal survival and post-thaw quality. The preservation method of choice should be compatible with the intended use of the cells, especially with regard to cryoprotectants used, method of cooling, and frequency of access. It must be recognized that low-temperature preservation of cells is not a panacea, and one cannot expect to recover from the process better quality specimens than were present prior to preservation.

Shipping of biological materials requires attention to the type of material being transported, adherence to regulatory requirements, packaging materials and proper assembly, and labeling and engaging reputable carriers. This article will provide a discussion of the current best practices for shipping of living cells and related materials.

References

- Barallon R, Bauer SR, Butler J, Capes-Davis A, Dirks WG, Elmore E, Furtado M, Kline MC, Kohara A, Los GV, MacLeod RA, Masters JR, Nardone M, Nardone RM, Nims RW, Price PJ, Reid YA, Shewale J, Sykes G, Steuer AF, Storts DR, Thomson J, Taraporewala Z, Alston-Roberts C, Kerrigan L (2010) Recommendation of short tandem repeat profiling for authenticating human cell lines, stem cells, and tissues. *Vitro Cell Dev Biol Anim* 46(9):727–732
- Baust JM (2007) Properties of cells and tissues influencing preservation outcome: molecular basis of preservation-induced cell death. In: Baust JM (ed) Baust JG. CRC Press, *Advances in Biopreservation*, pp 63–87
- Baust JM, Snyder KK, Van Buskirk RG, Baust JG (2009) Changing paradigms in biopreservation. *Biopreserv Biobanking* 7(1):3–12
- Capes-Davis A, Theodosopoulos G, Atkin I, Drexler HG, Kohara A, MacLeod RA, Masters JR, Nakamura Y, Reid YA, Reddel RR, Freshney RI (2010) Check your cultures! A list of cross-contaminated or misidentified cell lines. *Int J Cancer* 127(1):1–8
- Dirks WG, MacLeod RA, Nakamura Y, Kohara A, Reid Y, Milch H, Drexler HG, Mizusawa H (2015) Cell line cross-contamination initiative: an interactive reference database of STR profiles covering common cancer cell lines. *Int J Cancer* 26(1):303–304
- Freedman LP, Cockburn IM, Simcoe TS (2015a) The economics of reproducibility in preclinical research. *PLoS Biol* 13(6):e1002165
- Freedman LP, Gibson MC, Ethier SP, Soule HR, Neve RM, Reid YA (2015b) Reproducibility: changing the policies and culture of cell line authentication. *Nat Methods* 12(6):493–497
- Fuller BJ (2003) Gene expression in response to low temperatures in mammalian cells: a review of current ideas. *Cryo Letters* 24(2): 95–102
- Reid Y, Storts D, Riss T, Minor L (2013) Authentication of human cell lines by STR DNA profiling analysis. In: Sittampalam GS, Coussens NP, Brimacombe K, Grossman A, Arkin M, Auld D, Austin C, Baell J, Bejcek B, TDY C, Dahlin JL, Devanaryan V,

- Foley TL, Glicksman M, Hall MD, Hass JV, Inglese J, Iversen PW, Lal-Nag M, Li Z, Mc Gee J, Mc Manus O, Riss T, Trask OJ Jr, Weidner JR, Xia M, Xu X (eds) Assay Guidance Manual [Internet]. Eli Lilly & Company and the National Center for Advancing Translational Sciences, Bethesda (MD)
- Reid YA (2011) Characterization and authentication of cancer cell lines: an overview. *Methods Mol Biol* 731:35–43
- Snyder KK, Van Buskirk RG, Baust JM, Mathew AJ, Baust JG (2004) Biological packaging for the global cell and tissue therapy markets. *Bioprocessing* 3(3):39–45