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Mammalian Cell Culture: A Quality Approach

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Cell culture was first devised at the beginning of the 20th century as a method for studying the behavior of animal cells free of systemic variations that might arise in the animal both during normal homeostasis and under the stress of an experiment.[1] During the past 30 years, thousands of academic and for-profit organizations have come to rely on cultivation of animal cells as the basic foundation to perform biomedical research and large-scale biomanufacturing. Their success is directly dependent upon the reproducible production of high quality cell culture products. The complexity of the mammalian cell, its growth and storage requirements, and the need to maintain pure and uncontaminated cultures is a constant challenge to those involved with *in vitro* cell culture.

Biovest International and the National Cell Culture Center have provided the commercial and research communities with custom large-scale cell culture services for the past 15 years. During this time, we have cultured more than 1,500 different cell lines from our clients, providing whole cell pellets, cell pellet fractions, conditioned media and purified monoclonal antibodies manufactured under GLP, QSR, non-GMP, or cGMP regulations for Phase I, II, and III (and approved IVD products). This article describes some of our current practices and procedures. It is not meant to be comprehensive, but does present a basic approach that results in high quality and consistent cell culture products from a multiproduct cell culture facility.

Supply, purity, and growth

Three basic areas of focus for the foundation for any cell culture facility:

1. Cryopreservation of a cell line for a long term supply of a consistent cell population.
2. Ensuring that the population of cells remains pure and unadulterated by other cell lines or infectious contaminants (virus, bacteria, and mycoplasma).
3. Supporting the growth, propagation and production of whole cells or secreted products for further use.[1]

Cryopreservation

A major achievement permitting the development of animal cell technology has been the determination of parameters for routine cell cryopreservation. The importance of a stable, reliable, secure supply of material held at temperatures below –130° C cannot be overstated. In its most simple form, the technology is based on slow freeze and fast thaw, together with high protein concentration and the presence of an agent that increases membrane permeability (DMSO or glycerol).

General criteria for long-term stability of cryopreserved cells:

- Maintaining a temperature below –130° C
- Using an inventory system that allows efficient access to cryovials without compromising the temperature of adjacent vials
- Providing adequate safeguards to assure that constant storage temperatures are maintained

Pure cell population

Whether animal cell cultures are for research or commercial purposes, testing for cell line contamination should be a requirement — not a luxury. In purely financial terms it is more economical to establish rigorous safeguards against contamination, than to potentially allow contaminants into large-scale production systems.

Screening a cell line to authenticate its source and checking for cross-contamination can be accomplished with standard tests to determine the species of origin (i.e. cytogenetic analysis, isoenzyme testing, and DNA fingerprinting). To minimize the risk of cross-contamination, we suggest a few simple rules:

- One cell line in the hood at a time
- Do not share media bottles with other cell lines
- Do not stack T-flasks from other cell lines on top of or adjacent to each other in the same incubator

Bacterial contamination in the culture vessel is often detected as an unusual smell or cloudiness in the culture media. Typically, most bacterial contamination comes from operator technique or improperly cleaned equipment. When bacterial contamination is detected, it is best to bleach the culture fluid and dispose of the culture imme-

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diately along with the supporting media or buffers.

Virus and mycoplasma contamination or co-cultivation?

Many murine, rat, and hamster cell lines support suspected live virus that live in synergy with the cells. Most have little or no effect on the growth of cells or production of protein. These rodent viruses typically do not infect humans and are not a concern for *in vitro* diagnostic applications.

They are of concern, however, when the cell culture product is destined to be a human therapeutic. To ensure safety, the final product is cleared of virus load during downstream purification (heat inactivation, pH inactivation, and filtration). Viral clearance studies can validate the effective removal of these contaminants.

Human cell lines must also be stringently screened for the presence of HIV, hepatitis, and other pathogens. If these human viruses are detected it is typically better to start over with contaminant-free cells.

The most frequent source of cell culture contamination is usually mycoplasma. It is commonplace and not visible with standard light microscopes. Mycoplasma is a generic term given to organisms of the order Mycoplasmatales.

The first observation of mycoplasma infection of cell cultures was by Robinson *et al.* (1956). In the past two years, Biovest and the NCCC have received 181 different client cell lines, of which 35 (19%) have been contaminated with mycoplasma.

Mycoplasmas differ from other prokaryotes by their lack of a cell wall. They are unable to produce precursors of bacterial cell wall polymers. They are the smallest self-replicating prokaryotes with coccoid forms of only 0.3-µm diameter. Their genome is only one sixth the size of *E. coli's*.

The importance of mycoplasma detection in cell cultures should not be underestimated. The concentration of mycoplasmas in supernatant is typically within 10^6–10^8 mycoplasma/ml. Additionally, mycoplasmas will cytadsorb to host cells. They do not necessarily manifest themselves in the manner of most bacterial or fungal contaminants (e.g. pH change or culture turbidity.) Mycoplasmas have been shown to elicit various effects, including:

- Induction of chromosome aberrations
- Induction of morphological alterations, including cytopathology
- Interference in the rate of growth of cells
- Influence on nucleic acid and amino acid metabolism
- Membrane alterations and even cell transformation[2]

Cell growth and propagation

To culture mammalian cells *in vitro* requires nutrients, a container, a controlled atmosphere and trained hands and eyes. In other words, tissue culture media, a bioreactor, a pH-controlled warm humidified incubator, and a trained operator.

Animal cells, *in vivo*, grow in a highly controlled and complex environment. However, in cell culture they are dispersed from a histological configuration, where cell contact plays an important role, and placed into a simplified growth medium. This lacks hormonal and nervous system regulatory mechanisms and the thousands of intermediate metabolites present in body fluids. In addition, organisms have highly evolved oxygenation and detoxification systems ensuring that, except in conditions of stress, a consistent environment is maintained. Thus, a cell in *in vitro* culture is in a alien situation that can only be minimized by attention to environmental optimization.

Cell culture media were first developed with components identified in body fluids (such as amino acids and vitamins) plus serum to provide the multitude of unknown metabolites. The need for serum has since been reduced or replaced due to a greater understanding of cells' nutritional requirements. Thus, a range of growth factors, hormones, and inorganic ions can be added in place of serum to give a more standardized and optimized environment. This in turn not only means greater cell and product productivity, but allows more informative studies on cellular regulation of growth and metabolism. This allows greater maximization of performance in culture, whether to enhance secretion of therapeutic proteins or to respond to a drug toxicity assay.

An understanding of cell biochemistry and cell cycle stages is essential. Many nutrients play a key role in cell behavior (e.g. glucose, glutamine, oxygen). Inhibitory factors such as ammonia and lactate have also been identified. The nutrient balance is not the only factor affecting cell biochemistry; there also are many physiochemical interactions in a culture. The need to scale-up cell cultures increases the importance of such factors as agitation and mixing, and how oxygen and nutrients should be supplied in the least damaging and most efficient way.

Bioreactor selection is often determined by how the cell grows (suspension vs. attached), what the final product is (whole cells or secreted protein), and the scale needed. The most commonly used bioreactor systems are T-flasks, petri dishes, roller bottles, stacked plates, spinner flasks, stirred tanks, and hollow fibers.

The temperature, atmosphere, and humidity levels are similar for most mammalian cell culture needs. Typically, maintaining the bioreactor at 37° C +/- 0.5° with >90% humidity and 5–10% CO_2 (for most bicarbonate-based systems) will be satisfactory.

Mammalian cell culture woes

Although the focus of the laboratory staff may be on genomics, proteomics, genetics, molecular biochemistry, drug discovery, 3D protein characterization, or immunology, this work cannot proceed without cell culture. Even though the propagation of animal cells has been around for almost 100 years, recent advances in recombinant technology and hybridoma and monoclonal antibody techniques, have yielded a nearly infinite source of new cell lines.

Consider the following possible combinations:

Three basic mammalian cell types:

1. Suspension cells
2. Attachment-dependent cells
3. Suspension-adapted, attachment-dependent cells

Sources include:

1. Tissues such as kidney, bone, muscle, stomach, and tumors (including T or B cells, blood, ovary, skin, and brain)
2. Cells from humans, rodents, dogs, chickens, rabbits, goats, and horses

Cell culture media components:

1. Carbohydrates (glucose, sucrose, mannose, fructose, glutamine, GS system)
2. Vitamins
3. Amino acids (essential and non-essential)
4. Inorganic trace elements
5. Hormones
6. Serum sources (including bovine, equine, human, and chicken)

There are approximately 150–200 commercially available classical and chemically defined media. The trend is toward cell line-specific media (for example, CHO, HEK 293, and hybridoma cells.)

Culture methods:

1. T flasks
2. Roller bottles
3. Spinner flasks
4. Stacked plates
5. Bag technology
6. Stirred tank
7. Hollow fiber bioreactors
8. Airlift fermentors

The culturing of mammalian cells can be a very complex process when considering all of the variables stated earlier in this section. We have learned that the best way to make this an efficient process is to start with Quality (i.e. validation, training, and Standard Operating Procedures or SOP's) from the very beginning.

Standard Operating Procedures (SOPs)

Consistent SOPs that meet regulatory and industry standards are important for cell culture operators, second party reviewers, and regulatory approval processes. The SOP format should include:

1. Purpose
2. Scope
3. Responsibility
4. Applicable documents
5. Definitions
6. Equipment and materials
7. Procedures
8. Addenda

The next step is to ensure that four basic systems are in place before any cells are thawed: 1) validation procedures for equipment, 2) personnel training, 3) cell culture procedures, and 4) final product quality control. Whether your cell culture work is regulated under non-GMP, GLP, QSR, or cGMP guidelines, providing high quality, consistent batches of products will require some degree of investment in these areas.

I. Validation procedures (equipment and processes)
   1. Inspection Qualification (IQ)
   2. Operation Qualification (OQ)
   3. Performance Qualification (PQ)

Some equipment and processes to consider for validation are: autoclave, glassware washing, incubators, refrigerators, freezers, centrifuges, spectrophotometers, spinner plates, roller bottle racks, stirred tanks, hollow fiber bioreactors, and laboratory information management systems (LIMS).

II. Personnel training SOPs
   1. Accountability
   2. Identify who will train
   3. Identify who will monitor compliance
   4. Set policies for consequences

III. Cell culture procedures
   A. Facility SOPs
      1. Cleaning and environmental monitoring
      2. Equipment calibration and

The world of animal cell culture can be quite complex when considering all the possibilities presented above. In the 15 years that Biovest and the NCCC have been working with clients, we have heard many frustrated comments regarding animal cell culture difficulties. Here are just a few:

My PI told me to use the media and serum we have in the lab and to grow the cells just like they did last year. I found most of their old notes and thought I grew them the same way. Why is my protein not active?

We always use antibiotics in our lab and never have a problem. So what is mycoplasma and why do I have to test for it?

There were no documents to support the last batch of these cells. That was a different operator last year and he took his notes with him.

Only about half of my cell preps work with my transcription assays. I use the same buffers, the same equipment, and the same personnel. Why does this keep happening?

My cells did fine in a 100-ml spinner flask, why doesn't my culture reach the same density and viability in the 8,000-ml spinner flask?

I split my cells too far back on Friday because I did not want to come in this weekend. On Monday I counted them and they were all dead. They were very healthy when I seeded them at 10,000 cells per ml. Why did this happen?

Most of these questions can be addressed with the simple phrase, "It all starts with quality!"

monitoring
3. Water purification systems and water quality monitoring
4. Glassware washing and sterilization procedures
5. Ordering, receiving, and storing supplies, raw materials, and final products

B. Cell culture propagation SOPs
1. Raw material and reagent preparation
2. Thawing
3. Cell counting and viability determination
4. Aseptic techniques
5. Biological safety cabinet hood use and cleaning
6. Cell culture procedures for handling suspension, attachment-dependent, and suspension-adapted, attachment-dependent cells
7. Scale-up techniques (stirred tank, roller bottle, hollow fiber)
8. Collecting and processing final product
9. Handling, storing, and shipping cell culture products
10. Labeling
11. Documentation (data storage, notebooks, production documents, batch records, and electronic LIMS)

IV. Final product quality control SOPs
1. ELISAs
2. PCR
3. SDS-PAGE
4. Western blots
5. Mycoplasma and sterility
6. Endotoxin
7. Activity assays
8. Review and approval of production documents and batch records

Whether testing is performed in-house or outsourced, SOPs must be in place for each assay.

Know your cells

With each new cell line, the first step during the initial scale-up is to make a cell bank for your future production. It can be as simple as five to 10 vials or as elaborate as a fully characterized 200-vial master cell bank used for the entire life of the product. Regardless of the cell bank's purpose, (research or cGMP manufacturing), it is only valuable if you can recover viable cells. Before the test thaw is complete, make sure you have a backup flask of cells growing.

Once the call bank has been stored for more than two to three days, a test thaw should be performed. Typically a test thaw is considered acceptable if cells can be passaged within five to seven days. Hybridoma lines typically exhibit viabilities of 40%–90%, anchorage-dependent lines usually exhibit 70–90% viability, and suspension cell lines will be 60–100% viable. Having a reliable stock of cells helps ensure lot-to-lot reproducibility of your final product.

Your bank should be tested for adventitious agents (such as mycoplasma, bacteria, and virus) and clonality. The clonality of the cells can be tested by isoenzymes and karyology. The extent of testing is directly dependent on the development stage of your final product. Having a cell bank that can be revived and is known to be a pure population provides a strong foundation to further optimize your cell culture and protects against loss of desirable characteristics.

Once the bank is in place and you know it is pure, the next step is to understand the cell-specific growth characteristics. Determining the minimum and maximum density for your cell line will help with routine propagation. This can be accomplished with a series of small-scale culture vessels, each with a different seeding density (0.01, 0.05, 0.1, 0.3, 0.5, and 1.0 x 10^6 viable cells per ml). Count each vessel once a day until viabilities fall below 50%. This data can help characterize the range of your cells' growth potential. Once this data is established, it can be used as a guide for optimizing growth and production potential when changing media components, serum supplements, and production methodology. For hybridoma cell lines, media samples used to establish the growth curve can also be used to determine optimal antibody production. Poor antibody production can be used to determine if the cell line needs to be sub-cloned to achieve better expression levels.

To further characterize your cell line, it is important to continually passage them for two to four months. Daily cell counts and sample retention can be used to determine optimal doubling times and passage number. Because living organisms may not always exhibit the same characteristics or protein expression level over a long period of time, passages should be documented and characteristics that are important to your cell line should be monitored in relation to passage number.

Optimizing desirable characteristics

Whatever the product of interest is, optimization of the cell culture process makes for sound science and solid business sense. The three main areas of cell culture optimization are:

1. Cell culture media (basal with serum, serum-free, protein-free, chemically defined)
2. The bioreactor (spinner flasks, roller bottles, tanks, hollow fibers, stacked plates)
3. The culture process (optimal seeding, collection densities, and viabilities)

To start this process, contact several media vendors. Explain what type of cell line you have, how it is being cultured, and what type of product is being produced. They will often provide samples for evaluation.

In addition, the cell culture operator can examine several other options to further determine optimal media conditions:

- Serum supplementation of basal media
- Serum screening of reserve lots
- Fetal bovine serum (FBS), newborn calf serum (NCS), charcoal-treated, dialyzed, defined vs. characterized sera, low IgG FBS
- Serum-free or chemically defined media designed for specialized cell lines (such as HEK 293 cells, epithelial cells, keratinocytes,

neuronal cells, JEG3 cells, and hybridomas)
- Media buffering systems (i.e. phosphate/bicarbonate systems and CO_2 or HEPES, which is light sensitive)
- High- and low-glucose formulations
- Divalent cation composition
- L-glutamine concentration
- Non-essential amino acids and vitamins

Selecting the optimal bioreactor and culture process conditions takes time and patience — each cell line is unique. Once you have trudged through the countless passages and performed cell counts and product assays to determine the optimal clone with the appropriate media in the best bioreactor environment with the highest density and viability, make a new cell bank and perform a test thaw. The cell line you started with has been altered to either grow in a different media, or was subcloned along the way.

In addition, if you insert a different gene into the cell line, be aware that this is now considered a new cell line. What held true for the original cell line (optimal media conditions, plating efficiency, doubling time, and secretion rate of product of interest) does not guarantee the same performance for the new cell line.

When optimization is complete

Your cell culture and new cell line are well characterized with optimized production conditions. You have developed a means of producing your final product that meets your objectives for activity and cost-effectiveness. Now you need to ensure that someone else can repeat your work. It is time to format your entire process into a series of product-specific SOPs.

When doing so, keep in mind some aspects of cell culture that are often taken for granted by many cell culture operators:

- Use calibrated and monitored incubators
- Use calibrated pipettes
- Warm media to incubation temperature prior to passage
- Seed and harvest at standardized cell densities
- Set specifications for cell viability and passage number
- Set specifications for minimum and maximum cell density in culture
- Standardize methods for cell counting, centrifuging, and assays for product testing
- Standardize scale-up protocols (T flask to spinner flask to hollow fiber or stirred tank)
- Document the entire process
- Use glassware cleaned with autoclave-sterilized tissue culture-grade detergent
- Use tissue culture-grade reagents
- If possible, have backup vendors for your critical raw materials
- One cell line at a time during hood use (disinfect hood between users)
- Facility cleaning should be performed by trained personnel
- Routinely test for adventitious agents and cross-contamination
- Routinely evaluate the cost effectiveness of your process. Cell culture supply vendors are constantly updating supplies, equipment, media, and other raw materials
- Work with a well organized cell line storage system. If you can't find it, you can't grow it
- Use documentation software for rapid, legible data entry that can be referenced and audited

Cell culture tips

Creating cell banks

For cell lines that recover slowly from thaw, freeze larger quantities (use 3 to 5 ml cryovials instead of 1- to 2-ml cryovials) to reduce lag time upon recovery.

If you have difficulty recovering cell lines from frozen stock, use 10% DMSO + 90% FBS as your freezing solution. This can be used for lines cultured in serum-free or chemically defined media. This is only a storage solution and the FBS can be washed away after thaw by centrifugation.

Some human leukemic cell lines (Jurkat and HL-60) do not recover well (even when stored in liquid nitrogen) after three to five years in storage. Make frequent backup cell banks of these types of cell lines.

High pH spikes during cell bank preparation can damage many cell lines' ability to be recovered. This is especially true for hybridomas.

Freeze down cell cultures with densities of 3–10 x 10^6 cells/ml. Higher densities recover poorly due to the lack of cryoprotectant per cell. Most cell lines recover well using 5–15% cryoprotectant. A higher percent of DMSO or glycerol does not protect cells better when their density exceeds 2 x 10^7 cells/ml.

If you do not have access to a controlled rate freezer, place your vials in a styrofoam box with at least one inch thick walls at -80° C overnight (18–36 hours) then transfer them quickly into the liquid nitrogen dewar using dry ice in the box to avert temperature spikes. Alcohol baths also work well at the same temperature and time frame.

Thawing cells

Transfer a vial from the dewar to the thawing area on dry ice. Make sure any trapped liquid nitrogen has evaporated and vented from the vial. Ensure the cap is tight then swirl vial in a 37° C water bath of sterile water or an antimicrobial (Aquaclear, for instance). A 1 ml solution of cryopreserved cells will thaw within 1.5 to 2.5 minutes. When the last piece of frozen solution is about as big as a small pea, you can stop swirling in the water bath, spray the outside of the vial with 70% alsochol, wipe clean then transfer to the hood. The rest of the frozen solution will thaw in less than a minute.

Remove the cryoprotectant from the cell culture solution by centrifuging at low speeds (500 x g for five to 10 minutes at room temperature) or by simple dilution with media. If the cells are not washed by centrifugation and dilution is used, make sure the final DMSO concentration is <0.5%. Then change the media 24 to 48 hours post-thaw. Higher concentrations of DMSO upon thaw without washing out may cause cell death due to toxicity.

If the cell line exhibits low viability

upon thaw, gradually add media to the thawed cell solution. To one ml of cell suspension add one ml of media. Swirl, wait for 30 to 60 seconds, then add two ml. Repeat this process until the total volume is about 10–15 ml. This gradual volume change will help alleviate the large osmolarity change and maintain the integrity of the cell membrane.

During the first few days post thaw, do not rely solely on cell counts and viabilities to determine if your cells will recover or not. The use of an inverted microscope (allowing you to inspect the cells in culture), can provide valuable information relating to cell growth, confluency, and overall appearance.

Scale-up and passaging

Rotate the type of antibiotic used in your lab every three to six months. This helps prevent certain antibiotic resistant organisms from developing.

Passage cells daily to keep them in mid-log phase if you are looking for active nuclear proteins used for splicing or transcription related research. This is useful for cell lines such as Hela-S3, HL60, Jurkat, and K562. For attached cells, collect when they reach 50–70% confluency. Higher cell density does not necessarily translate into high levels of activity for specific nuclear proteins and transcription factors.

When weaning hybridoma cell lines from serum-based media to low protein, serum-free, or chemically defined media, it often works best to use a series of different defined media. Start with the one containing the highest amount of protein and work your way down to the one with the lowest amount of protein that still provides adequate cell growth and antibody production.

If adding large amounts of tissue culture media (>1 liter) to expand cell cultures, prewarm the media to incubation temperature to help reduce the lag phase in cell growth. For example, four liters of media at 4° C takes about 10 to 12 hours in an eight-liter spinner flask at 30–50 rpm to reach 37° C .

Also try different types of serum or serum supplements as an alternative to FBS. This seems to work well with HeLa, K562, Jurkat, MEL, and Raji cell lines. This type of screening can save time and expense down the road.

Clumps of anchorage-dependent cells do not seed and spread well. Use an automated pipette and tritiate the aggregates prior to expansion. This technique works well with cells such as SKNMC, HepG2, HEK 293, MDA breast carcinoma lines, MCF-7, and PC-12. Cell lines that have trouble reattaching to their culture surface upon seeding may benefit from a roller bottle technique where the bottle is allowed to turn intermittently (two minutes on, two minutes off) for the first few hours.

If you have suspension-adapted an anchorage-dependent cell line, keep the paddle speed slightly higher than for other suspension cell lines. Typically these cells can handle the increase in shear. This also helps reduce clumping in the spinner flask.

Maintain the paddle speeds lower for most suspension lines (such as Jurkat, MEL, HL-60, Raji, hybridoma, K562) which seem to be more sensitive to shear force.

Seeding densities for static conditions (T flasks) are typically about 5–10 x 10^3 cells/cm^2 . Increase this density to 3–8 x 10^4 cells/cm^2 when moving to more dynamic conditions like roller bottles. It helps the media conditioning phase and will help reduce the lag phase during cell expansion.

To maintain the log phase in adherent conditions, collect cells at 50–70% confluency.

Observing and recording morphological characteristics of growth patterns for adherent cells is sometimes more important than cell counts and viability stains. Cell counts can be skewed by clumping, trypsin damage, or incomplete removal of cells from the surface.

When using roller bottles to culture cells, use a seeding density of 1 to 4 (when comparing surface areas of old and new vessels). Use approximately 100 to 300 ml media/1,000-cm^2 roller bottle.

Precoat roller bottles with serum or attachment factor for those cells that need help sticking (this works well with the Hep2 and SA0S-2 cells). Another method is intermittent rolling (two minutes on, two minutes off) for the first few hours of culture. We have found this to be very helpful for a few cell lines (especially fibroblasts, i.e. SKNMC, 3T3, and SA0S-2).

Be cautious about reusing roller bottles. Some plastics will not maintain their electrostatic charge once cells have attached. Screen flasks or roller bottles from multiple vendors for attachment affinity. One type of roller bottle will not satisfactorily culture all types of adherent cells.

A variety of methods can be used to harvest adherent cells from T flasks or roller bottles. These methods depend on the nature of your product. If you are after surface receptors, chemical removal of cells may not be appropriate. The use of mechanical cell scrapers or physical tapping, although more labor intensive, is simpler and yields better quality membrane receptor preparations. We have done this frequently with SKNMC, HepG2, Hep2, and MCF-7 cells.

Trypsin, EDTA, and EGTA solutions work well when the end product is a protein or intracellular organelle. Be careful not to overexpose the cells and cause lysis. However, underexposure with chemical dissociation may cause the cells to come off in sheets or large clumps, which leads to reseeding and further expansion problems.

Some cell lines differentiate when the cell culture space limits expansion. We have seen this with MDCK, NIH3T3, L6, C2C12, and MRC-5. To maintain these cell lines, limit their confluency to 70–80%.

Some attachment cell lines (LnCap and Astrocytes) do not do well in roller bottles and as such are limited to strictly static conditions (large T flasks or stacked plates).

Adapting cell lines to grow as suspension-adapted, anchorage-dependent cells and still produce the protein of interest affects cost. Typically it costs about three to four times more per cell to be cultured as an adherent cell than a suspension cell.

Final product collection

When collecting whole cell preps for further processing, it is very important to chill the cells to 2–8° C as quickly as

possible to reduce further metabolic activity and decrease the action of protease. Using refrigerated centrifuges and removing cell pellets to an ice bath when performing large-scale harvests will help keep cell quality high.

After the culture has been harvested, wash the cell pellets in ice cold PBS. If they are adherent cells, make sure the PBS does not contain any divalent cations (they can cause the cells to clump and become difficult to process.) Cell pellets to be used for whole cell extracts can be aliquoted, snap frozen in liquid nitrogen and stored at –80° C for many months. Creating a large supply of homogenous cell pellets helps ensure consistent results.

When processing conditioned supernatant to collect and later purify proteins or monoclonal antibodies, it is imperative to keep the solution sterile, cold, and at the proper pH. A high pH (7.3–7.7) will help reduce the amount of acid protease activity in supernatant collected from a culture where much cell lysis took place. Storage of this conditioned media should be tested to make sure that 2–8° C or <–20° C freezing does not harm the protein of interest. Storing retention samples from different stages of production can help the operator troubleshoot when problems arise during product development.

Summary

The growth and propagation of mammalian cells *in vitro* requires manipulating and controlling complex set of conditions. In addition, the fact that every cell line is unique can require vast amounts of resources to produce a high quality, consistent product. This can be accomplished by focusing on the process, formulating SOPs to support cell culture production, investing in the facility's upkeep, monitoring, and validation, continual personnel training, and revising SOPs.

Taking the time to fully characterize the cell line, optimize for production, and create sound documentation through SOPs builds quality and consistency. In the long run, this saves time and money for the organization. It is never too early to build quality into a cell culture-based product.

REFERENCES

1. Freshney RI. Culture of Animal Cells: A manual of basic techniques. New York: Alan R. Liss Publishing; 1983.
2. Doyle A, Griffiths JB, Newell DG. Cell and Tissue Culture: Laboratory Procedures. West Sussex, England: Wiley Publishing; 1998.