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SUPPLEMENT TO LAB MANAGER MAGAZINE

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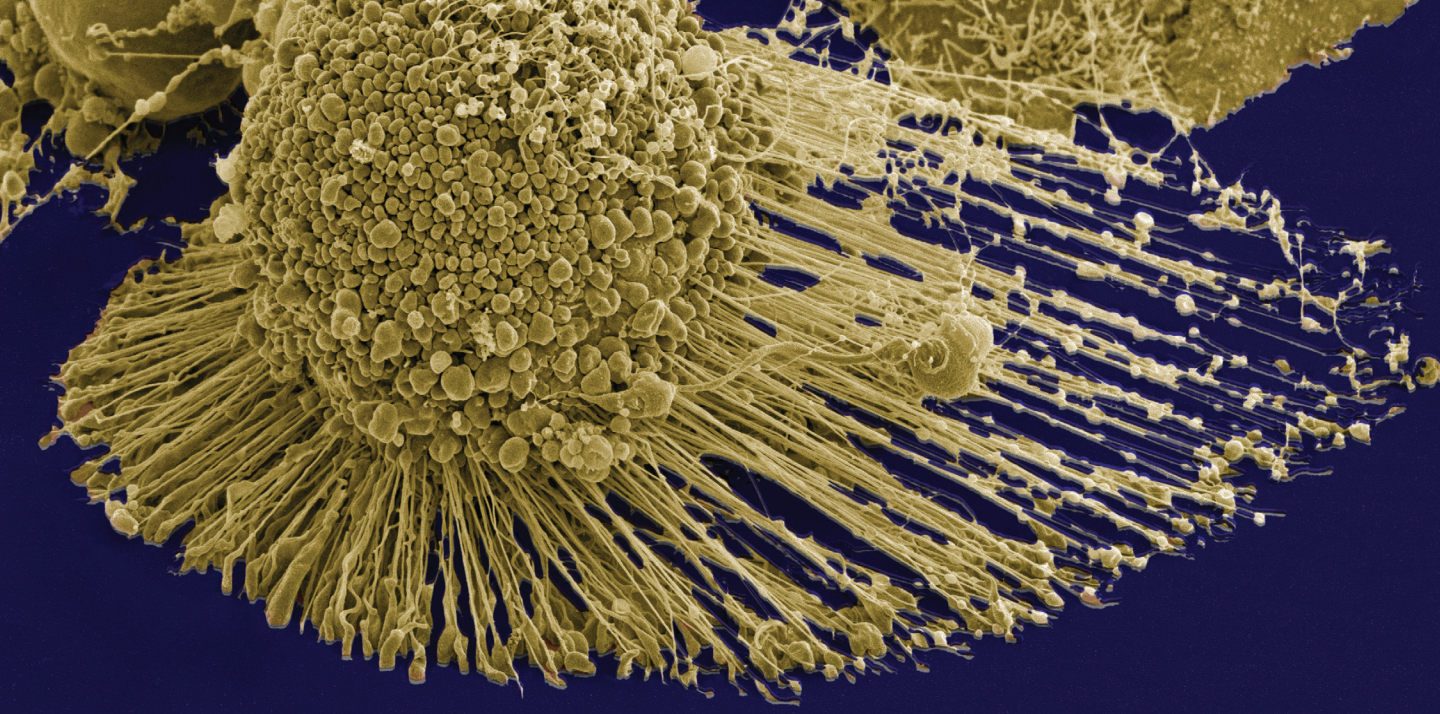

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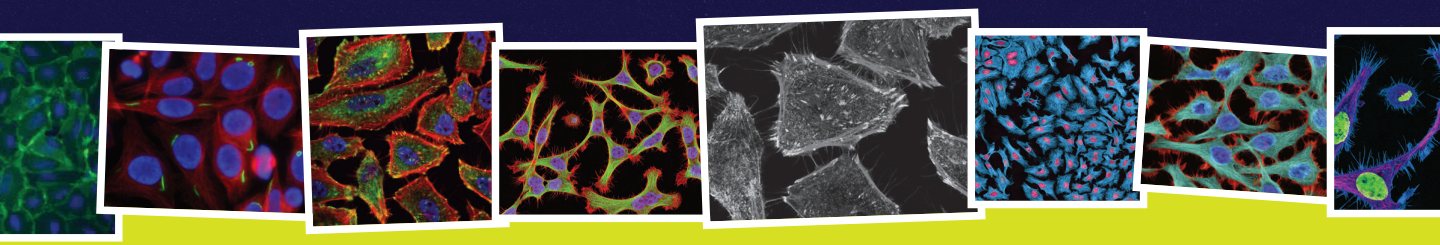

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In this Q & A, three experts from both academia and industry discuss the types of cell culture they perform in their labs, technologies they use, and the challenges they face.

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INSIGHTS
ON A CELL CULTURE LAB
STARTING THEM UP, KEEPING THEM RUNNING

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Cell culture incorporates diverse, broad-ranging operations for maintaining, expanding, and utilizing cells grown outside their natural milieu. All forms of cell culture share common operations, but the term "cell culture" has come to denote cultures derived from multicell, eukaryotic (possessing nuclei) organisms such as humans, animals, and, less commonly, insects. Bacterial and yeast cultures are often referred to as fermentations, the biological process through which beer (by yeast) and yogurt (bacteria) are manufactured. Fermentations tend to be of shorter duration than cell cultures because bacteria and yeast can double every 30 minutes, while animal cells take up to 24 hours to divide.

Cell culture supports myriad end-uses, and scales range from a few hundred microliters to 30,000 liters and higher. Among these applications are:

- Testing toxins or new pharmaceuticals
- Cell-based vaccines produced directly from killed cells
- Virus-based vaccines made by infecting cells and harvesting the virus
- Infectivity assays
- Basic biomedical research
- Production of genetically engineered drugs such as monoclonal antibodies
- Cell therapies, including stem cell treatments

Cell culture supporting biopharmaceutical manufacture is by far the highest-value-added activity. According to "Cell Culture Markets," an October 2011 report by Kalorama Information (New York, NY), the biopharm sector is driving 10 percent annual growth for cell culture, which will continue through at least 2015. The report estimates sales at $3.2 billion in 2012, rising to $4.3 billion at the end of the forecast period. North America and Europe dominated the 2010 market at $2.1 billion, or 83 percent of worldwide figures. The approvals of "biosimilars" (generic-class biopharmaceuticals) will spur further growth worldwide, but especially in Asia and Pacific regions, which currently account for just 12 percent of the market, and in the emerging economies of Eastern Europe and South America.

Although cell culture is more than 100 years old, it has only been applied to the manufacture of biological drugs for about 25 years. Today mammalian cell culture is the workhorse production platform for most of biotech's protein therapeutics and increasingly for cell- and virus-based vaccines. Chinese hamster ovary (CHO) cells are now the expression system of choice for monoclonal antibody therapeutics totaling tens of billions of dollars in annual sales. As a result of their monumental commercial significance, CHO cells are the subjects of significant small-scale cell culture as well, to support large-scale manufacturing.

Insect cells are a growing niche activity within cell culture. Insect cells are eukaryotic, but unlike mammalian cells they are not engineered directly to produce therapeutic proteins. The cells are instead infected by a baculovirus that takes over the cell's protein-producing machinery. No products derived from insect cell culture have been approved for human use, although several have been commercialized in veterinary markets.

Setting up and maintaining a cell culture laboratory from scratch requires expertise in workspace design, architecture, ergonomics, and engineering. While many groups have succeeded in converting chemistry or engineering suites into cell culture labs, workflows will not be optimized, particularly in labs densely populated with workers and equipment. After design and build, other key issues include equipment, workflow, contamination control, and consumables–primarily media and feed.

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After applications and processes, workflow optimization is the primary consideration when setting up a cell culture lab. Workflow relates to how samples and cultures move through the lab, the number of operations going on simultaneously, and chain of custody. "Particularly with cell culture, having a tight understanding of where and how things move through the facility protects you against cross-contamination and enables troubleshooting for unusual or unexpected occurrences," says Bryan Monroe, principal at Primus Consulting (Kingston, WA). Primus advises on cell culture facility design, process scaleup, and technology transfer. "Lacking that understanding makes it difficult to see how and why things are not going right with equipment, reagents, and everything affecting your process." Companies that overlook these issues will regret it later, Monroe adds. "A solid chain of custody is a top priority." Cell culture workflows break down into approximately five areas:

- Cell isolation and preparation, beginning with either tissue from a vial or from a cell bank
- Growth and passaging, when cell populations increase to reach levels that allow routine experimentation
- Harvest – removing cells from the dish for re-culturing or expansion
- Characterization
- Storage under freezing or refrigeration in convenient aliquots

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Optimizing cell culture workflows is an "inside out" exercise, involving three concentric workflow factors: facilities, large equipment, and operations (including personal equipment and consumables). "The point of cell culture is to mimic a cell's native physiologic environment," says Mary Kay Bates, global cell culture specialist at Thermo Fisher Scientific (Milford, MA)., "So that a culture represents what occurs inside the organism. Everything you use in the lab must contribute to that goal."

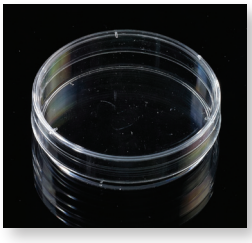
> "The point of cell culture is to mimic a cell's native physiologic environment."

Some layout concepts are common sense. Electrical outlets should be sufficient in number, and of adequate voltage, to power standard equipment. Designers should consider installing several circuits to avoid overloading, and providing extra outlets near benchtops, where a lot of equipment is used simultaneously. David Craig, national sales manager for BINDER (Bohemia, NY) says that lines, or tanks, should be located close enough to incubators (or other equipment requiring specialty gases) so that plumbing is minimized. "You'd be surprised how often incubators are located on one side of the room and CO_2 tanks on the other," Craig says. This layout requires the use of reinforced tubing to prevent pinching and crimping.

Others ideas must be learned, sometimes through experience. Craig also warns against installing incubators near heating or cooling vents. "During our seminars on contamination control we ask people to walk up to their incubator, take one or two steps back, and look up. The last thing you want to see is an air diffuser for heating or air-conditioning above your incubator. Every time you open the door the diffuser is blowing spores and bacteria into your work space." Craig dismisses the contention by some facility managers that air is filtered at intake. "Take some quilting fabric and leave it inside the vent for a couple of weeks, and it will emerge covered in black gunk."

When incubators are stacked, the bottom unit is significantly more likely to experience contamination than the upper boxes. According to Craig, that is because dirt and grime (read: microorganisms) accumulate in difficult-to-reach parts of the lab. He suggests placing stacked incubators on locking casters to facilitate cleaning below the units and to provide access for service. The cost of casters, about $350, is easily justified if they prevent just one contamination.

Insight into design and layout comes from a variety of sources. Sometimes managers don't have much choice. Academic groups tend to move into generically designed labs with legacy layouts and locations of utilities and fume hoods. Many companies do this as well.



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Well-heeled new facilities tend to hire engineering and design firms experienced in the demands of their particular workflows. Yet a surprising amount of information is available from equipment vendors, scientific meetings, colleagues' laboratories, trade publications, and regulators.

Vendors can provide invaluable information at the very earliest stages of lab setup, particularly if they talk to the designers and assist in planning with equipment dimensions, power requirements, and noise levels. Nothing short of a reciprocating saw will save the day when the designers have specified and built a 40-inch-wide space for a 48-inch-wide incubator.

"Labs designed or expanding on an ad hoc basis often crash."

Meetings, conferences, colleagues' facilities, and trade publications can provide valuable insight in the same way that garden magazines inspire do-it-yourselfers. The trick is to understand the differences and similarities between the pretty picture in the magazine or PowerPoint slide and your facility's actual requirements. And, like many amateur landscapers, many labs lack the will and resources to execute a do-it-yourself cell culture design project.

Regulated (FDA, EPA, OSHA) labs face an additional dimension of design complexity. While agencies do not specify how to conduct work, they set standards that must be met to remain in business. All the usual scientific standards apply, alongside the requirements of traceability, documentation, quality, and the potential for legal or regulatory repercussions.

Monroe cautions against laboratory "creep" or what he calls "organic facility growth," common in academic labs or companies inexperienced with cell culture. "They decide they need to do cell culture work, and the facility grows out organically as they assume more and more work." These labs add equipment and take on new processes, and eventually one group or process begins to overlap others next to it. "Labs designed or expanding on an ad hoc basis often crash due to contamination or instrument failure, requiring rework or redesign because they have not controlled the workspace or designed it to accommodate the workflows optimally. They're in the forest, but they can only see one or two trees at a time. Lab design must be approached with an overarching perspective."

Large, more experienced organizations tend to be more deliberate, Monroe says. They ask who will be using the facility, for what purposes, and conducting what processes, and then fit those factors in with the facility's existing footprint or within the newly designed workspace. "And by doing this they achieve not only higher quality and functionality, but the flexibility to accommodate different processes. Unfortunately, these labs are in the minority."

Regardless of the source of lab layout ideas, remember that all labs are different. "Forget about the cookie-cutter approach," Monroe warns. "The sheer variety of equipment and operations comprising a cell culture lab make one-size-fits-all approaches unworkable."

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INSIGHTS ON A CELL CULTURE LAB

USAGE AND LAYOUT OPTIMIZED TO WORKFLOWS IS CRITICAL

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Isolating facility and layout from equipment during planning and maintenance of a cell culture lab entails considerable risk, if for no other reason than the latter must fit into the former.

"With respect to layout, the biggest mistake a manager can make is to underestimate the space they will need for equipment," observes BINDER's David Craig. "People forget to account for door swing, leaving enough space for heat dissipation, placement of utilities, or glove box ports. You have to think one step beyond the nominal dimensions of equipment and available floor space, and consider heat dissipation as well." BINDER advises customers to allow six to nine inches between each incubator stack for circulating air to carry heat away from culture processes.

The best time to shop for new cell culture equipment is before that incubator or refrigerator dies. Purchases made out of necessity during emergencies often overlook new, potentially useful features, or over specify on bells and whistles. When the equipment arrives, Craig advises lab managers to go to the loading dock and inspect crates for damage. "One of our customers ordered six incubators, of which three were damaged during transit. The last thing you want is to get stuck between someone trying to sell equipment and the freight company."

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> "The best time to shop for new cell culture equipment is before that incubator or refrigerator dies."

Equipment usage and layout, and optimizing them to workflows, is critical. Most labs end up with equipment that many workers share on many different projects, as well as some devices dedicated to one process or to expert users. The former include dedicated incubators and refrigerators; the latter consist of common, everyday tools like microscopes and dishwashers. Equipment should not be scattered around the lab in ways that require users to bounce back and forth to accomplish routine tasks.

Frequency and criticality of usage dictate where both equipment types live. A highly sensitive balance does not belong next to a noisy, rumbling dishwasher, and a cell counter used just several times per month can probably go into an adjacent lab. Immediate, common workspace should be clutter-free to encourage good practices and discourage sloppy technique.

One of the most overlooked aspects of setting up a cell culture facility is the fickleness of biological systems, and how seemingly minute excursions can negatively affect the quality of results. Primus's Bryan Monroe cautions about taking anything for granted. For example, one of his clients assumed that the cell temperatures inside an incubator were identical to the air temperature readout on the LED. "The shaking table was generating heat, so the cells were actually at a higher temperature than the temperature sensor indicated." Monroe suggested putting a thermocouple into media on the shaker table, and adjusting the temperature setting down to compensate for the extra heating. Similarly, he notes that a poorly calibrated cell counter can provide seriously erroneous estimates of cell density, and poor choice of detergent can result in cells swimming in dish soap. "The learning curve for some of this equipment can be painful," he says. "You've killed your cells, and now you have to start over."

When in doubt about space requirements, Mark Bonyhadi, director of clinical business development for cell therapy for Gibco (Grand Island, NY), suggests specifying equipment that is easy to use, with an acceptable learning curve, and that fits on a desktop. "You want something that doesn't take up a lot of space and can operate in close proximity to other pieces of your normal workflow."

Quality assurance, long a hot topic in biotech and pharmaceutical development, is gaining significance in non-regulated work as well. Variability is the antithesis of quality and consistency.

Sources of variability are not limited to cells and culture conditions. As with sample and standards preparation in instrumental analysis, handling of ingredients, operator inconsistencies, and pipetting errors contribute to systematic and random errors. Robotics and automated liquid handling have contributed significantly to the consistency and quality of cell-based processes, particularly those

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involving aliquoting of suspended cells and multiple reagents. Robotics has been adopted in all high-throughput plate-based cell assays. Low-volume, high-throughput processes (e.g., microtiter plate-based assays) benefit the most from automated liquid handling, especially for operations like dilution, cell, and reagent addition.

Microbioreactors, scaledown, and design of experiment

Covering the hundreds of culture equipment options and combinations in a single article is impossible. Readers should check out separate articles in *Lab Manager Magazine* on biosafety cabinets, incubators, pipettes, refrigerators, etc. Here we focus on an emerging area of cell culture equipment, parallel microbioreactors, which have streamlined nearly all areas and application of cell culture.

Low-volume culture systems are becoming popular in biotechnology and pharmaceutical companies for their high parallelism, small volumes, and suitability for automated manipulation and liquid handling.

Microbioreactors monitor culture parameters like dissolved oxygen, carbon dioxide, pH, and others. Companies like Dasgip (Jülich, Germany), TAP Biosystems (Hertfordshire, UK) and Pall (Port Washington, NY) sell commercial microbioreactors with milliliter-sized reactors and varying levels of parallelism, monitoring, and control. The BIOSTAT system from Sartorius (Göttingen, Germany) uses one-liter bioreactors and is designed more for process optimization than for cell or media development. A system developed through a collaboration between Roche's (Basel, Switzerland) pharmaceutical and diagnostics divisions, the Cedex Bio, combines miniaturized bioreactors, electrolyte monitoring, and a 12-wavelength spectrophotometer, and measures up to 14 process parameters simultaneously.

When Bernd Reichl, head of the R&D lab at PAA Laboratories, now part of GE Healthcare Life Sciences (Pasching, Austria) joined his firm four years ago, his mission was to establish a new cell culture R&D laboratory based on T-flasks, microwell plates, and shaker/spinner flasks. The lab will support optimization of media, feed, and reactor conditions.

Reichl's lab will soon acquire a miniaturized, parallel mini-bioreactor system. His ideal system consists of at least 40 reactors, each with a volume of about 20 mL, which allows real-time monitoring and control, as well as daily removal of 0.5mL to 1mL aliquots for offline analysis. "Cell cultivation is a biological process. You see variability, even if you do your best to keep all parameters constant. That's why we require enough reactors to run everything in duplicate or triplicate."

Online-controlled parameters include pH, temperature, CO_2, and dissolved oxygen, with feedback capability to control the cultures if parameters fall from specification. Reichl is also interested in temperature gradients for the ability to test temperature as a variable or adjust it during culture. "And of course automated pipetting is an important feature you will need when you run so many cultures in parallel."

Offline analysis consists of glucose, lactate, ammonium, glutamine, glutamic acid, and potassium, plus amino acid analysis by HPLC, automated cell counting for viable cell density and viability, and HPLC analysis of protein titers. The GE group is also investigating advanced cell imaging techniques for assessing cell health and productivity.

"We are strengthening GE's capabilities in small-scale cell culture for testing media and screening modifications to media compositions and culture conditions in parallel using design of experiment," says Reichl. "Our goal is to shorten the timeframe for media and feed optimization."

Reichl believes that by adding analytics and modifying media development workflows, a similar "micro" approach may be taken toward cell line development–a task much more difficult and time-consuming than media development.

"Frequency and criticality of usage dictate where both equipment types live."

Another notable success for parallel bioreactors has been in "scaledown" modeling of large bioprocesses. The technique involves duplicating as comprehensively as possible bioreactor conditions, at very small scale, to troubleshoot or de-bottleneck large cultures or to test reactor conditions after cell line and media optimization. "Scaledown model qualification is a whole science in itself," observes Stuart McNaul, Ph.D., section leader for upstream process development at Fujifilm Diosynth Biotechnologies (Morrisville, NC). McNaul's lab conducts small-scale cell culture to support process development, in preparation to scaleup and manufacturing. The group maintains four cell culture labs and three microbiology labs operating at scales from 2 liters to 100 liters.

Scaledown cultures are generally larger than those used to optimize media, although that is changing. McNaul is closely looking at microbioreactors, which he describes as the "way of the future," and expects to see more of those in scaledown experiments and fewer of the multi-liter vessels. The 2-liter and 10-liter bioreactors, he says, are still needed to test purification and formulation, but might otherwise be replaced by reactors in the 2mL to 200mL range.

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INSIGHTS ON A CELL CULTURE LAB

CELL PRODUCTIVITY AND PERFORMANCE DEPEND ON MEDIA QUALITY AND CONSISTENCY

As Canadian scholar Marshall McLuhan famously noted, "The medium is the message." So it is with cell culture, where media and feed or supplementation strategies have been responsible for more improvement in cell productivity and performance than any other factors. Since cells receive all their nutrition from the medium, optimizing this critical factor is a top priority for both end-users and media vendors.

Through its Gibco business unit, Invitrogen specializes in media and supplements for cell culture-supporting pharmaceuticals, cell therapy, and research. Gibco's Mark Bonyhadi explains that most cell culture labs acquire information on media from published protocols based on their particular cell line. All significant media vendors maintain an extensive library on media applications, as well as ingredient traceability and quality data.

For years, cell culture media relied on animal derived components like bovine serum to supply the "magic dust" that kept cells viable and productive. Manufacturers of biological drugs have abandoned serum-based media for new processes, and have transferred older products to serum-free media. Serum is a source of variability because it is chemically undefined. More important, regulators are concerned about the possible transmission of infectious prions from animal sources to human patients.

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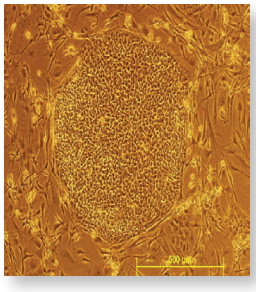
"Manufacturers of biological drugs have abandoned serum-based media for new processes."

One step up the value chain from serum-free media are chemically defined media, where every ingredient—as many as 100—is specified. Chemically defined media are the ultimate in consistency, providing reproducibility from culture to culture. Unfortunately, as one moves from serum-based to animal component-free to defined media it becomes more difficult to establish and expand a cell line and, in the case of recombinant proteins, to produce sufficient quantities to be economically viable.

Many labs use serum-free media for protein expressing cells, even for research-only products. Problems with serum go beyond inconsistency and potential infectivity, observes Steve Pettit, Ph.D., who directs cell culture development at Invitria (Fort Collins, CO). "Serum increases the purification burden because it contains so many high-abundance proteins of its own. Plus serum can mask the activity of a recombinant serum protein."

First-generation serum-free media underperformed, says Pettit, "but some of the newer media outperform serum." Invitria specializes in purified, recombinant serum components to help replicate the activity of serum in media, but as a defined component without the risks of animal component-derived ingredients. Two of the company's best sellers are recombinant albumin and transferrin. Albumin is a general-purpose nutrient for cell cultures, providing lipids and antioxidant activity, delivering metals required to maintain proper protein folding, and blunting the activity of cell toxins. Transferrin helps transport oxygen to cells in a biologically regulated manner. Serum-free media lacking this protein require the addition of iron compounds.



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Standard off-the-shelf media and supplementation suffice for most basic biological research. "Ninety percent of basic researchers, especially in academia, go with standard media and protocols," Bonyhadi explains. On the other hand, labs involved in the culture of cells that will eventually produce therapeutic proteins will often experiment with culture conditions, including media and feed, to optimize their process. Parallel microbioreactors are one approach to media optimization; some labs employ microtiter plate-based methods that are short on parameter control but high on parallelism. To the extent they are employed, media/feed optimization, as well as cell line engineering, occurs very early in development. Stem cell work always involves some type of optimization of media and supplementation with new cell types; afterward, labs will follow time-tested protocols.

Depending on their origin and ultimate use, media and feed requirements for industrially relevant cell lines can differ significantly. Over the past decade media/feed have been responsible for most of the growth and productivity enhancements of cells used to produce biological medicines. Arriving at the ultimate optimized media/feed strategy can take months and consume large volumes of materials. The modern approach involves testing many media/feed combinations in parallel in small bioreactors or even smaller plastic culture ware. This approach is known as "design of experiment" (DOE).

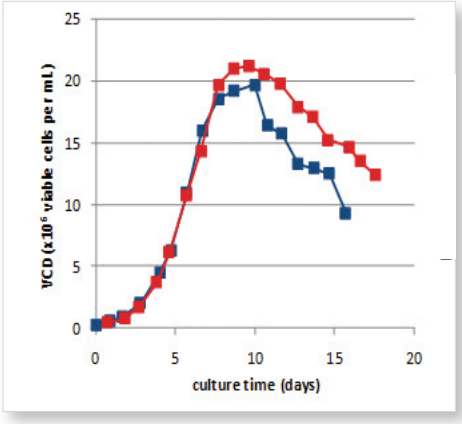
"Standard off-the-shelf media and supplementation suffice for most basic biological research."

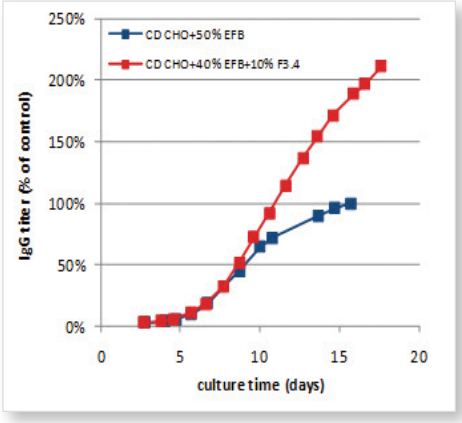
Tom Fletcher, who directs cell culture R&D at Irvine Scientific (Santa Ana, CA), believes media optimization could be even more widely adopted, especially for new cell lines. "If you have choices, a quick evaluation of several media in parallel is worth the time. Many labs settle on the least expensive choice, thinking they're the same from all vendors, but differences in performance can be significant, even when media contain the same basic standard ingredients."

Standardizing cell culture media has become a huge issue among development scientists in industry. The idea is catching on in nonprofit and academic centers as well. "Labs that screen cells for drug development are becoming more methodical about their processes," says Bonyhadi. "They look closely at components that make cell cultures more stable and reproducible."

"Cell culture media may contain up to a hundred different components," notes GE's Bernd Reichl. Each component contributes to a cell line's performance or productivity. "Taking a close look at the materials used during development can make scaling up much easier."

Specifying cell culture media for quality and consistency is just as important, if not more so, than careful selection of equipment. Reichl suggests conducting the usual vetting of ingredient and media suppliers, but simultaneously keeping the number of qualified vendors as low as possible. This helps ensure consistency from the earliest development stages through scaleup. "Look for suppliers who can cover large areas of your workflow or, better yet, who can supply an entire platform or process."





▲ *Additive for CHO Fed-Batch Processes | FunctionMAX™ TiterEnhancer | Life Technologies – Bioproduction Division | www.lifetechnologies.com/FunctionMAX*

INSIGHTS ON A CELL CULTURE LAB

SIMPLE CHANGES IN LAB POLICY COULD REDUCE CONTAMINATION SIGNIFICANTLY

Contamination, the bane of cell culture work, occurs at every level, from high school labs using Petri dishes to large-scale manufacturing plants. Given the ubiquity of microorganisms, saying that contamination is inevitable is not an understatement.

Humans carry approximately 10,000 microorganisms on every square centimeter of their bodies, and continuously slough off dead skin. Bacteria, mold, and fungi are present in air as well. Every one of these organisms is eager to settle into cultures exposed to the lab environment, even for a few seconds. Hurriedly moving the culture back to a secure location provides no guarantees; as Mary Kay Bates, global cell culture specialist at Thermo Fisher Scientific (Milford, MA) notes, "There's no magic place inside the BSC where nothing bad will happen. Each cell sample, each culture, is an investment not only in media, reagents, and plasticware, but in time. Contamination can be catastrophic because it's impossible to remediate."

Irvine Scientific's Tom Fletcher estimates that contamination occurs between one in one hundred and one in one thousand times a culture is manipulated. The most common contamination is fungus–"fuzzy balls floating in a culture or bottle of media."

"Lab workers lack a good understanding of what's involved in contamination and how to fight it," notes Bates. One of Thermo Fisher's objectives is to integrate its cell culture equipment–CO_2 incubators, biosafety cabinets, culture ware, serum, media, and lab equipment–to optimize cell growth and prevent contamination.

▲ *Ultra-Low-Temperature Freezer | MDF-U76V SANYO (now Panasonic) www.sanyobiomedical.com*

> "There's no magic place inside the BSC where nothing bad will happen."

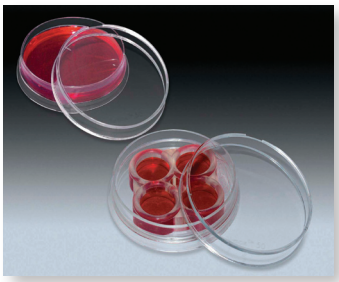
Openings to the outside world, such as doors and windows, are the bane of aseptic cell culture. "It makes no sense to have the incubator on one side of the room, carry cells to the other side of the room past a door to view them under a microscope, and back again to a biosafety cabinet," notes David Phillips, a BSC specialist at Thermo Fisher. "The equipment should be close together, away from the door."

Complacency is enemy number one for aseptic technique. "People get comfortable as they go through a project," Bates observes. "Things are going well, cells are cranking along, and you begin to relax, to skip steps. You assume another worker, with impeccable technique, has already wiped down a cabinet. And when you begin spreading contamination to colleagues, you have a very big problem."

▲ *CO2 Incubators | Galaxy Series | New Brunswick Scientific | www.nbsc.com*

Good cell handling practices involve storing cells in a freezer or under liquid nitrogen, using appropriate protective garb, and maintaining aseptic technique within the BSC. Shoes and air handling ducts are a common source of fungi, especially during warm, humid seasons. Contamination protection works both ways, says Fletcher. "It prevents losing a cell culture to microorganisms, but also protects operators from anything harmful."

Mycoplasma—very small bacteria lacking a bacterial wall, making them resistant to antibiotics—are a common and particularly dreaded form of contamination. Unlike more common microbes, mycoplasma do not cause changes that are immediately noticeable, such as drops in pH or cloudiness. Antibiotics are ineffective against mycoplasma, because the organisms lack a cell wall. The only way to guarantee that these organisms are absent is



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to test for them. Mycoplasma testing consists of a rapid PCR assay and/or confirmatory culturing, which takes a couple of weeks. Prevention methods include wearing a mouth covering to prevent human-to-culture transmission, and using a 0.1-micron filter to remove the organisms from media.

"When you begin spreading contamination to colleagues, you have a very big problem."

Simple changes in lab policy could reduce contamination significantly. "I'm amazed when I walk into a lab, and they let me touch the incubator, hood, and other equipment without washing my hands and donning gloves," says David Craig. Universities are particularly prone to lax contamination policy. "There should be a big 'do not enter' sign available when workers are working with a critical cell line."

Cleaning is another area that many labs overlook, particularly in shared facilities like cold rooms. Craig urges managers not to assign cleaning chores to the most junior workers, because they are unfamiliar with the lab's workflows and likely have very little personal investment in contamination control. "Have the junior workers clean out the microwave oven in the break room instead," Craig quips.

Industrial pharmaceutical and biotechnology laboratories avoid antibiotics as a strategy for contamination due to issues related to antibiotic allergies in patients. Academic laboratories still routinely employ antibiotics, but even basic researchers might rethink this strategy, says Fletcher. "Antibiotics lead to a false sense of security about bacterial contamination, which leads to sloppy technique." Instead of using aseptic technique to transfer cultures and media, labs may simply pour them from one vessel into another. And as noted, antibiotics do not eliminate mycoplasma, one of the most pernicious forms of contamination.

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Q&A WITH SELECT CELL CULTURE EXPERTS

OUR EXPERTS:

Simin Zaidi,
Director of Operations,
Stamford Bioprocess Technologies
Santa Ana, California

Claudia Zylberberg, Ph.D.,
CEO/CSO
Akron Biotech
Boca Raton, Florida

Aparna Oruganty,
Ph.D. Candidate in Biomedical Sciences
University of Massachusetts Medical School
Worcester, Massachusetts

Q: What type(s) of cell culture lab do you perform, for what industry?

A: **Simin Zaidi:** Production of proof-of-concept proteins from transient and stable cell lines to support process development, characterization and validation for biopharmaceuticals and biologics. Our lab is experienced in GMP production using mammalian cells in batch, fed batch, and perfusion cultures, as well as GMP microbial fermentation. Stamford Bioprocess Technologies is a small, customer-focused company. Our cell culture facilities are part of a 5600-square-foot process development (non-GMP), production, and GMP facility.

Claudia Zylberberg: We have two scales of work, research grade and cGMP grade in cleanrooms. For research grade we perform several bioassays and stem cell culture for our raw material qualification. We also conduct custom bioassay development. At large scale we manufacture other products, such as cellular fibronectin from human fibroblast, in one facility dedicated to human products. We also perform bioassays that require cell culture.

Aparna Oruganty: I work in an academic lab at a university with about ten other people. My work involves human and mouse cell lines as well as primary mouse neuronal cultures.

Q: What were your biggest challenges in setting up and/or managing a cell culture laboratory?

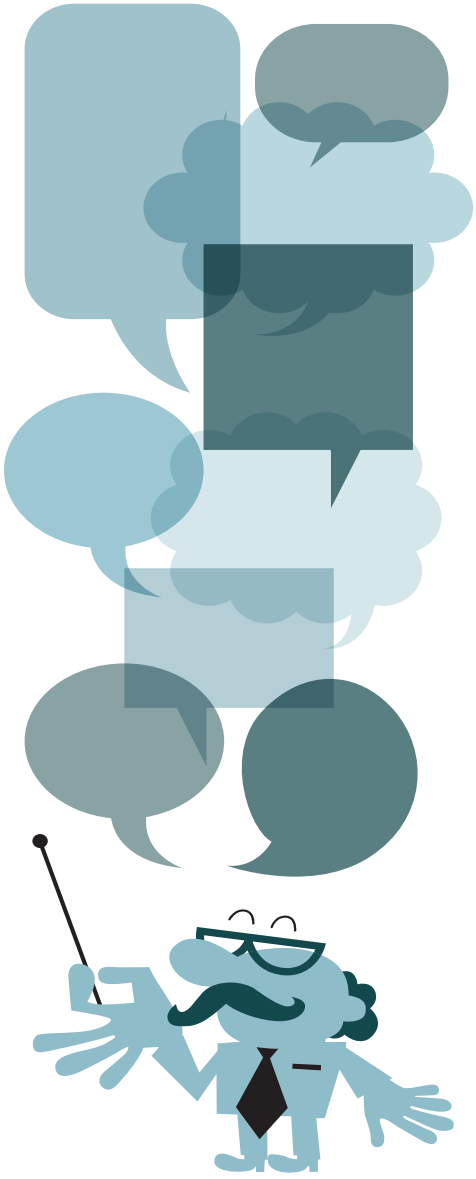
A: **Simin Zaidi:** As more vendors entered the cell culture space and existing vendors redefined their offerings, we found that vendors we had used historically were discontinuing certain equipment on which we relied. So we were forced to identify new trustworthy suppliers in a short timeframe.

Claudia Zylberberg: Maintaining records of all cultures and logging production electronically would be a top priority. I am not aware of anything other than Excel. Having electronic tools to track cell culture stage, media changes, schedule activities like testing and splitting, will help cell culture technicians maintain cleaning schedules and records together with cell culture activities—similar to a compliance software package that keeps track of the different activities carried out with different cell lines.

Aparna Oruganty: Our biggest challenge is maintaining our various cultures without contamination.

Q: What specific measures do you take to avoid/mitigate contamination?

A: **Simin Zaidi:** We insist that all mammalian cell lines be mycoplasma negative and should have documentation to support their status. During lab setup, the process development lab biosafety cabinets were professionally tested and we ran test cultures to ensure that they were operating as expected. We use disposable equipment as much as possible. Not just for small-scale cell culture but for bench-scale and larger controlled bioreactors. When clients are planning proof-of-concept studies, it is essential that their material never be contaminated by endotoxin, other proteins, or anything else that may lead them to the wrong conclusion about their product(s). There are many methods to remove endotoxin from a product; however, these are methods we have no plans to employ in our mammalian facility, as we minimize endotoxin contamination of the product from the outset. The way we see it is that endotoxin is an indicator of the level of contaminating microbial control in one's process. There are a myriad of other toxins that can affect animal studies that are not measured, so the best practice is to avoid their introduction in the first place. Major decisions are being made at this stage, so we ensure integrity of our client's products by utilizing single-use product contact materials and employ clean practices often comparable to the controls in GMP.



Claudia Zylberberg: We use our own line of cleaning and incubator products, the Cleansolutions spray, Cleansolutions Waterbath, and Cleansolutions Incubator CO_2, which come with cleaning validation support. We have not encountered contamination in the last six years, and many customers are committed to these products.

Aparna Oruganty: We follow a number of protocols to avoid contamination, above all being good laboratory practices. We employ separate incubators for primary cell culture and cell lines and an antibacterial liquid for the incubator water and water bath. Our lab extensively uses gloves and disposable pipettes, and we never pour media directly from the media bottle. Instead we use a pipette to aliquot media and other reagents. Finally, we use antibiotics in our cell culture media.

Q: What can vendors do better to improve your lab's workflow?

A: **Simin Zaidi:** We would like to see more disposable products for microbial fermentation at larger scales. We are very happy with the direction more vendors are moving in regarding single-use formats. We hope to see a decrease in costs of those materials as they become more widely used. It would be great to see a Nova Bioprofile (cell culture metabolite analyzer) type analyzer that can use smaller sample volumes so that it can be used for small-volume high-throughput culture.

Claudia Zylberberg: Generate electronic records and tablet software for documentation, traceability, and eliminating paper in cleanrooms that can be a source of contamination.

Aparna Oruganty: Media bottles could be designed more ergonomically, so they can be opened using just one hand. This will be particularly useful in speeding up the process of culturing cells. Another great product would be a sentinel cell line that would capture contamination earlier than most cell lines, thus helping in preventing contamination of important cells.

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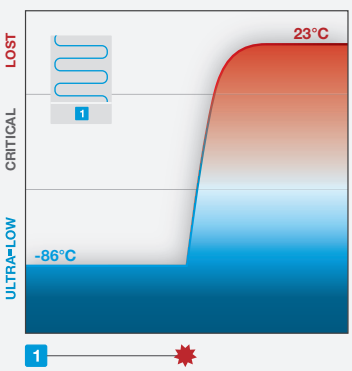

Freezer relies on a single system, causing a complete shut-down when failure occurs

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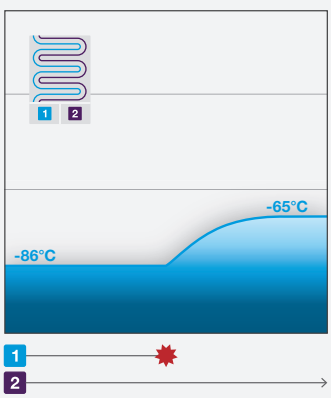
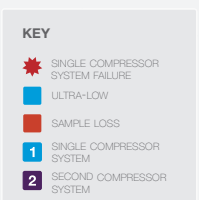


Second independent system kicks in to maintain ultra-low temperatures in the event of a failure



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