Cell Banking in the Spotlight

Advising Biologics Developers About Cell Bank Preparation and Characterization

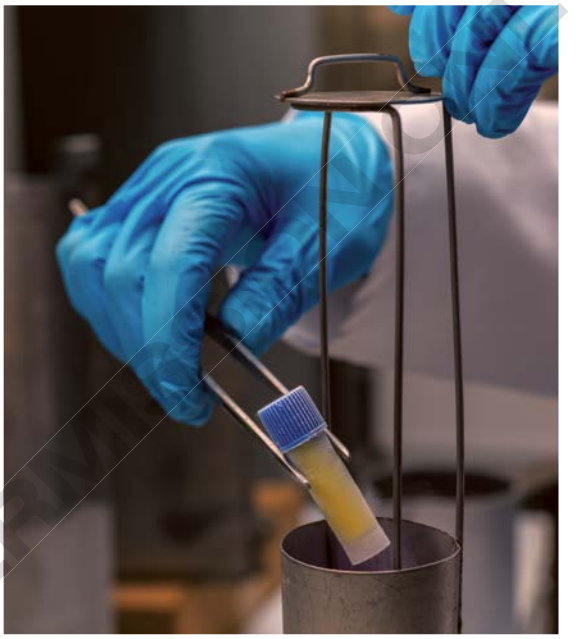
Brian Gazaille, with Francisco Castillo and Raymond W. Nims

Living cells are at the heart of biotechnology, and cell lines for production and testing of biopharmaceuticals are highly valuable assets. The process of banking cells generally moves from development of a research cell bank (RCB) based on a clone of interest to establishment of a master cell bank (MCB), from which working cell banks (WCBs) can be produced. Especially for biotechnology startups, preparation of an MCB can involve a significant jump from work performed in standard laboratory conditions to good manufacturing practice (GMP)-compliant operations. MCBs also must undergo rigorous characterization testing to ensure the purity, safety, functionality, and genetic stability of cells grown from those banks. For such reasons, biopharmaceutical developers usually delegate MCB preparation to contract development and manufacturing organizations (CDMOs).

What processes go into ushering cell banks from their earliest forms to those that enable GMP production of biologics? Under what conditions will contract partners select cell lines for bank preparation and testing? In what conditions should cell banks be prepared, stored, and ultimately handled? And what kinds of testing do end-of-production (EoP) cell banks undergo? Such questions have engrossed the BPI editorial team since a "Hot Topics" discussion about cell bank stability testing from CASSS's 2020 WCBP Symposium on the Interface of Regulatory and Analytical Sciences for Biotechnology Health Products. That event confirmed for us the importance of exploring cell banking in its own right rather than letting the topic serve as an occasional footnote in our upstream archives. In this featured report, we share some cell-banking best practices that we have gathered from biopharmaceutical developers, CDMOs, academic researchers, and industry consultancies.

The Consultant Perspective

I corresponded with Francisco Castillo this past spring to explore strategies for ushering RCBs through MCB preparation and characterization. Castillo is a



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managing director in the BioProcess Technology Group (BPTG) at BDO USA. He has 30+ years of experience in biopharmaceutical development and manufacturing, including work as a scientific director and head of fermentation and cell culture development at Berlex Biosciences (Schering AG) and positions with Xoma and the Venezuelan Institute for Scientific Research. He holds a PhD in microbiology from Rutgers University in New Brunswick, NJ.

I also spoke with Raymond W. Nims, a consultant at RMC Pharmaceutical Solutions. He has worked in the biomedical sciences since 1974, including directorships with Amgen's corporate quality control (QC) group and BioReliance's laboratories for biologics cell-line characterization, raw-materials testing, and product-release testing. A member of the International Cell Line Authentication Committee (ICLAC), Nims also has served on advisory boards for *United States Pharmacopeia* chapters 1237, 1050, and 1050.1. He holds a PhD in chemistry from American University in Washington, DC.

Castillo and Nims call attention to aspects of cell-bank preparation with which biologics developers sometimes struggle, especially with documentation of raw materials. The contributors also identify resources from which developers might benefit as they seek out contract partners for MCB preparation and characterization.

Basics of Cell Bank Characterization

When do biologics companies generally have MCBs and WCBs prepared and characterized, and what kinds of assays are required for cell banks?

Nims: RCBs are acceptable for preclinical work, but first-in-human (FiH) studies often serve as a trigger point for developing MCBs that comply with GMP guidelines. WCBs often are produced after a drug candidate has established proof of concept and initiated other activities within phase 1 clinical trials.

Castillo: Because GMP-compliant MCBs are part of the critical path to manufacturing clinical-grade drug substance and initiating clinical studies, I encourage clients to establish MCBs soon after clone selection. Development work can proceed in parallel while an MCB is prepared and characterized. Because WCBs are not required for initial or even subsequent GMP manufacturing, their preparation and testing can be postponed.

Nims: MCBs require thorough analysis. Some of that testing establishes that a cell bank is sterile and free from viral and microbial (e.g., mycoplasma, bacterial, and fungal) contamination. Other assays ensure that banked cells are genetically stable and have the wherewithal to generate a particular product. Compared with MCBs, WCBs receive abbreviated testing, although ICH Q5A(R1) requires that each WCB undergo one cycle of analysis using cells at the limit of in vitro cell age (LIVCA, also called *EoP cells* and *cells at the culture limit*). Cells are cultured through an entire production process, then harvested and evaluated for latent, stress-induced viruses. EoP testing is as rigorous as MCB characterization.

Quality guidelines from the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) drive cell-bank testing, specifically ICH Q5A(R1), Q5B, and Q5D (**1–3**). In the United States, analysts also can consult an aligned US Food and Drug Administration (FDA) points-to-consider document from 1993 (**4**). Those sources provide a detailed account of required testing. Because different organisms harbor different viruses of concern, regulatory guidance specifies what assays are required for a given cell-line species.

Castillo: Logistically, biologics developers can perform some requisite assays in house, including assessments of growth and production kinetics, titers, and yields. But characterization assays are handled primarily by third parties.

When Complications Arise

What cell-bank specifications are most difficult to meet?

Castillo: In my experience, companies struggle most with generating convincing data to establish banked cells' monoclonality. That concern applies specially to aging cell banks, for which monoclonality probability can be low. However, strategies exist for dealing with such concerns. One option is to reclone a cell bank, which is a time-consuming and costly endeavor. Other strategies include next-generation sequencing (NGS) to evaluate monoclonality and show convincing production robustness and reproducibility.

Nims: I find that large biopharmaceutical companies have little trouble getting their cell banks to meet safety specifications, especially when an RCB has been characterized well and when a CDMO has been commissioned to prepare the GMP bank. Genetic testing tends to be straightforward. Biologics companies spend a lot of time and money improving their cell lines' genetic characteristics and expression mechanisms. But cell-bank testing incurs large expenses. Complete safety and characterization testing costs can approach US$200,000.

Biologics companies respond differently to those cost concerns. Some of my clients have asked whether it is prudent to start with the assay that is likeliest to fail, believing that they could coast through the rest of testing — and maybe save some money — once that hurdle had been cleared. I've responded that they certainly could try that strategy, but it would take much longer to characterize their cells.

Are RCBs from universities and other non-GMP sources adequate for MCB development?

Castillo: Many RCBs come from such sources. If materials and processes for those RCBs are documented properly, including information about sources, storage, handling, and testing of plasmids and host cell lines, then such cell banks are suitable for preparation of an MCB.

Nims: MCBs are produced under GMP conditions, and contract laboratories and manufacturing organizations tend to be savvy about documenting raw materials and processes. Thus, few problems emerge when such companies are involved from start to finish of the cell-banking process. But regulatory agencies often ask biologics developers to find

documentation from the creation of RCBs to consider all opportunities for contamination by adventitious agents. That requirement can cause problems for biologics developers that did not have control over the materials used to generate their RCBs. Academic and research institutions typically don't generate their cell banks according to GMPs, and that can cloud the traceability of RCB raw materials.

I often encounter traceability concerns. I'll notice that a company used fetal bovine serum (FBS), trypsin, or some other animal-origin raw materials back in 2004, and I'll need to ask whether the company can provide certificates of analysis (CoAs) for those materials. Sometimes the company will have used an RCB produced with now-untraceable components. Similar documentation problems crop up with biotechnology startups that follow academic leads and seek out larger partners that can take their candidate therapies through clinical trials. When clients ask me how they can ease cell-bank characterization, I advise them to document all media components, especially animal-derived raw materials, as if their RCBs were produced within GMPs.

It always comes down to documentation. If a CoA can be produced for a particular component, then an auditor can assess that material for viral and microbial risks. A CoA can establish, for instance, that a lot of FBS used to bank cells was gamma-irradiated and thus poses minimal risk for viruses. Similarly, a certificate of suitability or certificate of origin can be used to assess the risk of transmissible spongiform encephalopathies (TSEs). But if no documentation can be produced, then assessing a cell bank for risk becomes guesswork — and biologics companies don't want to be in that situation.

Are companies making progress in adapting cell banks to chemically defined media? And if so, what bearing does that have on documentation concerns?

Nims: Companies are striving toward that goal, and they are trying to adapt cells to media free from animal-derived components as early as possible. Currently, I am working with a company that used an RCB developed by academics. The researchers had adapted it to serum-free media, and that was helpful. I noticed, though, that horse serum was applied during the adaptation period. Before that, the cell line had been grown in FBS, so I still needed to ask for all those related CoAs.

But having such information is helpful. At the MCB stage, raw-materials documentation helps determine what viral and microbial testing needs to be performed for a cell bank to meet specifications. If your raw materials are traceable, then you are more likely to gain regulatory acceptance. Problems come with being uninformed about your cell line's history.

Storage and Handling

What stability tests are performed on cell banks?

Nims: After generating initial rounds of WCBs, the companies that I have worked with generally have set aside their master banks for a couple years before revisiting them for production or testing purposes. Many companies' stability protocols stipulate evaluation of an MCB once every five years to ensure that those cells are alive and can generate requisite material for early upstream processing. Usually, that testing is simple, involving amplification in shake flasks, then monitoring a couple of relevant culture steps. WCBs don't require additional evaluation if they are thawed yearly or are used multiple times each year to generate drug substance. A successful production process, complete with requisite testing, establishes the stability of the WCB that supported it.

What factors relating to storage, handling, and transfer can compromise cell bank quality, and how can such problems be prevented?

Castillo: Cell banks must be prepared using dedicated laboratory space and equipment. Performing such activities in areas with concurrent projects can put cell bank purity/sterility at risk.

Microbial cell banks must be stored at temperatures below –70 °C, although it is recommendable to store them in a vapor-phase liquid-nitrogen (LN_2) freezer at temperatures below –130 °C. The lower temperature storage is a requirement for mammalian cell banks.

Shipping typically is performed using dry ice or LN_2 containers. Improper storage and shipping temperatures can affect cell viability and recoverability. Thus, biologics developers need to ensure appropriate maintenance of a cold chain. That includes identification of shipping containers that will maintain temperature specifications and preserve the integrity of the primary bank-storage containers. Temperature monitoring devices help to track environmental excursions.

Nims: We must remember that a cell bank represents a lot of time and money. The last thing that you want is to ship a cell bank and have it experience a temperature problem or other environmental excursion. Companies must mitigate shipment risks. Typically, that involves splitting a cell bank between two shipments that go to different locations. Large companies usually store cell banks at two sites; small companies often split their materials between a couple of repository organizations.

What concerns arise with aging cell banks, and how can companies handle such concerns?

Castillo: Legacy cell banks are likely to have fewer available vials than newly generated banks. Also, improper storage of old cell banks can diminish cell viability and recoverability.

Replacing an aging cell bank requires creation of a new bank, followed by the same kind of testing that corresponding banks undergo, including assessments of bank homogeneity, growth, productivity, and viral/microbial safety. Then, the new bank must undergo a comparability study to confirm that its product attributes match those from its predecessor.

Nims: Traceability is another challenge. The older the cell bank, the harder it is to reconstruct its production. Scientists move on to different organizations; papers get stuffed into cabinets. It can be difficult to report an MCB's history to the extent that a regulatory agency requires.

Regulatory agencies may work with companies that cannot reconstruct their cell banks' histories completely. I recently worked with a biologics developer that could not account for all the animal-derived components used during the evolution of its cell line. The FDA allowed that company to perform next-generation sequencing (NGS) in addition to the normally required viral testing of the MCB. The agency's rationale is that the broad detectability of the NGS method could mitigate risks conferred by gaps in the history of a cell line's evolution. Although regulators may offer such a path forward, biologics companies should not assume that extensive traceability gaps always will be resolvable in this way. Strong documentation of steps taken throughout cell line development is the best approach.

Selecting the Right CDMO

What advice do you have for biologics developers about working with contract partners to establish and characterize cell banks?

Nims: CDMOs almost always perform the viral and microbial testing required for cell-bank assessment because they can handle positive controls that biologics companies cannot introduce into their production facilities. Usually, a developer provides a CDMO with a few RCB vials, growth instructions, and if applicable, specialized media. Then CDMO scientists expand those cells to generate enough material for a selected panel of characterization tests. Contract laboratories know what they are doing, have the requisite testing methods "off the shelf," and operate under appropriate quality systems for GMP cell bank characterization. Few biologics developers have the range of assay capabilities required to perform MCB testing in house.

Although expensive, outsourcing cell-banking activities serves as a critical risk-mitigation strategy. A company must identify quality and contamination risks at the MCB stage. You don't want to start generating drug products and then discover that your cell line has issues. An MCB will stay with you for decades, so you must assess it thoroughly.

Biologics developers will find that CDMOs differ in their responsiveness to questions, flexibility for special testing needs, and time to reporting of results. But many CDMOs are fully capable of generating and characterizing cell banks that will pass muster with regulatory agencies.

Castillo: Cell-bank preparation and testing are straightforward activities for contract laboratories. They use platform processes for bank preparation, have implemented and validated a wide range of assays, and have abundant experience with preparation and testing of MCBs and WCBs. When selecting a contract partner for the first time, biologics developers should consider a laboratory's experience and reputation. It is prudent to request proposals from two or three organizations and then compare costs and timelines for cell-bank generation, testing, storage, and shipment.

Biologics developers also should inquire about cell-bank sizes to minimize the need for frequent rebanking. For instance, a sponsor might request >200 vials for an MCB and >300 vials per WCB.

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