Contractor Perspectives

Best Practices for Transfer, Handling, Testing, and Storage

S. Anne Montgomery, with Scott M. Wheelwright and H. Fai Poon

For comments about how contract development and manufacturing organizations (CDMOs) manage their cell-banking quality assurance (QA) practices. I contacted long-time member of BPI's Editorial Advisory Board Scott M. Wheelwright, PhD, for his perspectives. Wheelwright brings many years of experience to this discussion, with insights into the evolution of technologies and practices extending back to the early launch of the biopharmaceutical industry. Currently, he provides consulting support for companies with manufacturing and sourcing in China and other Asian countries. He also serves as chief operating officer for BioInno Bioscience Co., Ltd., based in Suzhou, China. BioInno is a CDMO for development and manufacture of antibodies, cell therapies, and gene therapies.

I also spoke with H. Fai Poon, chief operating officer of QuaCell Biotechnology, Co., Ltd., based in Zhongshan, Guangdong, China. His previous positions include chief scientific officer and director of cell culture at Zhejiang Hisun Pharmaceutical Co., Ltd. (Hangzhou, Zhejiang, China) and before that, senior scientist (Asia–Pacific technical lead) at Sigma Aldrich.

Together, their comments offer a glimpse into the ongoing harmonization efforts of global regulatory practices and the alignment of best practices for maintaining the quality and integrity of master and working cell banks (MCBs, WCBs).

Overview: Transfer and Preparation

What are your general goals and practices once you receive a client's cell bank or manufacture a cell bank for a client?

Wheelwright: As a CDMO, we are concerned about bringing clients' cell banks into our site. In particular we are concerned with mycoplasma and other contamination. Therefore, before accepting a cell bank for production, we require that our clients produce documents describing previous testing performed on the cell bank. We quarantine client

cell banks until they have met our QA department's requirements for review of testing and release.

We manufacture cell banks for clients, using either a primary cell bank (PCB) that we have created or one from the client. We recommend that the first good manufacturing practice (GMP) cell bank become the MCB, and enough vials should be generated so that this MCB never needs to be prepared again. Usually 200 vials is sufficient. We recommend that the WCB be made after an investigational new drug (IND) application is filed and clinical testing begins. Using two or three vials from the MCB for production of phase 1 clinical testing materials saves time and money at that urgent stage of production.

A WCB should be generated from an MCB in time for its use in production of phase 2 clinical testing material. Each time a vial of MCB or WCB is expanded, related data should be collected into a spreadsheet that documents the stability of the cell bank. A protocol for cell-bank stability should be prepared with a test-results table for data collection. That report should be updated each time a vial is used, or at least quarterly. Using such data to monitor cell bank stability is much less expensive than conducting separate cell bank stability testing.

GMP cell banks should be prepared using approved batch records. If a development

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department is preparing a PCB, we recommend use of a standard template that requires recording of all materials used in that process. If that preparation is recorded in laboratory notebooks, then a report should be generated describing the activities that took place; where, when, and by whom they were performed; and all materials used, identified by manufacturer, part number, and lot number. Notebook numbers and page numbers should be referenced in the report, and it should be signed, dated, and archived by QA. Inspectors will want to trace the preparation of cell banks back to original notebooks. Therefore, for each commercial product, a drug developer (or CDMO) must be able to demonstrate traceability.

What factors can compromise WCB quality during handling and transfer, and how can such problems be prevented?

Poon: The critical factor is temperature. Factors that influence temperature include container strength and pressure resistance (compressive capacity), temperature controls, refrigerants (whether liquid nitrogen or dry ice), and the heat-transfer capacity of the container material. Recommendations include choosing a contract manufacturer to perform an antidrop test, implementing a hollow vacuum as an interlayer to reduce heat transfer, making the outlet as small as possible, and using a material with poor thermal conductivity for the cover.

Refrigerant properties are important also. If liquid nitrogen is used as a refrigerant, its purity must be controlled by preventing other harmful gases from entering the freezing tube. If possible, try to use a liquid-nitrogen phase tank. If the refrigerant is dry ice, you should ensure that the dry ice bears no impurities.

The strength and tightness of cryopreservation tubes and the cleanliness of the containers should be considered. The inner cavity of a container must be easy to clean and drain. For the freeze-and-drop test, use cryogenic storage tubes and seals made of low-temperature–resistant materials.

During transportation, you need to verify the dynamic volatilization time and guide the replenishment time of refrigerant as needed. Be sure to investigate the qualifications and relevant experience of different transportation companies, fix container racks in place, and use a soft cushion to guard against the effects of violent transportation

What are the standard and preferred storage and shipping conditions for cell banks? Who bears responsibility for cell-bank quality?

Wheelwright: Control of access to cell banks is critical. Each cell-bank freezer should require two keys to open, and those keys should be held by different departments, such as Operations and QA. The room in which cell banks are stored should be locked, with restricted access. Cell-bank storage rooms should be outside of cleanroom areas to enable access without gowning.

Freezer temperatures should be monitored continuously by a calibrated system. Records should be stored in the QA archive for as long as a product is on the market. QA can expect regulatory inspectors to review temperature records for the entire time of storage, so those records must be available during inspections. If temperature monitors run on electricity, then backup power or an uninterruptible power supply (UPS) should be installed. If banks are moved between freezers, those transfers must be documented as a GMP activity. Freezers should have inventory control procedures for which the number of vials of each bank is documented and made accessible. Any gaps in the records must be documented and explainable. Excursions in temperature should be evaluated using a mean kinetic temperature analysis and documented as a GMP deviation.

All cell-bank freezers must be qualified. Workers who monitor and maintain freezers (including liquid nitrogen levels in liquid nitrogen freezers) must be trained, and training records must be made available for inspection. Freezers should have alarms with automatic callout procedures to notify a responsible person in the event of a temperature excursion.

Finally, we recommend that all cell banks be stored at two different sites, preferably in two different cities, but at least in two different buildings.

Poon: For storage and shipping conditions, the *Chinese Pharmacopoeia* recommends a temperature lower than –130 °C for liquid nitrogen. *The United States Pharmacopeia* (USP) specifies a temperature not warmer than –130 °C for nonclinical specimens or not warmer than –150 °C for clinical materials

(to give an adequate margin of error) in the vapor phase of a liquid-nitrogen freezer. During transport, cryopreserved cells typically are shipped in liquid-nitrogen–vapor shippers with temperature-monitoring systems to ensure that a unit does not become warmer than –130 °C for cell lines and –150 °C for clinical material while on route. For most cryopreserved cells, shipping in dry ice for a short duration may be adequate, but that shipping process should be validated, shown to have no adverse impact on the cells, and equipped with temperature monitors. However, some cells may require shipping in the liquid-nitrogen vapor phase (e.g., Dewar) containers.

So an advisable procedure is to ship cryopreserved cells in liquid-nitrogen–vapor shippers with temperature-monitoring systems. One inadequate procedure is to ship cryopreserved cells in dry ice without temperature-monitoring systems and without validating the shipping process.

Recommended Testing Practices

What sort of viral testing is performed on MCBs/WCBs? When and by whom?

Poon: Under normal circumstances, cells used in clinical trials need to be sent to a qualified testing agency for virus safety testing. In China, such agencies include the China National Institute of Food and Drug Control and the Wuhan Jiachuang Biotechnology Co., Ltd.

The 2020 *Chinese Pharmacopoeia* specifies the importance of testing for endogenous and exogenous viral factors. Such testing involves

- inoculation and culture of different cells in vitro
- inoculation and culture in vivo
- testing for retrovirus
- testing for species-specific viruses.

Regarding species-specific viruses, antibody tests are used with mouse-, rat-, and hamster-derived cell lines (see the "Viruses of Concern" box) to detect hemorrhagic fever virus, lymphocytic choroid meningitis virus, type III reovirus, Sendai virus, and foot-and-mouth disease viruses. Mouse-derived viruses include mouse adenovirus, mouse pneumonia virus, and retrovirus. If bovine serum is used in cell matrix establishment or passage history, the cell line must be tested for relevant viruses (e.g., transmissible spongiform encephalopathies, TSEs). If pig/porcine pancreatin is used in the cell matrix establishment or passage history, material should be tested for porcine-derived viruses. Other specific viruses include murine parvovirus.

Viruses of Concern for Rodent Cell Lines

Mouse Viruses

Ectromelia virus
Hantaan virus
Murine pneumotropic virus (K virus)
Lactic dehydrogenase virus (LDM)
Lymphocytic choriomeningitis virus (LCM)
Minute virus of mice
Mouse adenovirus (MAV)
Mouse cytomegalovirus (MCMV)
Mouse encephalomyelitis virus (Theiler's encephalitis virus, GDVII)
Mouse rotavirus (EDIM)
Pneumonia virus of mice (PVM)
Polyoma virus
Reovirus type 3 (Reo3)
Sendai virus
Thymic virus

Hamster Viruses

Lymphocytic choriomeningitis virus (LCM)
Pneumonia virus of mice (PVM)
Reovirus type 3 (Reo3)
Sendai virus
Simian virus 5

Rat Viruses

Hantaan virus
Kilham rat virus (KRV)
Mouse encephalomyelitis virus (Theiler's, GDVII)
Pneumonia virus of mice (PVM)
Reovirus 5ype 3 (Reo3)
Sendai virus
Sialoacryoadenitis virus

Source: ICH Q5A(R1): *Quality of Biotechnological Products — Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin.* European Medicines Agency: Amsterdam, The Netherlands, 1997; https://database.ich.org/sites/default/

The USP recommends extensive screening of the MCB and WCB for both endogenous and nonendogenous viral contamination. Retroviruses and other endogenous viruses can be detected using in vitro assays, in vivo assays, and antibody-production tests. Complete testing is performed to detect bovine virus contamination, porcine viral contaminants, and murine minute virus.

Do companies test MCBs for comparability and stability, or do they rely on concurrent validation of products derived from those banks?

Poon: The stability of a cell bank generally refers to its intrabatch stability. Related stability data can be obtained only indirectly from an annual quality

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review of the product. We generally set a period for random sampling. The cells undergo a simple resuscitation quality test, and randomly sampled cells are tested in a longer cycle for a more comprehensive test to monitor stability. Stability research is ongoing.

How many lots should be tested for characterization to ensure that a cell bank is the same as its progenitor and is well controlled?

Poon: There is no requirement for a specific number of batches. The more batches are run, the easier it is to be accepted by the regulatory authorities, but as mentioned above, the stability study is an ongoing process.

What concerns arise with cell bank container–closure systems, and what kinds of integrity testing do cell banks require? When and how often does such testing occur?

Poon: Containers are subject to material aging, seal aging, detection-probe inaccuracy, and data transmission failure. Under conditions of constant temperature, humidity, and pressure, a company can evaluate the thermal insulation capacity of container equipment by monitoring the frequency of replacement or filling of liquid nitrogen. An integrity test requires a professional pressure-hold test that ordinary companies cannot conduct. Generally, pressure vessels require monitoring of safety valves and rupture discs and of liquid-level gauges.

The frequency of monitoring is determined according to situations of use, usually once every two years. But the most important safeguards are regular inspections and backups.

Legacy Cell Banks

What risks do older MCBs introduce? What kinds of data will regulators need to accept a WCB derived from an older MCB? How do you advise companies facing that situation?

Wheelwright: A history of materials used may not be available for legacy cell banks. In such cases, most suppliers know the lot numbers of materials (such as cell-culture media) that were available in the local area at the time a bank was created, so copies of certificates of analysis for each lot that *could* have been used can be attached to the cell-bank preparation report. A risk assessment (RA) should be conducted on legacy banks and included in the report. That RA can explore any issues related to missing information and their potential impact on patient safety. Some testing of the bank can be done later to confirm freedom from viruses and other contaminants. Proof of clonality is expected, and if that was not performed as part of the original preparation, it should be discussed in the report.

In the past it was common to track and document each piece of DNA that went into making up a plasmid used for transformation, but that is no longer needed. The price of sequencing has dropped so far that it is now preferable to sequence an entire transfection plasmid or cut out the integration site, including some portions of DNA on each side, and sequence those sections to confirm the DNA codes for a desired amino-acid sequence.

The origin of the host cells used for transformation must be documented back to an original library (such as ATCC or ECACC), or the derivation of the primary cells and transformation must be traced back to a cell line. Of particular concern is whether animal-source materials (such as fetal bovine serum) were ever used with those cells. If such materials could have been used, then that should be documented. Again, the supplier should have a record of materials that were available at the time, the lot numbers, and the source of those materials. That information would be included in a cell bank report.

Poon: Under normal circumstances, if the storage temperature has been stable, it will not have made a great impact on the cells (consider, for example, a sperm bank model). But if the temperature fluctuated abnormally during storage of an old cell bank, that will affect cell stability and activity. Enzyme activity might be reduced as well. But in the case of a stable storage environment, most recovery problems that do occur are related to the freezing process.

Regulatory agencies will require commercial batches of cell banks to be tested by qualified testing agencies in accordance with the relevant laws and regulations of the reporting country. A company will need to explain in detail the passage, preparation method, and scale of seed banks at all

levels and provide a complete verification report that accounts for identification, microbial purity (exogenous factors), cell viability, expression levels, plasmid restriction endonuclease map and purpose, gene sequencing, and so on. At the least, you need to perform a sequencing report of the target gene in a master seed bank to confirm the correctness of the coding sequence of the polypeptide sequence, promoter, and operon-region–related elements. Clarify the storage locations, methods, conditions, and expected service life of seed banks at all levels, and document the stability of the host vector expression system under storage and recovery conditions.

The genetic stability of the host cell/vector system is verified by methods such as passage under pressure and nonpressure in the seed bank. Analyze and determine the maximum allowable cell doubling or passage number in the process of large-scale production. At the end of a production cycle, characteristics of the host-cell/vector system should be monitored, such as cell viability, plasmid (target gene) copy number, exogenous factors, restriction endonuclease digestion map, target gene expression level, and nucleic acid sequencing analysis. Evaluating those characteristics will confirm the genetic stability of cells during production. After that, it is necessary to compare the inspection data from the old MCB with those from the new WCB. Then data from the old WCB manufactured using the old MCB should be compared with data from the new WCB manufactured by the old MCB to make a risk assessment.

What issues arise when replacing cell banks (either for intermittent maintenance or wholesale replacement of a legacy bank)? How are companies handling those challenges?

Poon: When a developer replaces a cell bank, potential safety risks will arise, such as introduction of viruses, foreign genes, microorganisms, and active proteins. A company should simply prevent the need to replace an existing cell bank. I recommend that companies

- appropriately increase the number of MCB and WCB libraries in a single batch according to the amount of use and production capacity of the product
- institute remote storage to reduce the chances of needing to scrap an entire batch due to unpredictable factors
- pay attention to the storage conditions of cell banks, including maintenance of storage equipment, alternative plans, and early warnings, to ensure a stable supply of refrigerants; increasing the frequency of cell bank inspections installing a series of alarm systems can help ensure stability of storage conditions and reduce the risks for MCBs and WCBs
- test the function of the MCB (for example, through random resuscitation of cells once every two to three years, with a full inspection as required)
- prepare a new WCB in advance before an old WCB is used up, thereby reducing the time cost of preparation failure and change approval.

Regulatory agencies will require commercial batches of cell banks to be tested by professional testing agencies in accordance with the RELEVANT LAWS AND REGULATIONS of the reporting country. A company will need to explain in detail the passage, preparation method, and scale of seed banks at all levels.

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