SOP Title: Qualification of Cells and CGMP Cell Banks
SOP Number: 13200
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1. PURPOSE

This procedure lists the test requirements for Mammalian and Bacterial Cells to be utilized in various Current Good Manufacturing Practices (CGMP) projects by the Biopharmaceutical Development Program (BDP). This procedure also lists the minimum requirements for acceptance of incoming cells to the BDP (R&D, PA/QC, or GMP areas).

2. SCOPE

This procedure applies to BDP personnel responsible for receiving and/or testing incoming cells. The procedure does not apply to cells obtained from a patient or healthy donor (e.g., apheresis) that are intended for manufacture or development of a cell therapy product.

3. RESPONSIBILITIES

3.1 BDP Director of Regulatory Compliance (QA/RA)

- Defines procedure.

3.2 BDP Process Development Personnel

- Ensures that cells to be transferred to GMP manufacturing areas for banking have been tested per specifications in the applicable sections of this procedure.

3.3 BDP Production Manager

- Ensures adherence to this procedure for each particular project.

3.4 Project Scientist

- Ensures adherence to this procedure for each particular project.

3.5 Biopharmaceutical Process Analytics/Quality Control (PA/QC)

- Reviews and verifies test results
- Prepares Master Specifications and Certificates of Analysis.

3.6 Biopharmaceutical Quality Assurance (BQA)

- Provides quality oversight.

4. DEFINITIONS

- **Incoming Cells** – Cells that are received from an outside source, other than those intended to be used as Accession Cell Bank.
- **Accession Cell Bank (ACB)** – A pre-GMP cell bank used to establish the Master Cell Bank (MCB). The ACB requires a traceable, documented history that is sufficient to support the preparation of the MCB and eventual CMC section of the IND. It may be prepared by BDP personnel or received as a contract deliverable, provided the contractor provides sufficient documentation to comply with step 5.7 For the purposes of this SOP, a Research Cell Bank is equivalent to an ACB.
- **Master Cell Bank** – An aliquot of a single pool of cells which have been prepared in compliance with CGMP's, from the selected cell clone under defined conditions, dispensed into multiple containers and stored under defined conditions. The MCB is used to derive working cell banks.
- **Working Cell Bank** – The WCB is prepared in compliance with CGMPs from aliquots of a homogeneous suspension of cells obtained from culturing one or more vials of the MCB under defined culture conditions.
- **End of Production Cells** – EOP Cells are cells at the limit of In Vitro cell age or beyond the passage number used for production. For fermentation runs where induction is performed, take a sample immediately prior to induction. If the bacterial EOP bank is vialed within 1 hour of the time the sample is taken, the sample may be stored at 2-8ºC until it is vialed. Otherwise, continue incubating the sample at the temperature and agitation rate used for the last seed flask stage of the fermentation process until the bank is vialed.

5. GENERAL GUIDELINES FOR CELLS AND CELL BANKS COVERED BY THIS SOP

5.1 Cells must be tested per the requirements specified in the applicable section of this SOP for bacterial/yeast or mammalian/insect cells.

5.2 Test requests should be submitted to the BQC laboratory following **SOP 22002 - Request for Quality Control Testing.**

5.3 Outsourced tests shall be performed by BQA-qualified vendors whenever possible. Any testing not performed by BQA-qualified vendors will be reviewed by QA to ensure compliance with internal standards.

5.4 Cells obtained from audited CGMP or GLP-compliant facilities which have either provided a certificate of analysis or traceable, audited CGMP or GLP-compliant test results to the BDP do not need to have testing repeated, provided these tests were performed in a qualified CGMP/GLP laboratory according to current technical “state-of-the-art” standards.

5.5 Incoming cells can be cultured in non-GMP (e.g., research and development- R&D) cell culture areas within the BDP to generate sufficient culture (accession or R&D cell bank) for testing and/or evaluation.

5.6 The Project Scientist shall consult with the Director of Regulatory Compliance (or designee) regarding testing of incoming cell lines which were obtained from a commercial vendor prior to initiation of testing, to determine what, if any, further testing is required.

5.7 A technical report is required for the establishment and release of an accession cell bank that is to be used to establish a MCB. Preferably, the report should be provided by the source of the incoming cells but may also be prepared by the Development lab, at the discretion of QA, if one is not available.

- For stable cell lines, a history of the cloning performed should also be included.

5.8 All cells to be used in a GMP area must be quarantined and released in accordance with **SOP 20302, Receipt and Inspection of Materials**.

6. TESTING AND ACCEPTANCE CRITERIA FOR INCOMING CELLS AND ACCESSION CELL BANKS

6.1 I**ncoming mammalian or insect cells or accession banks:** Five-day incubation bioburden (BDP **SOP 22133** or equivalent), mycoplasma (by PCR), and viability testing are required for all incoming mammalian or insect cells or accession banks. Cells must be free of bacteria and mycoplasma and >90% viability. Additionally, sequencing must be performed on any incoming stable clones, prior to making or being used as an ACB to make sure the clone sequence matches the reference sequence. BDP QC will verify sequencing data from cell development vendors and will send a sample of the cell bank for confirmatory R&D sequencing.

6.2 **Incoming bacterial or yeast cells or accession banks:** Culture purity testing is required for incoming bacterial or yeast cells or accession banks. The cells must be a pure culture. In the event that a culture is impure, the receiving party may return the culture to the requestor and request a pure culture. If this option is not exercised, the receiving party is responsible for obtaining or preparing a pure culture prior to further work with the culture. Additionally, sequencing must be performed on any incoming stable clones, prior to making or being used as an ACB to make sure the clone sequence matches the reference sequence. BDP QC will verify sequencing data from cell development vendors and will send a sample of the cell bank for confirmatory R&D sequencing.

6.3 Product expression level testing may also be required for accession banks, at the discretion of the Project Scientist or QA.

7. TESTING AND ACCEPTANCE CRITERIA OF BACTERIAL AND YEAST ACCESSION, MASTER, WORKING, AND END OF PRODUCTION CELL BANKS

7.1 Recommended testing for the bacterial and yeast cell banks can be found in Attachment 1, Table 1 Bacterial and Yeast Cell Bank Testing Recommendations. These tests are performed in-house or outsourced to BQA qualified vendors.

7.1.1 If the final product is the plasmid DNA or is expressed from plasmid DNA, the entire plasmid must be sequenced. If the final product is a protein expressed by a chromosomally localized exogenous gene, critical regions for analysis include the inserted gene and its flanking regions encompassing critical regulatory elements must be sequenced (e.g., promoter, initiation and translation site, translation termination codons/transcription termination site, antibiotic resistance genes if any).

7.1.2 Restriction mapping of plasmids by agarose gel electrophoresis should include the following:

- Appropriately sized molecular markers.

- Undigested plasmid.
- Unique site digestion and plasmid linearization within the plasmid insert region (if reasonably possible).
- One or two restriction enzyme digestions (as appropriate) of the plasmid insert's 5' and 3' ends to demonstrate the ability to remove the therapeutic insert from the plasmid vector; and/or a 3-5 restriction site digestion(s) of the entire plasmid using multiple enzymes or a multi-site enzyme.
- Multi-site enzymes should be chosen so that no fragments are <100 bp or are within 200 bp of one another.
- Post-restriction fragment numbers and sizes should approximate the expected fragment size based on the plasmid sequence information.

7.2 Testing of an EOP bank is generally not required for an early-phase product. If the EOP bank is required to be tested (by a regulatory agency, IND sponsor or NCI), recommended testing is included in Attachment 1, Table 1. The BDP Project Scientist may request additional specific testing.

7.3 Acceptance Criteria for Bacterial and Yeast Master, Working, and End of Production Cell Banks.

7.3.1 Bacterial and Yeast Cell Banks must exhibit the correct cell morphology and Gram stain designation, as discerned by the purity and Gram stain assays. Minimum cell viability is determined on a project-by-project basis as determined by the BDP Project Scientist.

7.3.2 DNA sequence analysis will be performed on the MCB. If the DNA sequence analysis reveals mutations, insertions, deletions, or other sequence discrepancies, the BDP Project Scientist, Director of Regulatory Compliance, and the Program and Technical Director of the BDP must be notified immediately. The Project Scientist will also notify the NCI Project Officer to determine, with the Director of Regulatory Compliance and the Program and Technical Director of BDP, if the project can proceed. Upon approval, the MCB sequence will become the official BDP reference sequence. If tested, the EOP sequence should be comparable to the MCB and reference DNA sequences.

7.3.3 The restriction map should conform to expected fragment sizes. If tested, the EOP fragment sizes should be comparable to the MCB and the expected fragment sizes.

7.3.4 There must be no contamination with adventitious bacteriophages, toxins, or other adventitious agents, if applicable.

7.3.5 If tested, plasmid copy number and plasmid integrity, determined from the end of production cells, should be consistent with plasmid copy number and plasmid integrity of the master cell bank (and, if applicable, the working cell bank).

8. TESTING AND ACCEPTANCE CRITERIA OF MAMMALIAN/INSECT MASTER, WORKING, AND EOP CELL BANKS

8.1 Required QC Testing of Mammalian/Insect Master Cell Banks

8.1.1 The recommended QC tests for Mammalian/Insect Master Cell Banks can be found in Attachment 2, Table 2, Mammalian/Insect Cell Master Cell Bank Testing. These tests are performed in-house or outsourced to BQA qualified vendors.

8.1.2 Additional tests may be required depending on the cultivation history of the individual cells and whether cross-contamination opportunities by other cell lines existed. For example, primate cell lines that have been in contact with any simian materials should be screened for simian immunodeficiency virus (SIV), simian T lymphotrophic virus (STLV), and simian foamy virus. Cell lines from non-human primates should be additionally tested for the presence of simian retroviruses (SRV). Cell lines exposed to bovine or porcine components (e.g., serum, trypsin) should be tested for bovine or porcine adventitious agents. A requirement to test for Hepatitis A on human cell lines has also been suggested when the donor history is unknown for the cell line. Human Papilloma Virus (HPV) has been suggested for certain human epithelial cell lines. Please consult with the BDP Project Scientist and Regulatory Affairs to decide if any additional testing is required based on the derivation history of a particular cell line.

8.2 Recommended QC Testing of Mammalian/Insect Working Cell Banks

It is recommended that the Working Cell Bank be qualified by the following tests. (The WCB may be created before testing on the MCB is complete.)

- Sterility
- Mycoplasma
- Genetic Speciation Identity[1]
- Viability
- In-Vitro Adventitious Agents
- Bovine Adventitious Agents[2]
- Porcine Adventitious Agents[2]

1 This could be karotyping, isoenzyme analysis, STR/CODIS, or any currently accepted identity test to confirm the species of origin.

2 Bovine/porcine adventitious agent testing should be performed where there are components used that could possibly introduce such agents.

8.3 Testing of an End of Production (EOP) bank is generally not required for an early-phase product. If the EOP bank is required to be tested (by a regulatory agency, IND sponsor or NCI), the recommended testing is listed in Attachment 3 Table 3 Mammalian/Insect Cell End Of Production Cell Testing.

Additional tests may be required depending on the cultivation history of the individual cells and whether cross-contamination opportunities by other cell lines existed. For example, primate cell lines that have been in contact with any simian materials should be screened for simian immunodeficiency virus (SIV), simian T lymphotrophic virus (STLV), and foamy virus. Cell lines from non-human primates should be additional tested for the presence of simian retroviruses (SRV). A requirement to test for Hepatitis A on human cell lines has also been suggested when the donor history is unknown for the cell line. Human Papilloma Virus (HPV) has been suggested for certain human epithelial cell lines. If any viral tests were positive with the Master Cell Bank, it is also advisable to retest them at the EOP stage and perform a co-cultivation assay. Please consult with the Leidos Biomedical Research, Inc. BDP Project Scientist and Regulatory Affairs to decide if any additional testing is required based on the derivation history of the particular cell line.

8.4 Acceptance Criteria for Mammalian/Insect Master, Working, and End of Production Cell Banks

8.4.1 The CGMP cell banks must be sterile and free of mycoplasma contamination.

8.4.2 The identity test must conform to species specific identification.

8.4.3 The cell banks must be free of adventitious agents.

8.4.4 DNA sequence analysis will be performed on the MCB as specified in step 7.3.2.

8.4.5 Endogenous retroviruses and lysogenic virus sequences may be expected in certain cell lines. Viral contamination should be quantified and identified, if possible, to establish the extent of virus clearance achievable through process purification.

8.4.6 Material contaminated with LCM, reovirus, Sendai virus, Hantaan, HTLV-1, or HTLV-2 must not be used by the BDP. Consult with the Director of QA. PA/QC, and NCI Project Manager for use of cells contaminated with any viruses.

9. DOCUMENTATION AND RECORDS

9.1 Approved Master Specifications are prepared for CGMP Cell Banks by Leidos Biomedical Research, Inc., BDP Process Analytics with input from the Project Scientist.

9.2 An approved Certificate of Analysis is prepared by Leidos Biomedical Research, Inc., BDP Process Analytics that lists test results for CGMP Cell Banks.

10. REFERENCES AND RELATED DOCUMENTS

Document Number	Title
22002	Request for Quality Control Testing
22120	Digital Gel Imaging Using the Kodak 400 Image Station
22133	Bioburden Assay by the Membrane Filtration Method
22137	Preparation of a Gram Stain: Manual Method
22148	Restriction Endonuclease Enzyme Digestion of Plasmid DNA
22149	Agarose Gel Electrophoresis (A.G.E.) and Detection of Nucleic Acids
22708	Culture Morphology
22713	Microbial Contents
N/A	Code of Federal Regulations, Title 21, Part 610: General Biological Products Standards.
N/A	Guidance for Industry for the Submission of Chemistry, Manufacturing and Controls Information for a Therapeutic Recombinant DNA-Derived Product or A Monoclonal Antibody Product for In Vivo Use.
N/A	Guidance for Industry: Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications
N/A	Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use.
N/A	Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals.

Document Number	Title
N/A	Points to Consider in the Production and Testing of New Drugs and Biologicals Produced by Recombinant DNA Technology.
ICH Harmonized Tripartite Guideline	Derivation and Characterization of Cell Substrates Used for Production of Biotechnological/Biological Products.
ICH Harmonized Tripartite Guideline	Quality of Biotechnological Products: Analysis of the Expression Construct in Cells Used for Production of r-DNA Derived Protein Products.
ICH Harmonized Tripartite Guideline	Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin.

11. ATTACHMENTS

Attachment 1 Table 1: Bacterial and Yeast Cell Bank Testing Recommendations

Attachment 2 Table 2: Mammalian/Insect Cell Master Cell Bank Testing

Attachment 3 Table 3: Mammalian/Insect Cell End Of Production Cell Testing

Attachment 1 Table 1: Bacterial and Yeast Cell Bank Testing Recommendations

Test	SOP Number or Equivalent Method	Accession	MCB	WCB	EOP
Microbial Content on Antibiotic and Non-Antibiotic solid media[1]	22713 or Equivalent Vendor Method		X	X	X
Purity (cell morphology)	22708 or Equivalent Vendor Method	X	X	X	X
Gram Stain	22137 or Equivalent Vendor Method		X	X	X
Identity of Bacteria	Outsource Vendor Method		X	X	X
Identity of Plasmid DNA Sequencing- sequencing of Protein Coding Region is required[2]	Outsource Vendor Method	X	X		X
Restriction Mapping[3]	22149/22148 or Equivalent Vendor Method	X	X	X	X
Strain Typing/Ribo Typing[5]	Outsource Vendor Method		X		
Contamination with both lytic and Lysogenic bacteriophages[6]	Outsource Vendor Method		X		X
Shiga-like Toxin[4]	Outsource Vendor Method		X		X
Cell viability	22713 or Equivalent Vendor Method		X	X	X
Plasmid copy number	Outsource Vendor Method		X		X
Plasmid genetic stability (after fermentation)	Outsource Vendor Method		X		X
Product Expression Level	Product Dependent	X	X	X	

1 Applicable when antibiotic-resistant plasmids are utilized.
2 Refer to Section 7.1.1.
3 Refer to Section 7.1.2.
4 For gram negative organisms only.
5 Or other equivalent methods such as MALDI-MS.
6 Bacterial cells only

Attachment 2 Table 2: Mammalian/Insect Cell Master Cell Bank Testing

TEST	MURINE OR CHIMERIC HUMAN/ MURINE CELLS	MURINE/ WHOLE HUMAN CELLS	CHO CELLS (Hamster)	RAT/ MURINE CELLS	HUMAN CELLS	NON HUMAN PRIMATE CELLS	INSECT CELLS
Sterility	X	X	X	X	X	X	X
Mycoplasma	X	X	X	X	X	X	X
PCR Based or Enhanced RT (PBRT / PERT)	X	X	X	X	X	X	X
Transmission Electron Microscopy (TEM)	X	X	X	X	X	X	X
Extended XC Plaque Assay	X	X		X			
Extended S^+L^- Assay	X	X	X	X			
Murine Minute Virus			X				
MAP[1]	X	X		X[1]			
RAP[1]				X[1]			
HAP[1]			X				
In Vitro Adventitious Agents[2]	X	X	X	X	X	X	X
In Vivo Adventitious Agents	X	X	X	X	X	X	X
Genetic Speciation Identity Testing[3]	X	X	X	X	X	X	X
DNA Transgene Sequencing[4]	X	X	X	X	X	X	X
Human Immunodeficiency Virus I & II (HIV I & II)	X	X			X	X	
Human T Lymphotropic Virus I & II (HTLV I & II)	X	X			X	X	
Human Herpes Virus 6 (HHV-6)	X	X			X	X	

Attachment 2 Table 2: Mammalian/Insect Cell Master Cell Bank Testing

TEST	MURINE OR CHIMERIC HUMAN/ MURINE CELLS	MURINE/ WHOLE HUMAN CELLS	CHO CELLS (Hamster)	RAT/ MURINE CELLS	HUMAN CELLS	NON HUMAN PRIMATE CELLS	INSECT CELLS
Human Herpes Virus 7 (HHV-7)	X	X			X	X	
Human Herpes Virus 8 (HHV-8)	X	X			X	X	
HSV-1/HSV-2	X	X			X	X	
PCV-1/PCV-2	X	X	X	X	X	X	
B19 Parvovirus	X	X			X	X	
Epstein-Barr Virus (EBV)	X	X			X	X	
Cytomegalovirus (CMV)	X	X			X	X	
Hepatitis A Virus (HAV)	X	X			X	X	
Hepatitis B Virus (HBV)	X	X			X	X	
Hepatitis C Virus (HCV)	X	X			X	X	
MMV	X	X	X	X	X	X	
Human Papilloma Virus (HPV)[5]	X	X			X	X	
JC Virus	X	X			X	X	
BK Virus	X	X			X	X	
Co-Cultivation[6]	X	X	X	X			
Tumorigenicity[7]		X			X	X	X
Bovine Adventitious Agents[8]	X	X	X	X	X	X	X
Porcine Adventitious Agents[8]	X	X	X	X	X	X	X
SV40						X	

Attachment 2 Table 2: Mammalian/Insect Cell Master Cell Bank Testing

TEST	MURINE OR CHIMERIC HUMAN/ MURINE CELLS	MURINE/ WHOLE HUMAN CELLS	CHO CELLS (Hamster)	RAT/ MURINE CELLS	HUMAN CELLS	NON HUMAN PRIMATE CELLS	INSECT CELLS
SRV-1, 2, 3 and SMRV						X	
STLV						X	
SIV						X	
Simian Foamy Virus						X	
Viability	X	X	X	X	X	X	X

[1] The Rat Antibody Production (RAP) test is performed if rat cells are involved, the Mouse Antibody Production (MAP) test is performed if murine cells are involved, and the Hamster Antibody Production (HAP) test is performed if hamster cells are involved.

[2] The use of (monkey) Vero, and MRC-5 (human) cells are required. The third cell line for testing should be the same species as MCB line.

[3] This could be karyotyping, isoenzyme analysis, STR/CODIS, or any currently accepted identity test to confirm species of origin.

[4] Transgene sequencing is only required for integrated transgene where the source construct sequence cannot be confirmed or where it is desirable to determine the number and location of transgene integration sites.

[5] Specifically, oncogenic HPV strains 16 and 18 should be included in any HPV assay panel.

[6] If retroviral testing is shown to be infectious, co-cultivation is performed on human cells.

[7] Tumorigenicity is generally only performed for Human epithelial cells or live virus vaccines. Tumorigenicity is only required for cell lines where the tumorigenicity is unknown. If a cell line is known to be tumorigenic testing for this is not required.

[8] Bovine/porcine adventitious agent testing should be performed where there are components used that could possibly introduce such agents.

Attachment 3 Table 3: Mammalian/Insect Cell End Of Production Cell Testing

TEST	MURINE, MURINE/RAT OR MURINE/HUMAN HYBIDOMAS	HUMAN CELLS	CHO CELLS	INSECT CELLS
Sterility	X	X	X	X
Mycoplasma	X	X	X	X
In Vitro Adventitious Agents	X	X	X	X
Murine Minute Virus	X		X	
In Vivo Adventitious Agents	X	X	X	X
RT - PCR Based or Enhanced RT (PERT)	X	X	X	X
TEM	X	X	X	X
XC Plaque Assay	X			
S+L-Assay	X		X	
Genetic Speciation Identity Testing[1]	X	X	X	X
Tumorigenicity[2]	X	X		X
Bovine Adventitious Agents[3]	X	X	X	X
Porcine Adventitious Agents[3]	X	X	X	X
Viability	X	X	X	X

1 This could be karotyping, isoenzyme analysis, STR/CODIS, or any currently accepted identity test to confirm the species of origin.
2 Tumorigenicity is generally only performed for Human epithelial cells or live virus vaccines. Tumorigenicity is only required for cell lines where tumorigenicity is unknown .If a cell line is unknown to be tumorigenicity testing for this is not required
3 Bovine/porcine adventitious agent testing should be performed where there are components used that could possibly introduce such agents.