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1.0 Purpose

This procedure describes the steps to be followed in initiating and maintaining mammalian cell cultures in Process Analytics/Quality Control (PA/QC).

2.0 Scope

This SOP applies to PA/QC personnel who are performing cell culture.

3.0 Authority and Responsibility

3.1 The Director, PA/QC has the authority to define this procedure.

3.2 PA/QC personnel are responsible for accurate performance of this procedure.

3.3 Biopharmaceutical Quality Assurance (BQA) is responsible for quality oversight of this procedure.

4.0 Materials and Equipment

4.1 Disposable pipettes: 1 mL (BDP PN 20101), 2 mL (BDP PN 20103), 5 mL (BDP PN 20104), 10 mL (BDP PN 20100), 25 mL (BDP PN 20102), 50 mL (BDP PN 20105), or BDP approved equivalent.

4.2 Flasks, 25 cm2 BDP PN 21150, 75 cm^2 BDP PN 20745, 162 cm^2 BDP PN 20074, or BDP approved equivalent.

4.3 Pipetting device 4

4.4 15 mL centrifuge tubes, BDP PN 20006, 50 mL centrifuge tubes BDP PN 20140, or BDP approved equivalent.

4.5 Dispatch (BDP PN 10167), Sterile 70% isopropyl alcohol (BDP PN 30129), Clorox (BDP PN 20295), Sporocidin (BDP PN 30135), Cavicide (BDP PN 10168), or other BDP approved equivalent.

4.6 0.4% Trypan Blue, BDP PN 10095.

4.7 Water Bath.

4.8 CO_2 incubator: 37°C ± 1°C, 5% ± 1% CO_2; >70% relative humidity.

4.9 Centrifuge.

4.10 Hemacytometer, BDP PN 20739, or BDP approved equivalent.

4.11 Vacuum flasks for aspiration of medium and samples.

4.12 Phosphate buffered saline, calcium and magnesium free (BDP PN 30007), or BDP approved equivalent.

4.13 Aspirating pipettes 2 mL (BDP PN 21331) or 5 mL (BDP PN 21330), or BDP approved equivalent.

4.14 Terminal pipette keepers (BDP PN 21338), or BDP approved equivalent.

4.15 Trypsin/EDTA solution (BDP PN 30396), or BDP approved equivalent.

4.16 Medium appropriate for each cell culture.

4.17 Calibrated Thermometer within calibration date.

5.0 Procedure

Perform all steps aseptically in a certified Biological Safety Cabinet.

5.1 General Guidelines

These are general guidelines for maintaining cell cultures. Each cell line will have unique characteristics. Media formulations, subculture ratios and trypsin formulations (adherent cells only) will need to be determined for each cell line.

5.1.1 Before beginning work, disinfect the Biological Safety Cabinet and record disinfection and use per ***SOP 22909 - Use, Cleaning and Disinfection of Equipment and Laboratories in BQC/BD.***

5.1.2 Complete Medium Preparation Form - Disinfect all bottles and supplies (when possible) with a disinfectant listed in 2.5 and place inside the Biological Safety Cabinet. When preparing complete medium, use the Media Formulation Log (**Form 22140-02**). Add components necessary for complete media for the selected cell line. Use one pipette per component. Record the preparation of all media in the Medium Formulation Log (**Form 22140-02**).

NOTE: Prior to preparation of media, confirm which supplements need to be added (e.g., L-Glutamine, fetal bovine serum, etc.). It is recommended that a list of commonly-used media and supplemental components for all cell lines used be appended in the front of the Medium Formulation Log.

5.1.3 Label the bottle(s) of complete medium with the lot number (based on the date made and first letter of medium, code for type of serum and percent serum), date made, initials of preparer, and an expiration date based on the component with the shortest expiration date. To ensure sterility, incubate the bottles of complete

medium at room temperature for a minimum of four days in the dark. If the medium cannot be pre-incubated, it should be filtered through a 0.22 μm filter prior to use. Store all components at the recommended temperature and in the dark when not in use. After sterility testing of the complete medium, store at the recommended temperature.

5.1.4 Suspension Cultures

Suspension cells generally do not adhere to a substrate; however, the protein concentration in the growth medium and/or positive charge of the plastic may cause some cells to stick. These cells may be dislodged by gently tapping the flask.

5.1.4.1 The cells are incubated and examined microscopically, and counted when the cells are ready to subculture. Subculture ratio is based on density, good viability, and experience working with the cells.

5.1.4.2 Prepare the work area in the Biological Safety Cabinet before working with the cells. This includes disinfecting the media bottle and collecting the necessary supplies (flasks, pipettes, pipetter, centrifuge tubes, etc.).

5.1.4.3 Determine the optimal seed density for the cells by trying several concentrations. To calculate the volume of cells needed for each subculture, follow the formula below.

Volume of cells needed = desired cell seeding density X desired volume in new flask ÷ cell density in the original flask.

Aspirate the cells several times with a pipette to obtain a uniform mixture. Some suspension cells will stick to the flasks and can be removed by pipetting media directly onto the growth surface. Add the required volume of cells and fresh medium to a new flask(s) (suggested volumes: 25 cm^2 flask - 10 mL, 75 cm^2 - 20 mL, 162 cm^2 - 30-40 mL, 850 cm^2 roller bottle - 400 mL). The volume with each passage can be increased when more cells are needed (Example: cell banking, optimizing growth conditions, etc.).

5.1.5 Adherent Cells

Adherent cells normally stick to the surface of a growth substrate and must be dislodged by mechanical or chemical means. Growth is in the form of a monolayer or sheet of cells filling the growing surface of the vessel. Passaging the cells is usually judged on the percent confluence and cells/cm^2 not cells/ml.

After subculturing, the flask or other vessel is placed in a humidified incubator at 37°C ± 1°C, 5% ± 1% CO_2, (or what is optimal for the medium or cell culture used).

5.1.5.1 Passing Adherent Cells

Prepare the work area in the Biological Safety Cabinet before working with the cells. This includes disinfecting the media bottle and collecting the necessary supplies (flasks, pipettes, pipetter, centrifuge tubes, etc.).

5.1.5.2 Aspirate the supernatant from the flask. Add the Ca++ Mg++ free phosphate buffered saline (PBS) solution to the flask (suggested volumes: 25 cm^2 - 5 mL, 75 cm^2 - 10 mL, 162 cm^2 - 20 mL and 850 cm^2 roller bottle - 50 mL). Rinse the cell sheet once or twice with the PBS and remove. If the cell culture is difficult to trypsinize, wash the cell sheet with trypsin after the PBS wash or instead of the PBS wash. After the wash add a sufficient volume of appropriate trypsin solution to wet the cell sheet (suggested volumes: 25 cm^2 - 0.5 mL, 75 cm^2 - 1 mL, 162 cm^2 - 2 mL).

5.1.5.3 Incubate the cells at 37°C ± 1ºC for about 2-5 minutes; remove the vessel and tap the side to dislodge the cells. The vessel may be returned to the incubator for an additional 2-5 minutes or longer and the procedure repeated until the cells release from the plastic growth surface. Some cell cultures will rapidly dislodge from the surface and these cultures may be "incubated" in the BSC. Re-suspend the cells in fresh medium.

5.1.5.4 Aspirate the suspension until uniform and free of clumps, if possible. The cells may be counted, if desired, per ***SOP 13214 - Using a Hemacytometer to Determine Density, Viability, Generation Time and Doubling Time for Mammalian Cells***.

5.1.5.5 Transfer the required cell volume to a new flask(s). Adherent cell subcultures are either stated in ratios of flask surface area or cells/cm2. Example: at a ~1:6 split ratio, a 25 cm^2 confluent flask can make a 162 cm^2 flask. To subculture adherent cells based on cells/ cm^2 (surface area), use the formulation below.

Total cells needed = Seed Density (cells/ cm^2) x Surface area (cm^2)

Total volume suspension needed = Total cells needed ÷ Original Viable cell density

5.1.5.6 Add fresh medium and gently mix cells (working volume - 25 cm^2 flask - 10 mL, 75 cm^2 - 20 mL, 162 cm^2 - 30-40 mL 850 cm^2 roller bottle - 200 mL). The volume of media in the flask for adherent cells is not critical. Label the top or side of the flask with the cell line name, date and lot number (if applicable). Slightly loosen the cap (if not vented) and incubate in a humidified incubator at 37ºC ± 1ºC, 5% ± 1% CO2 (or what is optimal for the medium used).

NOTE: If the medium contains a buffer, i.e., HEPES, the flask cap need not be opened.

5.1.5.7 Disinfect and log out of the Biological Safety Cabinet per ***SOP 22909 - Use, Cleaning and Disinfection of Equipment and Laboratories in BQC/BD.***

NOTE: Some adherent cell lines respond well to refeeding between subcultures. Aspirate all media in the flask and add back the same volume of fresh complete growth media. The frequency is cell-line dependent.

5.2 Initiating Cells from Frozen Stocks

5.2.1 If the frozen vial(s) of cells are stored in the Central Repository, submit a request to withdraw the cells as per ***SOP 21707 - Deposit/Withdrawal of Product and Samples in the NCI-Frederick Repository***. Allow the Repository at least 30 minutes to prepare the vial(s) for pickup.

5.2.2 Prepare the work area in the Biological Safety Cabinet before thawing the cells. This includes disinfecting the media bottle and collecting the necessary supplies (flasks, pipettes, pipetter, centrifuge tubes, etc.).

5.2.3 Withdraw the cells from storage and transfer to the laboratory on dry ice.

5.2.4 Add complete medium to a 15 mL or 50 mL centrifuge tube or flask. The volume of media may be adjusted depending on the density of cells in the vial. Thaw the vial(s) of cells in 25ºC to 37ºC water. Do not immerse the seal of the vial in the water.

5.2.5 Immediately transfer the vial to the Biological Safety Cabinet and spray the vial with Sterile 70% isopropyl alcohol or other BDP approved disinfectant. Remove the contents with a pipette and add to the centrifuge tube or flask containing the medium from step 5.2.4. The vial may be rinsed with medium to increase the number of cells recovered.

5.2.6 If desired, a sample can be taken at this point, or after centrifugation, to determine recovery and viability of the cells (The Cell Subculture Record Sheet may be used to record this data) **(*SOP 13214 - Using a Hemacytometer to Determine Density, Viability, Generation Time and Doubling Time for Mammalian Cells*).**

5.2.7 Cells may be centrifuged at a setting of 1000 RPM, timer set to 10 minutes, using a calibrated centrifuge following the appropriate equipment SOP or manual. When the centrifugation is complete, aspirate the supernatant and re-suspend the cell pellet in complete medium. Place the cell suspension in a labeled flask(s). Alternatively, cells may be seeded directly from the vial into a flask(s) containing complete medium. If the latter is performed, the flasks should be refed the following day to remove residual cryo-preservatives (e.g., DMSO).

5.2.8 Label the top or side of the flask with the cell line name, date, and lot number (if applicable). Slightly loosen the cap (if not vented) and incubate in a humidified incubator at 37°C ± 1°C, 5% ± 1% CO_2 (or what is optimal for the medium used).

NOTE: If the medium contains a buffer, i.e., HEPES, the flask cap need not be opened.

5.2.9 Disinfect and log out of the BioSafety Cabinet per ***SOP 22909 - Use, Cleaning and Disinfection of Equipment and Laboratories in BQC/BD***

6.0 Documentation

6.1 Maintain records in laboratory notebooks or SOP record forms. Each page is numbered, dated, and initialed as per ***SOP 21409 - Good Documentation Practices*** and ***SOP 21408 - Laboratory Notebooks Control and Use***.

6.2 Record data for cell subcultures on **Form 22140-01**, and medium formulation on **Form 22140-02**.

7.0 References and Related Documents

7.1	**SOP 22909**	Use, Cleaning and Disinfection of Equipment and Laboratories in BQC/BD
7.2	**SOP 13214**	Using a Hemacytometer to Determine Density, Viability, Generation Time and Doubling Time for Mammalian Cells
7.3	**SOP 21707**	Deposit/Withdrawal of Product and Samples in the NCI-Frederick Repository
7.4	**SOP 21409**	Good Documentation Practices
7.5	**SOP 21408**	Laboratory Notebooks Control and Use
7.6	**Form 22140-1**	Cell Subculture Record
7.7	**Form 22140-2**	Media Formulation Log

8.0 Change Summary