Frederick National Laboratory for
Cancer Research, Frederick, MD

Biopharmaceutical Development Program

Standard Operating Procedure

# Title: Standard Aseptic Practices for Cleanrooms and Biological Safety Cabinets for Production Operations

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## 1.0 Purpose

This document provides guidance for aseptic technique within ISO 5 unidirectional flow cleanrooms and biological safety cabinets used for the production of Good Manufacturing Practices/Good Laboratory Practices (GMP/GLP) product.

## 2.0 Scope

This Standard Operating Procedures (SOP) applies to Biopharmaceutical Development Program (BOP) personnel who work in cleanrooms or in biological safety cabinets for the production of GMP/GLP product.


This procedure is made available through federal funds from the National Cancer Institute, NIH, under con [redacted]

## 3.0 Overview

**3.1** Infection can be life-threatening; therefore, contamination control is an essential part of drug manufacture. Drugs come in a variety of dosage forms (oral, injectable, eye/ear drops, lotions, etc.). Because injectables bypass the body's first line of defense (skin barrier), their manufacture requires more stringent controls to ensure a contaminant does not get injected into the body. The BDP manufactures injectable drugs.

**3.2** Many drugs can be sterilized AFTER they have been filled into the final drug container (terminal sterilization). However, for biologic drugs, terminal sterilization processes usually destroy the activity of the drug. Therefore, biologic drugs must be produced and filled using aseptic processes.

**3.3** Contamination controls (a range of engineering controls, chemical controls, and personnel-directed controls) are applied to minimize/prevent contamination. Strong controls for each of these components provides layers of confidence that the manufacturing operation will produce an aseptically safe drug product for patients.

**3.4** The major source of contamination in a controlled environment is the personnel. This procedure describes the considerations that personnel should give to the work they perform under aseptic conditions.

## 4.0 Authority and Responsibility

**4.1** The Director, Technical Operations, BDP has the authority to define this procedure.

**4.2** Manufacturing personnel are responsible for performing this procedure.

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

## 5.0 Aseptic Practices

**5.1** Personal Health and Hygiene

*5.1.1* Health: Criteria for the restriction of personnel from product contact environments due to adverse health of employees are presented in ***SOP 21706 - Personnel Health Restrictions in Product-Contact Environments***.

5.1.2 Hygiene

Personnel shall practice good sanitation and health habits. For specifics refer to ***SOP 19406 - Gowning Requirements for Personnel and Visitors: [redacted] Manufacturing and Support Areas*** and ***SOP 19410 - Gowning Requirements for Personnel and Visitors for the [redacted] Virus Production Facility***

**5.2** Gowning for Clean Room Environments

5.2.1 The gown should provide a barrier between the body and exposed sterilized materials and should prevent contamination from particles generated by, and microorganisms shed from, the body.

This procedure is made available through federal funds from the National Cancer Institute, NIH, under contract [redacted].

5.2.2 Specific gowning requirements are listed in the following SOPs:

- ***SOP 19406 - Gowning Requirements for Personnel and Visitors: [redacted] Manufacturing and Support Areas,***
- ***SOP 19410 - Gowning Requirements for Personnel and Visitors for the [redacted] Virus Production Facility***

5.2.2.1 Prior to and throughout aseptic operations, an operator may not engage in any activity that poses an unreasonable contamination risk to the gown and/or product.

5.2.2.2 Inspect gowns on a periodic basis for integrity. If any part of the aseptic garment has been compromised, exit the cleanroom area and re-gown as required.

5.2.2.3 Gowns must be monitored at the end of processing before tear-down of the filling equipment. Do not disinfect gloves or gowning immediately prior to microbial monitoring. After monitoring, wipe gloves with sterile 70% Isopropyl alcohol (Decon-Ahol, BDP Part # 30129 or Alcoh-Wipe, BDP # 22328) to remove residual media. If continuing to perform aseptic operations discard outer gloves and replace.

**5.3** Sterile Packaged Gloves

5.3.1 Sanitizing of gloves is not required immediately following sterile glove replacement. Sanitizing of gloves with sterile 70% Isopropyl alcohol (Decon-Ahol, BDP Part # 30129 or Alcoh-Wipe, BDP # 22328) before performing activities in critical areas, prior to performing any aseptic manipulations, or immediately prior to moving from the ISO 7 (Class 10,000) area to any ISO 5 (Class 100) area. If there has been a known potential for glove contamination, gloves should be replaced.

5.3.2 Disinfect the entire surface of the gloves so the sides, between the fingers, and the front and back of the gloves down to the wrist receive alcohol contact.

5.3.3 Position your hands such that overspray and room airflow is away from the filling machine, open components, and/or equipment such as the particulate counter.

**NOTE**: It may be preferable to use the sterile Alcoh-Wipe to avoid overspray in these situations.

5.3.4 Gloved hands are to be held in such a way during production so as to minimize/eliminate contact of the gloves with the operators gowning, carts, walls, etc., in order to minimize possible contamination. If contact does occur, disinfect gloves as in Step 5.3.2.

5.3.5 Inspect gloves frequently and replace if torn, punctured, or soiled.

5.3.6 Avoid unnecessarily touching non-sterile objects during aseptic operations. If necessary, spray, wipe or change gloves after touching a potentially non-sterile object before proceeding to another aseptic operation.

This procedure is made available through federal funds from the National Cancer Institute, NIH, under contract [redacted].

**5.4** Tools and Instruments

5.4.1 Contact sterile materials only with sterile instruments i.e., forceps, vial pusher, vial handling tools, pipettes, held in a manner that prevents contamination. Replace instruments as necessary throughout an operation.

5.4.2 Sterile instruments used must be sanitized prior to each use, preferably by dry heat sterilization or autoclaving or be single use disposable in nature. Use disposable sterile plastic ware and utensils when possible.

5.4.3 When a sterile instrument is not actively being used during an operation, it must be either placed within a sterile area such as an autoclave pouch or otherwise be stored to ensure its integrity. Replace the tool/instrument if the integrity is suspected to be compromised.

**NOTE**: Sterile conical tubes that have been placed in stainless steel brackets on the inside corners of the Flexicon plexiglass RAB are used for storage of tools used to manipulate vials during filling operations. These are treated as a single use item and replaced for each fill or if compromised during processing.

5.4.4 Non-routine tools (for example, repair tools) must be autoclaved or sanitized with an approved sporicide (Steri-Perox 6%, BDP part # 10665 is preferred as secondary wiping with alcohol is not required) and if a sporicide other than Steri-Perox 6% is used are wiped with sterile 70% Isopropyl alcohol (Decon-Ahol or Alcoh-Wipe) prior to use.

**5.5** Tables, Carts and Trash Containers

5.5.1 Avoid disrupting the air flow in the clean room areas by positioning tables, carts, and trash containers so they are a minimum of several inches away from walls. Use surfaces that are perforated, made of wire, or other materials that do not obstruct air flow whenever practical and possible.

**NOTE**: sterile trash can liners (BDP part #21561) Within the ISO 5 area, sterile trash can liners may only be used alone as no hard trash receptacles are permitted.

5.5.2 Do not store equipment or materials in front of room return air ducts. Materials should be stored at least one foot above the height of any air return duct. This is done to both ensure that airflow is not restricted and to avoid materials being in contact with air potentially carrying particulates.

**5.6** Movements in Unidirectional Flow Clean Rooms and Within BSCs

5.6.1 Movement in unidirectional air flow will disrupt air patterns. Move slowly and deliberately. Rapid movements can create unacceptable turbulence in a critical area presenting a challenge beyond intended clean room design and control parameters. Follow the principle of slow, careful movement throughout the ISO 5 (Class 100) environment.

5.6.2 ISO 5 environments create an aseptic environment – not a sterile environment. Consideration for how work is performed provides additional protection against contamination.

This procedure is made available through federal funds from the National Cancer Institute, NIH, under contract [redacted].

5.6.3 Unidirectional air flow design is used to protect sterile equipment surfaces, container-closures, and product. Disruption of the path of unidirectional flow air in the critical area can pose a risk to product sterility. Minimize actions within this critical area and limit to those interventions verified during validations.

5.6.4 Observe principle of "**First Air**" when working in ISO 5 areas. This principle emphasizes the need for HEPA filtered air moving in a unidirectional flow to contact critical components of the operation (open vials, vial closure components, open product, etc.) or aseptic processes prior to contacting any operator. Failure to follow "First Air" principles would result in HEPA filtered air flowing over part of the operator or other objects and then coming into potential contact with critical components or aseptic processes.

5.6.5 Any operator involved with the handling of open vials or making aseptic manipulations during operations is restricted to those operations and should not be involved with other operations that could increase the likelihood of glove or sterile gown contamination that could jeopardize the sterility of finished product. Other operations that would be prohibited would include but not be limited to the handling of batch records, performing concurrent EM, moving back and forth between different classification areas, handling of trash, removing outer layers of wrapping from sterile supplies etc.

5.6.6 Do not pass objects, including hands or non-sterile instruments, over open sterile materials. Perform aseptic manipulation from the side and not above the product.

5.6.7 Limit the time sterile containers are open to the environment whenever possible.

5.6.8 Avoid movement of used (dirty) items overtop of clean items. Separate clean and used (dirty) items within the workspace.

5.6.9 Refrain from unnecessary speaking when in direct proximity to the critical area.

5.6.10 Do not lean against walls or equipment such as tables, carts, and trash containers. The integrity of these items and your sterile gown can be compromised by contact with any of these items.

5.6.11 Do not kneel or lay directly on the floor. If necessary, place a sterile pad on the floor to protect the integrity of the gown or change your gown before proceeding to other activities.

5.6.12 Do not compress trash down into the waste container. This may force "dirty" air into the room environment. When the container is full, use another container.

5.6.13 Avoid picking up items that have fallen on the floor until the operation has been completed. When necessary, fallen items can be pushed out of the way with your foot.

5.6.14 Avoid unnecessarily touching non-sterile objects with your hands during aseptic operations. When not performing manipulations, keep hands in front of the body such that they are not in contact with anything. If necessary, spray or change gloves after touching a potentially non-sterile object and before proceeding to another aseptic operation.

This procedure is made available through federal funds from the National Cancer Institute, NIH, under contract [redacted].

5.6.15 To move through a curtain, part the curtain with your gloved hand. Sanitize gloves following entry to the area.

5.6.16 If you sneeze or cough, turn completely away from your work surface.

**5.7** Handling Supplies

5.7.1 Unwrapping Sterilized Components and Tools

5.7.1.1 Sterilized components and tools are wrapped to protect them during holding and storage. All sanitization is performed using sterile 70% Isopropyl alcohol (BDP part # 30129 or BDP part # 22328). Always spray in a location such that airflow will not carry aerosols into critical areas.

**NOTE:** When unwrapping items that have been sealed with autoclave tape it is best to open using sterile scissors to avoid getting tape residue on gloves.

- For double-wrapped items, sanitize and remove the outer wrap without touching the inner wrapping. Then move the item (now single-wrapped) into the critical area.
- For single-wrapped items, sanitize the item and remove the wrap within the critical area without touching the product-contact surfaces of the item.

**NOTE:** The unwrapping of sterile items is often performed using two people. One person removes the outer wrapping of double-wrapped items and moves the item into the critical area. The second person, in the critical area, removes the inner wrapping.

5.7.1.2 Do not re-enter a sterile bottle with a “used” pipette. Use a fresh, sterile pipette for each entry into a sterile bottle.

5.7.1.3 Do not return any excess material back into its original container.

5.7.2 Store sterilized items in such a way that they maintain first contact with clean laminar flow air and are not potentially contaminated by air that has first washed over other surfaces or items.

**5.8** Specialized Processes

5.8.1 Aseptic Connections

5.8.1.1 All aseptic connections are made in an ISO 5 (Class 100) environment.

5.8.2 Product Sampling

5.8.2.1 Product-sampling processes must occur in a manner to protect the purity and aseptic integrity of the bulk being sampled and the sample itself.

- Sample containers must protect the integrity of the sample.
- Do not re-enter a sterile bottle with a “used” pipette. Use a fresh, sterile pipette for each entry into a sterile bottle.

This procedure is made available through federal funds from the National Cancer Institute, NIH, under contract [redacted].

- Do not return any excess material back into its original container.

**5.9** Working in Biological Safety Cabinets (BSCs)

5.9.1 BSC Setup

5.9.1.1 Use the BSC including disinfection and cleaning per ***SOP 19102 - Routine Use and Disinfection of Biological Safety Cabinets, Incubators, Shakers, and Centrifuges).***

5.9.1 .2 Sanitize supplies and equipment with sterile 70% IPA prior to placing items into the BSC.

5.9.1.3 Organize materials within the hood so that air flow is not blocked. Do not obstruct the grills at the front and back of the BSC.

5.9.1.4 Maintain the sash at the proper height for optimized air flow within the BSC.

5.9.1.5 Following the guidelines for sterile packaged gloves in Section 5.3 add sterile sleeves and a new pair of sterile gloves within the BSC before beginning work. Sterile sleeves should be donned first and then the outer pair of sterile gloves added, taking care not to touch any outer portion of the second set of gloves with the original pair of gloves. Upper sleeve cuffs should be rolled up after donning the second pair of sterile gloves and done so by sliding the fingers of the opposite hand under the rolled cuff and sliding upwards to unroll the cuff.

5.9.2 BSC Operations

5.9.2.1 As much as possible, work in the middle of the BSC where conditions are optimized.

5.9.2.2 Limit movements in the proximity of the hood and the entry and exit of people into the area to those movements and staff required for the operation while the BSC is in use. Movement of objects and personnel behind the hood operator can disrupt the proper air flow patterns within the hood and allow room air to enter the hood environment. Use slow deliberate movements when working in the vicinity of BSCs in use.

5.9.2.3 Set pan lids etc., in the BSC in such a way that they do not block the grill area in the front or rear of the BSC or in any way significantly impede the air flow.

5.9.2.4 Passing materials in and out of the BSC during operation.

5.9.2.4.1 For double-wrapped items; an operator outside the BSC will sanitize the outer wrap and hands, open the outer wrap partially and present the package to the edge of the BSC so that the operator in the BSC may remove the inner wrapped package.

5.9.2.4.2 For single-wrapped items; an operator outside the BSC will sanitize the outer wrap and hands and present the package to the edge of the BSC so that the operator in the BSC may retrieve the package.

5.9.2.4.3 To hand materials out; decontaminate object (applies to virus processes only) and then present the object to the edge of the BSC where they can be retrieved.

5.9.2.4.4 During these operations, the hands of the outside operator should not enter the BSC and the hands of the operator within the BSC should not exit the BSC.

## 6.0 Environmental/Personnel Monitoring

**6.1** Environmental and Personnel Monitoring is performed a ified in SOP 22 ***Environmental Monitoring*** in ***BDP GMP Areas at the_ .***

**6.2** Do not change or spray gloves with sterile 70% IPA I (Decon-aholor equivalent) immediately before personnel monitoring. While changing and periodic spraying of gloves to reduce glove bioburden is an expected practice, spraying or changing gloves immediately before personnel monitoring does not generate data that is reflective of the actual condition of gloves during aseptic processing of product.

**6.3** Gowns should be monitored at the end of processing before any disassembly of equipment. Do not leave an ISO 5 area before being monitored, do not remove hands from the BSC until immediately prior to monitoring as this could negatively impact results.

**6.4** If continued work in the cleanroom or BSC is required after the processing of product and after personnel monitoring is completed, change the outer layer of gloves or wipe with sterile 70% Isopropyl alcohol (Decon-Ahol or equivalent) to remove residual media before proceeding.

## 7.0 References and Related Documents

7.1 **SOP 21706** *Personnel Health Restrictions in Product-ContactEnvironments*

7.2 **SOP 19406** *Gowning Requirements for Personnel and Visitors:- Manufacturing and Support* Areas

7.3 **SOP 19410** *Gowning Requirements for Personnel and Visitors for th Virus Production Facility*

7.4 **SOP 19102** *Routine Use and Disinfection of Biological* Safety *Cabinets, Incubators, Shakers, and Centrifuges*

7.5 **SOP 22315** *Environmental Monitoring in BOP GMP* Areas *at the• .*

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