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1.0 Purpose

This procedure outlines the process for BDP personnel performing the purification and handling of infectious and potentially infectious materials.

2.0 Scope

This procedure applies to BDP Purification personnel specifically trained in handling BL-2 and BL3 level agents. Late Process Sciences policies are specified in ***SOP 17109, Procedures for Safe Handling, Decontamination, and Spill Cleanup of Infectious Materials; SOP 19408, Cleaning and Disinfection of CGMP Areas*** [redacted].

Establishment of this procedure is based on Attachment 1, Risk and Mitigation Strategy.

3.0 Authority and Responsibility

3.1 The Safety Officer, BDP, has the authority to define the safety aspect of this procedure.

3.2 BDP personnel performing purification of infectious or potentially infectious material are responsible for the implementation of this procedure.

3.3 The Area Manager is responsible for ensuring that personnel under his/her supervision, who are performing the work, are trained in this procedure.

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

4.0 Laboratory Facilities and Safety Equipment

4.1 Refer to ***SOP 17109, Procedures for Safe Handling, Decontamination, and Spill Cleanup of Infectious Materials*** for requirements of laboratory facilities, safety equipment and procedures as needed.

5.0 Materials

5.1 Approved Cleaning/Sanitization Agents.

5.1.1 **Bleach Germicidal Disinfectant** (BDP PN 10167) or equivalent. May be used for surface disinfection in BSCs. May not be used in centrifuges or incubators.

5.1.2 **Decon-ahol** (BDP PN 30129) or equivalent. Use as a surface-sanitizing agent.

NOTE: Decon-ahol is not to be used as the primary or sole disinfectant for the decontamination of infectious or potentially infectious spills.

5.1.3 **Clorox bleach** (BDP PN 10579) or equivalent. Clorox with this BDP part number is supplied as 6.15% bleach. Used for disinfection of liquid waste.

NOTE: Aliquoted bleach should be <24 hours old. Do not use Cavicide with Bleach Germicidal Disinfectant or Clorox.

5.1.4 **Decon Spore** (BDP PN 30826) or equivalent. May be used for surface disinfection in BSCs, centrifuges, and incubators.

5.1.5 **Cavicide** (BDP PN 10168) or equivalent. May be used for surface disinfection in BSCs, centrifuges, and incubators.

NOTE: Cavicide is not approved for use as a disinfectant with non-enveloped viruses (such as AAV).

5.2 24 x 36 autoclave bag or equivalent, BDP PN 20728.

5.3 Povidone-iodine lotion and/or scrubs (Supplied by EHS).

5.4 Softcide hand soap (BDP PN 30137) or equivalent.

6.0 Virus Purification Procedure

6.1 Infectious or potentially infectious materials will be transported to the processing area per ***SOP 26101, Labeling, Transport, Submission, Storage, and Handling of Biohazardous Materials Within the BDP.***

6.2 If Applicable, allow material to thaw by placing product containers in a tray or pan located within a BSC. Secondary biohazard bags should remain on the product while thawing occurs.

6.3 Operations manipulating open containers of infectious or potentially infectious material will be performed in a BSC per ***SOP 17109, Procedures for Safe Handling, Decontamination, and Spill Cleanup of Infectious Materials.***

6.4 Virus Supernatant pre-purification (as applicable)

6.4.1 In a BSC, pipet magnesium chloride into the clarified virus supernatant to obtain a final concentration specified in the Batch Production Record (BPR).

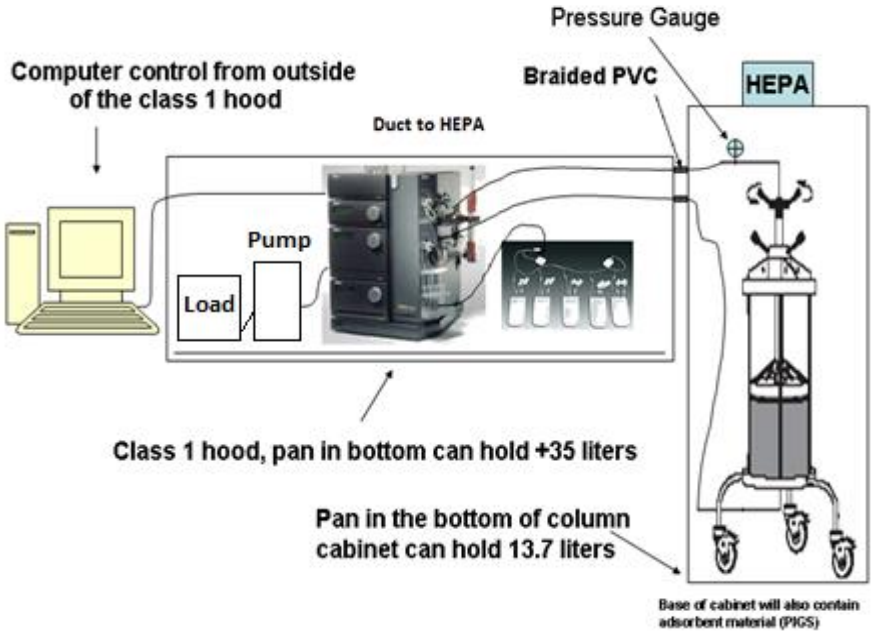
6.4.2 Remove DNA contaminants by pipetting Benzonase to the adjusted virus supernatant as per BPR.

6.4.3 Following the transport, decontamination and safety procedures found in ***SOP 17109, Procedures for Safe Handling, Decontamination, and Spill Cleanup of Infectious Materials*** incubate viral material as specified in the BPR.

6.5 Preparation of Purification Equipment

6.5.1 An ÄKTA Instrument from GE Healthcare-Biosciences and various column sizes and types of resins are used for the viral purification process specified in the Batch Production Record. Locations and connections of equipment are critical to safety procedures. Refer to Diagram 1 for system set up.

Diagram 1



6.5.1.1 The ÄKTA Instrument is housed in a custom designed class 1 BSC (see Attachment 2) or if desired, can be placed in a Class II BSC. The Class I or II biological safety cabinets ducted to a HEPA filtration unit on the top that provides the negative pressure to maintain the unit at >100 LFPM when one of the sliding doors is open. The custom designed class 1 cabinet has a metal base with 4 sidewalls designed to contain leakage in case of a spill. (The base of the cabinet can hold ~36 liters of liquid.) The class 1 cabinet has an emergency power cutoff button on the outside of the unit to easily cut power to the ÄKTA if required. The computer controlling the instrument is located outside of the BSC's.

6.5.1.2 Columns smaller than an XK50/30 are placed into the class I Biological Safety Cabinet that contains the ÄKTA Instrument.

6.5.1.3 Columns larger than a XK50/30 are placed into the "column cabinet" (see diagram 2). The column cabinet is a custom designed class I Biological Safety Cabinet that was built specifically to house large sized columns for virus purification. A HEPA unit is attached, maintaining the column cabinet at negative pressure to the room to ensure no aerosol escapes the cabinet. If a leak(s) were to occur, the floor of the cabinet functions as a pan with the ability to capture approximately 14 liters of liquid.

Tubing between the AKTA and the column cabinet will be contained in braided PVC tubing as an added measure of protection.

6.5.1.4 Prior to production, perform a pressure hold test on columns per ***SOP 14100 Packing Instructions for the GE BPG Series Columns, SOP 14121 Packing Instructions for Fineline Columns*** and ***SOP 14137 Packing Instructions for XK Series Chromatography Columns***.

6.5.1.5 Columns are to be pressure tested in excess of 3 - 5 bar[1] (0.3 - 0.5MPa, 44.1 - 73.5psi) to ensure that no visible leaks are apparent at any joints or connections.

6.5.1.6 The flow rates for cleaning the columns with the ÄKTA Instrument are operated at or above the target flow rates of the purification process to ensure there are no leaks observed from either the packed column or the ÄKTA Instrument. During this process pressure stabilization should be monitored for a minimum of 5 minutes, (refer to **Diagram 1** for location of pressure gauge). A pressure decrease ≤ 0.1 bar is indicative of a closed unit system.

NOTE: The maximum pressure of the ÄKTA purification system is set at the limit recommended by the manufacturer for the combination of column, resin and tubing being used; if during operation the pressure exceeds the set point the computer automatically shuts down the entire system and initiates an alarm.

6.6 Purification by Column Chromatography

6.6.1 Charge and equilibrate columns per BPR.

6.6.2 Load bulk virus onto chromatography columns.

6.6.2.1 Bulk material received in PETG bottles thawed and pooled, treated as described in Section 6.4, is loaded onto the chromatography column by using an external peristaltic pump (not controlled by the ÄKTA Instrument).

6.6.2.1.1 Disconnect column inlet tubing from the ÄKTA and attach to tubing and peristaltic pump. When loading a column with a peristaltic pump, the in-line pressure gauge (See **Diagram 1**) should be monitored based on specifics found in the BPR.

(The pressure reading captured is from the feed lines and the column pressure.)

6.6.2.1.2 Once loading is complete disconnect the column from the tubing / peristaltic pump and re-connect to the ÄKTA Instrument. Decontaminate the load container and tubing assembly with bleach solution per ***SOP 17109 Procedures for Safe Handling, Decontamination, and Spill Cleanup of Infectious Materials.***

6.6.3 Elute viral material as per BPR. Collect elution in either a sterile media bag or bottles within a BSC. Refer to ***SOP 17109, Procedures for Safe Handling, Decontamination, and Spill Cleanup of Infectious Materials*** for appropriate handling of materials.

6.6.4 Column/Resin decontamination

6.6.4.1 Decontaminate resin and internal components of the column by flushing with 0.5M NaOH as described in the batch production record.

6.6.4.2 Remove 0.5M NaOH by flushing WFI through column. All liquid waste produced will be disposed of per procedures in ***SOP 17109, Procedures for Safe Handling, Decontamination, and Spill Cleanup of Infectious Materials.***

6.6.4.3 External components of column (and associated tubing) are to be decontaminated with approved disinfectant, refer to ***SOP 17109, Procedures for Safe Handling, Decontamination, and Spill Cleanup of Infectious Materials.***

6.7 Concentration/Diafiltration (if required, refer to **Diagram 2**)

6.7.1 The viral concentration/diafiltration process must occur within an IBC approved containment system. Use “column cabinet” referred to in Section 6.3.1.3 for this process. Ensure that appropriate disinfectants (i.e. bleach Cavicide, etc.) and wipes are within the cabinet prior to bulk virus entry. The TFF process is to be performed as a closed system.

6.7.2 Preparation for Tangential Flow Filtration

6.7.2.1 Using hollow fiber cartridges specified in the BPR, attach both feed lines (buffer and load), permeate lines (with collection bags), and product containers with topworks assembly to the cartridges and peristaltic pump. In the presence of viral material, all connections to the TFF system are made with pumps turned off through the glove ports, refer to **Diagram 2**.

6.7.2.2 Pump buffer through the TFF system ensuring that air is removed from all lines as well as monitoring for any signs of leaks.

6.7.2.3 Perform a leak test per ***SOP 14125 Operation and Use of Cassette Filter TFF Systems***. Monitor pressure gauges, assuring limits do not exceed those that are specified in BPR, refer to gauge locations in **Diagram 2**.

6.7.3 Concentration/Diafiltration Procedure

6.7.3.1 Following safety procedures in ***SOP 17109, Procedures for Safe Handling, Decontamination, and Spill Cleanup of Infectious Materials***, transport purified virus bulk to "column cabinet" and place bags on hooks located at the back of the cabinet. Close the cabinet and make necessary connections to TFF system using glove ports.

6.7.3.2 Through the glove ports, position the tubing into the load pump. Using tubing, connect the buffer bag to the load. Buffer is to remain clamped shut until diafiltration process begins. Open the feed line of the bulk virus solution. Turn pump on at minimum speed to introduce bulk material to TFF system, gravity flow can replace the need for this pump if desired. Monitor pressure according to the BPR. Flow into the TFF system is controlled via pump; the feed line clamp will serve as a secondary control mechanism if needed.

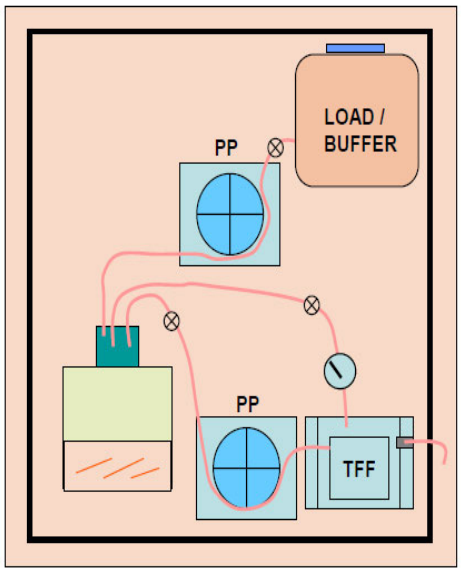
6.7.3.3 Continue process until targeted volume as per BPR is achieved. Feed line should be clear of bulk virus. If the line is not clear add buffer to chase the remaining virus out of the line.

6.7.3.4 Turn off the pump and allow pressure to dissipate.

6.7.3.5 Through the glove ports, open buffer line and allow desired volume to enter empty load container. Resume pumping buffer through TFF lines until lines are drained and diafiltration is complete. Remove topworks assembly and cap collection container. Place topworks assembly in a new container equivalent to collection for decontamination.

6.7.3.6 Decontaminate TFF system by pumping bleach solution through system components. Refer to ***SOP 17109, Procedures for Safe Handling, Decontamination, and Spill Cleanup of Infectious Materials*** for decontamination and disposal procedures.

Diagram 2: Concentration/Diafiltration (TFF System)



Concentration/ Buffer Exchange/ Diafiltration Step

"COLUMN CABINET "

Glove compartment for operation inside the Biological safety cabinet to minimize the exposure risk.

Biological safety cabinet made of Plexiglas isolates the TFF system.

7.0 References and Related Documents

SOP 17109 *Procedures for Safe Handling, Decontamination, and Spill Cleanup of Infectious Materials*

SOP 19408 Cleaning and Disinfection of CGMP Areas [redacted]

SOP 26101 *Labeling, Transport, Submission, Storage, and Handling of Biohazardous Materials Within the BDP*

SOP 14100 *Packing Instructions for the GE BPG Series Columns*

SOP 14121 *Packing Instructions for Fineline Columns*

SOP 14137 *Packing Instructions for XK Series Chromatography*

SOP 14125 *Operation and Use of Cassette Filter TFF Systems*

IBC Protocol P120902JLA01

NOTE: A mock TFF setup was demonstrated to the IBC on December 21st, 2005 for an amendment (1/17/06) to protocol P230902JLA01.

IBC Protocol P150703RHA01

NOTE: A mock purification setup using Riboflavin that included automatic shutdown was demonstrated on August 26th, 2005, for an amendment (Sept 15, 2005) to protocol.

Biological Safety in Microbiological and Biomedical Laboratories, Center for Disease Control and Prevention and National Institutes of Health. Current version.

Frederick National Laboratory Bloodborne Pathogen Exposure Control Plan, current version.

Frederick National Laboratory Environmental Health, and Safety Program. Health, Safety and Environmental Compliance Program Manual. Current version. http://web.ncifcrf.gov/campus/safety/compliance/index.stm .

Environment, Health and Safety. Safetygrams. Current version.

8.0 Documentation

8.1 Record equipment usage and cleaning in their respective logbooks.

8.2 Document process manipulations in an appropriate laboratory notebook or process-specific batch record.

9.0 Attachments

Risks and Mitigations

Engineering Drawing for Fan-assisted HEPA Filtered Containment Cabinet

10.0 Change Summary

[redacted]

Attachment 1: Risks and Mitigations

1) Column Purification Procedure, Positive Pressure

Risk	Mitigation
Accidental exposure of unrelated personnel	• Signs indicating that virus is being filtered are posted on the door to ensure that non-essential personnel do not enter the room.
System leak	• A Riboflavin trial run was performed and demonstrated to IBC Aug 26, 2005 (as part of protocol P150703RHA01 amendment Sept 15) to ensure system does not produce leaks. • Sanitary fittings are used wherever possible to prevent system leaks. If not possible, connections are flanged-type screw connections, finger tight. • The process is run with a pressure gauge in line, upstream of columns to monitor the system pressure at all times. • The pressurized parts of the system are contained within a class I BSC to prevent splatter in the case of a failure. • The AKTA Instrument is contained in the custom-built class I BSC. • Large size columns are contained in the Plexiglas Cabinet, a custom-made class I BSC. • Small columns that can fit into the class I BSC with the AKTA unit are contained in the same cabinet. • Both the AKTA class I BSC and the Plexiglas cabinet class I BSC are built within internal pans, the AKTA unit can contain -36 liters of material and the column cabinet can contain 14 liters of material, thus if either or both units experience catastrophic failure, infectious material is contained inside the class I cabinets.
Exposure in case of system failure	• The AKTA is connected to a computer and is controlled from outside the BSC. The custom-built class I BSC has an external power strip: in the event of a system failure the power is turned off from this switch.

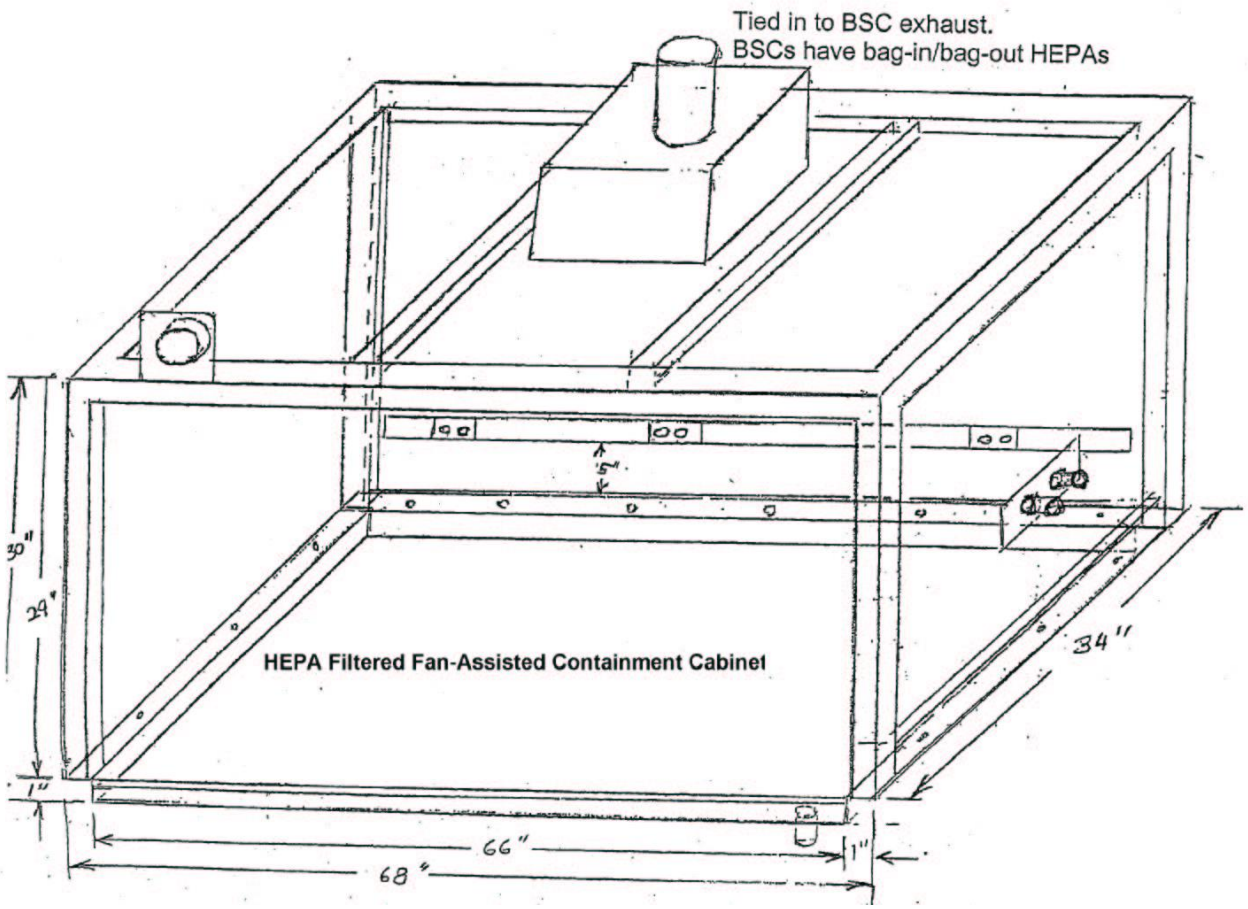
Attachment 1: Risks and Mitigations (Continued)

2) Concentration/Diafiltration Procedure, Positive Pressure

Risk	Mitigation
Accidental exposure	• Signs indicating that virus is being diafiltered / concentrated are posted on the door to ensure that non-essential personnel do not enter the room.
Equipment Foot Print	• The TFF system will be contained in the custom-built Plexiglas class I BSC. • The cabinet has four glove ports to facilitate access into cabinet and to control the TFF system. • Two operators monitor the system at all times.
System leaks	• A Riboflavin trial run was performed and demonstrated to IBC on Dec 21, 2005 (as part of protocol P230902JLA01) to ensure system does not produce leaks. This mock run was conducted at 30 psi without evidence of leaks in an attempt to test the system to failure. • A power switch is located outside of the custom-built class I BSC to enable the system to be turned off in case of any leak. • The TFF components are positioned inside an internal pan which can contain 35 liters of material and the outer cabinet can contain 13.7 liters of material, thus in the event of catastrophic failure, infectious material is contained. • Sanitary fittings are used wherever possible to prevent system leaks. If not possible connections will be flanged-type screw connections, finger tight. • Aerosolization of the product due to tube break is contained in the closed safety class I BSC cabinet. • **Tubing:** New MasterFlex tubing L/S- 16 (cat#06424-16) biopharmaceutical grade formulated for peristaltic pump applications is used for each operation. • **Filter:** pre-sterilized filter unit with tri-clamp inlet and outlet. • **System check:** prior to the introduction of virus a leak test is performed by pressurizing the system to 30 psi. A stable pressure must be observed for one minute.
Pressure	• **Operational pressure:** continuously visually monitored, initially maintained at ~8 psi, maximum operational pressure is 14- 15 psi. • The pressurized parts of the system are contained within a class I BSC to prevent splatter in the case of a failure. • **Pressure gauge:** Anderson 'mini' sanitary gauge with pressure range of 0-30 psi. Gauge is installed inline upstream of the filter using a sanitary 'T' connection at all times. • **Peristaltic pump:** variable speed control to enable adjusts to maintain operational pressure ~8 psi. • Working pressure for Master flex tubing is approximately ~15 psi with a Maximum continuous pressure of approximately 25 psi. Silicone tri-clamp fittings are molded onto appropriate tubing ends. • The system is de-pressurized prior to the cabinet doors being opened.
Exposure in event of system failure	• The TFF system is connected to power switch located outside of the custom-built class I BSC to turn off the system in the event of a system failure.

Attachment 2

Engineering Drawing for Fan-assisted HEPA Filtered Containment Cabinet



Attachment 2 (Continued)

Engineering Drawing for Fan-assisted HEPA Filtered Containment Cabinet