Frederick National Laboratory for
Cancer Research, Frederick, MD

RDD
Biopharmaceutical Development Program

Standard Operating Procedure

Title: Procedures for Safe Handling, Decontamination, and Spill Cleanup of Infectious or Potentially Infectious Materials

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Table of Contents

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

1.0 Purpose

This procedure outlines the process for handling infectious materials and infectious material spills by Biopharmaceutical Development Program (BDP) personnel.

2.0 Scope

This procedure applies to BDP personnel specifically trained in handling infectious or potentially infectious agents.

3.0 Authority and Responsibility

3.1 The Program and Technical Director, BDP, has the authority to define this procedure.

3.2 BDP personnel performing viral work are responsible for the implementation of this procedure.

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

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

4.0 Laboratory Facilities

4.1 When infectious materials are being handled, the laboratory door is posted with biohazard signs restricting access to the laboratory and is kept locked for limited access. A card key is required for entry. Identify the agent(s) being handled and the biosafety level on the sign. Only Institutional Biosafety Committee (IBC) approved personnel are permitted to work on a project that is being actively performed in IBC approved facilities. Personnel working on the project must be enrolled in the Environmental, Health & Safety Bloodborne Pathogen Program.

4.2 Mouth pipetting is prohibited; liquid transfers must utilize mechanical pipetting devices such as pipet aids. Mechanical pipetting devices must have a hydrophobic filter when used in pipetting potentially infectious materials.

4.3 Laboratory surfaces and furniture are made with non-porous material for easy decontamination.

4.4 An eyewash station is readily available in each work area.

4.5 Illumination is adequate for the activities performed in the room.

4.6 The location of the nearest shower is posted near the laboratory door.

4.7 Biological Safety Cabinets are located such that fluctuations of the room air supply and exhaust air do not cause the BSC to operate outside their containment parameters.

4.8 Treat water used in incubators for potentially-infectious materials as potentially infectious waste and disposed of per Section 11.0. Use alcohol-type thermometers (versus mercury) or digital resistance temperature detectors (RTDs) in water baths and incubators.

4.9 Restrict needles and syringes or other sharp instruments for use only when there is no alternative. The use of sharps must be justified in the IBC submission. Substitute plastic ware for glassware whenever possible.

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4.10 Used, disposable needles SHALL NOT be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; rather, they must be carefully placed in a proper sharps disposal container.

4.11 Broken glassware must not be handled directly by hand, but it must be removed by mechanical means such as a brush and dustpan, tongs, or forceps. Package containers of contaminated needles, sharp equipment, and/or broken glass in accordance with EHS guidelines and decontaminate via autoclaving prior to disposal. **Do not autoclave sharps containers.**

4.12 Although not considered sharps, pipettes and pipet tips can puncture autoclave or other hazardous waste bags and should be disposed of in terminal pipette keepers or other puncture resistant containers.

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.

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

NOTE: Aliquotted bleach should be <24 hours old. Do not use Cavicide with Dispatch, Clorox, or Spor-Klenz.

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.

5.1.6 Conflikt (BDP PN 30986) or equivalent. Process Analytics use only.

5.1.7 Steri-Perox 6% (BDP PN 10665) or equivalent.

5.1.8 Sporicidin (BDP PN 30135) or equivalent.

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

5.3 30 x 36 polypropylene autoclave bag or equivalent, BDP PN 20665.

5.4 Terminal pipette keepers (BDP PN 21338).

5.5 Terminal biohazard benchtop keeper (BDP PN 21491).

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

Handwash: Softcide (BDP PN 30137) or equivalent.

Vacuum Flasks (optional)

Aspirating pipettes (optional)

Filter for aspirating pipettes (PN 20667, optional)

Disposable lab coats

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Disposable lab gloves

Sleeve covers

Biohazard sharps container PN 20356

Hair nets

Booties

Coveralls

6.0 Safety Equipment (Primary Barriers)

6.1 Perform all open operations (i.e., those involving unsealed containers of potentially infectious materials) in Class II BSCs.

6.2 Where possible, all closed work with infectious agents that is NOT conducted in a flask, roller bottle, or cell factory within an incubator will be conducted within Class I biosafety cabinet or specially designed negative pressure HEPA filtered containment cabinet (see **Attachment I** for typical configuration and ***SOP 26104 – Design and Certification of HEPA-filtered Containment Cabinets***). This will typically include larger-scale production (1-10 L) in a disposable bioreactor and chromatography operations.

NOTE: All processes involving more than 10 liters of process fluid are considered BL-2-LS and require additional safeguards and approvals.

6.3 When working with infectious materials in a closed system outside of a BSC, incubator, or centrifuge where the infectious material is potentially above ambient pressure, personnel must wear either a Powered Air Purifying Respirator (PAPR) or HEPA filtered respirator to protect against a potential aerosol release. Any employee asked or requesting to wear respiratory protective equipment must be fitted and trained in the proper use of the equipment and medically cleared by Occupational Health Services.

6.4 Always wear eye protection (safety glasses or safety goggles) in the laboratory. Refer to area-specific gowning procedures (SOPs and/or posted signage) for additional gowning restrictions.

6.5 Protective laboratory coats or uniforms designated for laboratory use only are worn in the laboratory. This protective clothing is disposable. It is removed, deposited in the laboratory solid waste trash, and disposed of in accordance with the waste disposal procedures for each specific laboratory.

6.6 Wear two pairs of gloves when hands may contact potentially-infectious materials, contaminated surfaces, or equipment.

6.6.1 When working in a BSC with potentially infectious materials, disposable sleeve protectors and a second pair of gloves are worn, and both are removed to a suitable waste receptacle prior to exiting the BSC.

6.6.2 When working in a lab with potentially infectious materials, change the outer gloves whenever an operation that requires the handling of potentially infectious material is completed (e.g., transport of sealed centrifuge bottles to a centrifuge, or flasks, to an incubator).

6.6.3 The outer pair of gloves may be removed within the laboratory or in an adjacent airlock. Wash hands with Softcide or equivalent before exiting the airlock.

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6.7 Place all pipettes in a terminal pipette keeper box within the BSC prior to disposal by autoclaving.

6.8 Transfer of fluids containing infectious materials from one container to another can only be performed using a pipetting device or a pump. Transfer by pouring is not permitted. The discharge from the pipet or pump must be situated so as to minimize the potential for splashing.

6.9 If using a vacuum flask/aspirating pipette, to remove fluids from cultures use sterile, individually-wrapped plastic aspirating pipettes. Fluids are contained in the aspirating flask to which the decontamination agent (usually bleach) has been added. The aspirating flask containing culture material is in series with another flask containing decontamination agent (as an emergency overflow) and is in series with a 0.2µ fluid retention filter before connection to the vacuum system. Allow at least 30 minutes contact with the decontamination agent prior to disposing of the material in the vacuum flask. Alternatively, pipettes fitted with aerosol barrier tips can be used for handling small fluid volumes, or an aspiration device (such as Costar catalogue 4930) can be used with unplugged pipette tips to aspirate 96-well plates.

6.10 Conduct the transport of closed containers containing infectious materials within the laboratory using a cart. The process fluid will sit within a secondary container capable of handling the entire volume of fluid plus whatever volume of disinfectant is necessary to decontaminate a possible spill.

NOTE: A Rubbermaid-type container is appropriate for secondary spill containment on a cart. The container should be capable of completely containing the maximum volume of material being transported. All fluid transport at or above the 10 L scale within the laboratory will be a two-person operation.

7.0 Spills

7.1 Spills within a BSC (Class I or II) or Containment Cabinet

7.1.1 If spill volume is small enough to be absorbed on a single 12 X 12" absorbent pad the spill may be cleaned up by laboratory personnel.

7.1.1.1 Cover the spill with an absorbent pad.

7.1.1.2 Saturate the pad by slowly pouring Cavicide, Bleach Germicidal Disinfectant or bleach onto it to minimize splashing.

7.1.1.3 Wait an appropriate period of time to disinfect the spill (>3 minutes for Cavicide, >30 seconds for Dispatch).

7.1.1.4 Remove the absorbent pad, wipe up the residual liquid, and deposit all solid waste in an appropriate receptacle.

7.1.1.5 Contact the BDP Safety Officer [redacted] and Area Supervisor to report the spill. List the material spilled, the volume, location, any personnel involved in the spill, and the time of spill.

7.1.2 If the spill volume is not small enough to be absorbed on a single absorbent pad the spill cannot be cleaned up by laboratory personnel.

7.1.2.1 Absorbent pads may be used to contain the spill prior to evacuating the area.

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7.1.2.2 Disconnect power to all equipment inside the containment cabinet using the nearest power source OUTSIDE of the cabinet prior to evacuating the area.

7.1.2.3 Evacuate the area and notify the ATRF Safety Officer ([redacted]), the Area Manager, and the BDP Safety Officer ([redacted]).

7.1.2.4 Wait for EHS personnel to arrive and provide information regarding the nature of the spill.

7.2 Spills Outside of a BSC

7.2.1 If an aerosol was generated, immediately evacuate the area and close doors to the laboratory upon exit.

7.2.2 If personnel were exposed, immediately remove any contaminated laboratory clothing and exit the laboratory area.

7.2.3 After exiting the laboratory area, wash exposed skin with Povidone-iodine lotion followed by soap and water.

7.2.4 If an eye exposure is suspected, flush eyes with copious volumes of water at the eyewash station for 15 minutes.

7.2.5 If no aerosols are suspected, and a spill kit is readily accessible, spills may be contained before personnel exit the laboratory.

7.2.6 If a spill is noticed upon opening a centrifuge, keep all materials inside the centrifuge. Re-close the lid, evacuate the area, and close the door to the laboratory upon exit.

7.2.7 Contact the ATRF Safety Officer ([redacted]) and the BDP Safety Officer ([redacted]) to report the spill. List the material spilled, the volume, location, any personnel involved in the spill, and the time of spill.

NOTE: Exposed personnel must report immediately after emergency action to Occupation Health and Safety for a post-exposure evaluation! The Supervisor must complete an Accident Report Form and inform the BDP Safety Officer.

8.0 Cleaning/Sanitization of BSC

8.1 Clean/Sanitize the laboratory equipment (e.g., centrifuges, incubators, water baths, etc.) according to ***SOP 19102 - Routine Use and Disinfection of Biological Safety Cabinets, Incubators, Shakers, and Centrifuges*** or ***SOP 22909 - Use, Cleaning and Disinfection of Equipment and Laboratories in PA/QC.***

8.2 After working in the BSC or containment cabinet, disinfect with Bleach Germicidal Cleaner (Dispatch), Cavicide, Sporicidin, Steri-Perox 6% , or equivalent, based on the rotational schedule.

9.0 Centrifugation of Infectious Material

NOTE: Consult the manufacturer's specifications for the type of tube or bottle to be used with each type of rotor. The operator is responsible for ensuring that the selected tubes/bottles are suitable for the specific rotor used. Be sure to determine the

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maximum speed for use and determine whether the tube must be filled to avoid collapse during centrifugation. Place the tube in the centrifuge so that the seam will not be subjected to the full force of the centrifugation.

9.1 Centrifuge bottles/tubes must be filled inside the BSC per Step 4.8, while inside a bottle rack that provides support from tipping.

NOTE: 1) ALL centrifuge tubes must be gasketed.

2) Follow procedures for each specific centrifuge to ensure that tubes are properly balanced.

9.2 If possible, load the rotor in the hood and seal it prior to removal from the hood.

9.3 Disinfect the rotor and/or bottles and racks with Cavicide or Dispatch followed by Decon-ahol before removal from the BSC.

9.4 Transport materials to and from the centrifuge per Step 4.9.

9.5 The rotor bucket must be equipped with a gasketed cover.

9.6 If any unusual noises are noted during the centrifugation, shut down the centrifuge immediately, evacuate the laboratory and contact the ATRF Safety Officer ([redacted]) and the BDP Safety Officer ([redacted]). Do NOT attempt to open the centrifuge.

10.0 Handling Non-infectious and Infectious Cultures Simultaneously

10.1 Maintain separate bottles of medium for each non-infectious and infectious cell culture.

10.2 Always handle the non-infectious cultures first and disinfect the BSC.

10.3 Infectious cultures are handled after all non-infectious cultures have been manipulated.

11.0 Decontamination of Waste

11.1 Decontaminate liquids by addition of sufficient Clorox bleach (BDP PN 20295) to account for at least 10% of the final volume of liquids and mix. Allow contact for at least 30 minutes.

Untreated solutions containing potentially infectious materials cannot be discarded down a drain. All solutions that have come into contact with infectious materials must first be inactivated with bleach (Section 11.1). The solutions can then be discarded down a drain, followed with water.

11.2 Dispose of decontaminated liquids down a drain and follow with water. Alternatively, waste may be delivered to EHS for disposal.

11.3 Observe any applicable area-specific liquid waste handling procedures when transporting liquid waste.

11.4 Double bag, autoclave, and dispose of solid waste including gowning according to area-specific solid waste handling procedures. After autoclaving, place bag(s) in a black trash bag to be disposed of by maintenance staff.

11.5 Sharps containers are placed in a red bag taped shut and then placed in a biohazard box. The box is taped shut and labeled “Incinerate”. Do not place the box in a black trash bag. The custodial staff will transport the box to the Receiving department to be picked up by a contractor for incineration.

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

12.0 Documentation

12.1 Record equipment usage and cleaning in their respective logbooks.

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

13.0 References and Related Documents

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

13.2 **SOP 22909** *Use, Cleaning and Disinfection of Equipment and Laboratories in PA/QC*

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

13.4 **SOP 26104** *Design and Certification of HEPA-filtered Containment Cabinets*

13.5 **SOP 26106** *Spill Control and Clean-up in the BDP Production Areas of the* [redacted]

13.6 **SOP 26301** *Policies and Procedures for Registering Research with the NCI Frederick Institutional Biosafety Committee (IBC)*

13.7 BDP Infectious and Potentially Infectious Agent Handling Handbook located at [redacted]

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

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

13.10 Frederick National Laboratory Environmental Health, and Safety Program. Health, Safety and Environmental Compliance Program Manual. Current version. [redacted].

13.11 Environment, Health and Safety. Safetygrams. Current version.

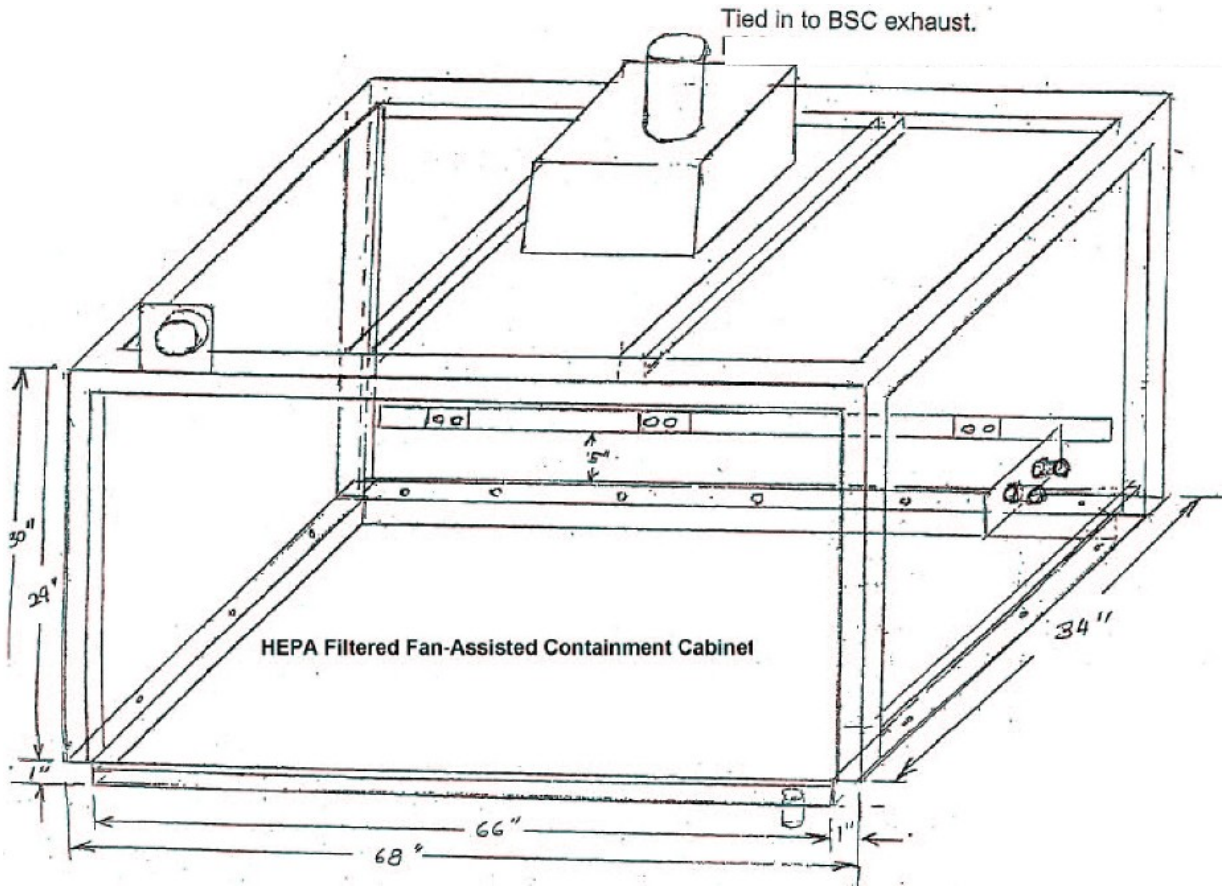
14.0 Attachments

14.1 **Attachment 1** Engineering Drawing for Fan-assisted HEPA Filtered Containment Cabinet

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

Attachment 1

Engineering Drawing for Fan-assisted HEPA Filtered Containment Cabinet



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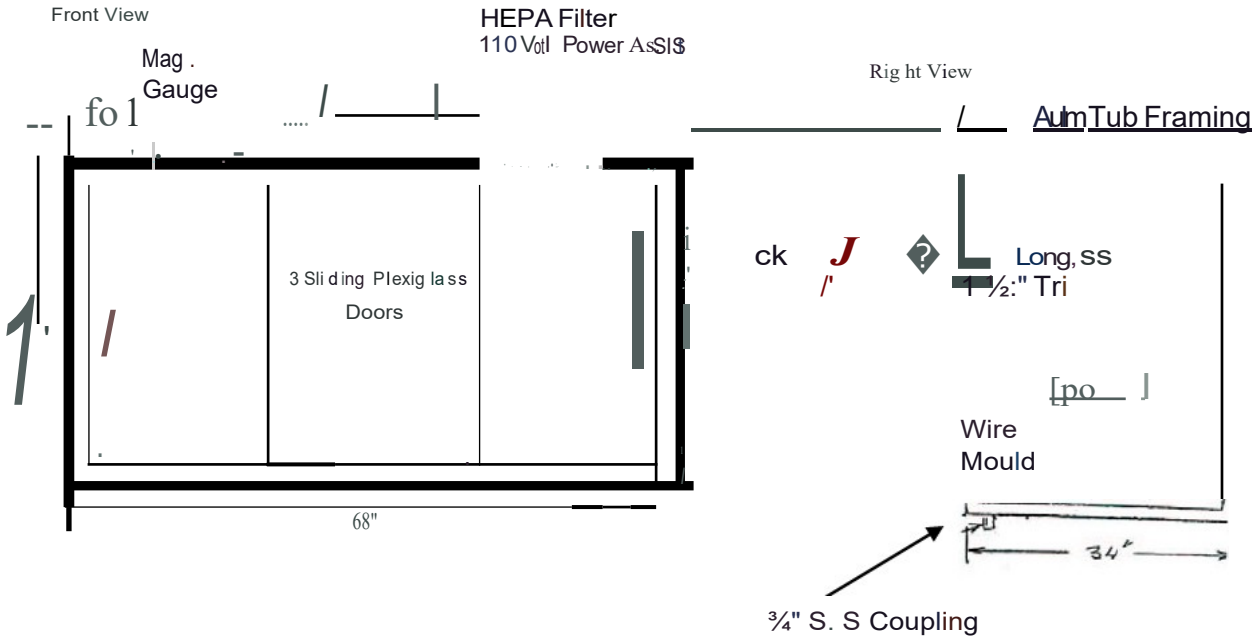
Attachment 1 (Continued)

Engineering Drawing for Fan-assisted HEPA Filtered Containment Cabinet



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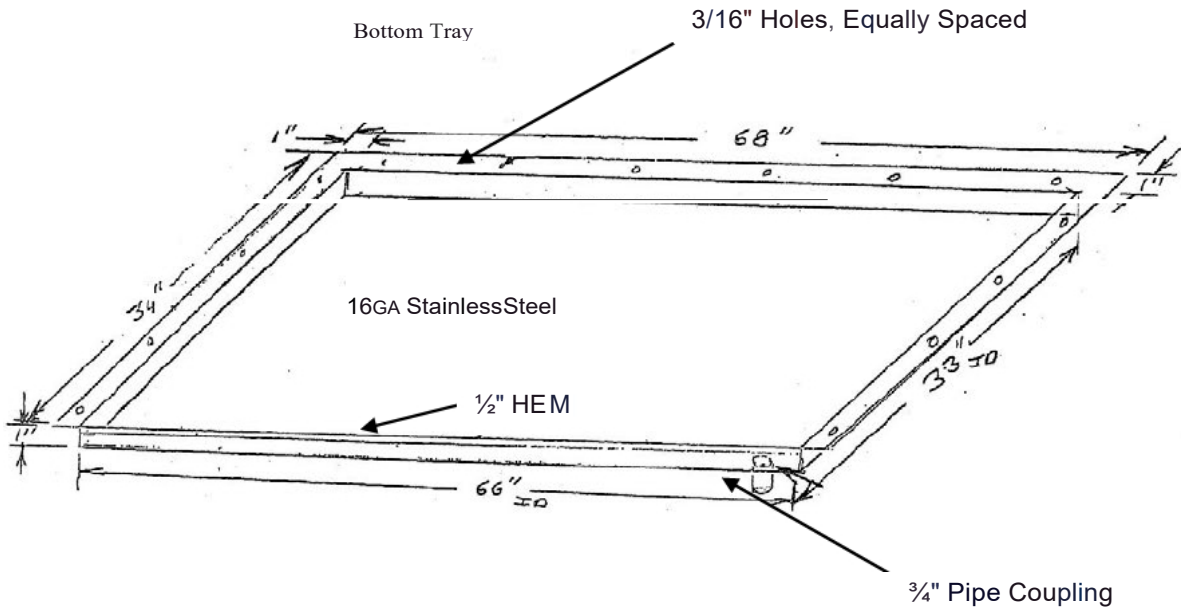
Attachment 1 (Continued)

Engineering Drawing for Fan-assisted HEPA Filtered Containment Cabinet



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