

# Standard Operating Procedure for Droplet-based Sorting of Biosafety Level 2 (BSL2) Samples

**Lab Location:** \_\_\_\_\_

**Original Issue Date:** 04/26/2010 **Revision Date:** 07/28/2010

**Machines/Facilities:** - Modified BD Biosciences FACS Aria with  
Aerosol Management System / Stem Cell Institute  
- SORP FACS Aria II with biosafety cabinet /  
Department of Microbiology

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**Approval Signature:**  \_\_\_\_\_

## 1.0 Introduction

To assure the assignment of proper safety practices and procedures, cytometry staff will collect detailed information about all samples prior to receipt. Facility specific Sorting Information Sheets and/or Biohazard Sort Forms must be completed and submitted to the Flow Cytometry Facility Manager prior to the scheduling the first sort of each project. The information will be reviewed by the facility and appropriate containment will be assigned based on risk in consultation with the Biosafety Officer. An approved IBC protocol which includes a cell sorting protocol must be on file with the IBC office.

## 2.0 Facilities

2.1 The facilities will comply with BSL-2 practices as per BMBL, including the following:  
Cell sorter will be contained within a Class II biosafety cabinet within a BSL-2 laboratory or the cell sorter must be located in a separate, lockable room where no other lab activity is performed.

2.2 Access to the BSL2 cell sorting facility is restricted to those persons whose presence is required for experimental support (operator plus a maximum of 2 others), as determined by flow cytometry staff.

2.3 The room should be under negative pressure between 2-10 Pascals (0.008 – 0.10 inches of H<sub>2</sub>O).

### 3.0 Samples

**Note: If BSL-2 cells are fixed, verification that fixative concentrations and durations effectively inactivate potential infectious agents (12) must be provided to the IBC. Otherwise, these cells will be considered BSL-2 and handled accordingly.**

3.1 BSL-2 samples must be contained in a leak proof container and clearly labeled with a sample identifier.

3.2 Samples should be transported on campus in sealed leak-proof primary and secondary containment. The secondary (outer) container should have the biohazard sticker.

3.3 Sample must be a single cell suspension and in an appropriate capped tube for sorting (12x75mm tube, 1ml tube, or 15ml conical tube).

3.4 All samples for sorting must be filtered prior to sorting to reduce the potential for clogging and decrease the risk of generating aerosols. Should the stream become clogged, customers will be asked to re-filter the sample in a Class II biosafety cabinet.

## 4.0 Stem Cell Institute BSL-2 Designated FACS Aria

### 4.1 BIO-CONTAINMENT AND INFECTIOUS CELL SORTING

4.1.1 The FACS Aria is equipped with an aerosol management system (AMS). It is the operator's responsibility to ensure that the AMS is turned on and functioning according to the manufacturer guidelines. Annual certification of the AMS will be performed by Brager Scientific, River Falls, WI, or alternate service provider.

4.1.2 The vacuum monitor on the AMS should be set to 20% and the vacuum gauge must read between 1.0 and 1.5 inches of H<sub>2</sub>O. If it is outside of this range, check to make sure that the filter is seated correctly. If vacuum gauge does not come into range, tubing will be inspected and the tubing and/or ULPA filter unit will be replaced if defects are detected.

4.1.3 The sheath waste tank must contain enough bleach to provide a final concentration of 10% when filled (1L bleach to a final 10L waste collected). The sheath waste tank will be emptied at the end of each sort. Fresh 10% bleach will be added at the beginning of each sorting procedure.

4.1.4 The droplet and sort stream camera systems must be functioning normally according to the manufacturer guidelines. These camera systems will be used to monitor the sort stream.

## 4.2 SORTING PROCEDURE FOR INFECTIOUS SAMPLES

4.2.1 Upon entering the BSL2 Sorter room, all personnel must wear personal protective equipment (PPE) according to BSL-2 guidelines if unfixed BSL-2 samples are to be sorted.

4.2.2 All personnel must be adequately informed of the risks of working with all agents within the room and the potential consequences of exposure to these agents.

4.2.3 Complete startup and QC procedure of FACS Aria will be performed, and the operator will confirm that the sheath tank is full.

4.2.4 Concentrated bleach will be poured into the waste tank making sure that there is 10% vol/vol bleach when the tank is full.

4.2.5 The waste tank will be emptied into the sink drain only after the waste liquid has been exposed to a final concentration of 10% bleach for at least 30 minutes.

4.2.6 All barriers around the sort chamber will be closed after the integrity of the gaskets has been confirmed.

4.2.7 Start the sort and visually monitor the sort performance using the camera systems. If during the sort the stream is deflected (due, for example, to a clogged flow cell tip), the sort will be stopped either by the operator or automatically by the FACS DiVa software. The sort will not restart until the operator has cleared the clog.

### 4.2.8 **The following procedure will be used to remove a clog from the Cytometer:**

4.2.8.1 Remove the sample from the sample chamber, recap the sample tube.

4.2.8.2 Turn stream off and then on again to attempt to clear the nozzle or sample line obstruction.

4.2.8.3 Run "Sample Line Back flush" for 20 sec followed by "Clean Flow Cell" using distilled water.

4.2.8.4 If the nozzle cannot be cleared, the system should be shut down and 5 minutes allowed to reduce the potential inhalation of aerosols generated during the sorting process.

4.2.8.5 Ensure that the high voltage deflection plates are turned off (view red light).

4.2.8.6 Open the collection chamber and remove the collection vials.

4.2.8.7 Remove the nozzle from the instrument, detach the o-ring from the nozzle and immerse the nozzle in a 10% bleach solution for 10 minutes,

then place it in a 50 ml centrifuge tube containing Contrad 70 solution. Sonicate the nozzle for 2-5 minutes in a bath sonicator.

4.2.8.8 After replacing the nozzle, gloves used for the cleaning procedure will be discarded and fresh gloves will be applied.

4.2.8.9 The area around the FACS sorter will be wiped with fresh 10% bleach for a contact time of 30 minutes, followed by 70% ethanol.

4.2.8.10 Sorting can be resumed after the obstruction is cleared such that the stream is stable and the droplet break-off and side streams are stable.

4.2.8.11 After sort is completed the sort collection tubes will be removed immediately after sample tube is unloaded from the sample station.

4.2.9 When the sort is finished, a 12 x 75 tube containing 4mL of 10% bleach solution will be loaded into the bulk injection chamber and run for 10 minutes at a flow rate of 10. Bleach will be run through the sample lines for 10 minutes (i.e. a long clean with bleach) and the inside and outside of the sort chamber will be sprayed and wiped down with 70% ethanol.

4.2.10 Areas around the FACSaria will be wiped with fresh 10% bleach solution. After 30 minutes, surfaces will be wiped with 70% ethanol.

4.2.11 All materials generated in the process of the sorting procedure or in the shutdown and cleaning procedure will be disposed of in a red Biohazard bag and disposed of appropriately.

## **5.0 Department of Microbiology BSL-2 FACSaria II**

### **5.1 BIO-CONTAINMENT AND INFECTIOUS CELL SORTING**

5.1.1 Access to the laboratory containing the SORP FACSaria II is contingent on specific approval by Dr. Ashley Haase. All personnel will be informed of the risks of working with all agents within the room and the potential consequences of exposure to these agents.

5.1.2 The SORP FACSaria II is contained within a Baker BioProtect III LE Class II biosafety cabinet and is equipped with an aerosol management system (AMS). The system operator will ensure that the biosafety cabinet and AMS are turned on and functioning according to the manufacturer guidelines. Annual certification of both the hood and the AMS will be performed by Brager Scientific, River Falls, WI, or an alternative service provider.

5.1.3 The vacuum monitor on the AMS should be set to a minimum of 10% and the vacuum gauge must read between 1.0 and 1.5 inches of H<sub>2</sub>O. If it is outside of this range, the system will be checked to make sure that the filter is seated correctly. If vacuum gauge does not come into range, tubing will be inspected and the tubing and/or ULPA filter unit will be replaced if defects are detected.

5.1.4 The sheath waste tank must contain enough of an appropriate germicidal solution to provide a final concentration of  $\geq 10\%$  when filled (example: 1L bleach to 9L waste collected to give a final volume of 10L). The sheath waste tank will be emptied at the beginning of each sort and rinsed with the appropriate germicidal solution. Fresh germicidal solution will be added at the beginning of each sort procedure.

5.1.5 The droplet and sort stream camera systems must be functioning normally according to the manufacturer guidelines so the camera systems will be used to monitor the sort stream.

## **5.2 SORTING PROCEDURE FOR INFECTIOUS SAMPLES**

5.2.1 Prior to initiation of sorting, specific sorting procedures for each BSL-2 organism will be approved by the Department of Microbiology BSL-2 Cell Sorting Facility advisory committee to verify the containment and decontamination procedures are appropriate for the BSL-2 organism to be sorted. Formal review and approval by the IBC is also necessary before experiments with infectious agents can be initiated ([www.research.umn.edu/ibc](http://www.research.umn.edu/ibc)). Alternate protocols may be developed in consultation with the advisory committee and the Biosafety Officer if the basic procedure outlined below is deemed insufficient for certain organisms.

### **5.2.2 Entry into the BSL-2 laboratory containing the SORP FACSaria II.**

5.2.2.1 All research personnel must be properly trained in the safety procedures outlined below. General guidance on safety procedures is available from Dr Peter Southern.

5.2.2.2 Dress in protective clothing is mandatory. Protective clothing should include a disposable gown, double gloves, shoe covers (closed toe shoes required), surgical mask and eye protection (safety glasses, shatter proof spectacles, or a full face shield).

5.2.2.3. Upon entry into the laboratory, empty sterilized waste from autoclave into appropriate containers.

5.2.2.4. Prepare fresh germicidal solution (use a final concentration of 10% bleach unless otherwise specified) in a 1L beaker and place inside hood for pipet tip and other liquid waste .

5.2.2.5. Set-up a dry biohazard waste bag inside the biosafety cabinet

5.2.2.6. The sheath waste tank will be removed, placed in a Class II biosafety cabinet and contents transferred to an autoclave safe container.

The waste tank will be rinsed with the germicidal agent and the rinse solution placed in an autoclave safe container. The autoclave safe container will then be placed in the autoclave to prepare for the subsequent autoclave run.

5.2.2.7. Wipe down the working surfaces inside the biosafety cabinet with 70% ethanol.

5.2.2.8. Fill sheath waste tank with the appropriate germicidal solution (use a final concentration of 10% bleach unless otherwise specified)

5.2.2.8. Spray secondary container with BSL-2 sample with 70% ethanol and place into biological safety cabinet.

5.2.3 Complete startup and QC procedure of FACS Aria II will be performed, and the operator will confirm that the sheath and ethanol tanks are full.

5.2.4 Run the “Aseptic Cleaning Mode” to ensure sterilization of the machine and tubing. This cleaning program involves a 45 minute series of ethanol and 10% bleach washes.

5.2.5 The BSL-2 samples will be uncapped and inserted into the machine. All barriers around the sort chamber will be closed and the integrity of the gaskets will be confirmed prior to sort initiation.

5.2.6 Start the sort and visually monitor the sort performance using the camera systems. If during the sort the stream is deflected (due, for example, to a clogged flow cell tip), the sort will be stopped either by the operator or automatically by the FACS DiVa software. The sort will not restart until the operator has cleared the clog.

**5.2.7 The following procedure will be used to remove a clog from the Cytometer:**

5.2.7.1 Turn stream off.

5.2.7.2 Remove the sample from the sample chamber, recap the sample tube.

5.2.7.3. Turn stream on again to attempt to clear the nozzle or sample line obstruction.

5.2.7.4. Run “Sample Line Back flush” for 20 sec followed by “Clean Flow Cell” using distilled water.

5.2.7.5. If the nozzle cannot be cleared, the system should be shut down.

5.2.7.6. Open the collection chamber and remove the collection vials.

5.2.7.7. Wait 5 minutes for potential aerosols to be cleared from the biosafety cabinet then remove the nozzle from the instrument. For integrated nozzles, wash nozzle with 70% ethanol, place nozzle in 3 ml tube containing deionized water, and sonicate the nozzle for 2-5 minutes in the bath sonicator in the hood. For non-integrated nozzles, detach the o-ring from the nozzle and discard, wash nozzle with 10% bleach, place nozzle in 3 ml tube containing 10% bleach, and sonicate the nozzle for 2-5 minutes in the bath sonicator in the hood, insert new o-ring prior to using the nozzle.

5.2.7.8. After replacing the nozzle, outer gloves used for the cleaning procedure will be discarded and fresh outer gloves will be applied.

5.2.7.9. Sorting can be resumed after the obstruction is cleared such that the stream is stable and the droplet break-off and side streams are stable.

5.2.8 After sort is completed, the sort collection tubes and sample tube will be removed and capped. The sort collection and sample tubes will be sprayed with the germicidal

agent and placed in the secondary container (appropriately labeled as containing BSL2 sample) prior to removal from the BSL-2 laboratory.

5.2.9 After all sorts of the organism have been completed, 10% bleach will be run through the sample line tubing for 10 minutes followed by the “Fluidics Shut-down” that replaces all fluidics in the machine with 70% ethanol until the next usage. The inside and outside of the sort chamber will be sprayed and wiped down with 70% ethanol.

5.2.10 Areas around the FACSaria will be wiped with fresh 10% bleach solution or other suitable germicidal solution followed by 70% ethanol for a total contact time of 30 minutes.

5.2.11 The dry and liquid biohazard waste containers in the hood will be loosely closed, sprayed with the germicidal agent, and removed from the hood to the autoclave after 30 minutes.

**5.2.12 Exit from the BSL-2 laboratory containing the SORP FACSaria II.**

- 5.2.12.1 Carry all dry and liquid waste containers to autoclave
- 5.2.12.2 Discard outer gloves into dry waste bag inside autoclave
- 5.2.12.3 Start autoclave
- 5.2.12.4 Enter name, time, and date on the autoclave log
- 5.2.12.5 Remove protective clothing and discard in metal waste container
- 5.2.12.6 Wash hands in changing room
- 5.2.12.7 Verify that autoclave has come up to pressure and temperature

5.2.13 The blower in the Class II biosafety cabinet will be left running continuously, even when experiments are not in progress.

**5.2.14 Autoclave procedures**

5.2.14.1 All waste materials must be autoclaved prior to disposal. The autoclave will be run for a minimum of 30 minutes at 121C on the liquid cycle and for solids, a minimum of 60 minutes at 121C or 125C. Once sterilized, waste is transferred from the autoclave into a plastic (tear-resistant) biohazard waste bag. No liquids are ever discarded in biohazard bags. When full, these biohazard waste bags are securely taped shut with duct tape and placed in the corridor outside the Containment Laboratory for collection by the janitors. After sterilization, glass and plastic ware not intended for disposal may be safely removed from the Containment Laboratory.

5.2.14.2 Chemical integrator strips are included at monthly intervals, to verify complete sterilization.

5.2.14.3 All items placed in the autoclave for sterilization should be prominently marked with autoclave tape. *It is the specific responsibility of the person unloading the autoclave to verify that the sterilization cycle has been completed successfully. As a routine practice, it is essential that the*

*autoclave should be emptied, and the contents appropriately stored, prior to initiating any experiments or new waste-generating procedures.* In this manner, newly created waste can be placed directly into the empty autoclave and there is no possibility of contaminating previously sterilized waste by contact with non-sterilized materials. Chemical integrator strips to verify the sterilization procedure are used at monthly intervals.

5.2.14.4 The autoclave log must be completed for every autoclave cycle.

5.2.14.5 The autoclave is serviced at regular intervals under a routine maintenance contract. Tim Leonard is the contact person in the Department of Microbiology (Mayo Room 953, Tel: 4-0977) for autoclave service problems. Under rare circumstances (e.g. steam supply problems) non-sterilized waste may be left inside the autoclave until it is possible to begin a complete sterilization cycle. If non-sterilized waste is left inside the autoclave, the autoclave door must be fully closed and a prominent warning sign complete with name, time and date, must be attached securely to the autoclave door handle. Waste should be left inside the biosafety cabinet, together with a warning sign if the autoclave is unavailable for immediate use. The person responsible for the waste in the biosafety cabinet should then return later in the day to autoclave their waste materials.

#### **5.2.15 Spill procedures**

5.2.15.1 Minor spills inside the biosafety cabinet should be covered with paper towels saturated with the germicidal solution. The spilled liquid and germicide-soaked towels are then collected inside dry paper towels and discarded into the dry waste bag inside the biosafety cabinet. The affected area is then washed with the germicidal agent, after 30 minutes the area is washed down extensively with 70% ethanol.

5.2.15.2 Minor spills outside the biosafety cabinet are surrounded by the germicidal solution and then dry paper towels are used to drag the solution into contact with the spilled liquid. It is important to minimize the formation of aerosols. After waiting 30 minutes to allow full inactivation of infectious materials by the germicidal solution, the saturated paper towels are collected inside dry paper towels and placed directly into an autoclave bag for sterilization.

5.2.15.3 In the event of a major incident or spill (FACS explosion) all personnel will immediately terminate all activities as rapidly and as safely as possible and will vacate the Containment Laboratory. Warning signs will be posted and the access door will be sealed. The Biological Safety Officer (BSO) will immediately be notified and nobody will be allowed to enter the Containment Laboratory until an appropriate plan of action has been designed and approved by the BSO.

5.2.15.4 In the event of an operating problem with the autoclave, waste will be stored inside one of the biosafety cabinets and experimental activities will be reduced to a minimum essential level until such time as

the autoclave is repaired and the waste can be properly sterilized and removed.

## **6.0 Hazard Identification and Risk of Exposure to the Hazards**

6.1 Hazards are possible exposure to infectious aerosols produced when:

- 1) transporting BSL-2 samples to the BSL-2 cell sorting laboratory;
- 2) sorting of BSL-2 samples on the Modified BD Biosciences FACS Aria.

6.2 Although many infectious organisms that require BSL-2 containment are not technically infectious via the aerosol route, there is a risk that aerosolized infectious agents may be ingested or come in contact with nasopharyngeal mucous membranes of persons working in the BSL-2 laboratory.

6.3 Risk of exposure may be high if there is a failure of the ULPA filter on the FACS Aria or the Class II biosafety cabinet. This potential risk will be determined via risk assessment for the infectious agent in question.

## **7.0 Exposure Controls Specific to Above Risk of Exposure**

See Sections 2, 3, 4, 5 above.

## **8.0 Waste Generated and Disposal Methods**

See Section 5 above and the attached Biological Waste Disposal Plan (Appendix A)

## **9.0 Spill and Accident Response Procedures**

See attached Biological Decontamination & Spill Clean-up Plan Template (Appendix B)

## **10.0 Immunizations**

All personnel working in the BSL-2 cell sorting facility must be offered a Hepatitis B vaccination by the employer for work with human cells. Consultation with Occupational Health and Safety should be made regarding any other immunizations that may be required.

## **11.0 Training**

All personnel who will be operating the Modified BD Biosciences FACS Aria will undergo training.

## 12.0 References

1. Merrill J.T. Evaluation of selected aerosol-control measures on flow sorters. *Cytometry* 1981; 1: 342-345.
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3. Karen B. Byers. Biosafety Tips. *Applied Biosafety* 2008; 13:(1)
4. Stephen Perfetto, Kevin Holmes. Biosafety Issues - Biohazard Sorting Update *ISAC E-News* MARCH 2009: [www.isac-net.org](http://www.isac-net.org)
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6. Schmid I, Nicholson J.K., Giorgi J.V., Janossy G., Kunkl A., Lopez P.A., Perfetto S., Seamer L.C. & Dean P.N. Biosafety guidelines for sorting of unfixed cells. *Cytometry* 1997; 28: 99-117.
7. Schmid I. & Dean P.N. Introduction to the biosafety guidelines for sorting of unfixed cells. *Cytometry* 1997; 28: 97-98.
8. Stephen P. Perfetto., David R. Ambrozak., Richard A. Koup., Mario Roederer. Measuring Containment of Viable Infectious Cell Sorting in High-Velocity Cell Sorters. *Cytometry Part A* 2003; 52A: 122–130
9. Guidelines C. 1997 revised guidelines for performing CD4+ T-cell determinations in persons infected with human immunodeficiency virus (HIV). Centers for Disease Control and Prevention. *MMWR Recomm Rep* 1997; 46: 1-29.
10. Schmid I., Kunkl A. & Nicholson J.K. Biosafety considerations for flow cytometric analysis of human immunodeficiency virus-infected samples. *Cytometry* 1999; 38: 195-200.
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13. Blood borne pathogens policies: <http://bmbl.od.nih.gov/>
14. NIH BL-2 safety guidelines: <http://bmbl.od.nih.gov/sect3bsl2.htm>
15. NIH BL-3 safety guidelines: <http://bmbl.od.nih.gov/sect3bsl3>

This SOP was adapted from NIAID Bio-Containment and Infectious Cell Sorting (FACS Aria) <http://www3.niaid.nih.gov/labs/aboutlabs/VRC/flowCytometryCoreLaboratory/BioContainmentandInfectiousCellSortingFACSAria.htm>

# APPENDIX A

## Biological Waste Disposal Plan

<b>P.I./Lab Supervisor(s):</b> Sue Keirstead, Kirsten Nielsen, Peter Southern	<b>Lab Location:</b> 2-419 McGuire Translational Research Facility, 1340 Mayo Building Department of Microbiology
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**Types of Biological Waste Generated:**

- Liquid Biological Waste
- Solid Biological Waste
- Animal Tissue, Carcasses & Bedding
- Toxins
- rDNA (including solid & liquid waste)
- Sharps
- Prions
- Cell Culture / Blood / Body Fluids (e.g., human / NHP / animal cells, blood, serum, body fluids, etc)
- Human Organs, Tissue or Body Parts

Please indicate any other types of biological, or mixed biological with other hazardous wastes that will be generated.

**NOTE: For each type of waste checked or listed above, indicate the disposal method below.**

**Liquid Biological Waste Disposal (including infectious and noninfectious, rDNA or cell culture / blood / body fluids):**

- Autoclave for 30 minutes at 121°C on liquid cycle.  
Test autoclave monthly with integrator per *Autoclaving Biological Waste Fact Sheet* below.

**OR**

- Disinfect with 10% (1:9 v/v) bleach for at least 30 minutes.
- List other proven effective disinfectant /concentration underneath: (minimum 30 minutes contact time).

**Solid Biological Waste Disposal (including infectious and noninfectious, rDNA or cell culture / blood / body fluids):**

- Autoclave for 60 minutes at 121°C or 125 °C. Test autoclave monthly with integrator

**OR**

- Place biological waste in the red biohazard bag and dispose of appropriately, fill to no more than ¾ full, seal and place in the designated waste area in the lab.

**Animal Tissue, Carcasses and Bedding:**

- Place animal tissue and carcasses in cooler designated by RAR.
- Handle animal cages, bottles, and bedding per RAR instructions.

**Toxins:**

- Treat with 2N NaOH for at least 1 hour.
- Describe other proven effective inactivating agent.

**Human Organs, Tissue or Body Parts:**

- Call Bequest Program 625-1111

**Sharps (contaminated):**

- Place sharps in sharps container, fill to no more than ¾ full, seal and place in the designated waste area in the lab.

**Prions (call waste management at 5-6481 for yellow bag and yellow barrel delivery & pick-up):**

- Place non-tissue low level solid waste (including animal bedding) in yellow waste bag in yellow barrel for incineration.
- Autoclave liquid waste at 134°C for 1 hour.
- Wipe instrument for re-use thoroughly clean, immerse in 2N NaOH for 1 hour, rinse with water, autoclave at 134-138°C for 18 minutes.
- Wipe instrument for disposal clean, soak in 2N NaOH for 1 hour at 20°C, then disposal.
- Place sharps in sharps container, fill to no more than ¾ full, seal and place in yellow waste bag for incineration.
- Dispose animal tissue and carcasses in animal digester.
- Describe other proven effective disinfectant.

**Other Waste Disposal:**

**Waste Disposal Reference:**

- Autoclaving Biological Waste Fact Sheet,  
<http://www.dehs.umn.edu/PDFs/autoclaveBioWaste.pdf>
- Chemotherapy Drug Disposal Fact Sheet,  
<http://www.dehs.umn.edu/PDFs/Chemo%20Waste%20Disposal%20fact%20sheet.pdf>
- Environmental Health and Safety's Waste Flow Chart,  
[http://www.dehs.umn.edu/bio\\_wastedisptble.htm](http://www.dehs.umn.edu/bio_wastedisptble.htm)
- Inactivation of Toxins, [http://www.dehs.umn.edu/bio\\_disposal.htm#inactivation](http://www.dehs.umn.edu/bio_disposal.htm#inactivation)
- Sharps Disposal, [http://www.dehs.umn.edu/bio\\_pracprin\\_su\\_ss.htm](http://www.dehs.umn.edu/bio_pracprin_su_ss.htm)
- Prion Waste Disposal, [http://www.dehs.umn.edu/bio\\_pracprin\\_prions\\_sp.htm](http://www.dehs.umn.edu/bio_pracprin_prions_sp.htm)
- Hazardous Chemical Waste Management Guidebook,  
[http://www.dehs.umn.edu/hazwaste\\_chemwaste\\_umn\\_cwmgbk.htm](http://www.dehs.umn.edu/hazwaste_chemwaste_umn_cwmgbk.htm)

## APPENDIX B

### Biological Decontamination & Spill Clean-up Plan Template

This template can be used in writing lab specific SOPs (Standard Operating Procedures). It should be posted in the lab for workers reference and reviewed with workers annually. This customized template is a required attachment when IBC forms are submitted. **The top section and any Lab Specific Requirements must be filled in.**

<b>P.I./Lab Supervisor:</b> <b>Lab Location: 2-419 McGuire</b> <b>Translational Research Facility, 1340</b> <b>Mayo Building Department of</b> <b>Microbiology</b>		<b>Emergency Contact Info: Sue Keirstead 612 626 2290</b> <b>Kirsten Nielsen 612 625 4979</b> <b>Peter Southern 612 625 2141</b>  (report all spills to P.I. or Lab Supervisor and Biosafety Officer)
Biological Agent (s)	Disinfectant / Concentration / Contact time	Cleanup Procedures (bench top, centrifuge, etc.)
BSL-2 unfixed cells	<input checked="" type="checkbox"/> Bleach / 10% / 30 minutes <input checked="" type="checkbox"/> Other proven effective disinfectant:	For spills or decontamination of area - 10% Bleach for 30 minutes  For decontaminating FACS Aria tubing, etc. between procedures – 10% bleach for 10 minutes

**Spill Response Equipment:**

- Written spill procedure including emergency phone numbers
- Disinfectant suitable for biological materials being used
- Paper towels, gloves, shoe covers, safety goggles
- Forceps to pick up sharps, including broken glass
- Sharps container for broken glass, etc.
- Squeegee & dust pan that can be decontaminated
- Biohazard bags (red bags or autoclave clear bags for 60 minutes at 121°C)

**Lab Specific Requirements (please describe below):****Small and moderate spills outside the biosafety cabinet:**

- Remove any contaminated clothing and put in autoclavable bag. Be aware that autoclaving may damage fabric.
- Notify other workers in the area of the spill and control traffic through area.
- Wear shoe covers and safety goggles if spill is on floor, may be splashed beyond immediate area of spill.
- Put on gloves and cover spill area with paper towels.
- Pour disinfectant over towels from edges of spill to center, be careful not to splatter.
- Decontaminate all objects in spill area.
- Allow 30 minutes of contact time.
- Pick up any sharps, including broken glass, with forceps and place in sharps container.
- Use squeegee and dust pan to recover any shards of broken glass in contaminated liquid.
- Wipe area with disinfectant and clean towels, mop if spill on floor.
- Remove gloves and foot covers before leaving area of the spill, put in biohazard bag, and wash hands.

**Lab Specific Requirements (please describe below):****Large spills (>100ml) in or outside of the biosafety cabinet:**

- Evacuate room, close doors, prevent others from entering, and wait 30 minutes for aerosols to settle.
- Follow procedures for small and moderate spills.

**Lab Specific Requirements (please describe below):****For small spills in a biosafety cabinet:**

- Wipe down all interior cabinet surfaces with appropriate disinfectant.
- Wipe down all supplies and equipment in cabinet.

**Lab Specific Requirements (please describe below):**

N/A

**For moderate spills in a biosafety cabinet, follow general spill procedures plus:**

- Leave the cabinet running.
- Wipe down all interior surfaces.
- Determine if spill has gone beyond the work surface such as in the grilles or side seams. Disassemble and decontaminate if necessary.
- If the cabinet has a catch basin below the work surface that may be involved in the spill, flood the basin with disinfectant. Do not use alcohol as a large quantity of alcohol presents a flammable hazard. Clean basin after 20 minutes.
- Autoclave or wipe down all items in cabinet with disinfectant.
- Let cabinet run for at least 10 minutes after cleanup.

**Lab Specific Requirements (please describe below):**

N/A

**For major spills in a biological safety cabinet:**

- Contact the Biological Safety Officer in DEHS (626-6002) to determine if professional decontamination is indicated

N/A

**For any spills of agents that are transmitted by inhalation, such as *Mycobacterium tuberculosis*, evacuate the lab immediately, close the door, do not allow any one to enter the lab, remove any contaminated clothing, wash exposed skin with soap and water, call the Biosafety Officer for assistance at 626-6002.**

**If Spill Results in a Hazard Exposure ( i.e. face or eye splash, cut or puncture with sharps, contact with non-intact skin):**

- Call 911 (a university phone will speed the dispatch process), wash exposed skin with soap & water, flush eyes for 15 min
- Seek medical attention.
  - During business hours DEHS suggests that you go to Boynton Health Service Urgent Care. See [Boynton's Web site for location and hours](#). This location is fully prepared to deal with laboratory hazard exposures.
  - After business hours, DEHS suggests that you go to the Emergency Room at Fairview University Medical Center. Boynton's Web site provides a [map and directions to Fairview's ER](#). This location is fully prepared to deal with laboratory hazard exposures.
  - If you are outside the Twin Cities area, or if you choose not to go to Boynton or Fairview, you may seek medical attention at the closest available medical facility or your own healthcare provider.
- Report the incident to your supervisor as soon as possible, fill out the appropriate paperwork. The University's Office of Risk Management and Insurance outlines [An Employee's Responsibilities](#), and [Supervisor Responsibilities](#) on their Web site.
- Report all biohazard exposures to the Biological Safety Officer (626-6002).

**Note:** It is important to fill out all of the appropriate paperwork in order to be eligible to collect workers compensation should any complications arise from the hazardous exposure in the future.

