Standard Operating Procedure for Droplet-based Sorting of Risk Group 2 (RG2) Samples that require Biosafety Level 2 (BSL2) Containment

Lab Location: _____

Original Issue Date: 04/26/2010 Revision Date: *03/31/2016*

Instruments/Facilities:

SORP FACSAria II with Biosafety cabinet (CCRB 1-209)
SORP FACSAria II with Biosafety cabinet (MRF 2-128A)
SORP FACSAria II with Biosafety cabinet (MCRB 695)

Prepared by:

Paul Champoux	**Director, University Flow Cytometry Resource (UFCR)**
Betty Kupskay	**Director, Biosafety & Occ Health Dept/UM Biosafety Officer**
Kirsten Nielsen	**Associate Professor, Microbiology Dept.**
Therese Martin	**Operations Manager, UFCR**

Approval Signatures:

Director, UFCR: Paul E. Champoux

UM Biosafety Officer: B Kupskay _____

1.0 Introduction

To assure the assignment of proper safety practices and procedures, UFCR 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 Operations 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 Institutional Biosafety Committee (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 BSL2 practices as per BMBL, including the following: Cell sorter will be contained within a Class II biosafety cabinet within a BSL2 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_2O).

3.0 Samples

Note: If RG2 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 BSL2 and handled accordingly.

3.1 RG2 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 BSL2 Designated SORP FACSAria II (CCRB 1-209A)

4.1 BIOCONTAINMENT AND INFECTIOUS CELL SORTING

4.1.1 The FACSAria II is equipped with an aerosol management system (AMS) which is connected to a port on the interior of the biosafety cabinet. A dedicated, two-stage motor is contained within the cabinet to draw air through the FACSAria II sort chamber area and filter the output using the HEPA filtration capability of the cabinet. This integrated cabinet functionality replaces that of a vendor supplied Buffalo Whisper module. It is the operator's responsibility to ensure that the cabinet fan motor is turned on and functioning according to the manufacturer's guidelines. Annual certification of the cabinet will be performed by Brager Scientific, River Falls, WI, or alternate service provider.

4.1.2 An alternate vacuum unit designed to be used with the FACSAria II will be kept onsite to be used in the event of a cabinet system failure. When in use, the vacuum monitor on the Buffalo Whisper Model XXX should be set to 20% and the vacuum gauge must read between 1.0 and 1.5 inches of H_2O. 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 beach 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's 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 BSL2 guidelines if unfixed RG2 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 Quality Control (QC) procedure of FACSAria II 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 nozzle), the sort and stream will be stopped either by the operator or automatically by the FACSAria II 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. If a normal stream results, continue with the sort restart procedure.

4.2.8.3 Turn the stream off and run a "Sample Line Back flush" procedure for 20 sec followed by a "Clean Flow Cell" procedure using an appropriate fluid.

4.2.8.4 If the nozzle cannot be cleared, the stream should be shut down and 5 minutes allowed to pass prior to opening the biosafety enclosure doors or sort chamber door. This will 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 Open the access cover to the sort chamber area and remove the nozzle from the instrument. Immerse the nozzle in a 10% bleach solution for 10 minutes for decontamination. Use other facility-approved cleaning measures or select an alternate nozzle to continue the sort procedure.

4.2.8.8 Open the sort chamber door to inspect the high voltage plates and chamber for conditions that may prevent proper operation. Properly discard any materials used to clean the sort chamber area.

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

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

4.2.8.11 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.

4.2.8.12 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. A fluidics shutdown protocol using 70% ethanol will be run to completion 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 II 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 BSL2 Designated SORP FACSAria II (2-128A MRF)

5.1 BIOCONTAINMENT AND INFECTIOUS CELL SORTING

5.1.1 The FACSAria II is equipped with an aerosol management system (AMS) which is connected to a port on the interior of the biosafety cabinet. A dedicated, two-stage motor is contained within the cabinet to draw air through the FACSAria II sort chamber area and filter the output using the HEPA filtration capability of the cabinet. This integrated cabinet functionality replaces that of a vendor supplied Buffalo Whisper module. It is the operator's responsibility to ensure that the cabinet fan motor is turned on and functioning according to the manufacturer's guidelines. Annual certification of the cabinet will be performed by Brager Scientific, River Falls, WI, or alternate service provider.

5.1.2 An alternate vacuum unit designed to be used with the FACSAria II will be kept onsite to be used in the event of a cabinet system failure. When in use, the vacuum monitor on the Buffalo Whisper Model XXX should be set to 20% and the vacuum gauge must read between 1.0 and 1.5 inches of H_2O. 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.

5.1.3 The sheath waste tank must contain enough bleach to provide a final concentration of 10% when filled (1L beach 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.

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

5.2 SORTING PROCEDURE FOR INFECTIOUS SAMPLES

5.2.1 Upon entering the BSL2 Sorter room, all personnel must wear personal protective equipment (PPE) according to BSL2 guidelines if unfixed RG2 samples are to be sorted.

5.2.2 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.

5.2.3 Complete startup and Quality Control (QC) procedure of FACSAria II will be performed, and the operator will confirm that the sheath tank is full.

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

5.2.5 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.

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

5.2.7 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 nozzle), the sort and stream will be stopped either by the operator or automatically by the FACSAria II software. The sort will not restart until the operator has cleared the clog.

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

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

5.2.8.2 Turn stream off and then on again to attempt to clear the nozzle or sample line obstruction. If a normal stream results, continue with the sort restart procedure.

5.2.8.3 Turn the stream off and run a "Sample Line Back flush" procedure for 20 sec followed by a "Clean Flow Cell" procedure using an appropriate fluid.

5.2.8.4 If the nozzle cannot be cleared, the stream should be shut down and 5 minutes allowed to pass prior to opening the biosafety enclosure doors or sort chamber door. This will reduce the potential inhalation of aerosols generated during the sorting process.

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

5.2.8.6 Open the collection chamber and remove the collection vials.

5.2.8.7 Open the access cover to the sort chamber area and remove the nozzle from the instrument. Immerse the nozzle in a 10% bleach solution for 10 minutes for decontamination. Use other facility-approved cleaning measures or select an alternate nozzle to continue the sort procedure.

5.2.8.8 Open the sort chamber door to inspect the high voltage plates and chamber for conditions that may prevent proper operation. Properly discard any materials used to clean the sort chamber area.

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

5.2.8.10 The area around the cell sorter will be wiped with fresh 10% bleach for a contact time of 30 minutes, followed by 70% ethanol.
5.2.8.11 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.
5.2.8.12 After sort is completed the sort collection tubes will be removed immediately after sample tube is unloaded from the sample station.

5.2.9 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. A fluidics shutdown protocol using 70% ethanol will be run to completion and the inside and outside of the sort chamber will be sprayed and wiped down with 70% ethanol.

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

5.2.11 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

6.0 BSL2 Designated SORP FACSAria II (695 MCRB)

6.1 BIOCONTAINMENT AND INFECTIOUS CELL SORTING

6.1.1 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's guidelines. Annual certification of both the cabinet and the AMS will be performed by Brager Scientific, River Falls, WI, or an alternative service provider.

6.1.2 The vacuum monitor on the AMS should be set to a minimum of 20% and the vacuum gauge must read between 1.0 and 1.5 inches of H_2O. 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. Replacement with an alternate AMS module would be the final step for remediation. A malfunctioning AMS system would result in the inability to use the system for RG2 sorting until repaired.

6.1.3 The sheath waste tank must contain enough bleach to provide a final concentration of 10% when filled (1L beach 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.

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

6.2 SORTING PROCEDURE FOR INFECTIOUS SAMPLES

6.2.1 Upon entering the BSL2 Sorter room, all personnel must wear personal protective equipment (PPE) according to BSL2 guidelines if unfixed RG2 samples are to be sorted.

6.2.2 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.

6.2.3 Complete startup and Quality Control (QC) procedure of FACSAria II will be performed, and the operator will confirm that the sheath tank is full.

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

6.2.5 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.

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

6.2.7 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 nozzle), the sort and stream will be stopped either by the operator or automatically by the FACS Aria II software. The sort will not restart until the operator has cleared the clog.

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

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

6.2.8.2 Turn stream off and then on again to attempt to clear the nozzle or sample line obstruction. If a normal stream results, continue with the sort restart procedure.

6.2.8.3 Turn the stream off and run a "Sample Line Back flush" procedure for 20 sec followed by a "Clean Flow Cell" procedure using an appropriate fluid.

6.2.8.4 If the nozzle cannot be cleared, the stream should be shut down and 5 minutes allowed to pass prior to opening the biosafety enclosure doors or sort chamber door. This will reduce the potential inhalation of aerosols generated during the sorting process.

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

6.2.8.6 Open the collection chamber and remove the collection vials.

6.2.8.7 Open the access cover to the sort chamber area and remove the nozzle from the instrument. Immerse the nozzle in a 10% bleach solution for 10 minutes for decontamination. Use other facility-approved cleaning measures or select an alternate nozzle to continue the sort procedure.
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6.2.10 Areas around the FACSAria II will be wiped with fresh 10% bleach solution. After 30 minutes, surfaces will be wiped with 70% ethanol.

6.2.11 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

7.0 Hazard Identification and Risk of Exposure to the Hazards

7.1 Hazards are possible exposure to infectious aerosols produced when:

1) transporting RG2 samples to the BSL2 cell sorting laboratory;
2) sorting of RG2 samples on the Modified BD Biosciences FACS Aria II

7.2 Although many infectious organisms that require BSL2 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 BSL2 laboratory.

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

8.0 Exposure Controls Specific to Above Risk of Exposure

See Sections 2, 3, 4, 5, 6 above.

9.0 Waste Generated and Disposal Methods

See Section 4, 5, and 6 above and the attached Biological Waste Disposal Plan (Appendix A)

10.0 Spill and Accident Response Procedures

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

11.0 Immunizations

All personnel working in the BSL2 cell sorting facility must be offered a Hepatitis B vaccination by the employer for work with human cells. Consultation with University Health and Safety – Biosafety and Occupational Health Dept. (UHS-BOHD) should be made regarding any other immunizations that may be required.

12.0 Training

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

13.0 References

1. Merrill J.T. Evaluation of selected aerosol-control measures on flow sorters. *Cytometry* 1981; 1: 342-345.
2. 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.
3. Karen B. Byers. Biosafety Tips. *Applied Biosafety* 2008; 13:(1)
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5. Ingrid Schmid, Janet Nicholson., Annalisa Kunkl. Biosafety Concerns for Flow Cytometric HIV Immunophenotyping: Questions and Answers. schmid@mednet.ucla.edu
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.
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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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12. Oberyszyn A.S. & Robertson F.M. Novel rapid method for visualization of extent and location of aerosol contamination during high-speed sorting of potentially biohazardous samples. Cytometry 2001; 43: 217-222.
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

Biological Decontamination & Spill Clean-up Plan Template

This template can be used in writing lab specific SOPs and posted in the lab for reference and annual review. This customized template is a required attachment when IBC forms are submitted. **The top section and any Lab Specific Requirements must be filled in. Note: all rDNA containing waste must be treated as biohazardous waste.**

P.I./Lab Supervisor: Paul Champoux, Kirsten Nielsen, Peter Southern **Lab Location: 695 MCRB, 1-209A CCRB, 2-128A MRF**	**Emergency Contact Info: Paul Champoux 612 625-7165 Kirsten Nielsen 612 625 4979 Peter Southern 612-625 2141** **(report all spills to P.I. or Lab Supervisor and Biosafety Officer)**	
Biological Agent (s) / rDNA / Biological toxin	**Disinfectant / Concentration / Contact time**	**Routine Decontamination Procedures (bench top, equipment, etc. not for spill clean-up)**
BSL-2 unfixed cells	☒ **Bleach / 10% / 30 minutes, OR** ☒ **Other proven effective disinfectant:** **Note:** some disinfectants are incompatible with bleach therefore should not be mixed.	**For spills or decontmination of area - 10% bleach for 30 minutes** **For decontaminating FACSAria II sample pathway and sort chamber,(i.e 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 121^0C)

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):

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):

For major spills in a biological safety cabinet:

- Contact the Biosafety Officer (BSO) (612-626-6002) to determine if professional decontamination is indicated.

For any spills of agents that are transmitted by inhalation, evacuate the lab immediately, close the door, restrict access, remove any contaminated clothing, wash exposed skin with soap and water, call the BSO for assistance at 612-626-6002.

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

- Encourage needle sticks and cuts to bleed, gently wash with soap and water for 15 minutes; flush splashes to the nose, mouth, or skin, with water; and flush eyes at the nearest eyewash station with clean water for 15 minutes.
- Call 911 or seek **immediate** medical attention if overtly exposed to recombinant or synthetic nucleic acid molecules or RG2 infectious agent(s) in a BSL2 lab.
  - For urgent care employees may go to HealthPartners Occupational and Environmental Medicine (M/F day time or Urgent Care after hours), or UMMC-Fairview Hospital (24 hrs). You may seek medical attention at the closest available medical facility or your own healthcare provider.
  - Follow-up must be done by HealthPartners Occupational and Environmental Medicine.
- Report the incident to your supervisor as soon as possible, fill out the appropriate documentation.
  - Employee First Report of Injury
  - Supervisor Incident Investigation Report
- Send Incident Report Form to the IBC if exposure has occurred during work on an IBC protocol. Any incident involving rDNA must be reported to the IBC within 24 hours to meet institutional requirements prescribed by the *NIH Guidelines for Research Involving rDNA Molecules*.
- Report all biohazard exposures to the Office of Occupational Health and Safety (612-626-5008) or uohs@umn.edu.

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

Revision Date: 4/3/2015

Biological Waste Disposal Template

P.I./Lab Supervisor: Paul Champoux, Kirsten Nielsen, Peter Southern

Lab Location: 695 MCRB, 1-209A CCRB,2-128A MRF

Types of Biological Waste Generated:

- ☒ Liquid Biological Waste (including plant/animal agents)
- ☒ Solid Biological Waste (including plant/animal agents)
- ☐ Animal Waste (e.g. tissue, carcasses, bedding, rDNA-containing animal waste, transgenic animal, etc.)
- ☐ Toxins
- ☒ rDNA (e.g. solid or liquid rDNA waste, transgenic plants & soil, rDNA-containing plant waste, etc.)
- ☐ Sharps
- ☐ Prions
- ☒ Cell Culture / Blood / Body Fluids (e.g. human / NHP / animal cells, blood, serum, body fluids, etc.)
- ☐ Human Organs, Tissue, Body Parts (Pathological Waste)

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 (e.g. rDNA, cell culture / blood / body fluids, any biological material including Risk Group 1-3, plant & animal agents, etc.):

- ☐ Autoclave for 30 minutes at 121°C on liquid cycle. Test autoclave monthly with integrator per *Autoclaving Biological Waste Fact Sheet* below.
- ☒ Disinfect with 10% (1:9 v/v) bleach for at least 30 minutes.

OR

- ☐ List other proven effective disinfectant /concentration underneath: (minimum 30 minutes contact time). **Note:** some disinfectants are incompatible with bleach therefore should not be mixed.

Solid Biological Waste Disposal (e.g. rDNA, cell culture / blood / body fluids, any biological material including Risk Group 1-3, plant & animal agents, transgenic plant & soil, gloves, etc.):

- ☐ Autoclave for 60 minutes at 121°C or 125 °C. Test autoclave monthly with integrator per *Autoclaving Biological Waste Fact Sheet* below.
- ☒ Place biological waste in the red biohazard bag, fill to no more than ¾ full, seal and place in the designated waste area in the lab.

Animal Waste:

- ☐ Place animal tissue and carcasses in cooler designated by RAR.
- ☐ Handle animal cages, bottles, and bedding per RAR instructions.
- ☐ Describe other animal waste disposal method.

Toxins:

- ☐ Treat with 10% (1:9 v/v) bleach for at least 30 minutes.
- ☐ Describe other proven effective inactivating agent.

Human Organs, Tissue, Body Parts (Pathological Waste):

- ☐ Call Bequest Program 612-625-1111

Sharps (contaminated with infectious material, rDNA, or biotoxins):

- ☐ 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 Recovery Services at 5-6481 for yellow bag & 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 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
- Inactivation of Toxins, http://www.dehs.umn.edu/PDFs/Toxins.pdf
- Sharps Disposal, http://www.dehs.umn.edu/bio_pracprin_su_ss.htm
- Prion Waste Disposal, http://www.dehs.umn.edu/PDFs/PrionResearchProcedures.pdf
- Hazardous Chemical Waste Management Guidebook, http://www.dehs.umn.edu/hazwaste_chemwaste_umn_cwmgbk.htm