

**Department/Lab:** SickKids-UHN TMDT Flow Cytometry Facility  
**Developed by:** C. Guidos/J.Yuan/J. Shannon/A. Monteath  
**Biosafety Procedures**

**Page:** 1 of 47

SOP #: FCF-BS1  
Version: 0.5  
Revision Date: 15.12.11

## Table of Contents

### 1. Overview of Facility

- 1.1. Introduction
- 1.2. Facility Locations
- 1.3. Biosafety levels and agents
- 1.4. User biosafety requirements
- 1.5. User training requirements
- 1.6. Security access

### 2. Biosafety Policies and Procedures

- 2.1. General laboratory practices
- 2.2. Use of Analyzers
- 2.3. Use of Sorters
- 2.4. Use of biosafety cabinets (BSC)
- 2.5. Decontamination procedures
  - 2.5.1. Decontamination procedures (sorters and analyzers)
  - 2.5.2. Spill response
  - 2.5.3. Biohazardous Waste disposal (sorters and analyzers)

### 3. Emergency Contact Info

### 4. Revision History

**Department/Lab:** SickKids-UHN TMDT Flow Cytometry Facility  
**Developed by:** C. Guidos/J.Yuan/J. Shannon/A. Monteath  
**Biosafety Procedures**

**Page:** 2 of 47

SOP #: FCF-BS1  
Version: 0.5  
Revision Date: 15.12.11

## 5. Appendices

Appendix I:	Table 1: FCF Biosafety Requirements for Analysis and Sorting of Different Sample Types
Appendix II:	Analysis Biosafety Information
Appendix III:	Sorting Biosafety Information
Appendix IV:	Decontamination Procedures For Analyzers
Appendix V:	Operating and Decontamination Procedures For Sorters
Appendix VI:	TMDT Emergency Response Plan – INJURIES
Appendix VII:	TMDT Biohazard and Chemical Spill Instructions

**Department/Lab:** SickKids-UHN TMDT Flow Cytometry Facility  
**Developed by:** C. Guidos/J.Yuan/J. Shannon/A. Monteath  
**Biosafety Procedures**

**Page:** 3 of 47

SOP #: FCF-BS1  
Version: 0.5  
Revision Date: 15.12.11

## 1. Overview of Facility

### 1.1. Introduction

The SickKids-UHN Flow Cytometry Facility (FCF) provides investigators, their trainees and technical staff with access to state-of-the-art analytical flow cytometers, high-speed fluorescence-based cell sorters, and magnetic cell separators. Our clients bring a wide array of fluorescently labeled human and animal cells into the FCF for analysis or cell sorting. Work site specific training on how to run samples on the analytical cytometers and magnetic cell sorters is provided to FCF users by FCF staff.

All users need to demonstrate a high level of understanding before they are allowed to use equipment without FCF staff assistance. Only FCF staff is allowed to use fluorescence-based cell sorters. The biosafety policies and procedures described in Section 2 are to be followed by all FCF staff and researchers using FCF facilities and equipment.

### 1.2. Facility Locations

FCF instruments are distributed across several laboratories in the TMDT East Tower of the MaRS complex at 101 College Street, Toronto, Ontario, Canada.

**Department/Lab:** SickKids-UHN TMDT Flow Cytometry Facility  
**Developed by:** C. Guidos/J.Yuan/J. Shannon/A. Monteath  
**Biosafety Procedures**

**Page:** 4 of 47

SOP #: FCF-BS1  
 Version: 0.5  
 Revision Date: 15.12.11

Instruments	Location	
	UHN	SickKids
Several analyzers, a magnetic cell separator and all fluorescence-based cell sorters	Rooms 2-504 and 2-203	
There are also satellite labs containing analytical instruments	Dr John Dick's lab Rm 8-604	Dr Cynthia Guidos lab Rm 14-404
Analytical cytometer <i>NB. Lab is a Containment Level (CL) 2 facility and CL3 operations are used</i>	Dr John Dick's lab: lentivirus isolation Lab Room 8-403	
Magnetic cell separator within a Class II Biosafety Cabinet (BSC)		SickKids Tissue Culture Room 14-403

### 1.3. Biosafety levels and agents

As per SickKids and UHN Research biosafety practices and/or policy, procedures used for handling biological samples within FCF labs are in compliance with the practices described in the *Laboratory Biosafety Guidelines* (3<sup>rd</sup> Edition, 2004, Public Health Agency of Canada-PHAC, as enforced by the *Human Pathogens and Toxins Act*) and *Containment Standards for Veterinary Facilities* (Canadian Food Inspection Agency-CFIA, 2007) Physical containment and risk group (RG) levels are as described in the above documents. Procedures must adhere to any additional SickKids and UHN Research biosafety

**Department/Lab:** SickKids-UHN TMDT Flow Cytometry Facility  
**Developed by:** C. Guidos/J.Yuan/J. Shannon/A. Monteath  
**Biosafety Procedures**

**Page:** 5 of 47

SOP #: FCF-BS1  
Version: 0.5  
Revision Date: 15.12.11

practices, manuals, policies or requirements as set out by SickKids and/or UHN Biosafety Committees.

All FCF labs operate in CL2 facilities using CL2 operations as a minimum standard of practice. Therefore FCF facilities are capable of handling samples designated as Risk Group 1 (RG1) or RG2. Agents assessed as RG3 or RG4 are not permitted. RG1 agents (or potential reservoirs of RG1) present low individual risk and low community risk. They are defined as biological agents/materials that are unlikely to cause disease in healthy workers or animals.

RG2 agents present moderate individual risk and low community risk. They are defined as pathogens/materials that can cause human disease, but under normal circumstances are unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures to RG2 agents rarely cause infection leading to serious disease; effective treatment and preventive measures are available and the risk of spread is limited. FCF labs are not equipped to handle samples known or likely to contain RG3 agents (agents that cause serious human disease, whether treatable or not) or RG4 agents (agents that cause serious human disease, are often untreatable and readily transmittable).

#### *Additional Risk Factors for Flow Cytometry*

High-speed cell sorters take the sample and under high pressure create a heterogeneous aerosol for cell sorting. This potentially

**Department/Lab:** SickKids-UHN TMDT Flow Cytometry Facility  
**Developed by:** C. Guidos/J.Yuan/J. Shannon/A. Monteath  
**Biosafety Procedures**

**Page:** 6 of 47

SOP #: FCF-BS1  
Version: 0.5  
Revision Date: 15.12.11

increases the risk of infectivity for many agents that might be contained in these aerosols. Many published studies document that even when high-speed cell sorters are equipped with manufacturer-installed aerosol containment units, there is substantial risk that aerosols containing infectious agents may escape and, if the equipment is not contained, these aerosols may escape into the general lab environment. Therefore, the Biosafety Working Group of the International Society for Analytical Cytometry (ISAC) has recommended that flow cytometry core facilities implement enhanced containment procedures when performing high-speed sorting of viable human and non-human primate (NHP) samples. FCF requires all RG2 sorts are conducted in a sorter contained within a certified Class II Type A2 Biological Safety Cabinet (BSC).

Analyzers, given the closed system under low pressures, are not considered a high risk for aerosol generation either during normal operations or due to possible failures in the fluidics. All RG2 biohazardous agents run on analyzers should be fixed, as outlined below, unless it is not possible due to the nature of the experiment (e.g. calcium flux assays). Users must don safety eyewear when running unfixed RG2 biohazardous agents on analyzers to minimize risk of exposure due to accidental splashing/droplets. The standard fixative used in TMDT FCF is 0.5-2 per cent paraformaldehyde for at least 30 minutes on ice, prior to running on a cell analyzer. Alternative fixation procedures may be required as outlined by SickKids Biosafety Permit or UHN Research Biosafety Certificate. Use of any other fixation procedure

**Department/Lab:** SickKids-UHN TMDT Flow Cytometry Facility  
**Developed by:** C. Guidos/J.Yuan/J. Shannon/A. Monteath  
**Biosafety Procedures**

**Page:** 7 of 47

SOP #: FCF-BS1  
Version: 0.5  
Revision Date: 15.12.11

must be pre-approved by the FCF and **must** be proven effective for agents being used. Prior notification (at least 1 week) and experiment details must be provided to the FCF manager by email.

All open manipulations of RG2 agents that may produce aerosol must be conducted within a certified class II Type A2 BSC. For example, vortexing of sample, if necessary must be conducted in a certified class II Type A2 BSC.

RG2 biohazardous agents that have been identified as requiring Level 3 operational containment (as per UHN and/or SickKids Biosafety Committee) must be analyzed and/or sorted within a certified class II Type A2 BSC. All open manipulations of RG2 agents requiring Level 3 operational containment must be conducted under a certified class II Type A2 BSC, irrespective of low risk of aerosol production. This is required as per PHAC *Containment Level 3 Operational Practices for use with specified RG2 organisms* and CFIA *Condition Requiring Level 2 Physical Containment and Level 3 Operational Procedures*.

#### **1.4. User biosafety requirements**

All SickKids users of the facility must have a valid SickKids Biosafety Permit as required by SickKids for use with biological agents prior to working in the FCF facility. All UHN and external users (including observers) must have a valid UHN Research Biosafety Certificate for use with biological agents prior to working in the FCF facility. SickKids

**Department/Lab:** SickKids-UHN TMDT Flow Cytometry Facility  
**Developed by:** C. Guidos/J.Yuan/J. Shannon/A. Monteath  
**Biosafety Procedures**

**Page:** 8 of 47

SOP #: FCF-BS1  
Version: 0.5  
Revision Date: 15.12.11

Biosafety Permit and UHN Research Biosafety Certificate must cover the work being conducted in the FCF facility.

### **1.5. User Training Requirements**

- 1.5.1. All users of the facility must provide documentation that they have completed SickKids Biosafety and Chemical Safety Training within the last 24 months or UHN Research Safety Training.
- 1.5.2. All users must attend a FCF Safety Orientation session. Completion of orientation will be documented by FCF staff.
- 1.5.3. All persons providing samples to FCF technical staff for processing and acting only as observers must attend a FCF Safety Orientation session. Completion of orientation will be documented by FCF staff.

### **1.6. Security access**

- 1.6.1. Access is restricted to FCF staff and registered users of the facility who have been issued a security access card. This security access card may not be used by or transferred to anyone other than the person to whom it is issued.

**Department/Lab:** SickKids-UHN TMDT Flow Cytometry Facility  
**Developed by:** C. Guidos/J.Yuan/J. Shannon/A. Monteath  
**Biosafety Procedures**

**Page:** 9 of 47

SOP #: FCF-BS1  
Version: 0.5  
Revision Date: 15.12.11

- 1.6.2. Contractors, service persons and vendors are permitted entry for service purposes and are required to undergo FCF Safety Orientation. Completion of orientation will be documented by FCF staff.
  
- 1.6.3. Other visitors may be permitted entry with prior permission of the FCF manager and are required to sign into the visitor's log.
  
- 1.6.4. Facility laboratory doors are closed and locked at all times.

**Department/Lab:** SickKids-UHN TMDT Flow Cytometry Facility  
**Developed by:** C. Guidos/J.Yuan/J. Shannon/A. Monteath  
**Biosafety Procedures**

**Page:** 10 of 47

SOP #: FCF-BS1  
Version: 0.5  
Revision Date: 15.12.11

## 2. Biosafety Policies and Procedures

### 2.1. General laboratory practices

- 2.1.1. Eating, drinking, and storage of food are not permitted in FCF labs.
- 2.1.2. Pipetting samples or any material by mouth is strictly prohibited.
- 2.1.3. All persons must wear a laboratory coats/front-closed gown while working in FCF laboratories. To facilitate compliance with containment requirements the FCF will supply laboratory coats/front-closed gowns to be donned upon entry into FCF labs and discarded upon exit.
- 2.1.4. All persons must wear laboratory gloves while handling samples in FCF laboratories. This includes running samples on cell sorters, analyzers or magnetic separators. Laboratory gloves will be provided by the FCF.
- 2.1.5. Sandals or any open toed shoes are prohibited. Users are to wear closed toe and heel footwear with non-slip soles while working in FCF labs.

**Department/Lab:** SickKids-UHN TMDT Flow Cytometry Facility  
**Developed by:** C. Guidos/J.Yuan/J. Shannon/A. Monteath  
**Biosafety Procedures**

**Page:** 11 of 47

SOP #: FCF-BS1  
Version: 0.5  
Revision Date: 15.12.11

- 2.1.6. Transportation of samples: All primary containers (FACS tubes, screw cap tubes, etc.) must be sealed (i.e. no open tubes in racks). In addition, all sealed primary containers must be transported to the facility in a secondary, sealed, leak-proof container. The leak-proof secondary container (e.g. 'tupperware'-type containers with tight fitting lid) should be able to contain the contents should the item be tipped/dropped in transit. Transportation of samples using public thoroughfares must be compliant with Transportation of Dangerous Goods Act (<http://www.tc.gc.ca/eng/acts-regulations/acts-1992c34.htm>).
- 2.1.7 Manipulation (pipetting, tube transfers, aliquotting, etc.) of all RG2 samples e.g., blood or tissues from humans, must be done within a FCF certified class II Type A2 BSC.
- 2.1.8 Persons must wash their hands with soap and water after handling samples containing viable cells and **before leaving the laboratory.**

## 2.2 Use of Analyzers

- 2.2.1 All users are required to be trained by FCF staff.

**Department/Lab:** SickKids-UHN TMDT Flow Cytometry Facility  
**Developed by:** C. Guidos/J.Yuan/J. Shannon/A. Monteath  
**Biosafety Procedures**

**Page:** 12 of 47

SOP #: FCF-BS1  
Version: 0.5  
Revision Date: 15.12.11

- 2.2.2 Personal login accounts on analytical instruments may only be used by the person whom the account belongs to.
- 2.2.3 Table 1 ('FCF Biosafety Requirements for Analysis and Sorting of Different Sample Types') in Appendix I outlines the biosafety requirements for running different types of animal and human cells on analytical cytometers and magnetic cell separators.
- 2.2.4. For every analysis appointment, users must indicate the types of samples to be analyzed and identify the Risk Group (RG) categories of any biological agents their samples contain, based on the Public Health Agency of Canada, *Laboratory Biosafety Guidelines*, 3<sup>rd</sup>. Ed. 2004 ( <http://www.phac-aspc.gc.ca/publicat/lbg-lmbml-04/index-eng.php> ) and Canadian Food Inspection Agency *Containment Standards for Veterinary Facilities* , 2007 ( <http://www.inspection.gc.ca/english/sci/bio/anima/convet/convete.shtml> ) and as assessed by SickKids Biosafety Committee/UHN Research Biosafety Committee.

This is done online by completing the Analysis Biosafety Information form (see Appendix II) when booking analysis appointments. This form must be completed to make the

**Department/Lab:** SickKids-UHN TMDT Flow Cytometry Facility  
**Developed by:** C. Guidos/J.Yuan/J. Shannon/A. Monteath  
**Biosafety Procedures**

**Page:** 13 of 47

SOP #: FCF-BS1  
Version: 0.5  
Revision Date: 15.12.11

analysis appointment. Only samples specified in the form can be analyzed.

- 2.2.5 As noted in Appendix I, Table 1 ('FCF Biosafety Requirements for Analysis and Sorting of Different Sample Types'), RG1 may be run unfixed. All RG2 biohazardous agents run on analyzers should be fixed, as outlined below, unless it is not possible due to the nature of the experiment (e.g. calcium flux assays). Users must don safety eyewear when running unfixed RG2 biohazardous agents on analyzers to minimize risk of exposure due to accidental splashing/droplets. The standard fixative used in TMDT FCF is 0.5-2 per cent paraformaldehyde for at least 30 minutes on ice after immunofluorescence staining is complete. Alternative fixation procedures may be required as outlined by SickKids Biosafety Permit or UHN Research Biosafety Certificate. Use of any other fixation procedure must be pre-approved by the FCF and **must** be proven effective for agents being used. Prior notification (at least 1 week) and experiment details must be provided to the FCF manager by email. RG2 samples, specified by UHN Research or SickKids Biosafety Committee as requiring

**Department/Lab:** SickKids-UHN TMDT Flow Cytometry Facility  
**Developed by:** C. Guidos/J.Yuan/J. Shannon/A. Monteath  
**Biosafety Procedures**

**Page:** 14 of 47

SOP #: FCF-BS1  
Version: 0.5  
Revision Date: 15.12.11

Level 3 Operations, must be analyzed on a sorter/analyzer contained within a FCF certified class II Type A2 BSC.

- 2.2.6 RG3 and RG4 biohazardous agents are not permitted in the FCF and may NOT be run on any sorter or analytical instrument.
- 2.2.7 Cells that are labeled with any radioactive material are not permitted in the FCF and may NOT be run on any sorter or analytical instrument.
- 2.2.8 Yeast and bacteria cannot be analyzed without FCF staff assistance. Prior notification (at least 1 week) and experimental details must be provided to the FCF manager by email. Special procedures may be required as assessed by SickKids Biosafety Permit /UHN Research Biosafety Certificate and/or FCF Managers.
- 2.2.9 Samples must be clump free and should be filtered immediately prior to being run.

## **2.3 Use of Sorters**

- 2.3.1 Appendix I, Table 1 ('FCF Biosafety Requirements for Analysis and Sorting of Different Sample Types') outlines the

**Department/Lab:** SickKids-UHN TMDT Flow Cytometry Facility  
**Developed by:** C. Guidos/J.Yuan/J. Shannon/A. Monteath  
**Biosafety Procedures**

**Page:** 15 of 47

SOP #: FCF-BS1  
Version: 0.5  
Revision Date: 15.12.11

biosafety requirements for running different types of animal and human cells on sorters.

- 2.3.2 For every sorting appointment, users must indicate the types of samples to be sorted and identify the Risk Group (RG) categories of any biological agents their samples contain, based on the Public Health Agency of Canada, *Laboratory Biosafety Guidelines*, 3<sup>rd</sup>. Ed. 2004 ( <http://www.phac-aspc.gc.ca/publicat/lbg-lbmbi-04/index-eng.php> ) and Canadian Food Inspection Agency *Containment Standards for Veterinary Facilities* , 2007 (<http://www.inspection.gc.ca/english/sci/bio/anima/convet/convete.shtml> ) and as assessed by SickKids Biosafety Committee/UHN Research Biosafety Committee.

Chemical risks must also be identified. This is done online by completing the Sorting Biosafety Information form (see Appendix III) when booking sort appointments. This form will be reviewed before FCF staff approves the sort appointment. Only samples specified in the form will be sorted.

- 2.3.3 Samples must be clump free and should be filtered immediately prior to being run.

**Department/Lab:** SickKids-UHN TMDT Flow Cytometry Facility  
**Developed by:** C. Guidos/J.Yuan/J. Shannon/A. Monteath  
**Biosafety Procedures**

**Page:** 16 of 47

SOP #: FCF-BS1  
Version: 0.5  
Revision Date: 15.12.11

2.3.5 Facility users are responsible for removing unused cell samples and sorted cells from the FCF. Policy for transport of unused samples is as in 2.1.6. Unused samples are to be treated (i.e. disposal, storage culture) as per federal, provincial, regional and host institutional requirements.

2.3.6 RG3 and RG4 biohazardous agents are not permitted in the FCF and may NOT be run on any sorter or analytical instrument.

2.3.7 Cells that are labeled with any radioactive material are not permitted in the FCF and may NOT be run on any sorter or analytical instrument.

## **2.4 Use of Biosafety cabinets (BSC)**

### **2.4.1 Testing, Certification and Decontamination**

There is a centralized biosafety cabinet program for SickKids and UHN equipment.

Biosafety cabinets must be tested and certified:

- at the time of installation
- once every 12 months after installation
- after corrective maintenance
- after being moved

**Department/Lab:** SickKids-UHN TMDT Flow Cytometry Facility  
**Developed by:** C. Guidos/J.Yuan/J. Shannon/A. Monteath  
**Biosafety Procedures**

**Page:** 17 of 47

SOP #: FCF-BS1  
Version: 0.5  
Revision Date: 15.12.11

Formaldehyde gas decontamination must be carried out on BSC:

- before routine testing and certification
- before corrective maintenance is carried out
- before the cabinet is moved to a new location.

#### 2.4.2 **BSC Start up Procedure**

- Always check that the drain valve is closed on your BSC so spills will remain in the cabinet and not drip onto the floor.
- Turn off UV lights and ensure that the sash is in the appropriate position.
- Turn on fluorescent light and cabinet blower.
- Remove any obstructions from the air intake grille
- If the BSC is equipped with an alarm, test the alarm and switch it to the 'on' position.
- Confirm inward airflow by holding a tissue at ½ way along the bottom of the glass sash.

**Department/Lab:** SickKids-UHN TMDT Flow Cytometry Facility  
**Developed by:** C. Guidos/J.Yuan/J. Shannon/A. Monteath  
**Biosafety Procedures**

**Page:** 18 of 47

SOP #: FCF-BS1  
Version: 0.5  
Revision Date: 15.12.11

- Disinfect the interior surfaces with a suitable, disinfectant (e.g., Virox). If you use bleach, after the appropriate contact time rinse it off with sterile tap water.
- Assemble all materials required for the procedure and load them into the BSC; do not obstruct the air grilles; segregate 'clean' items from 'contaminated' items.
- Wait 3 to 5 minutes to purge airborne contaminants.

#### 2.4.3 BSC Close down Procedure

- Allow the BSC to run for 5 minutes with no activity.
- Put on gloves.
- Close or cover open containers before disinfecting them out of the BSC.
- All solid wastes must be packaged inside the cabinet. The package is sealed and wiped (disinfected) out of the BSC
- Disinfect everything out of the BSC.

**Department/Lab:** SickKids-UHN TMDT Flow Cytometry Facility  
**Developed by:** C. Guidos/J.Yuan/J. Shannon/A. Monteath  
**Biosafety Procedures**

**Page:** 19 of 47

SOP #: FCF-BS1  
Version: 0.5  
Revision Date: 15.12.11

- Remove contaminated gloves and dispose of them in yellow bag waste; wash hands.
- Put on clean gloves.
- Wash the internal surfaces of the BSC (including sash) with an in-use dilution of a disinfectant (e.g., Virox). If you use bleach rinse off residue with sterile tap water.
- Turn off the fluorescent light and BSC blower.
- Close sash completely.
- Turn on the UV light if appropriate.

## **2.5 Decontamination procedures**

2.5.1 Decontamination procedures for analyzers and sorters are outlined in Appendix IV and Appendix V.

### **2.5.2 Spill Response**

#### **2.5.2.1 Spills inside a Biosafety Cabinet**

**General Tips when working in a Biosafety Cabinet (BSC) with biohazardous agents**

**Department/Lab:** SickKids-UHN TMDT Flow Cytometry Facility  
**Developed by:** C. Guidos/J.Yuan/J. Shannon/A. Monteath  
**Biosafety Procedures**

**Page:** 20 of 47

SOP #: FCF-BS1  
Version: 0.5  
Revision Date: 15.12.11

- Always check that the drain valve is closed on your BSC so spills will remain in the cabinet and not drip onto the floor.
- Prior to starting work always ensure that you have a prepared disinfectant solution close at hand and a supply of absorbent material e.g., paper towels.  
A 1 in 10 dilution of household bleach in tap water is a good broad spectrum disinfectant. **Do not use spray bottles.**
- If using bleach as disinfectant, ensure that you have some bottles of sterilized tap water – you will use this to remove chlorine residue after disinfection is complete. If you do not rinse the chlorine residue off the cabinet it will corrode and pit the stainless steel. Do not use deionized water, it will corrode stainless steel
- If using another disinfectant ensure that;
  - it is appropriate for the agent(s) you are working with
  - it is made up at the correct concentration
  - it is applied for the correct contact time to adequately disinfect.

**Department/Lab:** SickKids-UHN TMDT Flow Cytometry Facility  
**Developed by:** C. Guidos/J.Yuan/J. Shannon/A. Monteath  
**Biosafety Procedures**

**Page:** 21 of 47

SOP #: FCF-BS1  
 Version: 0.5  
 Revision Date: 15.12.11

- Ensure that while working at a BSC you wear your lab coat/ front-closed gown, laboratory gloves and, other appropriate personal protective equipment as may be specified by Occupational Health and Safety, Biosafety Officer, Supervisor or FCF management.

**Spill cleanup (contained within BSC)**

There is minimal risk of aerosol escape from the cabinet as long as:

- It occurs in a certified BSC
- The BSC is not overfilled
- The spill is all contained in the BSC and does not splash outside into the lab.

This spill needs to be cleaned-up immediately by the individual performing the work.

	<b>Procedural Steps</b>	<b>Learning Points</b>
1.	<ul style="list-style-type: none"> <li>• Leave the BSC running.</li> <li>• Immediately cover affected area with paper towels or other available absorbent material.</li> </ul>	<ul style="list-style-type: none"> <li>• Reduce the risk of containment breach by working carefully.</li> <li>• Do not move hands and arms in and out of the BSC unnecessarily.</li> </ul>
2.	<ul style="list-style-type: none"> <li>• Remove gloves, wash your hands and put on new gloves.</li> </ul>	<ul style="list-style-type: none"> <li>• Double glove so that you can remove top glove when necessary and still be</li> </ul>

**Department/Lab:** SickKids-UHN TMDT Flow Cytometry Facility  
**Developed by:** C. Guidos/J.Yuan/J. Shannon/A. Monteath  
**Biosafety Procedures**

**Page:** 22 of 47

SOP #: FCF-BS1  
 Version: 0.5  
 Revision Date: 15.12.11

	<b>Procedural Steps</b>	<b>Learning Points</b>
		protected.
3.	<ul style="list-style-type: none"> <li>Using a squeeze bottle of prepared disinfectant gently pour disinfectant on top of absorbent material covering the spill.</li> <li>Work from the perimeter towards the centre of the spill area.</li> </ul>	<ul style="list-style-type: none"> <li>Do not use spray bottles – sprays increase the potential for evaporation and contact times are difficult to achieve.</li> <li>Never use a spray bottle outside a cabinet. Sprays increase the risk of exposure of the eyes, face, other exposed skin and mucous membranes to the chemical disinfectant being used.</li> <li>Working from the perimeter to the interior of the spill prevents spreading of the hazard.</li> </ul>
4.	<ul style="list-style-type: none"> <li>Allow sufficient contact time (10 minutes minimum for bleach solutions).</li> </ul>	<ul style="list-style-type: none"> <li>If not using bleach check requirements of the disinfectant you are using to ensure adequate contact time is achieved.</li> </ul>
5.	<ul style="list-style-type: none"> <li>Place a discard bag, e.g., autoclave bag, inside the BSC.</li> <li>Pick up used absorbent material and place in discard bag.</li> </ul>	<ul style="list-style-type: none"> <li>Ensure that all clean up steps are carried out inside the BSC.</li> <li>Do not place discard bag outside of the cabinet.</li> </ul>

**Department/Lab:** SickKids-UHN TMDT Flow Cytometry Facility  
**Developed by:** C. Guidos/J.Yuan/J. Shannon/A. Monteath  
**Biosafety Procedures**

**Page:** 23 of 47

SOP #: FCF-BS1  
 Version: 0.5  
 Revision Date: 15.12.11

	<b>Procedural Steps</b>	<b>Learning Points</b>
	<ul style="list-style-type: none"> <li>If spill involves disposable glass ware or plastic ware place these in a rigid container, e.g. a cardboard box, before placing in the discard bag.</li> <li>If spill involves broken glass or sharps use tweezers or tongs to prevent cuts and puncture wounds to the hands and dispose in yellow biohazard sharps container.</li> <li><b>Do not seal discard bag yet.</b></li> </ul>	
6.	<ul style="list-style-type: none"> <li>Clean affected area again with disinfectant.</li> <li>Discard paper towels or other absorbent material into discard bag.</li> </ul>	
7.	<ul style="list-style-type: none"> <li>Rinse disinfected area again with disinfectant. If using bleach as your disinfectant, follow with a generous rinse of sterile tap water.</li> </ul>	<ul style="list-style-type: none"> <li>The water rinse prevents corrosion of the stainless steel by the bleach.</li> </ul>
8.	<ul style="list-style-type: none"> <li>Place used absorbent materials inside discard bag.</li> <li>Take off top pair of gloves</li> </ul>	

**Department/Lab:** SickKids-UHN TMDT Flow Cytometry Facility  
**Developed by:** C. Guidos/J.Yuan/J. Shannon/A. Monteath  
**Biosafety Procedures**

**Page:** 24 of 47

SOP #: FCF-BS1  
 Version: 0.5  
 Revision Date: 15.12.11

	<b>Procedural Steps</b>	<b>Learning Points</b>
	and place in discard bag. <ul style="list-style-type: none"> <li>Seal bag.</li> <li>Wipe outer surfaces of bag with disinfectant</li> <li>Remove the bag from the biosafety cabinet.</li> </ul>	
9.	<ul style="list-style-type: none"> <li>Place sealed discard bag in yellow bag waste stream.</li> </ul>	<ul style="list-style-type: none"> <li>Do not autoclave.</li> </ul>
	<p><b>Personal Contamination</b></p> <ul style="list-style-type: none"> <li>Remove any contaminated clothing or protective equipment.</li> <li>If skin has been contaminated, wash with soap and warm water.</li> <li>If eyes have been splashed, rinse under running water (eyewash or faucet) for at least 15 minutes.</li> <li>If medical attention is needed follow the Medical Emergency Procedure in Appendix VI.</li> </ul> <p><b>Follow-up:</b></p> <ul style="list-style-type: none"> <li>Report incident to your supervisor;</li> </ul>	<ul style="list-style-type: none"> <li>Known or suspected</li> <li>Do not scrub skin – scrubbing will compromise the protective barrier of the skin</li> <li>Response Plan - INJURIES</li> </ul>

**Department/Lab:** SickKids-UHN TMDT Flow Cytometry Facility  
**Developed by:** C. Guidos/J.Yuan/J. Shannon/A. Monteath  
**Biosafety Procedures**

**Page:** 25 of 47

SOP #: FCF-BS1  
 Version: 0.5  
 Revision Date: 15.12.11

	<b>Procedural Steps</b>	<b>Learning Points</b>
	<ul style="list-style-type: none"> <li>• Visit Occupational Health Services for your institution</li> <li>• Complete an incident report as outlined in Appendix VI</li> </ul>	

### 2.5.2.2 Spill Outside the Biosafety Cabinet

#### General Tips

- Spills of infectious agents, especially those requiring containment level 2 or higher, outside the biosafety cabinet present a higher risk of infection to laboratory workers.
- Drill your spill plan so that you are familiar with the response procedure.
- Know where your nearest spill kit is located.
- Nearest 2<sup>nd</sup> floor TMDT Spill kits are located:
  - 2<sup>nd</sup> Floor: 2-501 on the north west wall.
  - 2<sup>nd</sup> Floor: main corridor, across from the men's washroom.
- **Follow Spill response found in TMDT Spill Kit.** (also see Appendix VII ).

**Department/Lab:** SickKids-UHN TMDT Flow Cytometry Facility  
**Developed by:** C. Guidos/J.Yuan/J. Shannon/A. Monteath  
**Biosafety Procedures**

**Page:** 26 of 47

SOP #: FCF-BS1  
Version: 0.5  
Revision Date: 15.12.11

- Know which disinfectant and the correct concentration you are going to use.
- Know what contact time is required.
- If eyes have been splashed, rinse under running water (eyewash) for at least 15 minutes.
- If skin has been contaminated, wash with soap and warm water.
- **If anyone requires medical attention please follow the Medical Emergency procedure in Appendix VI.**
- **Report the incident as outlined in Appendix VI.**

### 2.5.3 Biohazardous Waste disposal

<b>Waste Type</b>	<b>Disposal –All waste must be disposed in accordance with UHN requirements as outlined below.</b>
-------------------	--

**Department/Lab:** SickKids-UHN TMDT Flow Cytometry Facility  
**Developed by:** C. Guidos/J.Yuan/J. Shannon/A. Monteath  
**Biosafety Procedures**

**Page:** 27 of 47

SOP #: FCF-BS1  
 Version: 0.5  
 Revision Date: 15.12.11

<b>Waste Type</b>	<b>Disposal –All waste must be disposed in accordance with UHN requirements as outlined below.</b>
<b>Solid Waste</b>	<p><b>Plastic Pipette tips</b>          Place in hard shell container such as yellow biohazard sharps container (waxboard container, plastic media bottles are also accepted). When <math>\frac{3}{4}</math> full seal container and place in yellow bag waste stream.</p> <p><b>Sharps (e.g., needles, glass, glass Pasteur pipettes)</b>          Place in puncture resistant yellow biohazards sharps container. When fill line reached close container and place in yellow bag waste stream.</p> <p><b>All other solid biohazardous Waste</b>          Place in yellow bag waste stream.</p>
<b>Liquid Waste</b>	<p><b>Disinfected liquid biohazardous Waste</b>          Put on face shield and pour treated liquid waste carefully (minimize risk of splashing) into designated sink. Rinse sink completely with tap water.</p> <p>See Appendices IV and V for disposal procedures for liquid waste in cytometer waste tanks.</p>

### 3. Emergency Contact Information

NAME	NAME	EXT.	MOBILE
<b>Principal Investigator</b>	Dr. John Dick	416.581.7470	
<b>Principal</b>	Dr. Cynthia Guidos	416.813.5026	

**Department/Lab:** SickKids-UHN TMDT Flow Cytometry Facility  
**Developed by:** C. Guidos/J.Yuan/J. Shannon/A. Monteath  
**Biosafety Procedures**

**Page:** 28 of 47

SOP #: FCF-BS1  
 Version: 0.5  
 Revision Date: 15.12.11

<b>Investigator</b>			
<b>Lab Contact 1</b>	Sheyun Zhao	416.581.7654	Pager 416.237.3754
<b>Lab Contact 2</b>	Pier Penttila	416.581.7610	
<b>TMDT Security</b>		416.581.7566	
<b>Research Facilities Coordinator Floors 2-3</b>	Tuyet Diep	416.581.7659	
<b>Research Facilities Coordinator Floors 4-10</b>	Jennifer Crosthwaite	416.581.7725	
<b>Research Facilities Planning Safety</b>	John Shannon Donna Johnston	416.581.7566 416.581.8589	
<b>Occupational Health &amp; Safety (UHN)</b>	Toronto General Hosp. Eaton 2-260	P: 416.340.3267 F: 416.340.3463	

#### 4. Revision History

Section	Revision	Date/Initials

**Department/Lab:** SickKids-UHN TMDT Flow Cytometry Facility  
**Developed by:** C. Guidos/J.Yuan/J. Shannon/A. Monteath  
**Biosafety Procedures**

**Page:** 29 of 47

SOP #: FCF-BS1  
 Version: 0.5  
 Revision Date: 15.12.11

## 5. Appendices

### Appendix I

**Table 1: FCF Biosafety Requirements for Analysis and Sorting of Different Sample Types**

Sample Type	Risk Group*	Analysis	Viable Cell Sorting
Primary cells from rodents or animals ( <b>excluding</b> humans and non-human primates (NHP)). Some human and NHP <u>cell lines</u> as determined by SK or UHN Research Biosafety Committees.	RG1 agents	Live/unfixed cells permitted.	Sorters without BSC permitted.
Primary cells or cell lines from human and animals (with RG2 agents, retroviral vectors, etc).	RG2 agents	Live/unfixed cells permitted. User must don safety eyewear. Aerosol producing steps of Live/unfixed cells in BSC only.	Sorters with BSC containment only.
Cells/biological agents requiring Level 3 operating procedures as determined by SK or UHN Research Biosafety Committees.	RG2, Level 3 Operating Procedures	Analysis of live/unfixed cells with BSC containment only. Analysis of fixed cells permitted outside of BSC.	Sorters with BSC containment only.
Other	RG3 or RG4 agents	Not Permitted.	Not Permitted.
Yeast or bacteria	RG1 or RG2 organism	Requires FCF Staff assistance and prior approval.	Not permitted.

**Department/Lab:** SickKids-UHN TMDT Flow Cytometry Facility  
**Developed by:** C. Guidos/J.Yuan/J. Shannon/A. Monteath  
**Biosafety Procedures**

**Page:** 30 of 47

SOP #: FCF-BS1  
Version: 0.5  
Revision Date: 15.12.11

\* Public Health Agency of Canada (PHAC) risk group (RG) classification scheme  
<http://www.phac-aspc.gc.ca/publicat/lbg-ldmbl-04/index-eng.php>

*Sample fixation: must be fixed with 0.5-2 per cent paraformaldehyde for at least 30 minutes on ice after immunofluorescence staining. Alternative fixation protocols must be pre-approved by the FCF. Alternative fixation protocols may be required as per SK or UHN Research Biosafety Committees subject to agent used and susceptibility to standard fixation protocol.*

**Department/Lab:** SickKids-UHN TMDT Flow Cytometry Facility  
**Developed by:** C. Guidos/J.Yuan/J. Shannon/A. Monteath  
**Biosafety Procedures**

**Page:** 31 of 47

SOP #: FCF-BS1  
Version: 0.5  
Revision Date: 15.12.11

## Appendix II

### Analysis Biosafety Information

All analysis must be performed in compliance with the SickKids-UHN TMDT Flow Cytometry Facility Biosafety Policy (<http://www.sickkids.ca/research/FCF/biosafety.asp>). For every analysis appointment, clients must indicate the types of samples to be analyzed and identify the Risk Group (RG) categories of any biological agents their samples contain, based on the *Laboratory Biosafety Guidelines* (3<sup>rd</sup> Edition, 2004, Public Health Agency of Canada-PHAC, as enforced by the *Human Pathogens and Toxins Act*) and *Containment Standards for Veterinary Facilities* (Canadian Food Inspection Agency-CFIA, 2007) Risk Group Classifications. A guideline for assessing RG categories of your samples can be found at <http://www.absa.org/riskgroups/>.

The SickKids-UHN TMDT FCF operates in CL2 facilities using CL2 operations as a minimum standard of practice. Therefore, FCF facilities are capable of handling samples designated as RG1 or RG2. **No RG3 and RG4 agents are permitted.**

Please consult TABLE 1: FCF Biosafety Requirements for Analysis and Sorting of Different Sample Types (<http://www.sickkids.ca/research/FCF/biosafety.asp>) to determine whether your samples need to be fixed prior to analysis. **In addition, please complete the table below.**

**Department/Lab:** SickKids-UHN TMDT Flow Cytometry Facility  
**Developed by:** C. Guidos/J.Yuan/J. Shannon/A. Monteath  
**Biosafety Procedures**

**Page:** 32 of 47

SOP #: FCF-BS1  
Version: 0.5  
Revision Date: 15.12.11

**Information about Samples to be analyzed in this appointment**

**SickKids Permit or UHN research Biosafety Certificate # issued to your Principal Investigator for this work:**

<<Type here>>

Risk Group of samples: RG1  RG2  RG3  RG4

*(Note: Users who identify their samples as RG3 or RG4 will receive a warning message that these agents are not permitted and they will not be able to proceed with the booking)*

Are cells fixed? Yes  No

Fixative (0.5-2 per cent paraformaldehyde for at least 30 minutes on ice) Yes  No

Alternative fixative used (please specify):

Do cells/biological agents require Level 3 operating procedures as determined by SK/UHN Research Biosafety Committee?

Yes  No

If yes, identify cells/biological agent(s) and Risk Group:

**I agree to abide by all SickKids-UHN TMDT Flow Cytometry facility Biosafety Policies (<http://www.sickkids.ca/research/FCF/biosafety.asp>) and**

**Department/Lab:** SickKids-UHN TMDT Flow Cytometry Facility  
**Developed by:** C. Guidos/J.Yuan/J. Shannon/A. Monteath  
**Biosafety Procedures**

**Page:** 33 of 47

SOP #: FCF-BS1  
Version: 0.5  
Revision Date: 15.12.11

**certify that the information provided above is true, correct, and complete.**

Yes  No

**I certify that these samples and procedures have been approved by the SickKids or UHN Institutional Biosafety Committee. Yes  No**

**Department/Lab:** SickKids-UHN TMDT Flow Cytometry Facility  
**Developed by:** C. Guidos/J.Yuan/J. Shannon/A. Monteath  
**Biosafety Procedures**

**Page:** 34 of 47

SOP #: FCF-BS1  
Version: 0.5  
Revision Date: 15.12.11

### Appendix III

#### Sorting Biosafety Information

All sorting must be performed in compliance with the SickKids-UHN TMDT Flow Cytometry Facility Biosafety Policies (<http://www.sickkids.ca/research/FCF/biosafety.asp>). For every sorting appointment, clients must indicate the types of samples to be sorted and identify the biological agents Risk Group (RG) categories and any chemical hazards their samples contain, based on the *Laboratory Biosafety Guidelines* (3<sup>rd</sup> Edition, 2004, Public Health Agency of Canada-PHAC, as enforced by the *Human Pathogens and Toxins Act*) and *Containment Standards for Veterinary Facilities* (Canadian Food Inspection Agency-CFIA, 2007) Risk Group Classifications. A guideline for assessing RG categories of your samples can be found at <http://www.absa.org/riskgroups/>.

The SickKids-UHN TMDT FCF operates in CL2 facilities using CL2 operations as a minimum standard of practice. Therefore, FCF facilities are capable of handling samples designated as RG1 or RG2. **RG3 and RG4 biohazardous agents are not permitted in the FCF and may NOT be run on any sorter.** High-speed cell sorters operate under high pressure and create aerosols that potentially increase the risk of infectivity for many agents that might be contained in these aerosols. Therefore, sorting of all RG2 samples must be performed within a FCF certified Class II Type A2 Biological Safety Cabinet (BSC).

In order to have your sorting appointment approved, please consult TABLE 1: FCF Biosafety Requirements for Analysis and Sorting of Different Sample Types (<http://www.sickkids.ca/research/FCF/biosafety.asp>) and ensure that you have booked a sorter with BSC if warranted by your sample type. **In addition, please complete a line in the table below for each sample to be sorted.**

**Department/Lab:** SickKids-UHN TMDT Flow Cytometry Facility  
**Developed by:** C. Guidos/J.Yuan/J. Shannon/A. Monteath  
**Biosafety Procedures**

**Page:** 35 of 47

SOP #: FCF-BS1  
 Version: 0.5  
 Revision Date: 15.12.11

**Information about Samples to be sorted in this appointment**

**SickKids Permit or UHN research Biosafety Certificate # issued to your Principal Investigator for this work:**

<<Type here>>

Species origin of cells  (e.g. human, mouse, rat)	Primary cells (from what tissue) or cell line (give name)	Risk group	List any chemical treatment (e.g. PMA & Ionomycin or other chemical mitogens, lipofectamine or other transfection reagents) and it's associated risk (e.g. carcinogen)	List any Infectious Biological Agent cells have been exposed to and Risk Group for each (e.g. bacteria, retrovirus, lentivirus, adenovirus, EBV, etc.)	Have the cells been infected or transfected with any gene transfer vector(s)? If yes, list vector(s) and gene(s) transferred and Risk Group for each
e.g. murine	Bone marrow	RG2	None	Lentivirus, RG2	pLEN (H1GFP), shRNA cmyc, RG2
1.					
2.					
3.					
4.					

**I agree to abide by all SickKids-UHN TMDT Flow Cytometry Facility Biosafety Policies (<http://www.sickkids.ca/research/FCF/biosafety.asp>) and certify that the information provided above is true, correct, and complete. Yes  No**

**I certify that these samples and procedures have been approved by the SickKids or UHN Institutional Biosafety Committee. Yes  No**

**Department/Lab:** SickKids-UHN TMDT Flow Cytometry Facility  
**Developed by:** C. Guidos/J.Yuan/J. Shannon/A. Monteath  
**Biosafety Procedures**

**Page:** 36 of 47

SOP #: FCF-BS1  
Version: 0.5  
Revision Date: 15.12.11

## Appendix IV

### Decontamination Procedures For Analyzers

#### A. BD Analytical Instruments

The following procedures must be carried out at the end of **each** appointment.

1. Run bleach for 1 min. in STANDBY mode with SIP arm away (this step is not applicable for Cantoll).
2. Run bleach for 5 min. in RUN mode on HIGH with SIP arm at rest.
3. Run rinse solution for 1 min. in STANDBY mode with SIP arm away (this step is not applicable for Cantoll).
4. Run rinse solution for 5 min. in RUN mode on HIGH with SIP arm at rest.
5. Run ddH<sub>2</sub>O for 1 min. in STANDBY mode with SIP arm away (this step is not applicable for Cantoll).
6. Run ddH<sub>2</sub>O for 5 min. in RUN mode on HIGH with SIP arm at rest.
7. Fill up sheath tank.
8. Put on face shield
9. Empty waste tank carefully (to avoid splashing) into designated sink and flush sink with cold water.
10. Add 500 mL of 20 per cent bleach to empty waste tank.
11. Wipe down keyboard, mouse, benchtop and instrument panel with Virox wipes.

#### B. BD Analytical Instruments with HTS unit

The FCF Daily Cleaning procedure must be carried out at the end of **each** appointment.

FCF Daily Cleaning

**Department/Lab:** SickKids-UHN TMDT Flow Cytometry Facility  
**Developed by:** C. Guidos/J.Yuan/J. Shannon/A. Monteath  
**Biosafety Procedures**

**Page:** 37 of 47

SOP #: FCF-BS1  
Version: 0.5  
Revision Date: 15.12.11

1. Prepare plate by filling wells A1-4 with 70 per cent ethanol, wells B1-4 with bleach, wells C1-4 with Rinse and wells D1-4 with water.
2. Choose HTS > Clean
3. Select FCF Daily Clean- 96 well U-bottom template, OK.
4. Load plate, put on cover and put machine in RUN mode.
5. Click OK. (takes ~20 minutes).

#### Placing HTS Unit into Long-Term Storage

If you are not using the unit for a week or more place HTS unit into long-term storage.

1. Slide HTS unit slightly toward you to access the tubing at the back.
2. Detach (clear) sheath line from HTS unit.
3. Connect purging assembly line to the Sheath (B) port.
4. Put the end of the purging assembly line into a beaker containing ddH<sub>2</sub>O.
5. Put the safety cover on the HTS unit.
6. Choose HTS> Prime, repeat nine times. (each prime take ~2 minutes).
7. Remove the purging assembly line from the ddH<sub>2</sub>O.
8. Disconnect it from HTS unit.
9. Remove HTS unit, replace DCM sleeve and return cytometer to tube mode.
10. Wipe probe and syringe with kimwipe dampened with water and place HTS unit back on shelf.

#### **C. FC500 Instrument Decontamination Procedure**

1. Clear the work list (Click on 'New worklist' icon, just right of cytometer control icon).
2. Click on the panel tab (just beside protocol tab in Resource Manager).
3. Select 'common' folder.
4. Open 'After Acquis Wash.PNL' panel (drag it into the worklist area).
5. Enter Carousel number in the work list. Make sure MCL MANUAL MODE and SINGLE TUBE MODE buttons are deselected.

**Department/Lab:** SickKids-UHN TMDT Flow Cytometry Facility  
**Developed by:** C. Guidos/J.Yuan/J. Shannon/A. Monteath  
**Biosafety Procedures**

**Page:** 38 of 47

SOP #: FCF-BS1  
Version: 0.5  
Revision Date: 15.12.11

6. Put 1 tube of bleach and 2 tubes of ddH<sub>2</sub>O (place tubes in order into the location specified on the worklist).
7. Click Start/Play.
8. Wait for the panel to complete and exit the CXP software. (approx. 15 mins)
9. Fill sheath tank when the alarm sounds.
10. Put on Face shield
11. Empty waste tank when the alarm sounds. Empty waste tank carefully (to avoid splashing) into designated sink and flush sink with cold water. Add 20 per cent bleach to empty waste tank up to the red line.
12. Wipe down keyboard, mouse, benchtop and instrument panel with Virox wipes.

**Department/Lab:** SickKids-UHN TMDT Flow Cytometry Facility  
**Developed by:** C. Guidos/J.Yuan/J. Shannon/A. Monteath  
**Biosafety Procedures**

**Page:** 39 of 47

SOP #: FCF-BS1  
Version: 0.5  
Revision Date: 15.12.11

## Appendix V

### Operating and Decontamination Procedures For Sorters

The following procedures are to be carried out by trained FCF Operators only.

#### Routine Maintenance

- Check sheath line and waste line daily for leaks. Replace any leaking lines immediately.
- Rinse sheath tank with clean sheath if there is debris or crystals inside the tank.
- Replace waste tank caps monthly or if filter gets wet.

#### Sample handling

- Gloves and lab coats/gowns are worn at all times.
- Sample tubes must be uncapped and recapped inside the BSC.
- Collection tubes should be capped and wiped with Virox before removing from BSC.
- Visually inspect samples to be sorted to ensure samples appear clump free (filter samples if necessary in the BSC).

#### A. Aria Sorters

1. At the beginning of the day, put on face shield and empty waste tank carefully (to avoid splashing) into designated sink, flushing sink with cold water.
2. Replace with alternate tank containing 500 mL of 20 per cent bleach.
3. If sorter is located inside a BSC, open cabinet doors and turn on water bath if required.
4. Close cabinet doors and turn on BSC ensuring gauge (which displays the static pressure within the pressure plenum supplying the downflow and exhaust filters) reads 0.5" w.g.  $\pm$  0.1" w.g. (wait at least 15 minutes to establish circulation before sorting).
5. Wipe down keyboard, computer mouse, workstation, sorter, sorter table

**Department/Lab:** SickKids-UHN TMDT Flow Cytometry Facility  
**Developed by:** C. Guidos/J.Yuan/J. Shannon/A. Monteath  
**Biosafety Procedures**

**Page:** 40 of 47

SOP #: FCF-BS1  
Version: 0.5  
Revision Date: 15.12.11

- and interior surface of cabinet with Virox.
6. Turn on sorter.
  7. Perform fluidics start up if required.
  8. Remove closed loop nozzle and insert correct size nozzle.
  9. Start stream and proceed with sort setup.
  10. Upon termination of sort, wait 2 minutes before removing collection tubes. (Collection tubes should be capped and wiped before removing from BSC).
  11. Between appointments, run a full 15 mL conical tube of bleach for 5 minutes and a full tube of ddH<sub>2</sub>O for 10 minutes. Wipe down sort block, sort collection chamber, sample collection tube holder, and loading port including tube holder with Virox. Change gloves.
  12. At the end of the day run full 15 mL conical tube of bleach for 15 minutes and a full 15 mL conical tube tube of ddH<sub>2</sub>O for 15 minutes. Perform 'clean flow cell' one time with FACSRinse and two times with ddH<sub>2</sub>O or if the instrument is not being used the next day perform 'fluidics shut down' with 70 per cent ethanol. Rinse nozzle with ddH<sub>2</sub>O and dry with compressed air. Wipe down sort block, sort collection chamber, sample collection tube holder, loading port including tube holder and clean the surface of inside biosafety cabinets with Virox. Turn off sorter and BSC. Refill sheath tank.
  13. If a clog occurs during sorting, sort will terminate automatically and stream will shut off: Wait 2 to 3 minutes before opening sort chamber door. Using Virox wipe down sort block, sort collection chamber, sample collection tube holder, loading port including tube holder and clean the inside surfaces of the biosafety cabinet. Follow standard procedures to remove the clog and resume sort.

## **B. Moflo Sorters**

At the beginning of the day, turn on the water cooling systems for the I-70 and I-300C Coherent lasers and the lasers, if needed. Turn on the power supply of the 488 laser first, wait for 5 minutes then turn on the laser by switching the key on the power supply. While the laser warms up, proceed with the following:

1. Put on Face shield. Empty waste tank carefully (to avoid splashing) into

**Department/Lab:** SickKids-UHN TMDT Flow Cytometry Facility  
**Developed by:** C. Guidos/J.Yuan/J. Shannon/A. Monteath  
**Biosafety Procedures**

**Page:** 41 of 47

SOP #: FCF-BS1  
Version: 0.5  
Revision Date: 15.12.11

- designated sink, flush sink with cold water, add 500 mL of 20 per cent bleach.
2. Wipe down keyboard, computer mouse, workstation, sorter, sorter table and interior surface of cabinet with Virox.
  3. Turn on the Moflo-XDP BSC, clean nozzle tip with ddH<sub>2</sub>O using Q-tips, tighten the sheath relief valve, turn on sorter, start stream and proceed to de-bubble and back flush at least 3 times respectively.
  4. Clean deflection plates with ethanol swabs, install the plates and proceed with sort setup.
  5. Upon termination of sort, wait 2 minutes before removing collection tubes. (Collection tubes should be capped and wiped before removing from BSC.)
  6. Between appointments: For the Moflo-XDP, run a full 15 mL conical tube of bleach for 5 minutes and a full tube of ddH<sub>2</sub>O for 10 minutes. Wipe down sort chamber, cyclone, deflection plates, sortRescue or sample cooling kit and sample loading port including sample holder of the SmartSampler with Virox. Change gloves. For the Moflo, run a full 5 mL of bleach for 5 minutes and full tube of ddH<sub>2</sub>O for 10 minutes. Wipe down sort chamber, Cyclone, deflection plates, sortRescue or sample cooling kit and manual sample station with Virox. Change gloves.
  7. At the end of the day turn off laser power supplies for the Coherent lasers to let the lasers cool down and then proceed with the following daily cleaning procedures.
  8. Run full 15 mL conical (or 5 mL FACS tube for the MoFlo) tube of bleach for 15 minutes, run 15 mL of FACSRinse for 15 minutes and a full 15 mL conical tube of ddH<sub>2</sub>O for 15 minutes. Shut down instrument by running a tube of 70 per cent ethanol in the sample line. Wipe down sort chamber, cyclone, deflection plates, sortRescue or sample cooling kit and sample loading port including sample holder of the SmartSampler or manual sample station and clean the surface of inside biosafety cabinets with Virox. Turn off sorter and BSC.
  9. Remove deflection plates and rinse with water and dry the plates with paper towel immediately after cleaning and keep it at a designated place.
  10. Refill sheath tank, release sheath relief valve and turn off the water cooling system for the Coherent lasers.

**Department/Lab:** SickKids-UHN TMDT Flow Cytometry Facility  
**Developed by:** C. Guidos/J.Yuan/J. Shannon/A. Monteath  
**Biosafety Procedures**

**Page:** 42 of 47

SOP #: FCF-BS1  
Version: 0.5  
Revision Date: 15.12.11

11. If a clog occurs during sorting, for the Moflo-XDP, sort will terminate automatically and stream will shut off: Wait 2 to 3 minutes before opening sort chamber door. Wipe down sort chamber, cyclone, deflection plates, sortRescue or sample cooling kit and sample loading port including sample holder of the SmartSampler with Virox and clean the inside surfaces of the biosafety cabinet. For the Moflo, turn off stream immediately first. Wait 2 to 3 minutes before opening sort chamber door. Wipe down sort chamber, cyclone, deflection plates, sortRescue or sample cooling kit and manual sample station with Virox. Follow standard procedures to remove the clog and resume sort.

### **C. Influx Sorter**

At the beginning of the day, turn on the main power supply to the lasers first, wait 5 minutes, then turn on the blue laser (488nm) by switching the key to the 'on' position. Next, turn the keys for the red (633nm) and violet (403nm) lasers from the 'standby' to the 'on' position to activate the laser power. While the lasers warm up, proceed with the following:

1. Put on Face shield. Empty waste tank carefully (to avoid splashing) into designated sink, flush sink with cold water, add 500 mL of 20 per cent bleach.
2. Wipe down keyboard, computer mouse, workstation, sorter, sorter table and interior surface of the biosafety cabinet with Virox.
3. Turn on the Influx BSC, clean nozzle tip with ddH<sub>2</sub>O using Q-tips, turn on sorter, start stream and proceed to de-bubble and back flush at least 3 times respectively.
4. Clean deflection plates with ddH<sub>2</sub>O and ethanol swabs and proceed with sort setup.
5. Upon termination of sort, wait 2 minutes before removing collection tubes. (Collection tubes should be capped and wiped with Virox before removing from the BSC.)
6. Between appointments, run a full 5 mL polypropylene tube of bleach for 5 minutes and a full 5 mL tube of ddH<sub>2</sub>O for 10 minutes. Wipe down sort chamber, deflection plates, sample loading port and sample holder device

**Department/Lab:** SickKids-UHN TMDT Flow Cytometry Facility  
**Developed by:** C. Guidos/J.Yuan/J. Shannon/A. Monteath  
**Biosafety Procedures**

**Page:** 43 of 47

SOP #: FCF-BS1  
Version: 0.5  
Revision Date: 15.12.11

- with Virox. Change gloves.
7. At the end of the day, turn off laser power for Coherent lasers to allow them to cool down and proceed with the following daily cleaning procedures.
  8. Run a full 5 mL tube of bleach for 15 minutes, then a full 5 mL tube of FACSRinse for 15 minutes, and finally a full 5 mL tube of ddH<sub>2</sub>O for 15 minutes. Shut down instrument by running a tube of 70 per cent ethanol in the sample line. Wipe down sort chamber, deflection plates, sample loading port, sample holder device and the surfaces inside the biosafety cabinet with Virox. Turn off sorter and BSC.
  9. If a clog occurs during sorting, terminate the sort and shut the stream off. Wait 2 to 3 minutes before opening the sort chamber door. Wipe down the sort chamber, deflection plates, sample loading port, sample holder device, and the interior surfaces of the biosafety cabinet with Virox. Follow standard procedures to remove the clog and resume the sort.

**Department/Lab:** SickKids-UHN TMDT Flow Cytometry Facility  
**Developed by:** C. Guidos/J.Yuan/J. Shannon/A. Monteath  
**Biosafety Procedures**

**Page:** 44 of 47

SOP #: FCF-BS1  
 Version: 0.5  
 Revision Date: 15.12.11

## Appendix VI

### TMDT Emergency Response Plan- INJURIES

If exposure to potentially infectious material (cuts, needle sticks, punctures, scratches, animal bites, etc.) occurs, the injured area must be immediately washed thoroughly with soap and water, disinfected and the cut then covered with a sterile bandage. Report the incident and obtain assistance as indicated in the matrix below:

<b>Injury Matrix-UHN Personnel*</b>			
<b>Injury Severity</b>	<b>Examples</b>	<b>Monday to Friday 7:30 a.m. to 3:30 p.m.</b>	<b>Weekends, Holidays or clinic off hours</b>
Injuries that do not require immediate treatment by a physician	Sprains, strains, Needle sticks, minor cuts, infectious aerosols exposure, abrasions or burns etc.	UHN Occupational Health Clinic <b>TGH Eaton 2-260 ext. 14-3267 fax. 14-3463</b>	Toronto General Emergency Department
Injuries requiring treatment by a physician	Fractures, cuts requiring stitches, major burns, etc.	Toronto General Emergency Department	Toronto General Emergency Department
<b>All Personnel:</b>			
Severe Injury requiring immediate assistance	Unconsciousness, cardiac arrest, etc.	<b>Dial 9-911 then 416.581.6161</b> Follow instructions in: <b>'TMDT Emergency Call Procedures'</b>	

All incidents must be reported to the senior investigator as soon as possible. Incidents must be reported to UHN Occupational Health and Safety within 24 hours. Fax the completed and signed '*Employee Incident Report*' form to Occupational Health and Safety.

**Department/Lab:** SickKids-UHN TMDT Flow Cytometry Facility  
**Developed by:** C. Guidos/J.Yuan/J. Shannon/A. Monteath  
**Biosafety Procedures**

**Page:** 45 of 47

SOP #: FCF-BS1  
Version: 0.5  
Revision Date: 15.12.11

\*Non-UHN Personnel should seek medical attention as necessary and abide by institutional requirements for incident/injury reporting.

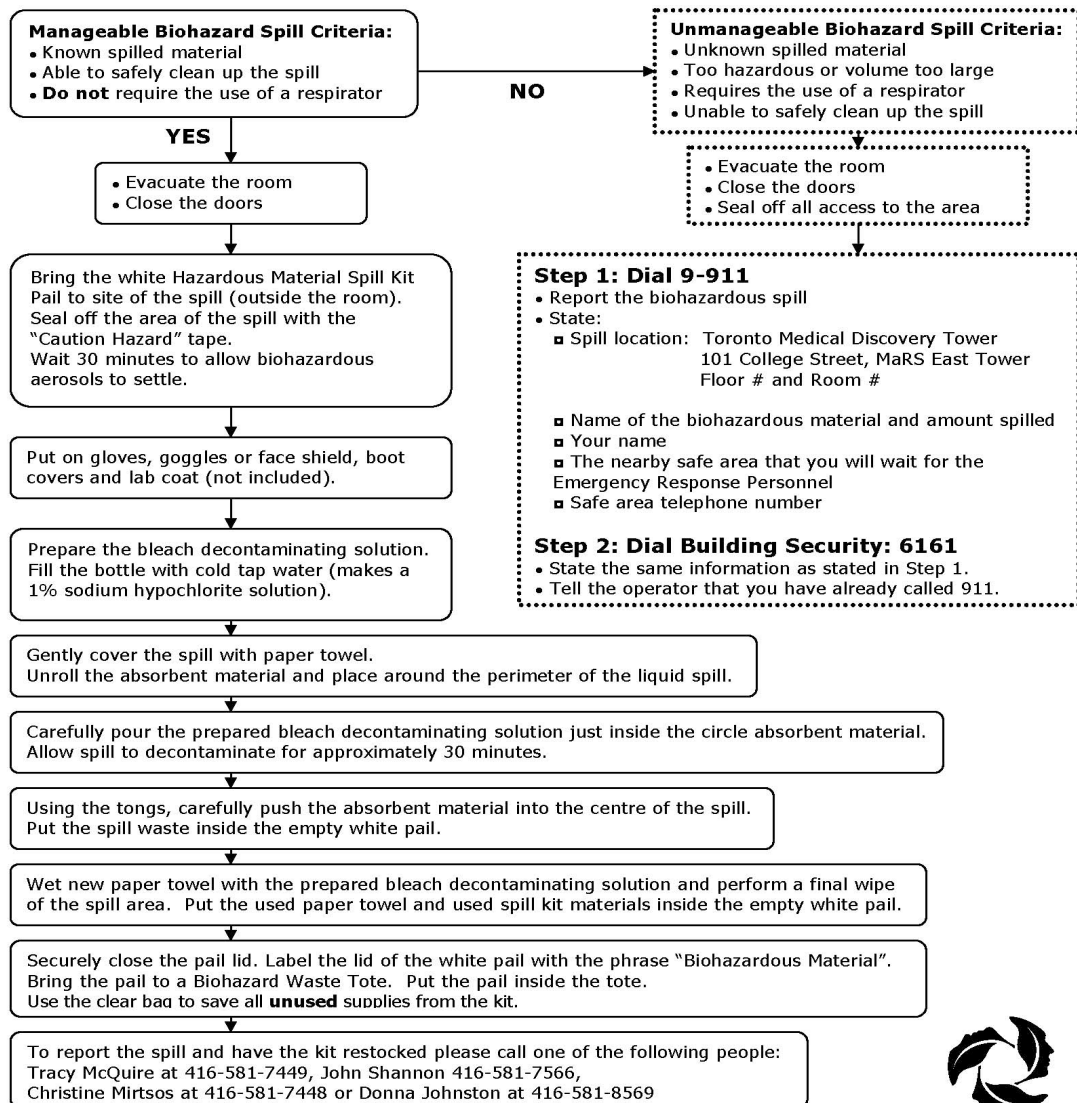
## **Appendix VII**

**Department/Lab:** SickKids-UHN TMDT Flow Cytometry Facility  
**Developed by:** C. Guidos/J.Yuan/J. Shannon/A. Monteath  
**Biosafety Procedures**

**Page:** 46 of 47

SOP #: FCF-BS1  
 Version: 0.5  
 Revision Date: 15.12.11

## Biohazard Spill Instructions



**Department/Lab:** SickKids-UHN TMDT Flow Cytometry Facility  
**Developed by:** C. Guidos/J.Yuan/J. Shannon/A. Monteath  
**Biosafety Procedures**

**Page:** 47 of 47

SOP #: FCF-BS1  
 Version: 0.5  
 Revision Date: 15.12.11

## Chemical Spill Instructions

