ADDENDUM

TO

NYUSOM
SMILOW DUAL AGENT BSL3 FACILITY
SAFETY MANUAL

STANDARD OPERATING PROCEDURES

FOR

CELL SORTING
WITH iCYT® SYNERGY

November 2011
Amendment to NYUSOM Smilow Dual Agent BSL3 Facility Safety Manual
Standard Operating Procedures for Cell Sorting with iCyt Synergy – Nov. 2011
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<th><strong>Issue date</strong></th>
<th>11/2011</th>
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1 High-speed cell sorting and biosafety

High-speed droplet based cell sorters have the potential to generate aerosols, which can, if not properly contained, expose operators to infectious agents. The heightened concern in the case of cell sorting arises from the possibility that cells or microorganisms may be delivered directly into the lungs of personnel in the vicinity of a cell sorter. In theory, this could increase the risk of infection with an occult pathogen, transfer of genetic material, sensitization to antigens or other potentially harmful effects. Although such adverse effects have not been documented as a consequence of exposure to aerosols during cell sorting, there is sufficient concern about this to warrant the implementation of procedures to eliminate any excess risk to personnel. “Complete BSL-3 containment is required when agents known to be transmitted by the inhalation route are sorted since the greatest potential of exposure or infection from these agents is from aerosolization (e.g., M. tuberculosis) and they are known to cause substantial morbidity and mortality.” (Schmid, I. et al. (2007). International Society for Analytical Cytology Biosafety Standard for Sorting of Unfixed Cells. Cytometry Part A, 71A: 414-437).

2 Containment

2.1 Facility

The iCyt Synergy cell sorter is located in the TB laboratory (room SML1009E, see layout below) of the NYUSOM Smilow Dual Agent BSL3 Facility, on the tenth floor of the Smilow Research Center building, on the NYU Langone Medical Center main campus.

For general facility layout, air handling system and containment procedures, refer to the NYUSOM Smilow Dual Agent BSL3 Facility Safety Manual.

2.2 Biosafety cabinets (BSC)

The iCyt Synergy cell sorter is housed in a dedicated four-foot SterilGARD® III Advance BSC (The Baker Company, Inc.). An additional four-foot Baker Class II Type A BSC is available in the room for sample preparation when the iCyt Synergy cell sorter is in use or for other experiments when sorting is not being conducted. NYULMC Environmental Health and Safety (EH&S) retains a vendor (Technical Safety Services, Inc. www.techsafety.com) who certifies each BSC semi-annually. The certification is conducted in accordance with NSF Standard 49 and currently accepted best practices.

2.3 Aerosol management system (AMS)

The iCyt Synergy cell sorter is equipped with an aerosol management system to remove and filter aerosols from the sorter’s interior compartments. Aerosols from these compartments are
drawn directly into the HEPA filter of the machine’s BSC. Function of the AMS can be confirmed using the aerosol containment test described in section 4.5.

3 Facility entry and exit

Entry into and exit from the BSL3 facility is through the Clean Vestibule (see NYUSOM Smilow Dual Agent BSL3 Facility Safety Manual), which provides a safe environment for personnel to gown (upon entry). All personnel must sign the entry/exit log.

3.1 Entry protocol

The operator entering the Dirty Vestibule through the Clean Vestibule must be wearing the following:
Personal Protective Clothing | Comments
--- | ---
NIOSH-approved N95 particulate respirator or hooded PAPR | For personnel with beards or other factors that limit the efficacy of an N95 respirator, a hooded PAPR unit must be used to ensure safety. Unless a hooded PAPR is available in the Dirty Vestibule.
Tyvex suit | Unless one is available in the Dirty Vestibule.
Inner gloves
Outer gloves
Inner shoe covers
Outer shoe covers
Hair covers/eye protection | Recommended but not compulsory.

Approved hooded PAPR include, without being limited to:
1. Air-Mate Respirator (3M)
2. 700 Shroud System (Maxair Systems)

For general operating procedures of hooded PAPR, see NYUSOM Smilow Dual Agent BSL3 Facility Safety Manual and manufacturer’s recommendations.

### 3.2 Exit protocol

When leaving the TB Laboratory to enter the Dirty Vestibule remove personal protective clothing in the following order:

<table>
<thead>
<tr>
<th>Personal Protective Clothing</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyvex suit</td>
<td>Used Tyvex suits can be kept for up to 15 days if dated and marked with the user’s initials, hung on dedicated hooks on the left side of the TB Laboratory exit door.</td>
</tr>
<tr>
<td>Outer shoe covers</td>
<td>Must not be worn in the Dirty Vestibule.</td>
</tr>
<tr>
<td>Outer gloves</td>
<td></td>
</tr>
<tr>
<td>Hair covers <em>(if worn)</em></td>
<td></td>
</tr>
</tbody>
</table>

A biohazard waste receptacle (a covered plastic bin lined with a biohazard autoclave bag) is available inside the TB Laboratory for disposal of protective clothing.

In the Dirty Vestibule, the hooded PAPR if worn can be removed, plugged for charging and stored in a container located near the common bench.

When leaving the Dirty Vestibule to enter the Clean Vestibule, remove the NIOSH-approved N95 particulate respirator if worn and the inner shoe covers. A biohazard waste receptacle is available at the Dirty Vestibule exit area for disposal of protective clothing. Workers enter the Clean Vestibule wearing the inner gloves.
In the Clean Vestibule, workers discard inner gloves in a biohazard waste receptacle and wash their hands using the sink in the Clean Vestibule before exiting the facility.

4 iCyt Synergy cell sorter operation

Considering the containment measures in place for the iCyt Synergy cell sorter (see section 2), the sorter’s use is not considered a high-risk procedure. However, due to the layout of the TB laboratory and to ensure optimal safety, unrelated experiments cannot be conducted during operation of the iCyt Synergy cell sorter. A web-based calendar ensures that all BSL3 work is adequately scheduled.

The iCyt Synergy cell sorter is operated according to the manufacturer’s manual (available at NYUSOM OCS Flow Cytometry Facility Core).

4.1 Startup protocol

Machine startup steps will be performed in the following order:

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Check sheath, waste and diH20 levels.</td>
<td>Sheath will not be added unless waste tank is empty. See section 4.4.</td>
</tr>
<tr>
<td></td>
<td>for emptying waste. Add sheath and diH20 as necessary.</td>
</tr>
<tr>
<td>Turn on Air and Vacuum lines.</td>
<td>Ensure vacuum filter is dry. Replace if necessary.</td>
</tr>
<tr>
<td>Turn on BSC and iCyt Synergy cell sorter.</td>
<td>Machine and blower will be on for 15 minutes before use.</td>
</tr>
<tr>
<td>Run “Fluid Start” using the iCyt software.</td>
<td>Verify vacuum system is clearing waste stream</td>
</tr>
<tr>
<td>Align and setup sorter as per the manufacturer’s user manual.</td>
<td></td>
</tr>
</tbody>
</table>

No sample will be placed on the sorter until these steps have been completed.

4.2 Clog or failure protocol

In the event of a clog, the stream may lose stability and generate an aerosol. The stream will be turned off immediately and the sample removed. Any aerosol should be contained within the sort block and filtered out by the AMS. If the stream restarts correctly, the sort can continue. If the clog persists, the nozzle must be removed and cleaned or replaced with a secondary stand-by nozzle.

If the nozzle must be removed for cleaning, it will be treated as biohazardous. After determining that the nozzle needs to be removed, the operator will wait 60 seconds for any aerosol to clear.
from the interior compartments. The stream will be turned off, and the nozzle taken out of the machine. The nozzle will be placed into a 5ml tube, filled with 10% Contrad™ detergent, which is then capped. This tube is then placed into the sonicator within the BSC for cleaning. After sonication, the capped tube is opened and the cleaned nozzle can be removed.

Prevention of clogs by filtering all samples through a 70um cell strainer (or smaller) will greatly reduce the likelihood of aerosol generation, and is therefore required. Samples should be filtered prior to delivery to operator, though strainers will be available in the TB room to re-strain particularly problematic samples.

In the event that the vacuum line fails, an aerosol can be created where the stream enters the waste catcher. Any aerosol should be contained within the sort block and filtered out by the AMS. Turn the stream off immediately and attempt to resume vacuum. If unable to, the sorter will not be used until Facilities Operations & Maintenance has resolved vacuum issue.

### 4.3 Shutdown protocol

After sorting is completed, follow the protocol below for sort shutdown:

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shutdown stream using Nozzle Source, Off, from HAPS Startup page</td>
<td>Should turn off instantaneously</td>
</tr>
<tr>
<td>Run decontamination procedure in section 4.3.1</td>
<td></td>
</tr>
<tr>
<td>Remove Collection tubes from sort chamber.</td>
<td>Wait at least 60 seconds after stream is turned off before opening sorting chamber in order to let aerosol evacuation.</td>
</tr>
<tr>
<td>Run the “Fluid Stop” command within the software.</td>
<td></td>
</tr>
<tr>
<td>Remove all sample and infectious material from sorter and hood.</td>
<td>All tubes will be capped and placed in waste bag. Waste bag will be sealed and sprayed before removal.</td>
</tr>
<tr>
<td>Wipe down all surfaces with Vesphene IISe followed by 70% isopropanol</td>
<td>Both surfaces inside and outside the hood will be decontaminated as in Section 4.4.2.</td>
</tr>
<tr>
<td>Empty waste tank.</td>
<td>Waste needs to be decontaminated before disposal. See section 4.4.</td>
</tr>
<tr>
<td>Shutdown software, Synergy, and air and vacuum lines.</td>
<td></td>
</tr>
</tbody>
</table>

#### 4.3.1 Sample line decontamination procedure
In the HAPS Service window of the iCyt Synergy Software, click the fluidics tab. In this tab, ensure the sheath flow is off. Load a tube filled with 10% concentration of household bleach to a volume greater than that of the sample just run. Close the sample station and turn sample flow and sample pressure on. Toggle Nozzle on and off to ensure that the entire nozzle is disinfected. Repeat above procedure with a tube of diH2O to rinse out the bleach.

This procedure ensures that ALL tubing that is exposed to sample during regular operation is disinfected. These surfaces include the sample tubing, the pinch valve tubing and surfaces in the flow body and nozzle.

4.4 Maintenance/decontamination

4.4.1 iCyt Synergy cell sorter waste disposal

All material entering the sorter’s waste tank will be considered biohazardous and must be disinfected before disposal down the sink in the TB Laboratory. To accomplish this, enough bleach will be added to the waste tank before use to result in a 10% concentration when full. Bleach solution must be in contact with contaminated waste fluid for a minimum of 30 minutes before disposal. Since bleach is corrosive to stainless steel, the inside of the tank will be visually inspected after emptying to confirm integrity has not been compromised.

4.4.2 Surface disinfection

After sorting all surfaces inside the hood and in the Synergy’s interior compartments will be disinfected with Vesphene IISe followed by 70% isopropanol.

Surfaces outside the hood that may have accidentally become contaminated will be decontaminated with Vesphene IISe (or Staphene spray) as well. This includes computer desk surfaces, as well as the keyboard and mouse. If the protective covers on the keyboard and mouse have become soiled, they will be disposed of and replaced before the next sort.

4.4.3 Synergy fluidics decontamination

This procedure will be run on a monthly basis or when the machine’s fluidics have become contaminated.

The procedure is initiated by running the Aseptic Prep command in the iCyt Synergy software. The Rinse tank will be filled with 10% bleach for this procedure. The procedure is automated and is described in section 16.3.1 of the iCyt Synergy cell sorter manual.

4.5 Aerosol containment test
In order to ensure containment of aerosols by the Aerosol Management System (AMS) and BSC, the aerosol containment testing protocol will be used at a minimum after service of the iCyt Synergy cell sorter.

The equipment used in this protocol include a fluorescence microscope, and Glo Germ™ beads, as well as an E-Lite Pump™ and an Air-O-Cell™ cassette, both supplied by EMSL Analytical, Inc. The test protocol is adapted from the sampling guide available from the manufacturer. The protocol follows:

- Prior to sampling, calibrate the pump to 15 liters per minute.
- Remove and retain tape seal covering Air-O-Cell™ inlet and outlet.
- Attach the outlet (round hole) to the supplied tubing adapter, positioning the inlet within 1 foot of the sort chamber. The sort chamber cover should be removed.
- Begin sorting Glo Germ™ beads at 20,000 events per second and simulate an aerosol causing clog by moving the waste catcher.
- Start the sampling pump, and sample for 10 minutes.
- Remove Air-O-Cell™ from tubing, and reseal with the original tape. Label sample. For a positive control, the above steps should be repeated with the aerosol containment tubing pinched closed.
- Extract coverslip from inside of cassette, lay on slide and image using the fluorescent microscope. Count Glo Germ™ beads seen.

Aerosol containment is considered maintained when less than 2 beads are present per coverslip. Ensure that the positive control is run AFTER the test sample.

4.6 Service

Field service engineers when working on the Synergy are required to abide by the entry criteria described in the NYUSOM Smilow Dual Agent BSL3 Facility Safety Manual.

5 Biohazardous sample transportation

For biohazardous samples generated in a location distinct from the NYUSOM Smilow Dual Agent BSL3 Facility, e.g. NYUSOM ABSL3 Facility, and requiring on-campus transportation, specific training and packaging requirements apply.

5.1 Training requirements

Personnel who want to transport biohazardous samples on-campus must be current with training requirements.
EH&S provides the self-study packages: *Introduction to Shipping Hazardous Materials* and *Shipping and Receiving Biological Materials*, which are available at:

http://redaf.med.nyu.edu/shipping-and-receiving-biological-materials-training

A training certificate is issued upon successful completion of the post-test and is valid for two years.

### 5.2 Packaging instructions

- Place the sample in a primary container, which is sealed and leak-proof (e.g. tear-resistant ziplock bag, polypropylene conical tube with threaded lid).
- Place the primary container in a secondary, aerosol-tight container, which is easy to decontaminate (e.g. Saf-T-Pak STP-104 or STP-710 Pressure Vessel).
- Liquid samples will be surrounded by enough absorbent pads in the secondary container to contain any spill and absorb any shock during transport.
- Place the secondary container in a shock-resistant tertiary container (e.g. Nalgene BioTransport carrier).
- Containers can only be opened inside one of the BSCs of the NYUSOM Smilow Dual Agent BSL3 Facility.
- All samples and containers must be labeled. Label information must include the identity of the biological material or agent, the universal biohazard symbol and laboratory identification (e.g., Principal Investigator’s name, building and room number).

### 5.3 Transportation and delivery to operator

- Avoid crowded areas whenever possible.
- The container must be carried directly to the intended laboratory - do not take the container to offices, cafeterias or other public or inappropriate locations.
- The package must be carefully inspected for signs of leakage or other contamination and, if necessary, decontaminated before opening.
- Samples for sorting in the BSL-3 must be handed off to the operator prior to setup of the instrument, enclosed in a tertiary container (see section 5.2). Sorted samples will be returned to the investigator in this enclosure.