FHCRC HVTN Endpoint Assay Laboratory
Standard Operating Procedure for:
LSR II Flow Cytometer

SOP #: FH-HVTN-E0022 ***Version:*** 6.0

Name: LSR II Flow Cytometer

Effective Date: July 21, 2010

Owner(s): **Stephen DeRosa,** HVTN R & D Co-Investigator

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Signature Date

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Signature 7/20/2010 Date

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Signature 7/20/10 Date

Revision History:

Version	Description	Revised by	Revision Date
1.0	Initial Version		4/21/06
2.0	Updated procedures	Donald Carter	12/12/06
3.0	Updated procedures and modified Cleaning & Maintenance and QC logs	Donald Carter	2/24/09
4.0	Clarification of experiment templates. Changes to methods for exporting data.	Cass Beckham	4/28/10
5.0	Changed bi-monthly clean and added quarterly maintenance.	Nathaniel Chartrand	6/9/10
6.0	Added additional information for the new LSR II.	Nathaniel Chartrand	7/20/10

Purpose

This standard operating procedure (**SOP**) describes how to use the LSR II Flow Cytometer.

Scope

This SOP applies to the use of the LSR II Flow Cytometer within the FHCRC HVTN Endpoint Assay Laboratory.

Background

The LSR II is a flow cytometer that is capable of measuring fluorescence in multiple channels simultaneously, which is often referred to as multi-color capability. This instrument is used to analyze the cells that are processed using the Intracellular Cytokine Staining (ICS) procedure and other flow cytometric assays. This multi-color capability allows for the identification of CD4 and CD8 cells in addition to multiple cytokines produced by each cell type. All these measurements are made on a cell-by-cell basis as the cells flow past one or more laser beams.

The LSR II can be optionally equipped with a High Throughput Sampler (HTS), an instrument that automatically loads cells into the LSR II from a 96-well plate. This is convenient since the staining procedures for the ICS assay are performed in the 96-well plate format. If the HTS is not available, the cells from each well of the plate must be transferred into FACS tubes and the operator must load each tube individually into the LSR II.

The software used for the collection of samples on the LSR II and HTS is named DIVA. A detailed understanding of this software is not required in order to use these instruments. Basic information is provided in this SOP.

Protocol-specific templates will be prepared in the software prior to each study and these templates will include most of the identifying information required for each sample that is analyzed.

This instrument SOP describes the maintenance of the LSR II and the HTS. It also describes the Quality Control and standardization of the instrument. These are performed using standard particles that have signals detectable in all fluorescence channels. It does not describe the collection of the samples for the particular study. This is described in the general ICS SOP (FH-HVTN-A0002.GEN).

Authority and Responsibilities

1. The FHCRC HVTN Endpoint Assay Laboratory performing this procedure has the authority to establish this procedure.

2. Quality Assurance is responsible for the control of this SOP.

3. The Laboratory Manager is responsible for the implementation of this procedure and for ensuring that all appropriate personnel are trained.

4. All technicians working on HVTN studies are responsible for reading and understanding this SOP prior to performing the procedures described.

Materials

1. FACS tubes, 5mL polystyrene round bottom tubes, 12 X 75mm, BD Falcon, Cat # 352052.
2. Assay plate, 96 well, round bottom, sterile, Tissue Culture Treated, Non-Pyrogenic, Polystyrene
3. Wash Bottles, 500 mL or 250 mL
4. 0.005" diameter bare stainless steel wire.
5. Tube with coupler, cat#335452
6. Tube from probe to pump 2, cat#335454
6. Probe for HTS, cat#34389017
7. Filter for HTS, cat#335710

Reagents & Solutions

Recommended vendors are listed. Unless otherwise specified, products of equal or better quality than those recommended ones can be used. Follow FH-HVTN-S0007, Reagent Preparation and Storage, when preparing, labeling and storing reagents.

1. BD FACS Sheath Solution with Surfactant

1.1 Vendor: Becton Dickenson (BD) Biosciences, Cat# 336524

2. Bleach

2.2 Preparation of 10% bleach solution

2.2.1 Add 50 mL bleach to 450 mL dH_2O in labeled 500 mL wash bottle as needed.

3. Ethanol

3.2 Preparation of 70% ethanol solution

3.2.1 Add 175 mL ethanol to 75 mL dH_2O in labeled 250 mL wash bottle as needed.

4. Coulter Clenz

4.1 Vendor: Beckman Coulter, Cat# 8546931

4.2 Preparation of Coulter Clenz referred to as detergent.

4.2.1 Add undiluted Coulter Clenz to labeled 500 mL wash bottle as needed.

5. Deionized Water

6. Dulbecco's Phosphate-Buffered Saline solution w/o Ca^{++} and Mg^{++}

6.1 Vendor: Gibco BRL Life Technologies, Cat # 14190-144

7. Sphero Ultra Rainbow Fluorescent Particles, 3.0 μm, 2 mL

7.1 Vendor: Spherotech, Cat#RCP-30-5A-7

7.2 Preparation of Ultra Rainbow beads working dilution referred to as 7th Peak Rainbow Beads:

7.2.1 Add 1 mL 1x PBS to a 5 mL FACS Tube.

7.2.2 Vortex stock vial of Sphero Ultra Rainbow Fluorescent Particles.

7.2.3 Add 3 drops of the stock beads to the PBS in the FACS Tube.

7.2.4 Label tube with "7th Peak Beads", the lot number and size of beads, your initials and the expiration date (1 week from preparation date.)

7.2.5 Store at room temperature in minimal light.

8. Sphero Rainbow Calibration Particles (8 Peaks), 3.0 μm, 5 mL

8.1 Vendor: Spherotech, Cat# RCP-30-5A

8.2 Preparation of working dilution referred to as 8x Rainbow beads:

8.2.1 Add 1 mL 1x PBS to a 5 mL FACS Tube.

8.2.2 Vortex stock vial of Sphero Rainbow 8 Peak Calibraton Particles.

8.2.3 Add 3 drops of the stock beads to the PBS in the FACS tube.

8.2.4 Label tube with "Rainbow 8x", the lot number and size of beads, your initials and the expiration date (1 week from preparation date.)

8.2.5 Store at room temperature in minimal light.

Instrumentation

1. Vortex
2. LSR II
3. HTS (High Throughput Sampler)

Procedure

1. Daily Initial Setup and Cleaning

1.1 Turn on power to the LSR II.

1.2 Log onto the attached computer. Double click BD FACS Diva software icon. Pop-up requests password for Administrator. Just click "Ok." Diva software opens.

1.3 On LSR L (22-01-02), after log on is complete, open the icon "Green 150mw laser.ht". This icon is on the desktop. A Hyper-Terminal window will appear. Type "On" in this window to turn on the green laser. **Note:** "On" must be typed exactly as above – capital O, lower-case n, or you will get a syntax error. If this occurs, just retype "On" correctly. If successful, "OK" will appear on the screen. This step is not necessary for LSR M (22-01-03). See section 8 for special instructions for LSR II "Yoshi."

Note: The green laser requires at least 20 minutes to warm up and be ready for use.

1.4 Check the level of sheath fluid in the pressurized tank. The tank rests on a scale that is tared to the weight of the empty tank. A scale readout of 1 kg roughly corresponds to 1 L of sheath fluid. The tank has a capacity of 6 L, and should not fall below 1 L. It should be filled at the end of each run, and before the run if it was not filled beforehand.

1.4.1 To fill the sheath fluid tank, unhook the two lines, and release the pressure valve. The lid can then be unscrewed.

1.4.2 Add BD FACS Sheath Solution to tank to upper indentation line on tank interior.

1.4.3 Replace lid, and screw in tightly. Replace two lines and set on the scale.

1.4.4 Inspect the sheath fluid filter located near the connection to the tank for bubbles. If bubbles have appeared, run sheath fluid through the filter by loosening the roller clamp at the top of the line and let the fluid flow into a small container. Tap the filter as needed to remove bubbles. When no bubbles remain, retighten roller clamp.

1.4.5 If sheath fluid tank has been run dry, open roller clamp located on the right hand side of the LSR II for 5 seconds to ensure sheath fluid is running through all the lines.

1.5 Check the level in the plastic waste container.

1.5.1 The waste tank should be checked after each run and emptied if it is ≥½ full using the following steps. If it needs to be emptied, follow these steps before starting a run.

1.5.2 Remove the line from the LSR II and the alarm sensor.

1.5.3 Empty contents into the sink.

1.5.4 Run faucet to rinse out the sink.

1.5.4 Add bleach to the waste container (enough to have ~1/2 inch deep on the bottom)

1.5.5 Replace line and sensor.

1.6 To begin daily clean, place a FACS tube with at least 1ml of 10% bleach on the sample port. Turn the LSR II to “RUN” and set flow rate to “HI”. Run for 5 minutes.

1.7 Repeat above with tubes in the following order: detergent, 70% ethanol and dH_2O, each for 5 minutes.

1.8 When finished with clean, leave the tube of dH_2O on the sample port and set LSR II to STNDBY.

2. Bi-monthly Maintenance

To perform the monthly cleaning you will need DI water and a fresh 10% bleach solution (1 part bleach in 9 parts DI water).

2.1 Bypass the cytometer sheath filter:

2.1.1 Press the quick-disconnectors on both sides of the filter assembly.

2.1.2 Remove the filter assemblies. This includes the in-line sheath filter and the HTS sheath filter (see picture on pg. 15)

2.1.3 Reconnect the two fluidic lines.

2.2 Remove the sheath tank and replace the sheath fluid with a freshly prepared 10% bleach solution. Replace the tank. Save sheath fluid in designated receptacle

2.3 Open the roller clamp on the side of the cytometer for approximately 10 seconds.

2.4 Close the roller clamp.

2.5 Prime the cytometer two to three times to bring the cleaning fluid to the flow cell.

2.6 Place the cytometer in Run mode.

2.7 Choose HTS > Prime; repeat the prime.

2.8 Choose HTS > Monthly Cleaning.

2.9 Click Continue.

2.9.1 A dialog appears while cleaning is in progress; this can take up to 30 minutes.

2.10 Remove the tank and replace the cleaning solution with DI water.

2.11 Reinstall the tank.

2.11.1 A progress dialog appears while rinsing is in progress; this can take up to 30 minutes.

2.12 Click OK when monthly cleaning is complete.

2.13 When finished, turn the LSR II to “STNDBY”. Remove sheath fluid tank, empty remaining water, and pour saved sheath fluid back in. Refill if necessary.

2.14 Spray outside of sheath fluid container with 70% ethanol and wipe off with a paper towel.

2.15 Reattach sheath fluid container to LSR II, and replace the sheath fluid filter.

2.15.1 Inspect the sheath fluid filter located near the connection to the tank for bubbles. If bubbles have appeared, run sheath fluid through the filter by loosening the roller clamp at the top of the line and let the fluid flow into a small container. Tap the filter as needed to remove bubbles. When no bubbles remain, retighten roller clamp.

2.16 Run fluid through the roller clamp located on the right side of the LSR II for at least 5 seconds to ensure sheath fluid is running through the lines.

2.17 LSR II should be in "STNDBY" mode with the tube of dH_2O attached to the sample port.

3. High-Throughput Sampler (HTS) Daily Clean

3.1 The HTS unit should be cleaned before each set of plates in an experiment is run, and after the final run on the unit.

3.2 In order to run the clean procedure, an experiment must be open in the FACS Diva software. This can be the daily QC experiment, or the experiment that is to be run. See below for procedures to create a new experiment.

3.3 Connect the HTS unit to the LSR II.

3.3.1 Remove the tube of dH_2O from the sample port.

3.3.2 Turn the collection switch located above the LSR II power button from tube to plate.

3.3.3 Connect the HTS to the LSR II by screwing the sample line from the HTS to the sample port on the LSR II.

3.3.4 Turn on the HTS unit.

3.4 Open a new 96 well culture plate or reuse a plate previously used for the cleaning procedure. Prepare cleaning reagents as follows:

3.4.1 Fill wells A1 and A2 with 10% bleach.

3.4.2 Fill wells A3 and A4 with 70% ethanol.

3.4.3 Fill wells B1 and B2 with detergent.

3.4.4 Fill wells B3 and B4 with dH_2O.

3.5 Place the cleaning plate on HTS unit. Well A1 should be closest to the sample port. Replace HTS cover, and set LSR II to "RUN" on "LO".

3.6 In the FACS Diva software, choose HTS from the menu bar, and select Clean. Choose the Daily Clean option. The system will prompt you to load the clean plate on the HTS. Select OK.

3.7 When cleaning is finished, place the LSR in "STNDBY" mode.

4. Daily Quality Control /Standardization Procedure

4.1 From the Experiment submenu, choose New Experiment.

4.2 The Experiment Template window will appear. In this window, select the QC-General-Endpoints Template on **Luigi**, but the HVTN-Endpoints-QC Template on **Mario** and then, click Ok.

4.3 Right click on the experiment name (which will appear in the list of experiments in the left pane), and rename according to protocol. Typically the naming convention follows this format, e.g., 0500-L-069-QC

- "0500" The unique batch number given to each experiment
- "L" Refers to the LSR being used for collection (L for Luigi and M for Mario)
- A three digit code for the study or protocol (e.g., 069 in this example)
- QC identifies this as the QC file

The protocol-specific SOP may specify a different naming convention.

4.4 Briefly vortex a FACS tube of 7th Peak beads, and place on sample port.

4.5 Click on + syringe, then 7th Peak beads. Set the LSR II to "RUN" and "LO" setting. Begin acquiring 7th Peak beads by clicking on the 7th Peak Beads tube, which is the collection file for the 7th Peak Rainbow beads, and click Acquire in the Acquisition window. The acquire button will turn green, as well as the icon to the left of the collection file under the experiment folder.

4.5.1 The beads should collect at a rate of 150-350 events per second. If they are collecting faster than that, remove the beads from the LSR and add PBS to dilute them as needed. If they are collecting more slowly, make up new beads if the beads being used were not made up today.

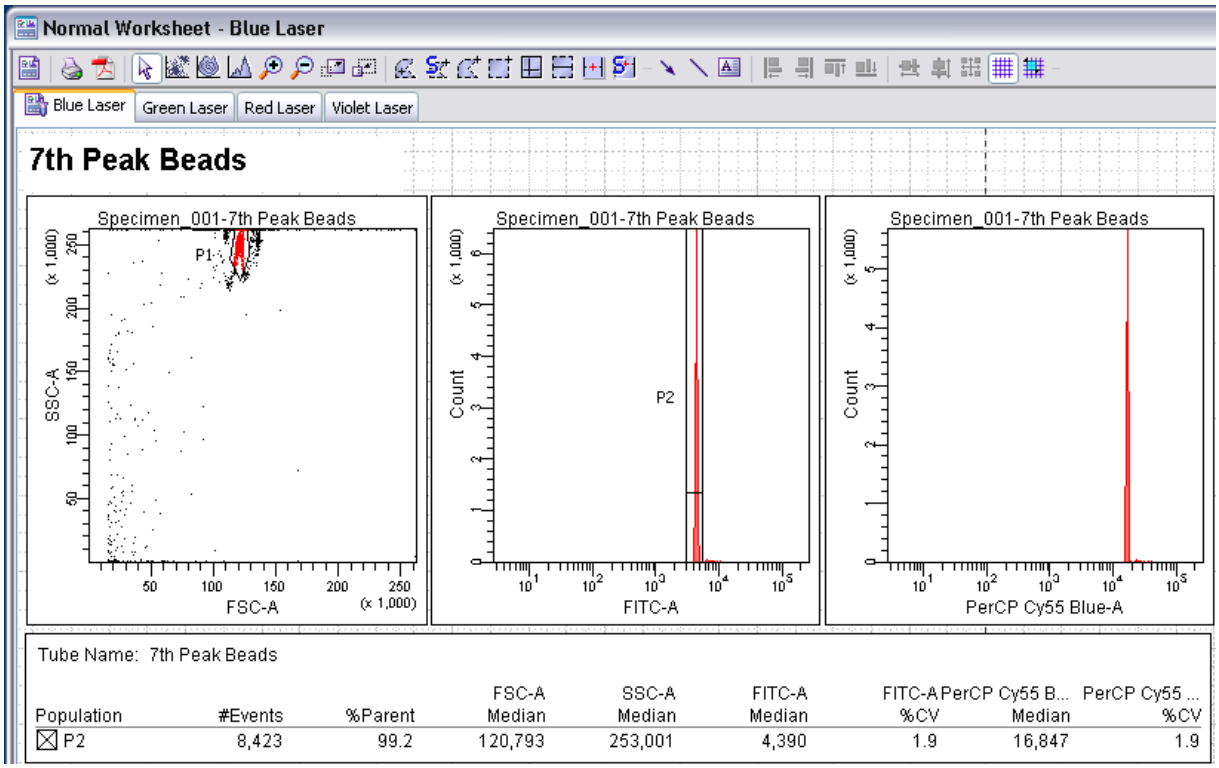
4.5.2 If beads made up today are collecting too slowly, it is likely there is a clog. Take the beads off the LSR II, leave the arm off to the side and press "PRIME". Place 1ml of DI water on the sip and prime again. Then attempt to collect the beads again.

4.5.3 If the beads continue to collect slowly, run a 1 minute clean cycle (1 minute each for bleach, detergent, ethanol and H_2O) on the machine. Then repeat step 4.5.2.

4.5.4 If the LSR II continues to collect too slowly, contact the study director or other qualified supervisor.

4.6 In the worksheet window (on the right screen), click on the Blue Laser tab, and adjust the P1 gate to fit around the singlets, if necessary (see example below)

4.7 In the FITC histogram, adjust the P2 gate to exclude the secondary fluorescence peak (to the right of the main peak) if necessary (see example below).



4.8 Check the coefficient of variation (CV) for each channel to ensure they all fall below the acceptable protocol specific CV ranges as defined in the SOP or SSP.

4.8.1 If CV values are too high, remove the beads and press the prime button that is located on the fluidics control panel. This will initiate a purge of the flow cell. When this is complete, the instrument will be automatically placed in stand-by mode.

4.8.2 Place a FACS tube containing at least 1ml of DI water on sample port and run on "HI" for 1 minute.

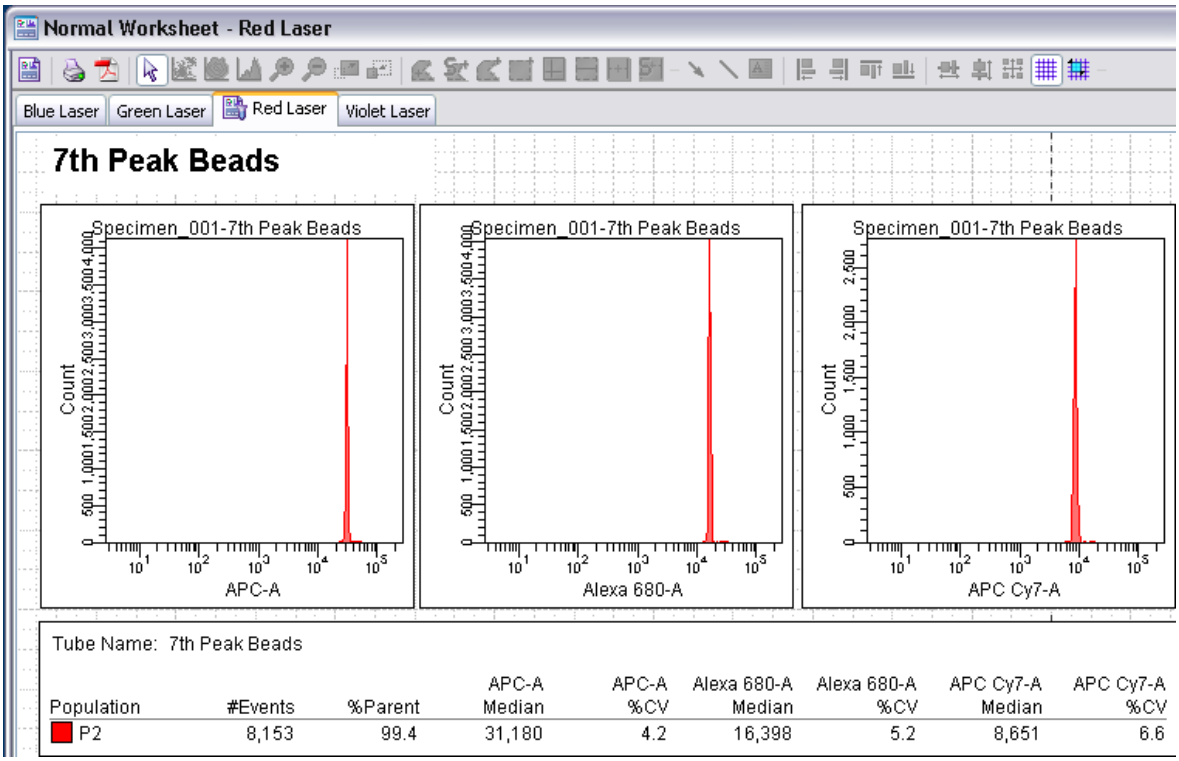
4.8.3 Put the LSR II back on "LO" and place the tube of rainbow beads on the sample port and check to see if the CV values are within range.

4.8.4 If this fails to fix the problem, make a new tube of 7th Peak beads, and repeat from step 4.4.

4.9 If CV values remain too high, remove the beads and run a 1 minute clean cycle (1 minute each for bleach, detergent, ethanol and H_2O) on the machine.

4.9.1 If this fails, request assistance from the study director or other qualified supervisor.

4.10 If all the CV values are acceptable, then for each laser tab, adjust the PMTs for each channel so that the median fluorescence matches the target values specified in the SOP or SSP. The P1 and P2 gates may need additional adjustment as the FSC, SSC, and FITC PMT voltages are adjusted, so these PMTs should be set first. In the worksheet window, there are collection tabs for the four different lasers. Each tab allows you to view the channels collected from that laser. An example for the red laser is shown below.



4.11 Click Record in the Acquisition window and collect 10,000 events. PMT voltages should not be readjusted after the beads are collected unless specified by protocol.

4.12 Briefly vortex the tube of 8X Rainbow beads and place on the sample port.

4.13 Click Next in the Acquisition window to select and open the 8 X Rainbow bead collection file.

4.14 Adjust the P1 gate to include only the singlets, if necessary. Note: it may be necessary to scroll down in the worksheet window to view the histograms for the 8x beads.

4.15 Click on Record to acquire 10,000 events.

4.16 Remove tube from sample port, replace with tube of dH_2O and place LSR II in "STNDBY".

4.17 Right click on the Instrument Settings icon, and select Copy. These will be pasted onto the instrument settings for the protocol-specific experiment (see below).

5. Creating a New Experiment for Collection of Samples

Refer to ICS General SOP for specific details concerning collection of samples.

5.1 From the Experiment submenu, choose New Experiment.

5.2 The Experiment Template window will appear. In this window, select the protocol-specific experiment template and click Ok. If the protocol template is not found, check in the **OLD** templates or a new one needs to be made.

5.3 Right click on the experiment name (which will appear in the list of experiments in the left pane), and rename according to protocol. Typically the naming convention is similar to that used for the QC file, e.g., e.g., 0500-L-069, but the "QC" is omitted.

5.4 Right click on the global Instrument Settings icon (immediately below the experiment name), and select paste (make certain you have copied the Instrument Settings from that days QC file first).

5.4.1 Remove the tube of dH_2O from the sample port.

5.4.2 Turn the collection switch located above the LSR II power button from tube to plate.

5.4.3 Connect the HTS to the LSR II by screwing the sample line from the HTS to the sample port on the LSR II.

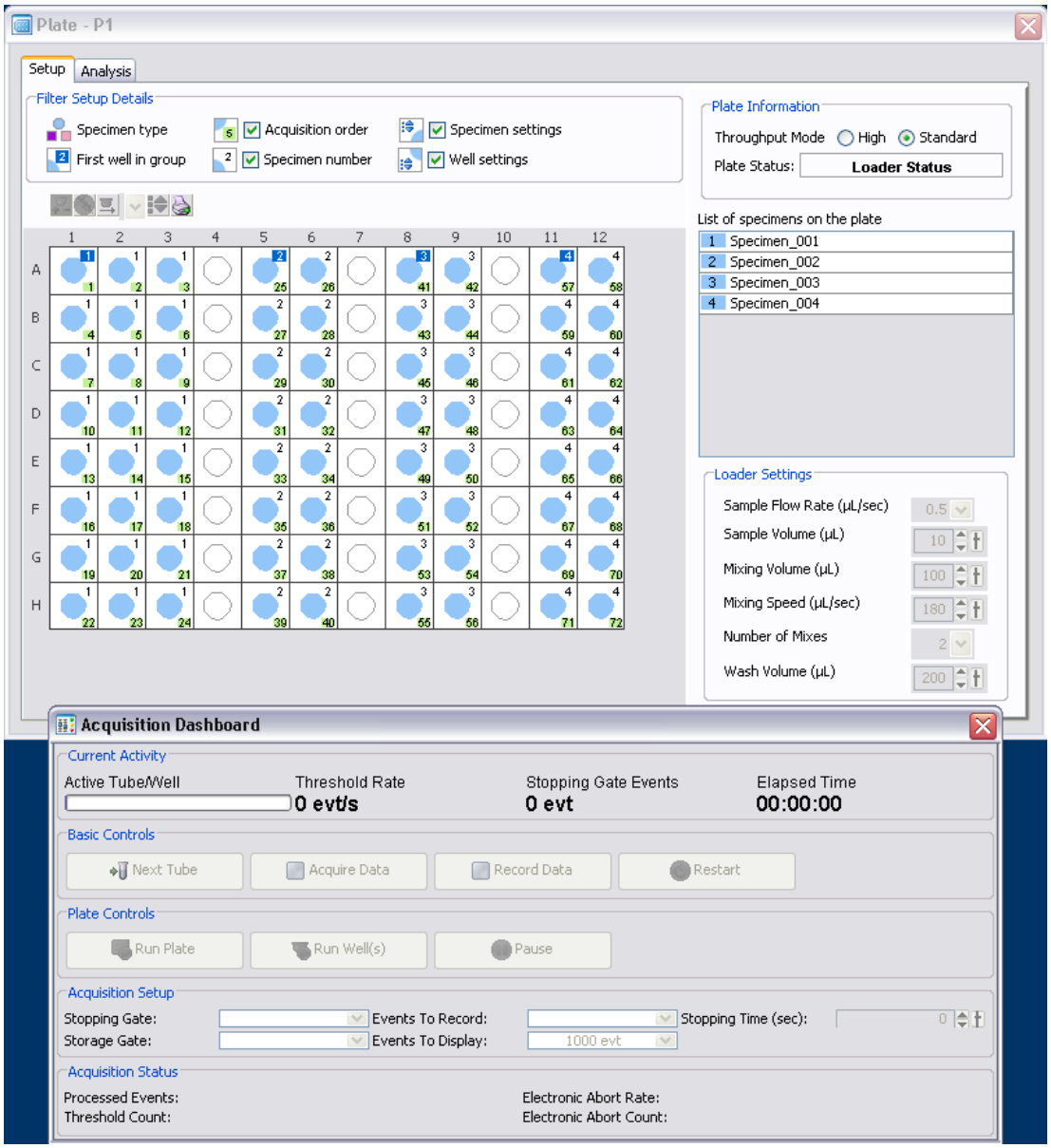
5.4.4 Ensure that the HTS unit is switched on.

5.5 Individual plates within the experiment will be labeled (P1, P2, etc.). Double-click on the first plate to open the Plate window.

5.6 In the Plate window, click the setup tab. Select the well numbered 1 in the layout (not necessarily well A01 on the plate itself.)

5.6.1 Note that there are two numbers in each well (see figure below). The number in the upper right corner corresponds to the group numbers listed on the right hand side of the window (see figure below). This number will appear on a blue background if it is the first well of that group to be collected.

5.6.2 The number in the bottom right corner indicates the order of collection, with "1" being the first collected and continuing sequentially though all the wells on the plate.



5.7 Place the plate on the HTS unit so that well A01 is closest to the sample probe, and be sure to remove tissue culture cover.

5.8 Replace HTS cover.

5.9 Put LSR II on “Run” (from “STNDBY”) and set flow rate to “LO.”

5.10 With well 1 selected, click Run in Plate Layout window.

NOTE: If Run button is grayed out, check the right screen to see if the Global Worksheet is visible. If not, click the left-hand green icon in the Worksheet window. When Global Worksheet is visible, the Run button will no longer be grayed out.

5.11 As plate collects the first well, observe the FSC/SSC cell profile and check that the lymphocyte population falls within the P1 gate. If not, P1 gate can be adjusted while the sample is being collected.

5.11.1 Also observe the number of events being collected per second. This number should be above 5000. If not, collection will need to be paused and the machine primed.

5.11.2 If collection needs to be paused, click pause in the Plate Layout window. The HTS will complete collection of the sample currently being collected and then will stop.

5.11.3 Remove the plate from the HTS and disconnect the HTS from the LSR. Prime the LSR with no tube on the sip, prime it with 1ml DI water, then reconnect the HTS and prime the HTS (select Prime from the HTS menu).

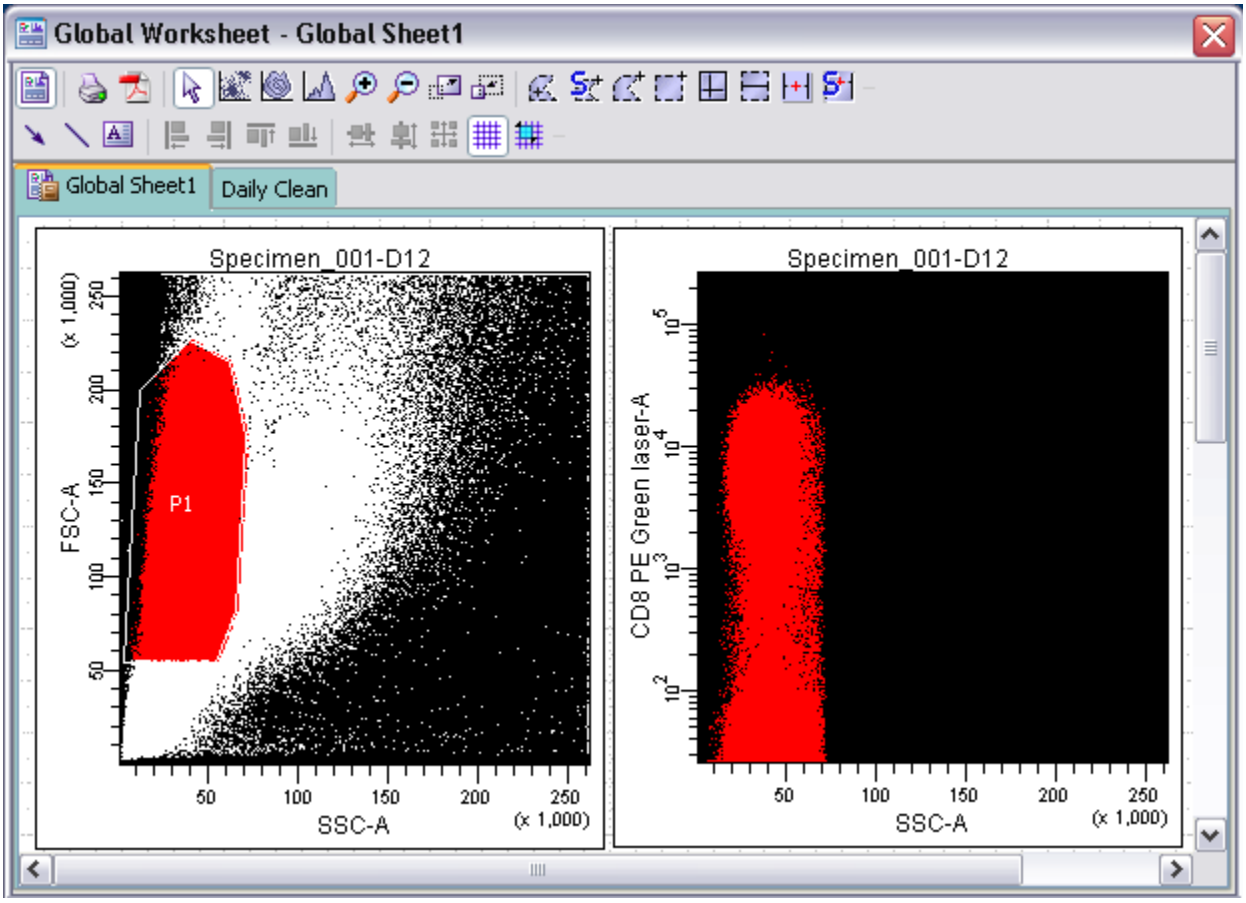
5.11.4 Add PBS to well 1 from the Plate Layout window (the one well that has been collected). The amount to add depends on how much was collected. Check the sample volume in the plate window (generally 90µl). Return the plate to the LSR, select well number 1 in the Plate Layout window, and hit run.

5.11.5 The number of events collected from well 1 will be low; watch well 2 to see how many events are collected. If the machine is still collecting slowly, pause collection again and contact the study director or other qualified supervisor.

5.12 At the end of a plate, a window will show that the run is complete. Click Ok.

5.13 If there are additional plates, double-click on the next plate in the experiment to open the Plate Layout window. Repeat steps 5.6 to 5.13 for this and any additional plates in the experiment.

5.14 When all plates from the experiment have been run, check the compensation wells to be sure that all of them have been stained. Double-click on a well and look at a plot in the Global Sheet, see example below with a lymphocyte gate (P1) in the first plot and the CD8 PE staining on the lymphocytes in the second plot. If any comps are inappropriately stained or were not stained, they should be replaced (either with comps from a previous run or by staining new comps).



5.14.1 The replacement comps can be collected over the original data. Simply pipette the replacement comps into the appropriate well(s) of a 96 well plate and select the well(s) to be replaced on the Plate window, then hit Run Well(s). You will receive a warning that prior data will be over-written; click Ok to proceed.

5.14.2 While it is possible to simply use the data from a comp collected on a different day to replace missing comps in FlowJo, this is sub-optimal. Day to day variation in the machine can result in problems attempting to compensate samples with comps run at a different time.

5.15 Remove the last plate and proceed with an HTS clean, and record this in the logbook. When cleaning is complete, place LSRII into “STNDBY” mode.

5.16 To export data, click on the experiment and ctrl-click on the QC experiment to highlight both, and then right click on the experiment folder. Choose Export, and then choose Experiments. In the Export window, browse the directory to the desktop. Open **exports** file, then choose export. When exporting is completed, copy file to secure server. Typically the data are exported to a hierarchical folder structure by date with a folder for day, within a folder for month within a folder for year. These folders are in a secure folder on a secure server. The file path is:
Sluf50/Vaccine/FACS_Data_for_ICS/[year]/[month]/[day] with [year] [month] [day] replaced with the 4 digit year and 2 digit month and day. The SOP may specify a

different path. After the files are copied to the secure server, delete the files in the Exports file on the desktop.

5.17 If you are the final user of the instrument, proceed to the Shut-down procedure. If not, place instrument in "STNDBY" mode.

6. Shut-down Procedure

6.1 Before shutting down the instrument, run a 5 minute clean procedure using 10% bleach, detergent, 70% ethanol, and dH_2O (5 minutes each). If an HTS clean has just been performed, a 1 minute clean in the same order is acceptable. Leave the instrument with the water on the sample port.

Note: Even if a clean procedure has been performed on the HTS, before shutting down the LSR, disconnect the HTS tubing, perform the 1 minute clean using tubes and **leave a tube of water on the sample port before shutting down**.

6.2 Close FACS Diva program, and log out.

6.3 Turn off power to LSR II and the HTS every evening.

7. Quarterly Maintenance

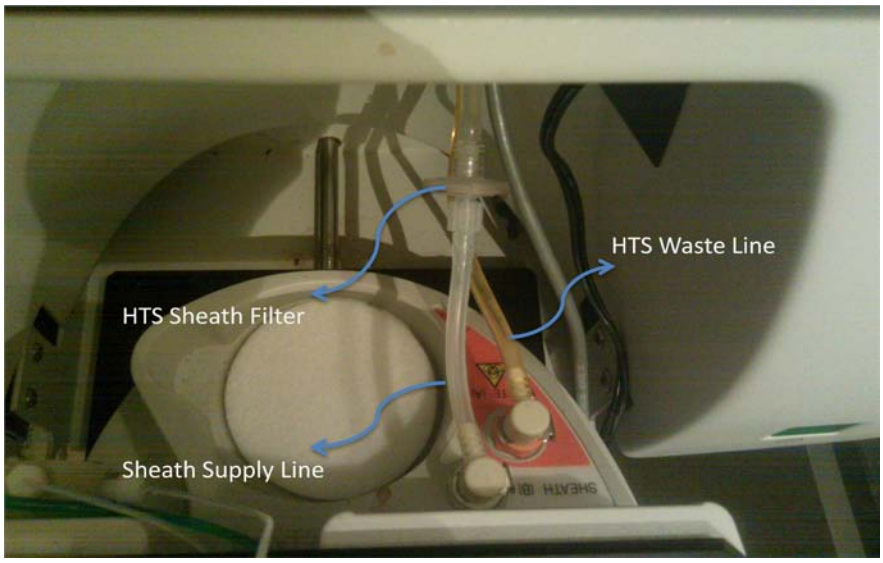
7.1 Every 3 months the tubing and sheath filter on the HTS needs to be replaced.

7.1.1 The tubes running from the HTS probe to pump 2 and from pump 1 to the sample injection port carry samples and should be replaced quarterly. (See picture on page 16)

7.1.2 Unscrew the plastic fittings from the pumps and dispose of the old tubing.

7.1.3 Install the new tubes ensuring a nice tight seal at the pumps and probe

7.1.4 Gently slide the HTS away from the LSR. Replace the filter on the main sheath line to the back of the HTS. (See Picture Below)



7.2 The probe on the HTS should be cleaned every 3 months.

7.2.1 Unscrew the probe from the HTS.

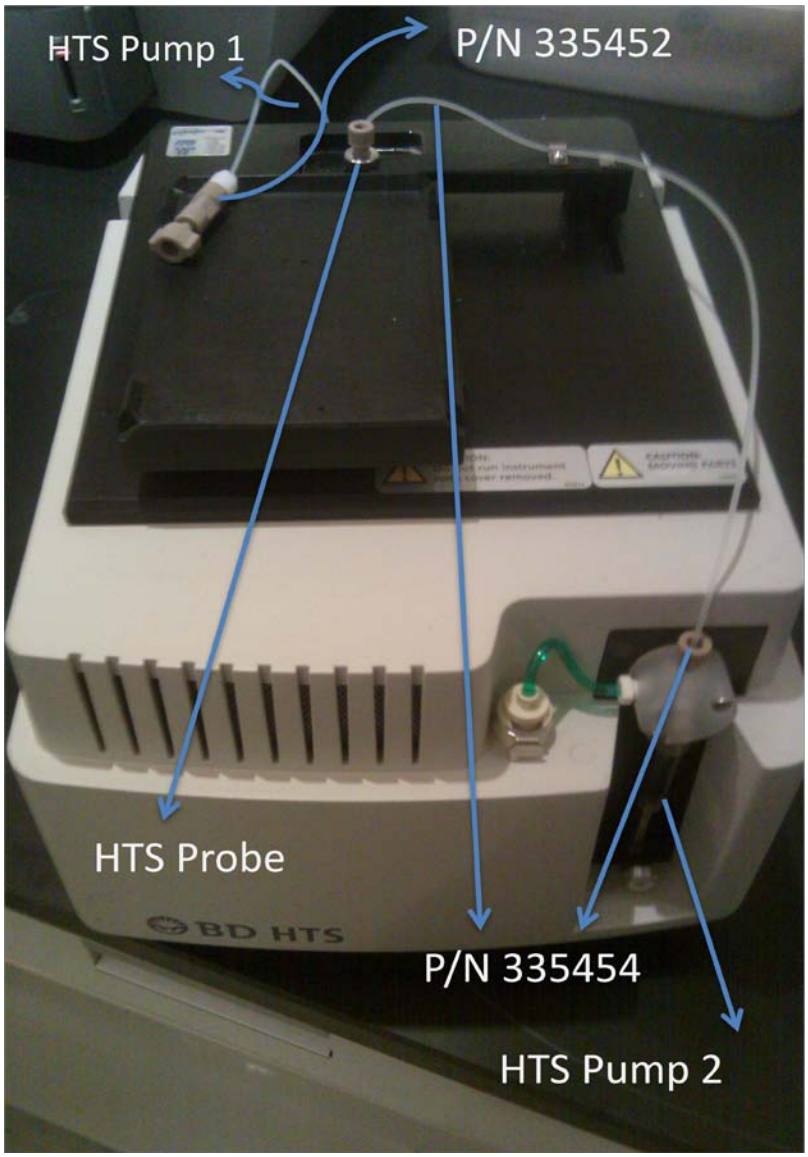
7.2.2 Run 0.005" diameter wire through the probe and run it back and forth to clear any debris.

7.2.3 Thoroughly rinse probe with 70% Ethanol.

7.2.4 Clean the HTS arm the probe screws into with 70% Ethanol to clear any dried sheath fluid or other debris.

7.3 Prime the HTS 2 times to get fresh fluids into the new tubing

7.3.1 Check for any leaks coming from the newly replaced tubing and tighten the fittings if necessary



8. Special Instructions for LSR II "Yoshi"

8.1 Turning on the Red and other Lasers

8.1.1 Open the "GUI-VFL" software by double clicking.

8.1.2 Click on the com port on the upper left side of the window and switch to com 4.

8.1.3 In the lower left of the window turn the power to the laser to the "On" position.

8.1.4 Set the laser power to 200mW and click "Activate."

8.1.5 Red laser should now be turned on to the appropriate voltage.

8.1.6 Open the "BD Coherent Connection" software for the other lasers.

8.1.7 Once the software is opened, the laser windows will automatically pop up and lasers will turn on.

8.2 Fluidics System

8.2.1 Sheath fluid and waste should be checked and changed regularly by manually checking the weight.

8.2.2 Sheath is supplied directly from the 20L box

8.2.3 Waste is drained into an empty 20L sheath box labeled "Waste." Be sure to cross out the sheath solution label.

8.2.4 There are alarms for waste overflow, sheath low (1.5L), and sheath empty.

8.2.5 When changing the sheath fluid or emptying waste these alarms will sound if the fluidics station has power.

8.2.6 Silence the alarm by pressing the "alarm" button on the fluidics station before changing the sheath fluid or emptying waste.

8.2.7 Once the fluids are at an acceptable level press "restart" and all alarms will be reset.

Attachment 1
LSR II Usage Log

Date	Sample Description	Time in	Check sheath / waste	Cleaning (Y/N)			QC Bead CVs ok (Y/N)	Fill sheath / empty waste	Time out	By
				5 min tubes / HTS clean	HTS clean	1 min tubes				

Reviewed by _____ Date _____