Core Equipment ID: 0714444

Description: SONY LE-MA900

Room: BPS 5115

Champion: Daniel Vocelle

1.0 Purpose:

Standardize the process of control, maintenance, and ownership of the SONY LE-MA900 instrument located in BPS Room 5115.

1.1. SONY LE-MA900 Capabilities

The SONY LE-MA900 series is a benchtop, high-speed, multilaser, flow cytometer and cell sorter designed for research laboratory use. It uses a novel, replaceable, microfluidics, cell "sorting chip" that features high reliability and greatly simplifies cell sorting setup. It can be easily and quickly exchanged as needed for quick turnaround between measurements to minimize system downtime and to improve user workflow.

The SONY LE-MA900 interrogates cells in samples using up to four lasers on two axes, 488 nm/561 nm (5 detectors) and the 405 nm/638 nm (7 detectors). It measures forward scatter, back scatter, and a maximum of 12 fluorescent channels. The excitation wavelengths support the use of a wide variety of common fluorochromes for use in cell analysis. Multiple parameters enable real-time identification of selected cell subpopulations at speeds of up to 70,000 events per second (eps) during analysis. Populations of interest can be sorted into 0.5 mL/1 mL Eppendorf tubes, 5 mL tubes, or 15 mL conical tubes. Populations can also be sorted directly into multi-well plates, including: 12-well, 24-well, 48-well, 96-well, and 384-well.

1.2. MA900 Cell Sorter Software Capabilities

a. Streamlines laboratory workflow in a multi-system environment by enabling users of SONY platforms to use a single software application for acquisition and analysis.

b. Provides easy-to-use instrument setup and quality control (QC) when used with SONY Automatic Setup Beads.

c. Enables standardization across SONY platforms for both inter- and intra-site experiments with use of application settings.

d. Provides common feature sets that allow users to transition easily across SONY platforms and from analysis to sorting applications. Provides flexible data management tools for users to export data for use with other third-party analysis software tools.

2.0 Reason for Issue

Maintain a document that describes the Standard Operating Procedures that allows for the standard safe and optimal use of the SONY LE-MA900 instrument within the MSU Flow Cytometry Core Facility.

3.0 Process Description

Allow Core Facility Users to properly and effectively use the SONY LE-MA900 instrument. The process description details the standard use of the SONY LE-MA900 instrument. The controlled standard must maintain and adhere to proper and approved research and regulatory qualitative conditions.

3.1. SOP: 0714444.5115.001 for SONY LE-MA900 instrument, authored by Matthew Bernard and Daniel Vocelle, created on 02/21/2024, issued on 11/01/2024.

3.2. SOP: 0714444.5115.001. applies to any User and / or Trainer of the SONY LE-MA900.

3.3. **Responsibilities:** All Users are responsible for obtaining the proper approval and training before the use of the SONY LE-MA900 instrument. All Users are responsible for the proper use, according to defined protocol, when using the SONY LE-MA900 instrument

a. **New Users** need a SONY Cell Sorter Software user account created for equipment access, before initial use. New accounts are authorized and created by the Equipment Champion and / or the Core Facility Staff. A new account may be created after training and equipment approval has occurred.

b. All Users are expected to have completed EHS training programs Bloodborne Pathogens (BBP) and Biosafety Principles (BSP), as required for respective research projects.

c. All Users receive EH&S/IBC approval of a Cell Sorting Addendum in the HURON Click system. Once approved, all users are required to complete a Biosafety Questionnaire for each approved cell type prior to scheduling use of instrumentation in the facility.

d. **All Users** must schedule equipment using the iLab Solutions portal.

e. Only covered samples may enter Room 5115. Samples must be brought to the facility in a standard **spill control box/leak-proof secondary container** that will contain any multiple tube or plate spill, per EHS standards (see Section 3.4.g). All tubes and plates should be capped to maintain containment of samples. Seal multi-well plates with plate sealer or parafilm. Spill control boxes must be labeled with Biohazard identification for BSL-2 or BSL-2+ samples.

f. The last user for the day must shutdown the SONY LE-MA900 flow cytometer immediately after use (see Section 4.10).

3.4. Equipment Safety Issues

a. **Safety Issues** – The Core Facility operates up to BSL-2 plus. Biosafety level and limitations for this facility are restricted to WHO and NIH risk groups defined as:

Risk Group 1 – Agents that are not associated with disease in healthy adult humans (no or low individual or community risk)

Risk Group 2 – Agents that are associated with disease which are rarely serious and for which preventive or therapeutic interventions are often available (moderate individual risk but low community risk).

Examples of risk groups 1 and 2 which may be analyzed include: 1) Plasma or serum from non-primate animals; 2) cell supernatants from cell lines of ATCC origin and those tested negative for human immunodeficiency virus (HIV), hepatitis C virus (HCV), hepatitis B virus (HBV), and Epstein-Barr virus (EBV); 3) primary human serum or plasma if tested for HIV, HBV, HCV, and EBV; 4) Supernatants from primary human cells if tested for HIV, HBV, and HCV; 5) Supernatants from genetically modified cell lines using third generation lentivirus systems.

Research involving BSL-3 or BSL-4 requirements are not supported, which includes WHO and NIH risk groups 3 and 4.

Summary of Biosafety Practices for cell sorting in the Core Facility:

Type of Cell/Procedure	**Exempt BSL-1**	**BSL-1**	**BSL-2**	**BSL-2+ (enhanced precautions)**
Examples of cells	Wild-type cells from murine or other non-human/non-primate species that have **NOT** been exposed to any microbial agent (e.g., viral, bacterial, fungal, protozoan, or parasitic) and have **NOT** been genetically modified. Or Cells determine by EH&S to be recombinant NIH-exempt BSL-1.	Cells from murine or other on-human/non-primate species that have not been exposed to any microbial agent, but have been genetically modified using non-viral methods (e.g., cells from transgenic animals or cells treated with nucleic acids). **Or** Cells determined by EH&S to be approved as non-recombinant BSL-1 or recombinant BSL-1.	Cells of human or non-human primate origin or Cells that have been genetically modified using viral methods or Cells exposed to microbial agents (e.g., viral, bacterial, fungal, protozoan, or parasitic) and **have been** approved by EH&S for BSL-2 containment and sorting.	Cells of human or non-human primate origin or Cells that have been genetically modified using viral methods or Cells exposed to microbial agents (e.g., viral, bacterial, fungal, protozoan, parasitic) and **have been** approved by EH&S for BSL-2+ containment and sorting.
EH&S BMR Form Update Requirement	Not Required	**Required**	**Required**	**Required**
BBP Training	Not Required	Not Required	Initial and annual renewal required for work with human cells and other cells exposed to BBP.	Initial and annual renewal required for work with human cells and other cells exposed to BBP. N95 respirator fit test also required.
Sort Sign-up	Required. Complete sign-up in iLab calendar and provide a clear BSL-1 notation.	Required. Complete sign-up in iLab calendar, sample description, and provide a clear BSL-1 notation	Required. Complete sign-up in iLab calendar, sample description, and a clear BSL-2 notation.	Required. Complete sign-up in iLab calendar, sample description, and provide a clear BSL-2+ notation.
Sample Transport	No Requirement	Leak-proof secondary container	Leak-proof secondary container	Leak-proof secondary container

Type of cell/Procedure	**Exempt BSL-1**	**BSL-1**	**BSL-2**	**BSL-2+ (enhanced precautions)**
Room Restriction	None	None	Yes, door to 5115 must be closed during sort and the BSL-2 Biohazard sign posted on the outside.	Door to 5115 must be **locked** during sort and the BSL-2+ Biohazard sign posted on the outside. Room 5115 will be monitored for negative pressure prior to initiating sort.
PPE	Lab attire is required (Closed-toed shoes and long pants). When manipulating samples (e.g., loading and unloading) gloves and lab coat are recommended. Spills (required): Lab coat, and nitrile gloves.	Lab attire is required (Closed-toed shoes, long pants and lab coat). When manipulating samples (e.g., loading and unloading) gloves and lab coat are required. Spills (required): Lab coat, and nitrile gloves.	Lab attire is required (Closed-toed shoes, long pants, lab coat, and gloves). When manipulating samples (e.g., loading and unloading) face protection is also required. Spills (required): Lab coat, nitrile gloves, and goggles.	Lab attire is required (Closed-toed shoes, long pants, lab coat, and gloves). When manipulating samples (e.g., loading and unloading) an N95 respirator is also required. Spills (required): front-closed gown, nitrile gloves, goggles, and N95 respirator.
Waste	Contaminated materials must be decontaminated prior to disposal.	Gloves and other waste must be disposed of as biohazardous.	Gloves and other waste must be disposed of as biohazardous.	Gloves, gown, and other waste must be disposed of as biohazardous.

b. Aerosol Risk:

The SONY LE-MA900 designated for the acquisition and sorting of fixed or unfixed samples up to the BSL-2 plus level. High-speed cell sorters use higher system pressures and higher drop drive frequencies, which produce smaller droplets and satellite drops. During instrument failure (e.g., partial blockage of the nozzle) the generation of secondary aerosols can occur (*Schmid I, Lambert T, Ambrozak D, Marti GE, Moss DM, Perfetto SP. International Society Cytology and Biosafety Standard for Sorting of unfixed Cells. Cytometry 2007; 71A:414-4371*). The potential exposure to escaped aerosols may be a health risk to sort operators, although this is mitigated by the design of the sort chip vs a sort nozzle. The SONY LE-MA900 has been installed in a Baker BCC300AMS Class II Biosafety Cabinet (BSC), specifically designed for this instrument, in order to mitigate exposure to aerosols when present, however aerosol containment must be properly assessed (Section 3.8.c).

c. **All samples exposed to or infected with bacterial or viral agents must be approved by EH&S/IBC on a case-by-case basis.** A related Cell Sorting Addendum must be submitted through the PI's HURON Click Biosafety Protocol and approved by the IBC/EH&S prior to scheduling a sort. Failure to adhere to this rule can result in a lab being permanently prohibited from utilizing the Core facility.

d. Decontamination of SONY LE-MA900 post-operation:

Following the Shutdown procedures (Section 4.10) will result in appropriate daily decontamination of the flow cytometer between uses. In between users and during shutdown, the Bleach Cleaning cycle should be run with 30 mL of a freshly prepared solution containing 10% Bleach and 5% Contrad, followed by the DI Rinse cycle with 15 mL of sterile filtered DI/MilliQ water.

e. Decontamination of work surfaces:

External surfaces in front of the SONY LE-MA900, inside the Biosafety cabinet and inside cabinet door can be cleaned with Envirocide (or equivalent) or wiped down with Sani-Cloth Plus germicidal wipes (or equivalent). Wipe metal surfaces down with 70% ethanol after decontaminating to prevent corrosion.

f. Radioactively labeled samples are prohibited.

g. Spill control:

Samples must be brought to the facility in a standard spill control box that will contain any multiple tube or plate spill. All tubes and plates should be capped to maintain containment of samples. Seal multi-well plates with plate sealer or parafilm.

Report spills to the Core Facility Staff.

In the event of a spill for BSL-2 samples, the spill should 1st be covered with absorbent paper towel, which will then be saturated with 10% bleach and allowed to soak a minimum of 10 minutes. The wet towel should be placed in a biohazard waste container after contact. The spill area will then be covered with 10% bleach, allowed

to soak briefly, and then wiped up with an absorbent towel. After cleaning the spill, dispose of the absorbent material and gloves into a biohazard waste container. Squeeze or spray bottles made up of 10% bleach, resulting in a final concentration of 0.5% sodium hypochlorite, are made fresh daily for spill control.

h. Ensure that the SONY LE-MA900 waste container is filled with enough fresh bleach (1L) to result in 10% bleach solution following use (the maximum volume of the waste tank is 10 liters or 2.6 gallons). After a sort, the waste container should be removed from the fluidics cart and agitated enough to ensure proper mixing. The waste solution needs to sit for at least 20 minutes before transferring it into an EHS approved 5 Gallon carboys.

3.5. Laboratory Conditions

a. BPS 5115 is a BSL-2 research lab with negative pressure air flow. Negative room pressure is indicated by a green light on the Setra Air Pressure Monitor, located on the inside of the lab above the door. Positive room pressure is indicated by a red light on the Setra Air Pressure Monitor. The lab door must be closed at all times. The room contains a sink for hand washing, germicidal soap, emergency eye wash station, and spill control kit/equipment.

b. **Signage:** Current BSL-2 and Chemical safety signs having laboratory practices and emergency contact information will be found at the door of Rm 5115 **A temporary sign must be posted on door during a BSL-2 or BSL-2+ sort to notify laboratory personnel and indicating only appropriate individuals are allowed to enter.**

c. **Access:** Access is limited to people with permission to run samples on the SONY LE-MA900, which has been booked through the iLabs web portal. Only individuals involved in training exercises, running samples on the cell sorter, or retrieving data should be in Rm 5115. The room will be locked during a BSL-2+ sort.

d. **PPE Requirements:** Standard laboratory PPE must be used at all times, which includes gloved hands, long-sleeve lab coat over full coverage shirt and pants, and full coverage shoes with intact soles. Safety glasses and N95 respirator may also be worn. For sorting samples with a BSL-2+ designation, a front-closure gown, face shield and safety glasses and/or goggles, and N95 respirator are also required (PAPR may be worn in place of N95 and face shield, following appropriate EH&S training).

e. All samples will be handled with BSL-2 precautions, including proper handling, storage, transportation, disposal, and decontamination according to the MSU Biosafety Manual and BBP Exposure Control Plan.

f. **Negative Pressure:** Room 5115 is set up to maintain negative air pressure. For BSL-2+ sort, the room will be monitored for negative air pressure prior to sort initiation using the Setra pressure monitor and/or smoke bottle.

g. **Exposure Control Plan:** Please refer to the Exposure Control Plan available on the MSU EH&S website for instructions regarding what to do in the event of exposure. The MSU Exposure Response Procedure is posted in Room 5115.

i. **Eye/Mucous Membrane Exposure:** Flush immediately at nearest eyewash station for 15 minutes.

Wounds/Needlesticks: Wash the area immediately, use warm water and sudsing soap to scrub the area for 15 minutes.

ii. Notify your supervisor immediately if he/she is available.

iii. Print Authorization to Invoice MSU Form to take to care facility.

https://www.hr.msu.edu/benefits/workers-comp/documents/InvoiceMSU.pdf

iv. Report to a Lansing Urgent Care facility for post-exposure follow-up as soon as possible.

https://www.lansingurgentcare.com/

v. Be prepared to provide information about the agent or cells involved in the accident. Additionally, route of exposure, dose/concentration, unusual characteristics of the agent, animal infection, cell line, and PI contact information.

Note: Any required follow-up visits must also take place at Lansing Urgent Care. The location in Frandor is open 24 hours.

vi. Follow up by completing the Report of Claimed Occupational Injury or Illness Form with your supervisor within 24 hours.

h. Sample handling and decontamination within BPS Rm 5115 is covered in Section 3.5. All tubes, pipettes, plates, etc. that represent a biological hazard must be removed by the user and returned to their lab. Waste containers are available for non-hazardous waste. A biological waste container for waste generated during a biohazard cleanup is available in the lab. **No needles are permitted in the Core Facility.**

i. **Eating, drinking, or use of personal care products are prohibited in the facility.** Use of personal electronics will not be allowed if that use interferes with proper operation of the instrumentation in the facility. Those operating flow instrumentation in the facility must remove gloves and wash their hands before using any personal electronic device. Sani-Cloth Plus germicidal wipes are available for wiping **keyboards and personal electronic devices if cross-contamination accidentally occurs.**

j. Dispose of PPE appropriately in the Core Facility. Remove disposable lab coat and place it in biohazardous waste. Gloves should be discarded in the biohazardous waste container. Remove eye protection and wipe with Sani-Cloth Plus wipes.

k. Wash hands thoroughly before exiting the Core Facility.

l. **Medical:** Users of the facility should have all current vaccinations, including those for HepB. Anyone who may be immune compromised should visit Occupational Health before working in the facility.

3.6. **Contact Information**

a. **Matthew Bernard: Core Director,** Office, IQ Building, Rm 2315 (517)-355-4076

b. **Daniel Vocelle: Assistant Director,** Office, BPS Building, Rm 4198 (517)-355-1536

c. **Environmental Health & Safety:** (517) 355-1053

d. **Occupational Health (University Physician's Office):** (517) 353-8933

e. **MSU Police:** (517) 355-2221

3.7. **Quality Measures**

a. **Daily:**

i. **Autocalibration:** When prompted run an auto calibration with SONY Automatic Setup Beads (20 drops, approximately 1 mL) to ensure the system is optimized for sorting before running samples. Autocalibration establishes chip alignment, laser delay, droplet calibration, side stream calibration, and sort delay calibration on the SONY LE-MA900. See Section 4.1 for detailed description.

ii. **Daily QC:** The Daily QC should be run at least once per day, by the first user scheduled on the instrument. See Section 4.2 for a detailed description.

b. **Monthly**:

i. **Performance QC:** A Performance QC should be run on the SONY LE-MA900 flow cytometer. Run 8-peak beads, SONY #LE-AE700522, (4 drops in 1 mL of sheath fluid) to establish a baseline. Performance QC measures the linearity and separation index(S) parameters of the QC target channel using 8-peak beads.

ii. **Sterility Test:** At least once a month a sterility test should be conducted on the instrument. After instrument startup and QC, run the Bleach Cleaning Cycle (30 mL) and the DI Cleaning cycle (15 mL). See **MSU Flow Cytometry Core Sterility Test SOP** for details.

3.8. **Assessment of Aerosol Containment**

a. The following procedure is for the purpose of measuring the effectiveness of the Aerosol Management System (AMS) on a droplet based, high pressure cell sorting flow cytometer as well as to ensure compliance to proper safety practices and procedures.

b. Aerosol Management: For proper aerosol containment, the following guidelines must be followed while sorting viable infectious material under high pressure. All sort operators must be trained by an equipment champion prior to any cell sorting:

i. The Baker BCC300AMS Class II biosafety cabinet must be on and functioning according to the manufacturer guidelines. Using this system, the AMS should be set to **LOW** and the vacuum gauge should read between -0.2 and -0.4. If

these values are outside of these ranges, the HEPA filter should be replaced and biosafety cabinet inspected.

ii. The waste tank must contain enough sodium hypochlorite (bleach) to provide a final concentration of 10% when filled (1L bleach to a final 10L waste collected).

iii. When sorting the sort control must be designated with a green light, found at the top right of the droplet formation viewer. Following calibration and setup the SONY software monitors the stability of the sort stream and alerts the operator to potential sort stream disruption, which can lead to increased aerosols. The SONY LE-MA900 is also equipped with droplet breakoff monitoring technology, with the Control Breakoff active (box checked), which is used during all sorting operations and can detect stream drifts due to possible clogs and automatically shuts down the stream.

c. **Measurement of Containment:**

i. Setup instrument with a clean 70-micron nozzle chip.

ii. Run the *Bleach Cleaning* cycle with 30mL bleach option, followed by the *DI Rinse* cycle with 15 mL of water to clean the sample path.

iii. Prepare Dragon Green 1 micron diameter microspheres (Bangs Labs, #FSDG004), or equivalent, for testing: Vortex vigorously and add 10 µL of beads to 1 mL of 1x PBS and 1 µL of Tween-20 (0.1%).

iv. Set up vacuum system for micro5, cyclex-D, or equivalent filters.

v. Set up template for beads, triggering on green fluorescence (FL1).

vi. The Baker BCC300AMS Class II biosafety cabinet must be tested under simulated worst case failure mode. In this mode, the instrument is set with the stream hitting the waste catcher to create excessive aerosols. Cover waste catcher in the sort chamber with tape to simulate clog conditions.

vii. Place an appropriate container lined with paper towels at the bottom of sort chamber to catch liquid.

viii. Don non-permeable back-close gown, sleeve protectors, N95 respirator, goggles, and close inner facility door for testing, and put a sign on the door to prevent anyone from entering.

ix. Run samples and make sure the event rate is approximately 50,000 events per second.

x. Run test and record results on Containment Test Record (Appendix I)

1 Control: Sort Chamber

- Baker Biosafety Cabinet AMS: **LOW**
- Micro5, cyclex-D, or equivalent filter
  — Vacuum: 20L/min.

—— Location: Inside BSC near sort chamber
- Sort Chamber:
  —— Front Cover: Closed
  —— Waste Catcher: Uncovered (normal operation; no clog)
- Collection time: 5 minutes

2 Simulated Clog: Sort Chamber

- Baker Biosafety Cabinet AMS: **LOW**
- Micro5, cyclex-D, or equivalent filter
  —— Vacuum: 20L/min.
  —— Location: Inside BSC near sort chamber
- Sort Chamber:
  —— Front Cover: Closed
  —— Waste Catcher: Covered (simulated clog)
- Collection time: 5 minutes

3 Simulated Clog: Outside BSC

- Baker Biosafety Cabinet AMS: **LOW**
- Micro5, cyclex-D, or equivalent filter
  —— Vacuum: 20L/min.
  —— Location: Outside BSC near workstation
- Sort Chamber:
  —— Front Cover: Closed
  —— Waste Catcher: Covered (simulated clog)
- Collection time: 5 minutes

4 Positive Control: Sort Chamber

- Baker Biosafety Cabinet AMS: **OFF**
- Micro5, cyclex-D, or equivalent filter
  —— Vacuum: 20L/min.
  —— Location: Inside BSC near sort chamber
- Sort Chamber:
  —— Front Cover: Open
  —— Waste Catcher: Covered (simulated clog)
- Collection time: 1 minutes

xi. After testing is complete, unload beads, remove the tape from the waste catcher, and run *Bleach Cleaning* cycle with 30mL bleach option, followed by the *DI Rinse* cycle with 15 mL of water to clean the sample path.

xii. While running cleaning, turn the AMS on **HIGH** for at least 5 minutes.

xiii. Decrease the AMS to **LOW**.

xiv. Discard the paper towels in the bottom of sample chamber into a waste container.

xv. Prepare 4 microscope slides, one each per test: Add one drop of immersion oil to the center of the slide. Label slide with sample name or number/letter. Take apart micro5, cyclex-D, or equivalent filter, and add coverslip to slide on top of immersion oil (make sure the collection side faces down on oil). Repeat for each test. (You can add clear nail polish around coverslip if wanting to keep these slides for an extended period of time).

xvi. Analyze on an appropriate microscope with an appropriate filter (make sure to schedule an appointment on the microscope several days before testing).

d. **Acceptable Tolerance**: Acceptable tolerances for the measurement of containment using the Dragon Green beads (or equivalent) protocol are listed below:

i. Beads outside BSC = Zero tolerance, no particles on the entire slide. Any positive result must be investigated, resolved, and the instrument retested before proceeding with sorting potentially infectious samples.

ii. Beads inside (positive control) = Greater than 100 per slide.

4.0 Procedure: SONY LE-MA900 Use

4.1. Startup & Autocalibration

a. Biosafety Cabinet & Fluidics Check

i. Turn on the Baker BCC300AMS Class II biosafety cabinet main blower and turn on AMS to low. The BSC must be in operation for a minimum of 5 minutes prior to running any samples. The AMS should be set to **LOW** and the vacuum gauge on the BSC should read between -0.2 and -0.4.

ii. Check fluid levels, ensuring that the waste tank is empty and the Sheath tank is FULL (filled to weld line, 10L). Wear gloves when filling the Sheath tank, as to avoid contamination of the tank/sheath fluid. Autoclave sheath tank [or minimally clean with 70% ethanol and rinse with sheath], as appropriate.

iii. Add an appropriate amount of bleach (1L) to waste tank, which will result in ~10% final bleach concentration (note that the maximum fill volume of the waste tank is 10 liters or 2.6 gallons). Hold the metal fittings in the cap stable and turn the outer cap only to remove. When removing cap rest with filter on cap in upright orientation, do not invert, as clogging the filter in the cap could result in a pressurization issue.

iv. Ensure that the sheath-line (blue) is connected to the sheath filter and the air line (clear) is connected to the sheath tank prior to startup.

b. Sort Chamber & Air Supply

i. Using a lint free wipe, wipe down sort chamber and deflection plates with 70% ethanol.

ii. Remove any sort collection devices from the sort chamber.

iii. Turn on the air compressor to pressurize the sheath tank. Pressure gauge on tank compressor should read between 80 and 100 psi. Make sure the in-line air valve (blue) is turned to the ON position.

c. **Instrument & Software Startup**

i. Turn on the Cytometer (top silver button on right side of cytometer), computer.

ii. Log into the computer. Sign in under User Account Name and password, as appropriate.

iii. Click on the Cell Sorter desktop icon to start the software. This software runs the SONY LE-MA900 flow cytometer. Sign in using appropriate username and password.

iv. When resuming from ethanol shutdown, software will prompt user to load an empty 15 mL tube. **Do not use a 15 mL tube.** Instead, load a 30 mL tube. Failure to use a 30 mL tube will result in sheath fluid being sprayed inside the sample chamber.

v. When prompted, scan QR code of the appropriately sized sorting chip using the camera mounted on the computer monitor. Check the date and time of the chip in the cytometer to see if it can be reused (<24 hours). Otherwise, use a new chip and write the date and time on chip packaging. Place the chip packaging in the clip on the outside of the BSC.

d. **Load Sorting Chip & Auto-Calibrate**

i. Follow the wizard to remove the old chip (cleaning or previously used), then load the new sorting chip, being careful to ensure that the front of the chip is facing forward. Make sure that chip is handled aseptically, being careful to not touch the ports or the front/back where the lasers pass through. Additionally, check the sample line as instructed.

ii. Discard chips >24 hours old in biohazard or retain cleaning chip.

iii. In 'Initial Instrument Setup' only turn on the lasers needed for the day's sort by toggling checkboxes. Next select 'Standard' optical configuration. Fluidics check will automatically run.

iv. If droplet shape is stable click 'Next', if unstable perform a "Sheath Filter Debubble" following the prompts. ***If it has been more than a week since the instrument was started, or if you are starting the instrument after an ethanol shutdown, run a "Sheath Filter Debubble".**

v. Select 'Autocalibration' if sorting will be performed. If ANALYSIS ONLY will be performed, ''Autocalibration' may be deselected.

vi. Prepare 1 mL of SONY Automatic Setup Beads (LE-B3001) (20 drops = ~1 mL) in a 5 mL tube and load the tube on the SIP. Follow onscreen instruction to run the autocalibration. Store the bead suspension at 4°C in the dark until you

are ready to use them (Note: beads are stable from 2°C - 25°C for no more than 20 minutes in direct light, and up to 8 hours if protected from light).

vii. Select 'Standard' sort mode. 'Targeted' sort mode is also available on the 100 µm nozzle and can be used to sort larger cells at a lower pressure.

viii. Automated chip alignment, laser delay, droplet calibration, side stream calibration, and sort delay calibration will automatically be optimized.

ix. Save a screenshot of the Autocalibration results each day and save to: C:\Users\fcm\Michigan State University\MSU Flow Cytometry Core - User Data\Core Staff

4.2. Daily Instrument Qualification

a. Once Autocalibration has completed successfully, select the *Daily QC* on the Cytometer tab to initiate the Daily QC Wizard.

b. Place a sample tube containing at least 0.5 mL of the SONY Automatic Setup Beads in the sample loader and follow the on-screen instructions.

c. Check the appropriate optical filter set is selected (*Standard*) and click *OK*.

d. Select *All Setup Beads* in [LE-B3001] and click *OK*.

e. The results are displayed when Daily QC is completed.

f. Verify that the performance passed on the *Daily QC* Screen. The Cytometer Performance Results should display a green Pass next to it each filter. If any parameters did not pass, refer to the cytometry supervisor or the SONY Multi-Application Cell Sorter Operator's Guide for help troubleshooting.

4.3. Preparing for a Sort

a. Prior to sorting, run *Bleach Cleaning* with 30 mL of a solution containing 10% bleach and 5 % contrad.

b. Run *DI Rinse* with 12 mL of sterile water in a 15 mL conical tube.

c. Open the *Droplet Viewer* by clicking on the image of the stream.

d. Wait until the *Status Icon* is solid green (this indicates the stream is stable), then click *Control Breakoff*.

4.4. Setting up an Experiment

a. Select the *File* tab of the ribbon and click *New.* Select a template from the Public Template or My template section (top left). If you do not have an existing template, select "Blank Template" from the Public Templates.

b. Select the appropriate settings for your experiment under *Measurement Settings* (e.g., Markers, Fluorophores, Area/Height/Width). You will always want FSC (A, H, W) and BSC (A, H, W) turned on.

c. When finished building the experiment, click *Create New Experiment* in the bottom right-hand corner of the window.

4.5. **Acquiring Data**

a. Press the sample loader door button to open the sample loader door.

b. Place a sample tube in the corresponding sample tube holder, then place it in the sample loader.

c. Press the sample loader door button to close the sample loader door.

d. Specify the measurement stop conditions under *Sample Stop Condition*

e. Click *Start* to begin acquiring the sample.

f. Click on *Detector & Threshold Settings* then optimize the FSC and BSC voltages to place the population of interest on scale.

g. Optimize fluorescent parameter voltages to ensure positive populations are on scale. If a positive population is off scale, decrease the PMT voltage for that parameter until the positive population is entirely on scale. Negative populations may be adjusted within gray boxes as a guide. Acquisition can be stopped, and sample unloaded when gains have been adjusted appropriately.

4.6. **Compensation Setup**

a. If compensation is required, click *Compensation Wizard* on the *Compensation* tab of the ribbon to launch the compensation wizard. ***An unstained tube is required for compensation on the MA900.**

b. Click Next. A screen will pop up with a green *Start* button displayed showing the operating procedure and how to record the control. A control tube is automatically assigned as the active tube and the worksheet for the tube will open. Follow the on-screen instructions until the compensation wizard has been completed.

c. After recording all single stained control tubes, click *Calculate Matrix* on the *Compensation* tab of the ribbon. The *Calculation Compensation Settings* dialog appears.

d. Check the target compensation panel, then click *Calculate*. The spillover matrix compensation values are calculated and applied to the experiment.

e. Check the fluorescence compensation values, and if acceptable, click *Close*.

f. Click Finish to exit the Compensation Wizard.

4.7. **Performing a Tube Sort**

a. Check that data acquisition and recording are stopped or paused.

b. Place the collection tubes into the corresponding holder

c. Open the collection area door, place the holder on the collection stage, and close the door.

d. Click *Load Collection* in the *Sort Control* pane.

e. Select the correct tube type in *Method*. To start recording at the same time as sorting, place a check mark in the *Auto Record* checkbox.

f. Select a sorting mode in *Mode*.

g. Select *Sort Settings* then set the *Sort Gate* and *Stop Count* for each collection tube. *Entering a "0" disables the stop condition.

h. Change the *Sample Stop Condition* to **Recording and Sorting**, which will stop data acquisition when both sorting and recording.

i. Click *Start* or *Resume* in the data acquisition control pane to begin acquiring data.

j. Adjust the *Sample Pressure* to acquire sample at an acceptable event rate, being careful not to exceed maximum event rate for nozzle or sort mode setup being utilized.

k. **DO NOT SORT** if the *Status Icon* is flashing green or gray. If the Status Icon is flashing green or gray, pause the sort and wait for it to stabilize.

l. Click *Sort Start* or *Sort & Record Start* in the *Sort Control* pane to begin sorting.

m. Recording will stop automatically when the stop condition is met but sorting will continue. Sorting and recording are independent.

n. Once sorting conditions are met and sort is complete, Stop or Pause sample acquisition, and click the *Unload Collection* in the *Sort Control* window.

   i. As appropriate, run AMS on High for at least 1 minute before unloading BSL-2 or higher samples from sorting chamber.

   ii. Open sort chamber to remove samples using aseptic technique. Collection tubes should be capped inside the BSC, wiped on the outside with appropriate disinfectant, and placed in a biohazard transport container, as appropriate. Gloves should be changed and discarded into a biohazardous waste container.

   iii. To pause/stop the stream click *Settings* on the *Cytometer* tab of the ribbon. Click *Advanced Settings*. Click on *Pressure Options*, then *Standby*.

4.8. Performing a Plate Sort

a. Check that data acquisition and recording are stopped or paused.

b. Attach the splash guard.

c. Place the collection plate into the corresponding holder.

d. Open the collection area door, place the holder on the collection stage, and close the door. Make sure to securely mount the holder adapter to ensure successful sorting. Check orientation of the plate (well A1) to ensure correct sorting.

e. Click *Load Collection* in the *Sort Control* pane.

f. Select the correct plate type in *Method*. To start recording at the same time as sorting, place a check mark in the *Auto Record* checkbox.

g. Select *Sort Settings* then configure the settings for sorting into wells on the *Plate Sort Setting* tab*.*

h. Select *Index Sorting* if desired.

i. Select the sorting sequences in *Sort Layout Settings*.

j. Select the target well to set

k. Select the gate whose population you want to sort into the well in *Sort Gate*, then select a *Sort Mode.*

l. Enter the number of events to sort per well in *Stop Count.*

m. When finished click *Close*.

n. **DO NOT SORT** if the *Status Icon* is flashing green or gray. If the Status Icon is flashing green or gray, pause the sort and wait for it to stabilize.

o. Click *Start* or *Resume* in the data acquisition control pane to begin acquiring data.

p. Adjust the *Sample Pressure* to acquire sample at an acceptable event rate, being careful not to exceed maximum event rate for nozzle or sort mode setup being utilized.

q. Click *Sort Start* or *Sort & Record Start* in the *Sort Control* pane to begin sorting.

r. Recording will stop automatically when the stop condition is met but sorting will continue. Sorting and recording are independent.

s. Once sorting conditions are met and sort is complete, Stop or Pause sample acquisition, and click the *Unload Collection* in the *Sort Control* window.

    i. As appropriate, run AMS on High for at least 1 minute before unloading BSL-2 or higher samples from sorting chamber.

    ii. Open sort chamber to remove samples using aseptic technique. Collection tubes should be capped inside the BSC, wiped on the outside with appropriate disinfectant, and placed in a biohazard transport container, as appropriate. Gloves should be changed and discarded into a biohazardous waste container.

    iii. To pause/stop the stream click *Settings* on the *Cytometer* tab of the ribbon. Click *Advanced Settings*. Click on *Pressure Options*, then *Standby*.

4.9. **In the Event of a Clog or Stream Destabilization**

Partial or Full Clog: Observe the event rate while performing a sort. Wild swings in event rate, dropping near zero or to zero events per second can be an indication of a partial or full clog.

a. **Any time there is a concern that a partial clog or full clog has formed:**

i. Unload the sample.

ii. Turn the Baker BCC300AMS Class II BSC AMS to ***HIGH***.

iii. Wait **5 minutes** for any potential aerosols to clear from the sort chamber before opening the sort chamber.

iv. Remove the sample tube from the sample loader and the sample collection tubes and cap them. When removing collection tubes, be aware that the outside of the tube is potentially contaminated, therefore, wipe with Sani-Cloth Plus wipes to decontaminate. Use 10% bleach, PDI wipe or 70% Ethanol to decontaminate the sort chamber, sample chamber, and immediate surrounding surface.

v. If the event rate fluctuates wildly or drops near zero, this may indicate a partial clog.

1. Click *Pause* and *Unload Sample* to initiate a backflush

2. Under the *Cytometer* tab, click on the *Probe Was*h to repeat backflush

3. Refilter the sample prior to loading and resuming sort.

b. **If the event rate drops to zero, indicating a possible full clog, and the droplet viewer indicates the breakoff/stream is STABLE the following steps may be considered:**

i. Unload the sample

ii. Turn the Baker BCC300AMS Class II BSC AMS to ***HIGH***.

iii. Wait **5 minutes** for any potential aerosols to clear from the sort chamber before opening the sort chamber.

iv. Remove the sample tube from the sample loader and the sample collection tubes and cap them. When removing collection tubes, be aware that the outside of the tube is potentially contaminated, therefore, wipe with Sani-Cloth Plus wipes to decontaminate. Use 10% bleach, PDI wipe or 70% Ethanol to decontaminate the sort chamber, sample chamber, and immediate surrounding surface.

v. Run a *Probe Wash*.

vi. Evaluate whether clog has cleared and the stream restabilizes. You should attempt this at least three times before proceeding to the next step. If this stabilizes the droplet breakoff, refilter the sample prior to loading and resuming sort.

vii. If *Probe Wash* is insufficient, run a *Chip Debubble*. Evaluate whether clog has cleared and the stream restabilizes. You should attempt this at least three times before proceeding to the next step.

viii. If this stabilizes the droplet breakoff, refilter the sample prior to loading and resuming sort.

c. **<u>Stream Destabilization</u>: A warning pop-up window will appear if the instrument is unable to maintain the drop delay with the addition of a FLASHING green light indicator in the Droplet Viewer. If this occurs, consider the following**:

i. Unload the sample.

ii. Turn the Baker BCC300AMS Class II BSC AMS to ***HIGH***.

iii. Wait **5 minutes** for any potential aerosols to clear from the sort chamber before opening the sort chamber.

iv. Remove the sample tube from the sample loader and the sample collection tubes and cap them. When removing collection tubes, be aware that the outside of the tube is potentially contaminated, therefore, wipe with Sani-Cloth Plus wipes to decontaminate. Use 10% bleach, PDI wipe or 70% Ethanol to decontaminate the sort chamber, sample chamber, and immediate surrounding surface.

v. Try running a *Chip Debubble*. Evaluate whether clog has cleared and if the stream restabilizes. You should attempt this at least three times before proceeding to the next step. If this stabilizes the droplet breakoff, refilter the sample prior to loading and resuming sort.

vi. If *Chip Debubble* is insufficient, run a *Sheath Filter Debubble*. Evaluate whether clog has cleared and the stream restabilizes. You should attempt this at least three times before proceeding to the next step. If this stabilizes the droplet breakoff, refilter the sample prior to loading and resuming sort. Note that if this is insufficient, attempt to alternate between *Chip Debubble* and *Sheath Filter Debubble*.

vii. If the green light is still flashing perform a *Sort Calibration*. If this solves the problem, and stabilizes the drop delay (indicated by a solid green light) clean with 10% bleach and water.

viii. If after Sort Calibration the green light is still flashing, bleed the sheath filter and perform a *Sheath Filter Debubble*.

ix. If the drop delay does not stabilize, proceed to Section 4.9d.

d. **If the stream is no longer visible** (or steps performed above were unsuccessful in stabilizing the droplet breakoff) **the nozzle is FULLY CLOGGED and/or the chip will need to be replaced.**

i. In the Cytometer tab, click on Chip Exchange to eject the clogged nozzle and discard the chip in the biohazard waste.

ii. Scan a new chip QR code and perform the *Chip Alignment* and *Sort Calibration.*

iii. Re-filter the sample prior to loading and resuming sort.

e. **If one is unable to replace the chip or perform any functions in the software**:

i. Press and hold the power button the front of the instrument until the instrument turns off.

ii. Restart the desktop.

iii. **It may be necessary to clean the sort chamber following a clog or stream destabilization:** Wait **5 minutes** after turning the **AMS to HIGH** until opening the sort block. Disinfect/Clean the sort collection chamber and tube/plate holder as necessary with 70% ethanol, 10% bleach, and/or Sani Cloth Plus wipes.

iv. Manually open the sort chamber and remove the sample.

v. Manually remove the chip using the instruction found in the MA900 User Guide (Ejecting the sort chip manually).

vi. Once cleaning is complete, change and discard gloves into a biohazard waste container prior to closing the sort chamber, close the sort chamber door.

vii. Attempt to turn on the instrument and desktop. Follow the steps for instrument startup (section 4.1).

viii. Filter the sample again through an appropriately sized cell strainer, as appropriate, and resume sorting.

4.10. **Shutdown/Decontamination**

a. **Cleaning between samples, as appropriate:**

i. Run AMS on High for at least 1 minute before unloading BSL-2 or higher samples from sorting chamber.

ii. Minimally, an automatic Probe Wash will execute between samples. Maximally, run *Bleach Clean* with 30 mL of a solution containing 10% bleach and 5 % contrad. Run *DI Water Rinse* with 12 mL of sterile water in a 15 mL conical tube.

b. **Cleaning between users or sample types, as appropriate:**

i. Run AMS on High for at least 1 minute before unloading BSL-2 or higher samples from sorting chamber.

ii. Run *Bleach Clean* with 30 mL of a solution containing 10% bleach and 5 % contrad. Run *DI Water Rinse* with 12 mL of sterile water in a 15 mL conical tube.

c. **Daily Shutdown:**

i. Click *Software and Hardware* in the *Shutdown* group on the *Cytometer* tab to open the Shutdown Wizard. Select the 'Careful Cleaning' mode to perform shutdown with 30 mL of a solution containing 10% bleach and 5 % contrad.

ii. After bleach cleaning click next and select the 'Normal Cleaning' option to run DI water rinse with 12 mL of sterile water in a 15 mL conical tube. Keep the box checked to keep the DI water inside the sample probe. This will shutdown the system with DI water in the sample probe.

iii. After Cleaning completes, click *Next* and *OK* to perform the shutdown. This will automatically turn off the SONY-MA900 and depressurize the system.

iv. Open the sort collection chamber and disinfect with 70% ethanol, 10% bleach, or equivalent disinfectant. Allow to sit for appropriate disinfection time. Ensure that enough time has passed after sorting a sample/clog has cleared and that appropriate PPE is being worn before opening the sort block. Close the sort block once liquid has been fully aspirated.

v. Turn off the air compressor.

vi. Log off of the computer.

vii. Disconnect and empty the waste tank, containing ~10% bleach and empty **into large carboy provided by EH&S, as no liquids containing fluors or beads can be disposed of down the drain**. Wear appropriate PPE (e.g., lab coat, safety glasses, and gloves).

viii. Reconnect the waste tank.

ix. Clean the keyboard, mouse, and work surfaces in front of the SONY LE-MA900 with Envirocide (or equivalent) or with Sani-Cloth Plus germicidal wipes (or equivalent). Subsequently, wipe metal surfaces with 70% ethanol to avoid corrosion of the surfaces.

x. Turn off the Baker BCC300AMS Class II Biosafety Cabinet, as appropriate.

d. **Weekly (when in use) Ethanol Shutdown:**

i. Run *Bleach Clean* with 30 mL of a solution containing 10% bleach and 5 % contrad. Run *DI Water Rinse* with 12 mL of sterile water in a 15 mL conical tube.

ii. When cleaning with water is complete, select Click *Shutdown with Ethanol* in the *Shutdown* group on the *Cytometer* tab to open the Shutdown Wizard.

iii. After Cleaning completes, click OK. This will automatically turn off the SONY-MA900 and depressurize the system.

iv. Open the sort collection chamber disinfect with 70% ethanol, 10% bleach, or equivalent disinfectant. Allow to sit for appropriate disinfection time. Ensure that enough time has passed after sorting a sample/clog has cleared and that appropriate PPE is being worn before opening the sort block. Close the sort block once liquid has been fully aspirated.

v. Turn off the air compressor.

vi. Exit Cell Sorter software and log off of the computer.

vii. Disconnect and empty the waste tank, containing ~10% bleach and empty **into large carboy provided by EH&S, as no liquids containing fluors or beads can be disposed of down the drain**. Wear appropriate PPE (e.g., lab coat, safety glasses, and gloves).

viii. Reconnect the waste tank.

ix. Clean the keyboard, mouse, and work surfaces in front of the SONY LE-MA900 with Envirocide (or equivalent) or with Sani-Cloth Plus germicidal wipes (or equivalent). Subsequently, wipe metal surfaces with 70% ethanol to avoid corrosion of the surfaces.

x. Turn off the Baker BCC300AMS Class II Biosafety Cabinet, as appropriate.

5.0 Maintenance

The following preventative maintenance should be performed according to the MA900_Operators_Guide (see page 164).

a. **Monthly:**

1 Replace the waste tank air filter (MA900_Operators_Guide page 200).

2 Autoclave the DI water tank, sheath tank, and waste tanks (MA900_Operators_Guide page 172).

b. **Every 3 Months:**

1 Change the PEEK sample line (MA900_Operators_Guide page 175).

2 Autoclave the probe adapter (MA900_Operators_Guide page 175).

3 Change the sheath filter (MA900_Operators_Guide page 178).

4 Run waste line A maintenance (MA900_Operators_Guide page 193).

5 Clean the sample loader O-ring (MA900_Operators_Guide page 196).

6 Autoclave the sheath filter (MA900_Operators_Guide page 200).

7 Run “Waste Line Maintenance” (MA900_Operators_Guide page 193).

c. **Every Year:**

i. **BSC Certification:** Have licensed a professional certify the Baker BCC300AMS Class II biosafety cabinet.

ii. Preventative Maintenance:

1 Change the DI water tank and ethanol tank air filters (MA900_Operators_Guide page 200).

2 Change the waste catcher O-ring (MA900_Operators_Guide page 190).

3 Clean the optical fibers (MA900_Operators_Guide page 202).

d. **Approximately every 12 month: Schedule preventative maintenance with Service Contract provider.**

i. Preventative maintenance and service must be performed periodically on the SONY LE-MA900.

ii. All surfaces must be decontaminated before the service engineer performs any maintenance on the instrument. The following surfaces should be wiped down with appropriate disinfectant at least 20 minutes prior to servicing:

iii. All outer surfaces of the cytometer.

iv. All table surfaces, keyboard, and mouse.

v. The inner and outer surfaces of the BSC near the front with which the service engineer might come in contact.

vi. Once this is done the BSC can be turned off.

vii. Complete the Equipment Release Form and attach to the exterior of the BSC.

6.0 Procedures for Sorting BSL-2+ with Enhanced Precautions

a. Perform assessment to ensure that Room 5115 is set up to maintain negative air pressure. For BSL-2+ sort, the room will be monitored for negative air pressure prior to sort initiation using the Setra system and/or smoke bottle.

b. Following startup, empty the waste tank. Add enough fresh bleach to the waste tank to provide a final concentration of approximately 15% when filled.

c. Place a sign on the outside of the door indicating what agent is in use, potential hazards posed by the infectious agent, and what protection should be worn when working with the infectious agent. The door should remain locked at all times.

d. Put on PPE: This includes a back-closure gown, gloves, face shield, goggles/safety glasses, and N95 respirator for BSL2+ sorts.

e. Prepare the proper disinfectant (e.g., 20% bleach) and wipes in case of a sample spill. Tubes will be vortexed inside the BSC with caps on.

f. Check and clean the sort collection chamber, and collection peripherals with 70% Ethanol prior to sorting. Pay specific attention to any salt deposits that can interfere

with the proper function of the components involved in sorting. Clean these off with sterile water.

g. Turn the AMS on High for at least 5 minutes after completion of sorting before opening sorting chamber.

h. **After sorting is completed and AMS has been run for appropriate amount of time**, collection tubes should be capped and wiped on the outside with appropriate disinfectant and placed in a biohazard transport container. Gloves should be changed and discarded into a biohazardous waste container.

   i. Run *Bleach Clean* with 30 mL of a solution containing 10% bleach.

i. **Run a second round** of *Bleach Clean* with 30 mL of a solution containing 10% bleach and 5 % contrad, followed by a *DI Water Rinse* with 12 mL of sterile water in a 15 mL conical tube. Alternatively, run Daily or Weekly Shutdown as described in Section 4.10.c or 4.10.d, respectively.

j. Open the sort collection chamber disinfect with 70% ethanol, 10% bleach, or equivalent disinfectant. Allow to sit for appropriate disinfection time. Ensure that enough time has passed after sorting a sample/clog has cleared and that appropriate PPE is being worn before opening the sort block.

   i. After decontamination of the system's fluidics is complete, ensure the waste tank is mixed. Allow the waste tank to sit overnight before transferring it into an EHS approved 5 Gallon carboy.

   ii. Clean the keyboard, mouse, and work surfaces in front of the SONY LE-MA900 with Envirocide (or equivalent) or with Sani-Cloth Plus germicidal wipes (or equivalent). Subsequently, wipe metal surfaces with 70% ethanol to avoid corrosion of the surfaces.

k. Wipe all surfaces down with disinfectant, including the outside of the cytometer and the table surface.

l. Disinfect and clean the surfaces around the cytometer, especially near the sort chamber, with proper decontaminant and treat all work surfaces with appropriate disinfectant.

m. With a clean pair of gloves, clean the keyboard, mouse, and work surfaces in front of the SONY LE-MA900 with Envirocide (or equivalent) or with Sani-Cloth Plus germicidal wipes (or equivalent). Subsequently, wipe metal surfaces with 70% ethanol to avoid corrosion of the surfaces.

n. Before leaving the room, place all disposable PPE in the biohazardous waste container and wash hands thoroughly. **Leave the BSC turned on overnight.**

o. Records

p. **Error Messages / System Issues** – All error messages and system issues must be relayed to the Equipment Champion and the Core Staff and appropriately recorded in the Equipment Maintenance Logon the same day as equipment use.

7.0 Resource Index

a. SONY LE-MA900 Operator's Guide:

i. SONY LE-MA900 flow cytometer software literature and resources for the following items can be found at the links below. Printed versions of these resources can also be found with the SONY LE-MA900 flow cytometer in room 5115.

ii. Link for SONY LE-MA900 Operator's Guide

b. SONY Biotechnology Technical Support:

SONY Technical Support is available to users in the U.S. by calling 1.800.275.5963, select 2.

SONY Biotechnology Company Representative:

Jerry Aultz
Midwest Sales Representative
Tel: 815.355.7162
e-mail: jerry.aultz@sony.com

c. Transport of Biological Materials:

For detailed information about the transport of biological materials, see the EHS recommended procedures available at:

https://ehs.msu.edu/lab-clinic/shipping/bio-transport-local-vehic.html

8.0 Competencies, Authorization and Training

8.1. New Users must receive proper authorization from either the Equipment Champion and / or Core Facility Staff before equipment use. A new User may contact the Equipment Champion or Core Facility Staff to schedule training. Training includes SOP and instrument familiarization and any additional required or specialized training. Once training is complete authorization may be issued and a system account and password may be setup. All Users are individually responsible for current SOP familiarization. All New Users must refer to 3.4a during new SONY LE-MA900 instrument account creation.

9.0 SOP Performance and Equipment Review

9.1. The effectiveness of the SOP: 0714444.5115 will be monitored by the Core Facility Staff, Equipment Champion and All Users. Any procedural or qualitative deviations will be reflected within an updated SOP. Any Approved User should aptly report any procedural or qualitative issues and / or errors to the Core Facility Staff or Equipment Champion. The Core Facility Staff and Equipment Champion's name and contact information can be found on the Pharmacology and Toxicology Core Laboratory in iLab. Updated SOPs will be published and Approved Users will be notified. SOP: 0714444.5115 reviews will occur approximately every two years.

10.0 Definitions

10.1. **SOP:** Standard Operating Procedure, which is a standard guide that officially standardizes the process of control, maintenance, and ownership of the SONY LE-MA900 instrument. The SOP number stands for (xxx . xxx . xxx) equipment serial number . room number . SOP version number.

10.2. **Originator / Author:** The individual representing the Pharmacology and Toxicology Core Facilities that created SOP: 0714444.5115.

10.3. **Stakeholder**: Any individual that uses or performs the task of which is the subject of the SOP, including the Pharmacology and Toxicology Core Facilities Department.

10.4. **New User:** An individual who has not completed the requirements in section 3.4.

10.5. **Approved User:** An individual who uses the LE-MA900 instrument and has fulfilled the requirements in section 3.4. This title may only be given by the Equipment Champion and / or the Core Facility Staff.

10.6. **Champion:** An individual whose direct expertise with the SONY LE-MA900 instrument has been recognized by the Pharmacology and Toxicology Core Facility Staff. This title may only be awarded by the Pharmacology and Toxicology Core Facility Staff.

11.0 Approval

The below signatures and dates are required for full SOP approval and implementation.

This SOP was written, authorized, and approved by:

Dr. Matthew Bernard  01Nov2024

Dr. Daniel Vocelle  11/13/2024

Issue Date: November 13th, 2024

Appendix I

Containment Test Record

Sample 1: Normal Operational Mode
AMS: **LOW**
Micro5/Cyclex-D Filter: **20 L/min**
Filter Location: **On top of the sort collection chamber**
Collection Time: **5 minutes**
Sort Chamber Door: **Closed**
Waste Catcher Covered: **No**

Result_____

Sample 2: Simulated Clog
AMS: **LOW**
Micro5/Cyclex-D Filter: **20 L/min**
Filter Location: **Inside BSC, near sort chamber door**
Collection Time: **5 minutes**
Sort Door: **Closed**
Waste Catcher Covered: **Yes**

Result_____

Sample 3: Simulated Clog
AMS: **LOW**
Micro5/Cyclex-D Filter: **20 L/min**
Filter Location: **Outside BSC, at workstation**
Collection Time: **5 minutes**
Sort Door: **Closed**
Waste Catcher Covered: **Yes**

Result_____

Sample 4: Simulated Clog (Positive Control)
AMS: **OFF**
Micro5/Cyclex-D Filter: **20 L/min**
Filter Location: **On top of the sort collection chamber**
Collection Time: **1 minute**
Sort Door: **Slightly open**
Waste Catcher Covered: **Yes**

Result_____

Date:

Operator: