1. Purpose

This SOP is designed to provide assurance that personnel operating with FLOW CITOMETRY and CELL SORTING use the proper precautions to minimize the biohazard exposure associated with these activities. Adherence by staff to both these policies and general guidelines for safe practices, will minimize the risks, though no guarantee can be given that these guidelines will ensure absolute protection.

1.1 Synonyms and Abbreviations

BSC – Biological Safety Cabinet
CEDOC – Chronic Diseases Research Centre
HBV – Hepatitis B Virus
HCV – Hepatitis C Virus
HIV – Human Immunodeficiency Virus
ILO – International Labour Organization
NMS – NOVA Medical School
PPE – Personnel Protective Equipment
SARS-CoV-2 – Severe acute respiratory syndrome coronavirus-2 (COVID 19)
WHO – World Health Organization

2. Scope

These Biosafety recommendations apply to all personnel working in NMS / CEDOC Laboratories, and particularly to all operations of FLOW CITOMETRY and CELL SORTING involving the manipulation of:

- human cells of any type (includes human cell lines, immortalized cells, as well as primary cells)
- cells with recombinant DNA vectors (e.g. lentivirus, retrovirus).

3. Framework

n.a.

4. Flowchart

n.a.

5. Development

5.1. Potential Health Hazards

The use of flow cytometers and particularly, cell sorters, for the analysis of samples containing viable infectious organisms places the facility users and operators at high risk for Laboratory-Associated Infections (LAI).
These instruments, working by electrostatic drop deflection, can easily generate aerosols which cannot be contained during analysis and sorting.

Transmission of known or unknown pathogens can occur through percutaneous or mucous membrane exposure or inhalation due to occupational exposures to droplets or aerosols. The deposition within the respiratory tract is determined by the aerodynamic diameter of aerosols: specifically, aerosols in the range of 2-4 um preferentially deposit in the alveolar region of the lung, but larger aerosols deposit in the upper respiratory tract and nasopharynx. Moreover, aerosols may remain suspended in the air for long periods of time and can easily contaminate equipment, ventilation systems, and the human skin. The risk is even higher in case there is a clog in the cell sorter nozzle, as the aerosol production in the sorting chamber can become very intense, increasing the chance of exposing the operator to uncontained aerosols.

A sign clearly displaying the universal biohazard symbol must be posted at the entrance of the laboratory.
Posted information must include: the laboratory's biosafety level, the supervisor's name (or other responsible personnel), telephone number, and the required PPE for entering the laboratory.

Additionally, many dyes used for staining in flow cytometry protocols are toxins, mutagens or carcinogens, which further increase the risks to the users/staff/researchers.

5.1.1. Laser Hazards

Lasers are used in many flow cytometers. With few exceptions, these machines include hard to-disable safety devices that prevent user exposure to the beams in normal use. Analytical flow cytometers only present a laser hazard if the safety covers are removed or the interlocks are defeated.

5.2 Risk Assessment

A risk assessment should be conducted for all samples/agents prior to the manipulation, and the appropriate biosafety level determined in collaboration with **the NMS|FCM Biosafety Committee**. The results of a comprehensive risk assessment determine the appropriate procedures and practices for flow cytometry and cell sorting procedures. Risk analysis takes into account the Risk Group of the agent and the procedures to be performed using the agent.

The Principal Investigator is responsible for assessing and managing the potential risks associated with any sample to be processed in the Flow Cytometry Facility.

In general, the work with biological samples should be conducted at Containment level 2 laboratories (BSL2) or higher, following Standard Laboratory Precautions. All activities with biological samples must be processed in a Class II BSC until they have been properly inactivated (by any type of process - physical or chemical).

Please consult SOP NMS.BS.PR.01 for further information.

5.3 Biosafety Recommendations for Flow Analyzers (Flow Cytometers)

Benchtop analytic flow cytometers are fully enclosed systems. The biological hazards associated with them relate to sample preparation and handling rather than the instrument itself. The main problem lies in the contamination of external surfaces or a sample spill onto a work surface or floor in an environment that is not adequately setup to handle these risks.

5.3.1 PERSONAL PROTECTIVE EQUIPMENT (PPE)

Operators must wear PPE appropriate for the procedures and the materials to be analyzed. This includes at a minimum:
- surgical mask,
- gloves,
- lab coat or disposable gown.

If live tests are necessary (see below in sample restrictions), the operators must reinforce the PPE to wear by using:
- two pairs of gloves,
- safety glasses or visor,
- FFP2 respirators should also be available for high-risk situations.

5.3.2 SAMPLE CONSIDERATIONS

The fixation of biohazardous samples to be acquired on all flow analyzers is recommended, whenever live tests are not necessary.
Examples of samples that preferably SHOULD NOT BE ACQUIRED in a viable state (i.e., without undergoing fixation):

- Human cells of any type (includes human cell lines as well as primary cells).
- Cells with recombinant DNA vectors (e.g. lentivirus, retrovirus).

If such a protocol is needed, extra containment measures may be required, such as FFP2/3 respirators.

If you are in doubt about the sample biohazard status or if it is unknown, please discuss this with the Flow Cytometry Core Manager (claudia.andrade@nms.unl.pt) or contact the NMS|FCM Biosafety Committee (biosafety@nms.unl.pt).

Although the information about SARS-CoV-2 is unclear in some aspects, there is evidence that the samples can be partially inactivated with reagents containing paraformaldehyde.

Recommended for Surface staining: adding hemolysis solution with at least 1% paraformaldehyde inactivates possible pathogens (e.g. BD FACS Lysing solution).

Recommended for Intracellular staining: add paraformaldehyde fixation solution (e.g. solution A of Fix & Perm reagent).

Inactivation of samples does not seem to be effective if other hemolytic solutions without paraformaldehyde (e.g. BD PharmLyse solution), which may require the use of reinforced PPE.

Non-inactivated biological samples cannot be opened, pipetted, strained or vortexed (unless sealed) outside of a class II biosafety cabinet and must be transferred between lab spaces within leak-proof secondary containment.

If you wish to learn more about Antibody fixation, please consult the links below:

- https://www.biolegend.com/en-us/fixation

-https://www.thermofisher.com/pt/en/home/life-science/cell-analysis/cell-analysis-learning-center/cell-analysis-resource-library/ebioscience-resources/antibody-fixation-considerations.html

- Chow, Sue, et al. “Whole Blood Fixation and Permeabilization Protocol with Red Blood Cell Lysis for Flow Cytometry of Intracellular Phosphorylated Epitopes in Leukocyte Subpopulations.” Cytometry Part A, vol. 67A, no. 1, 2005, pp. 4–17., doi:10.1002/cyto.a.20167.

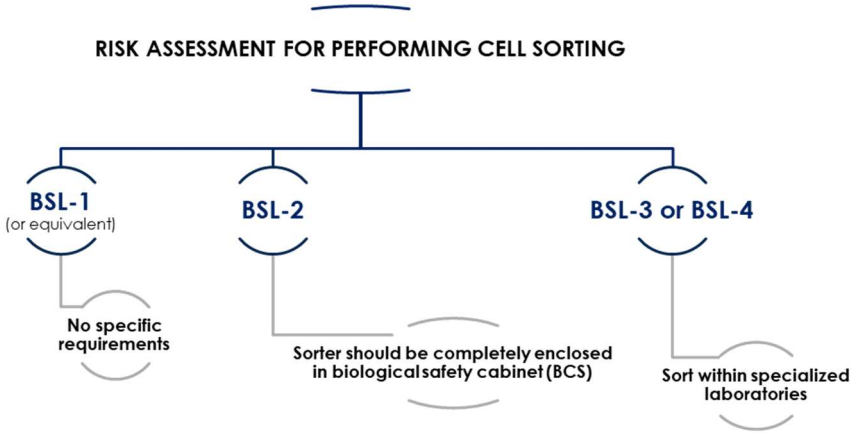
- Law, Jacqueline P., et al. “The Importance of Foxp3 Antibody and Fixation/Permeabilization Buffer Combinations in Identifying CD4+CD25+Foxp3+Regulatory T Cells.” Cytometry Part A, vol. 75A, no. 12, 2009, pp. 1040–1050., doi:10.1002/cyto.a.20815.

5.3.3 ADDITIONAL PROCEDURES:

- All surfaces (flow cytometers, computers, …) within the Facility must be manipulated with gloves.
- Follow the cytometer cleaning procedure according to the manufacturer's instructions, using at least one bleach solution (e.g. BD FACS Clean) to ensure the disinfection of the flow chamber and sample probe.
- After using the equipment, it is mandatory to disinfect the computer keyboard, mouse and screen, as well as the cytometer front panel, tube dock and PBS tank, by spraying these surfaces with 70% ethanol.

5.4 Biosafety Recommendations for Cell Sorters

Cell sorting intentionally generates aerosols that under most of the conditions are more or less controlled, but in some cases, when there is a partial clog in the nozzle tip in the stream, the quantity of aerosols that can be generated is tremendous. Therefore, cell sorters used for biological samples should be enclosed in certified class II biosafety cabinets or similar containment structures. Moreover, sorting biological samples may require special procedures for operation and decontamination of the cell sorters. Please make sure that you inform the facility staff on the type of sample you need to sort before starting your experiment.



- The International Guidelines recommend whenever any samples requiring BSL-2 containment are to be sorted, that the cell sorter should be placed in a separate, lockable room where no other laboratory activity is performed and enclosed within a certified Class II BSC.
- If possible, air flow in the room should be balanced to create negative airflow into the room. It is recommended that a visual monitoring device is located at the door to measure negative airflow.

  According to the biosafety recommendations, until the cell sorter is installed in a certified class II BSC, samples requiring BSL-2 containment should not be sorted, unless all users in the shared laboratory use adequate PPE, including FFP2/3 respirators or equivalent.

5.4.1 PERSONAL PROTECTIVE EQUIPMENT (PPE)

- **Personal Protective Equipment (PPE) recommended:**
  - lab coat and disposable gown;
  - 2 pair of gloves;
  - eye protection: safety goggles, face shield, splatter guard, or integral respirator/face shield which provide mucous membrane protection as required for anticipated splashes or sprays of infectious or other hazardous materials;

- Respirators:
  Filtering face piece respirators must be worn by all personnel in the cell sorter laboratory during operation of the cell sorter using biological samples.
  Approved respirators include EN-certified FFP2 or FFP3, or NIOSH-approved N-95, N-99, or N100 filtering face piece respirators.
  Note that fit testing should be performed for all personnel wearing respirators.
  Respirators must remain on during all procedures associated with sample manipulation, including sample tube cap removal and loading of sample on instrument, or when removing collection tubes or other procedures where the sort or collection chamber is opened.
  Note that respirator protection may otherwise be removed during the sorting process providing the aerosol management system is active and all sort chamber and collection chamber doors are closed.
  For Cell Sorters enclosed in a certified BSC, the use of respirators as outlined above is recommended during instrument/sample manipulation within the BSC but can otherwise be removed during sorting procedures providing the BSC is operational, aerosol management system is active, and all sort chamber and collection chamber doors are closed.

- Disposable protective equipment MUST be removed when leaving the laboratory and disposed of with other contaminated waste (Group III Waste – Biological risk).

5.4.2 SAMPLE CONSIDERATIONS

For all the projects that require the use of the cell sorter, the Principal Investigator MUST complete the request cell sorter form, showed in Annex I, to previously assess the potential biohazard(s) associated.

5.4.2.1 PRE-SORT PROCEDURES

- Samples must be completely prepared prior to arrival to the cell sorting room:
  - **Ressuspended in saline buffer,**
  - **Filtered,**
  - **Placed on ice.**

  Users are responsible for contacting the facility staff to discuss proper sample preparation before sorting.

- **Transporting Samples to the Flow Facility:**
  - All samples need to be packaged prior to transport at least with one layer of packaging, which should be leak-proof.

- All samples must be transported to and from the cell sorting room in an appropriate container: **double-package, leak-proof, puncture-resistant plastic carrier with a secure lid, which would be able to contain the specimen in case of breakage of the primary container.**

- **The facility staff will perform these procedures before starting to sort:**
  - Place concentrated bleach into the waste tank;
  - Make sure the sheath tank is full;
  - Start the flow cytometer system;
  - Dispose sample tubes and sort collection tubes inside the proper chambers;
  - Start the sorting procedure and monitor the sorting performance using the AccuDrop camera.

5.4.2.2 POST-SORT PROCEDURES

- The daily shutdown procedures of usual equipment are specified in the SOP - Standard Operating Procedure of FACSAriaIII instrument setup.
- **Restrictions for BSL-2 samples:**
  - After cell sorting is finished, wait a few minutes to allow any aerosols to be cleared;
  - Carefully remove, re-cap and decontaminate the outside of samples containers;
  - Place the experimental sample and collection tubes in the transporter container;
  - Run 5 ml of 10 % bleach for 10 minutes followed by 5 ml of sterile distilled water to clean the FACSAria III sample line;
  - Use the Long Clean command of FACSAria III to decontaminate the internal sheath path with 70 % ethanol:
    - Choose Instrument > Cleaning Modes > Long Clean,
    - If you have not already turned off the stream, a message appears reminding you to do so,
    - Select a Long Clean option and click OK,
    - Wait 8–10 minutes for the cleaning cycle to finish.
  - Decontaminate the sort chamber, sample chamber and any other surfaces which were used (i.e. computer workspace) with a solution of 70 % ethanol;
  - Discard outer gloves and sleeves in the biohazardous waste containers;
  - Remove disposable laboratory coat and place it in biohazardous waste container;
  - Remove the waste tank and transfer the contents to a Neutralab safe container. Rinse the waste tank with concentrated bleach.

UNEXPECTED NOZZLE OBSTRUCTION

Sorting with an appropriate nozzle is a critical safety aspect to consider, because a mismatch between cell and nozzle size may create a partial or complete clogging of a sort nozzle. During a partial clog, the aerosol production in the sort chamber can become very intense, increasing the chance of exposing the operator to uncontained aerosols. If the nozzle aperture is completely obstructed, the sorter is designed to stop automatically and block the sorting tubes.

In both cases, the manipulations required to return to sorting in normal operational mode, enhances the risk of operator exposure to pathogens contained in the sort sample motivated by accidental splashes and/or escape of sort aerosols. Only facility staff is allowed to troubleshoot the problem and clear a clog.

Nozzle Cleaning Procedure:

- Remove the sample from the sample chamber and recap the tube;
- Turn off the stream. FACSAria III has a safety device that will stop the sorting process as soon as a clog occurs. If the automated function fails, the stream has to be turned off manually;
- Run “Sample Line Back flush” for 20 seconds and clean the flow cell with distilled water. If the nozzle cannot be cleared, the system should be shut down;
- Wait 5 minutes to allow the reduction of the potential inhalation of aerosols generated during the cell sorting process;
- Ensure that the high voltage deflection plates are turned off;
- Remove the nozzle from the instrument and sonicate the nozzle immersed in pure detergent for 5 minutes in a bath sonicator;
- Clean the nozzle with distilled water and place it back in the instrument;
- After replacing the nozzle, the gloves used for the cleaning procedure needs to be discarded and substituted by new ones;
- The area around the sorter will be decontaminated with 70% ethanol;
- Sorting can be restarted after the stabilization of the stream and droplet break-off.

CELL SORTER DECONTAMINATION PROCEDURE

In a flow facility, a cell sorter is regularly used by many operators for the analysis and sorting of a variety of samples, including human or animal derived blood products and cultured cells. These samples can be contaminated with bacteria, viruses, or other pathogens that can potentially cause contamination of subsequent samples. Another cause for contamination is insufficient cleaning and maintenance of the cytometer and the use of fluids (sheath fluid, deionized water, bleach or ethanol) that have become contaminated.

The decontamination procedure (specified in the SOP - Standard Operating Procedure of FACSAriaIII instrument setup) can be used after a known bacterial contamination, immediately prior to an aseptic sort, or as a routine maintenance procedure to minimize or prevent the occurrence of bacterial contamination (every 15 days).

5.5 Emergency Procedures

5.5.1 SPILLS

If a spill of a sample occurs it is very important to decontaminate the sort chamber, the sample chamber, the surrounding surfaces, and other additional surfaces which were affected, with a solution of 2 % clearsol in 70 % ethanol.

After waiting for 30 minutes to allow full inactivation of possible infectious materials by the decontamination solution, the area needs to be extensively washed with 70% ethanol. The used paper towels have to be placed directly into a biohazardous waste container.

After cleaning the spills, change the gloves and any other personal protective equipment.

In the case of a major incident all personnel should immediately terminate all activities as rapidly and safely as possible and evacuate the room. The advice signs have to be posted and the access to the room interrupted. Notify the NMS|FCM Biosafety Committee immediately.

5.5.2 DECONTAMINATION OF SURFACES AND EQUIPMENT

A diluted bleach solution (1/10) should be prepared daily to be available in the laboratory.

Ethanol should also be available in all laboratories in a 70% solution.

Work Surfaces:

During work, surfaces must be frequently disinfected with 70% ethanol to control infectious risks.

At the end of the day, and whenever there is evidence of contamination, work surfaces should be cleaned with the bleach solution diluted 1/10 and then passed through 70% ethanol.

BSC surfaces should also be cleaned with 70% ethanol and then, at the end of the day or after the work sequence, there must be a 20-minute UV sterilization cycle.

Glassware:

All glassware contaminated with fluids should be fully dipped into a strong solution of sodium hypochlorite (≈0.5% available chlorine): common use bleach: dilute 1 / 10.

Centrifuges:

Whenever there is evidence of centrifuge contamination, it should be cleaned with 70% ethanol.

In the event of a breakage, place the potentially contaminated material in a disinfectant solution for 24 hours and disinfect the interior of the centrifuge with a non-corrosive agent (*eg*. 70% ethanol). Disinfect the undamaged tubes as well, so that the sample can then be recovered.

Monthly, the interior of the centrifuges and their components must be cleaned and disinfected with 70% ethanol.

Thermal baths:

Whenever there is evidence of water contamination, the bath should be washed, and the water replaced.

Monthly, the thermal baths in use must be maintained:

- Create a thermal shock at 70ºC, for 30 minutes,
- Remove water and wash the bath after thermal shock,
- Refill the bath with ultrapure water,

Record the operation on the Maintenance Map.

Micropipettes:

After each work cycle and whenever there is evidence of contamination, the micropipettes should be disinfected with ethanol at 70%.

5.5.3 EMERGENCY PROCEDURES AND PROTECTION AGAINST EXPOSURE

Exposure: An exposure that might place health-care or research personnel at risk of SARS-Cov-2, HBV, HCV, or HIV infection is defined as a percutaneous injury (e.g. a needle stick or cut with a sharp object) or the contact of mucous membrane or non-intact skin (e.g. exposed skin that is chapped, abraded or afflicted with dermatitis) with blood, tissue or other body fluids that are potentially infectious. (ILO/WHO).

Exposure prevention is the primary strategy for reducing occupationally acquired infections. However, there will always remain a risk of occupational exposure from blood-borne pathogens.

5.5.3.1 IMMEDIATE ACTIONS

Immediate care to the injured individual should be based on the most current WHO guidelines on post-exposure prophylaxis, which include referral of the designated individual for risk assessment of infection transmission and provision of post-exposure prophylaxis or another needed medical follow-up. This evaluation should be performed immediately in the nearest Hospital or Emergency Department.

General procedures for exposures to a potentially infectious material:

1. Stop work and immediately wash or flush the exposed area with soap and water for 10 minutes.

2. If exposure is to the eyes, flush eyes (holding open) using the eyewash station for 10 minutes.

- ***Puncture wounds, cuts and abrasions***

  The affected individual should remove protective clothing, wash the hands and any affected area(s) with soap and water, apply an appropriate skin disinfectant and seek medical attention as necessary.

  The cause of the wound and the organisms involved should be reported, and appropriate and complete medical records kept.

- ***Ingestion of potentially infectious material***

  Protective clothing should be removed, and medical attention sought. Identification of the material ingested, and circumstances of the incident should be reported, and appropriate and complete medical records kept.

5.5.3.2 FOLLOW-UP ACTIONS

Designated individuals should ensure that full reports on the injury and immediate treatment provided are completed in a timely manner. This includes referral of the exposed individual for counselling and testing and another follow-up. An investigation of the exposure incident, including identification of potential actions to prevent similar exposures in the future, should be completed in a timely manner.

5.5.3.3 ANALYSIS AND RECORD KEEPING

All exposure incidents and respective analysis should be recorded, maintained in the Accident/Incident report form and sent to the Biosafety Committee (biosafety@nms.unl.pt).

5.6 Occupational Health

1. All laboratory staff working in the Flow Cytometry Facility should be enrolled in an occupational medicine program.
2. Staff with chronic diseases or immunosuppressed may have some restrictions in their normal laboratory work, and medical advice may be necessary in some cases.
3. Pregnant women, women in puerperium or breastfeeding may also have restrictions in their normal laboratory work. Medical advice may be necessary in these cases.

5.7 Waste Management

All samples and potentially contaminated materials (tubes, Petri dishes, pipette tips, gloves) must be treated as **group III waste**:

- samples and materials handled in BSC - collected in a double white bag at BSC.
- contaminated liquids - collected in a biological waste container inside the BSC.

Sharp materials and materials / solutions with chemical risk must be treated as **group IV waste**:

- sharps waste (needles, scalpels, broken contaminated glass) - waterproof, watertight, rigid, cut and puncture proof containers
- chemical hazardous waste - collected in a red bag.

All bags / waste containers that are inside the BSC must be rinsed with a disinfectant solution before being removed from the BSC, and then placed in the respective container (70L) in the waste room.

References:

- Biosafety in Microbiological and Biological Laboratories, 5th Ed. USA, NIH/CDC. Available at http://www.cdc.gov/biosafety/publications/bmbl5/

- OSHA Bloodborne pathogens USA, "Universal Precautions" for handling samples containing potentially infectious material. Available at https://www.osha.gov/SLTC/bloodbornepathogens/worker_protections.html

- Pathogen Safety Database: Public Health Agency of Canada Listing of Pathogens with risk assessment criteria. Available at http://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment.html

- World Health Organization Laboratory Biosafety Manual: third edition of WHO biosafety manual in several languages. Available at https://www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/

- Laboratory biosafety guidance related to coronavirus disease (COVID-19). Available at https://www.who.int/publications/i/item/laboratory-biosafety-guidance-related-to-coronavirus-disease-2019-(covid-19)

- Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19). Available at https://www.cdc.gov/coronavirus/2019-nCoV/lab/lab-biosafety-guidelines.html

- Flow Cytometry Biosafety Course at CYTO U.
Available at http://cytou.peachnewmedia.com/store/seminar/seminar.php?seminar=26508

- Revision to ISAC Cell Sorter Biosafety.
Available at https://cdn.ymaws.com/isac-net.org/resource/resmgr/docs/revision_to_isac_cell_sorter.pdf

- SRL Best Practices Series, Part 4: Laboratory Safety in a Flow Cytometry SRL. Available at http://www.cytou.org/store/seminar/seminar.php?seminar=90813

- Holmes KL, *et al*. International Society for the Advancement of Cytometry cell sorter biosafety standards. *Cytometry A*. 2014;85(5):434-453. doi:10.1002/cyto.a.22454

- Perfetto S.P., *et al*. Novel Impactor and Microsphere-Based Assay Used to Measure Containment of Aerosols Generated in a Flow Cytometer Cell Sorter. Cytometry A. 2019 Feb;95(2):173-182. doi: 10.1002/cyto.a.23680

Annex I

BIOSAFETY ASSESSMENT - CELL SORT REQUEST FORM

PURPOSE: To assess the potential biohazard(s) associated with any material to be sorted using a jet-in-air cell sorter (e.g. FACS Aria III). Complete this form for any variation that will need to be considered separately (cell type, pathogen, etc.)

Currently, the sort instrument and Facility cannot accommodate any BSL-3 or BSL-4 material.

Information about the sample sources and potentially infectious agents is critical for effective biosafety measures. Consequently, this sample information form must be filled out completely and signed by the Principal Investigator who is requesting samples to be sorted in the Flow Cytometry Facility before experiments or projects are started. The same biosafety assessment questionnaire will be kept on file provided none of the information it contains has changed. It is the responsibility of the Principal Investigator to make sure that an up-to-date questionnaire is on file. **Failure to do so may jeopardize future use of the facility.**

From now on, any requests to run/sort undocumented samples will be denied.

The International Society for Advancement of Cytometry revised its Cell Sorter Biosafety Standards in 2014. J. of the International Society for the Advancement of Cytometry, Cell Sorter Biosafety Standards, 2014. This table provides a concise summary of recommended biocontainment levels for cell sorting.

Cell Type	Fixed or Unfixed	Source, Experimental Condition or Modification	Biocontainment for Flow Sorting
Any	Fixed	Any source or experimental condition With adequate paraformaldehyde or formalin fixation	BSL2
Mouse (or other non-primate)	Unfixed	Transduced with replication-deficient lentivirus or retrovirus vector (and vector stock tested negative for replication competent virus)	BSL2
Mouse (or other non-primate)	Unfixed	Transduced with replication-competent lentivirus or retrovirus vector or Transduced with replication-deficient lentivirus or retrovirus vector (and vector stock NOT tested for replication competent virus)	BSL2+ / biological safety cabinet
Human or non-human primate	Unfixed	Human cell lines (regardless of source or testing for pathogens).	BSL2+ / biological safety cabinet
Human or non-human primate	Unfixed	Includes all primary cells (regardless of source or testing for pathogens). Includes cells from humanized mice	BSL2+ / biological safety cabinet
Any	Unfixed	Infected with risk-group 2 agents requiring BSL-2 containment. Examples: LCMV, Vaccinia, Listeria, Malaria, KSHV, Aspergillus, Cryptococcus, etc.	BSL2+ / biological safety cabinet
Any	Unfixed	Infected with agents requiring BSL-3 containment. Example: Mycobacterium tuberculosis	BSL3

Your Name: _____

Laboratory (PI): _____

Date of Sort (estimation for project duration): _____

1. What is the appropriate biosafety level for the samples to be submitted for sorting (check one)? (for routine in vitro procedures, not the sorting procedure)

☐ BSL-1 ☐ BSL-2 ☐ BSL-2+

2. Will the samples be fixed, with all potentially infectious agents inactivated?

☐ Yes ☐ No If **YES**, describe the fixation method:

3. Will the samples be of human origin (or another primate)?

☐ Yes ☐ No If **NOT** of human origin, please identify the cells to be sorted:

4. Will the samples contain known infectious agents?

☐ Yes ☐ No If **YES**, list the infectious agent(s):

5. Will the samples contain recombinant or synthetic nucleic acids (r/s NA)?

☐ Yes ☐ No

If **yes**, list the vector by name and describe the method of delivery of the r/s NA molecules (e.g. transfection with expression plasmid, lentivirus transduction):

6. If a viral vector will be used for transduction of cells, was the original viral vector able to infect human cells?

☐ Yes ☐ No ☐ N/A

7. If a viral vector will be used for transduction of cells, was the vector stock tested and shown to be free of replication-competent virus?

☐ Yes ☐ No ☐ N/A

8. Will exogenous genes be transferred into the cells?

☐ Yes ☐ No ☐ N/A If **YES**, list the genes:

9. Will any of these genes be oncogenes or toxins?

☐ **Yes** ☐ **No** ☐ **N/A** If **YES,** list the genes:

Principal Investigator signature: _____

Date: _____