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SRL COMMUNICATION

Establishing a biosafety plan for a flow cytometry shared resource laboratory

Jessica B. Back[1] | Lola Martinez[2] | Lauren Nettenstrom[3] | Dagna Sheerar[3] | Sherry Thornton[4]

[1]Microscopy, Imaging, and Cytometry Resources Core, Karmanos Cancer Institute, Wayne State University, Detroit, Michigan, USA

[2]Flow Cytometry Core Unit, Spanish National Cancer Research Center (CNIO), Madrid, Spain

[3]Carbone Cancer Center Flow Cytometry Laboratory, University of Wisconsin, Madison, Wisconsin, USA

[4]Division of Rheumatology, Department of Pediatrics, Cincinnati Children's Hospital Medical Center, University of Cincinnati, Cincinnati, Ohio, USA

Correspondence
Sherry Thornton, Division of Rheumatology ML4010, Cincinnati Children's Hospital, 3333 Burnet Avenue, Cincinnati, OH 45229, USA.
Email: sherry.thornton@cchmc.org

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Abstract

A biosafety plan is essential to establish appropriate practices for biosafety in a shared resource laboratory (SRL). A biosafety plan will contain the essential information for the use of biological samples on specific instrumentation, their apparent risks, and the steps that should be taken to mitigate these risks. Establishment of a biosafety plan can be a daunting task as the variety of pathogens that come through the SRL is highly diverse and may change over time; however, having a plan that can adapt to this variety will provide a framework for addressing concerns and educating personnel and users on biosafety practices. Using resources available at your institution and developing a robust relationship with health and safety personnel at your institution is key to generating an effective biosafety plan. Here we provide a basic underlying structure for a biosafety plan to aid SRL personnel in generating or maintaining their biosafety procedures, and provide guidance for establishing a dynamic, living biosafety plan.

KEYWORDS
biosafety, biosafety plan, shared resource laboratory, SRL operations

1 | INTRODUCTION

Shared resource laboratory (SRL) facilities serve tens to hundreds of investigators studying widely varying cells and pathogens; however, SRL directors and managers may not have experience writing a biosafety plan for a laboratory that handles such a diverse set of samples. A biosafety plan contains the fundamentals for operational decisions regarding the use of these biological samples on specific instrumentation, their apparent risks, and the steps that should be taken to mitigate these risks. A biosafety plan is a living document and should be approached as an evolving, ongoing process as investigators and pathogens come and go through the facility. It is a useful starting point for

Statement of Purpose: To provide a structure for SRL personnel to establish a biosafety plan.

development of SRL biosafety best practices [1]. Having a biosafety plan in place will aid in quickly adapting to new biosafety risks, such as the COVID-19 pandemic, that required SRLs to adjust to the unusual situation of their users becoming a significant biohazard risk to the SRL personnel [2]. For consistency within this manuscript, we refer to this document as the biosafety "plan"; however, several other comparable terms, such as biosafety "protocol" or "procedure," are also widely used when describing biosafety within an SRL or laboratory.

Biosafety plans can be organized in a variety of ways and each institution may have a specific format they prefer; however, every plan should provide a framework for biosafety guidelines and include the following components: i) scientific description, ii) specific cell or pathogen information and risk assessment, iii) instrument maintenance, training, and compliance, and iv) interactions with biosafety officers and/or committees. It is important to note that biosafety plans are living documents and should be created, maintained and regularly reviewed collaboratively between the SRL and their local biosafety officer/committee. Final decisions regarding acceptance of samples should follow local guidelines and comply with the SRL's capabilities and infrastructure. In the following sections we have provided a structure for the basic information that should be included in each of these components, as well as useful tools to assist with plan generation, including reference guides, appointment forms, and an example of a biosafety plan used by an established SRL.

2 | BIOSAFETY PLAN COMPONENTS

2.1 | Scientific description

An SRL biosafety plan should start with an overview of the mission and purpose of the facility. Information regarding expertise of facility personnel, services and instrumentation available within the facility should be included. For personnel, information regarding their role, years of training, and contact information allows for quick reference should biosafety concerns arise. Services and instrumentation should be described in a general manner including any biosafety concerns for operation of specific equipment and biosafety levels that would require defined personal protective equipment (PPE) for performing specific services. Additionally, the scientific description should broadly describe the agents utilized, types of sample manipulations, and containment conditions implemented to protect SRL personnel, while specific details should be included in later sections of the plan.

2.2 | Specific cell or pathogen information and risk assessment

A risk assessment should be performed on any agent coming into the laboratory by SRL staff with guidance from institutional biosafety personnel. Holmes et al. [3] provides a working framework that can be utilized for risk assessment of cell sorting or other laboratory activities within the SRL, but specific local or national regulations should be taken into account when carrying out the risk assessment. The five steps in this process involve i) identification and evaluation of specific agent hazards, ii) identifying risks intrinsic to the laboratory procedure, iii) determining the biosafety level and assignment of additional precautions, iv) evaluation of staff proficiencies and the integrity of safety equipment, and iv) review of the risk assessment with a biosafety professional.

A complete set of hazardous agent characteristics should be included in the biosafety plan. Many institutions may have a database of risk assessments that the SRL and investigators can readily utilize, which may be highly advantageous. However, both the SRL and the individual research lab(s) personnel need to be conscious of risk group classifications and biosafety levels and be comfortable with handling these agents. While these may vary by region and/or country, a number of resources are available to identify both risk groups and biosafety levels, including a comprehensive list by the World Health Organization (WHO) and on the International Society for Advancement of Cytometry (ISAC) website (See Table 1). For the SRL, some agents may need to be broadly classified. For example, while it is good practice to ask customers what they are expressing in their viral vectors, it is not likely feasible that an SRL would be able to list all of these genes in their biosafety plan. It is important to note that some variations of viruses or other pathogens may require different biosafety precautions, so a frontline method for vetting pathogens coming into the facility may be necessary. Following risk assessment, it is suggested that a table with these agents, their risk group, and effective disinfectants and PPE be posted in various locations throughout the SRL and attached as Supporting Information to the biosafety plan. This table can serve as an effective quick reference tool for SRL personnel and users to act quickly complying with biosafety practices (see example Table 2). As an example, lentivirus is used for a variety of projects, particularly by users that sort cultured cells; however, the genes that are encoded in the lentiviral vector likely vary from user to user making it impossible to list all the genes that may come into the facility. In this case listing lentivirus as the hazard, and the handling precautions required for samples containing this hazard, may be appropriate. All viral risks are not the same. For example, adenovirus may pose different risks from lentivirus, thus based on their risks they should be listed as separate hazards. Other hazard characteristics that require consideration include reagent stability at various temperatures, hazard infectivity, and oncogenicity. Importantly, when using sodium hypochlorite a freshly prepared solution made from a non-expired stock solution should be utilized for decontamination.

In a flow cytometry SRL, several different types of physical manipulations of the samples may take place, including vortexing, aerosolization during cell sorting, and tissue handling (with or without fixatives). Each of these manipulations should be examined in light of the pathogen that will be utilized and its associated biosafety risk group, and succinctly detailed in the scientific description. All SRL personnel should be familiar with the inherent biosafety risks for each

TABLE 1 Resources for determining risk group appropriate biosafety levels and disinfectants to use for various parts of the world

Category	Country	Organization	Link	Reviewed
Biological	Australia and New Zealand	Australian/New Zealand Standard	https://shop.standards.govt.nz/catalog/2243.3:2010(AS%7CNZS)/scope https://www.nzms.org.nz/uploads/9/9/5/6/99562762/102745_as_nzs_2243.3_public_commenting-draft.pdf	2019
	European Union	European Centre for Disease Control	https://www.ecdc.europa.eu/en https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32000L0054&from=EN	2000
	North America (United States and Canada)	US Centers for Disease Control and Prevention (CDC)	https://www.cdc.gov/labs/pdf/CDC-BiosafetyMicrobiologicalBiomedicalLaboratories-2009-P.PDF	2009
		NIAID	https://www.niaid.nih.gov/research/emerging-infectious-diseases-pathogens	2018
		American Biological Safety Association (ABSA) Risk Group Database		
	International	World Health Organization (WHO)	https://www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf?ua=1	2004
Chemical	Australia and New Zealand	Australian/New Zealand chemical and environmental agencies	https://www.epa.govt.nz/database-search/chemical-classification-and-information-database-ccid/ http://hcis.safeworkaustralia.gov.au/	2020
			https://www.tga.gov.au/sites/default/files/consult-disinfectants-changes-080312.pdf	2008
			https://www.hazardoussubstances.govt.nz	2017
			https://www.worksafe.govt.nz/topic-and-industry/hazardous-substances/	2021
	European Union	European Chemicals Agency	https://echa.europa.eu/	1998
			https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:31998L0024&from=EN	2014
			https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2014:065:0001:0007:EN:PDF https://echa.europa.eu/documents/10162/23036412/clp_en.pdf	2017
	North America (United States and Canada)	United States and Canada Environmental Protection Agencies	https://www.epa.gov/environmental-topics/chemicals-and-toxics-topics	2021
			https://www.canada.ca/en/environment-climate-change/services/canadian-environmental-protection-act-registry.html	2020
		Occupational Safety and Health Administration (OSHA)	https://www.osha.gov/aboutosha https://www.ccohs.ca/	
	International	Chemical Hazard and Alternative Toolbox	https://www.chemhat.org/en/worldwide-regulation	

TABLE 2 Example table of biosafety containment measures for cell sorting involving various pathogens

Agent	Type	Infectious to humans?	RG level	Containment level	Biosafety level for cell sorting[a]	Method of decontamination	Contact time (minutes)	Waste disposal requirements
Adeno-associated virus	*Parvoviridae*	YES	RG1	BL1	BL1	10% Bleach (sodium hypchlorite)	5 min	Double-bag
Adenovirus, human[b]	*Adenoviridae*	YES	RG2	BL2	BL2+	10% Bleach (sodium hypochlorite)	5 min	Double-bag
Helicobacter pylori	*Gram negative bacteria*	YES	RG2	BL2	BL2+	5% Bleach (sodium hypochlorite)	5 min	Double-bag
						Clorox germicidal wipe	5 min	
Histoplasma capsulatum	*Yeast form*	NO	RG2	BL2	BL2	SuperSanicloths	2 min	Double-bag
HIV	*Retroviridae*	YES	RG3	BL2	BL2+	SuperSanicloths	2 min	Double-bag/ autoclave[c]

[a]BL2+ level sorts must be performed on cell sorters housed inside biosafety cabinets.
[b]Any virus demonstrated to be free of detectable replication competency or cells in which transduction was performed >48 h prior to the sort can be sorted using BSL-2 containment (outside of the hood).
[c]Autoclave the bagged waste at 121°C for 40 min in a plastic tub. Dispose of the waste in large biohazard container.

sample entering the lab and the scientific description can provide a useful overview for this purpose.

Biosafety considerations are heightened when considering cell sorting, and its production of droplets and aerosols [4, 5], specifically containment level 2 experiments may need to be upgraded to containment level 3 if droplet sorting is required. If an agent or pathogen is aerosolized, it may require re-categorization to a higher risk group and/or require heightened biosafety precautions. Some cell sorters have built-in containment, such as an aerosol management system behind the closed door of the sort block, but others are stream-in-air systems that provide no inherent containment to the sort stream when destabilization occurs. Regardless of the presence or absence of an aerosol management system, when a stream destabilization occurs during cell sorting, it is advisable to let the biosafety cabinet (BSC) and/or other aerosol management system run for a specified, predetermined amount of time [3] and to don appropriate PPE prior to opening the instrument to interact with the sort block. A clear description for handling stream destabilization during cell sorting should be included in the biosafety plan.

Containment conditions should also be clear and overarching in a biosafety plan. While an entire standard operating procedure (SOP) is not necessary, a brief description of proper disinfection and containment protocols relative to the procedures being used should be included (Table 2). It is advisable to attach detailed SOPs to your biosafety plan and have them reviewed by your biosafety officer or committee on an annual basis. The plan should include an action plan in the event of a laboratory accident or exposure, including the policies and procedures for communication to institutional officials for reporting and treatment. Institutional guidelines are usually in place for a spill or accident and should be communicated to SRL personnel. It is advantageous to have a spill kit (i.e., gloves, masks, absorbent paper, shoe covers) ready for use for containment. While a research cytometry facility may encounter more diverse sample types, these guidelines are equally applicable to clinical and translational labs, especially with regards to human patient samples where universal precautions are practiced.

An additional, non-trivial point to consider is the risk inherent to disposal of the biological and chemical waste that is generated in the SRL and the impact it may have on the environment. This disposal-associated risk may vary depending on governmental policies. Different types of waste (e.g., cytotoxic and biological waste) require special treatment and disposal, and the correct precautions should be followed to ensure this disposal complies with all local biosafety and environmental regulations and laws. In many cases institutional environmental health offices or specialized third-party companies deal with such waste disposal. SRLs should partner with their institutional biosafety officer or biosafety committee to ensure these rules are being followed and to determine if any of the associated waste generated, such as non-contaminated plastic, glass, and paper, could be recycled.

2.3 | Instrument maintenance, training, and enforcement

Another important step in good biosafety practice that should be detailed in the biosafety plan is regular testing and maintenance of containment equipment such as BSCs, other instrument enclosures, centrifuges, and incubators. Ideally these items are subject to a scheduled maintenance protocol that matches the frequency of service with the risk group and biosafety level of the samples passing through the SRL. Appropriate quality controls, instrument cleaning and maintenance, and sample preparation will help minimize the risk of a cell sorter stream destabilization event or "clog" that can produce unwanted aerosols and other hazards. Daily and weekly cleaning procedures for all instrumentation in an SRL should be established and strictly adhered to. Compliance with the biosafety guidelines by SRL personnel and users should be enforced by the SRL manager or

director with support from principal investigators and institutional leadership and should include clear procedures for reporting and addressing non-compliance. This could be especially challenging for SRLs in smaller institutions or departments but is worthwhile to establish.

SRL users have varying levels of training and expertise in flow cytometry and cell sorting; therefore, it is important for SRL personnel to provide thorough training on the inherent biosafety risks of cytometry and cell sorting, and to obtain detailed information on the experiments being performed at the SRL to ensure the plan is carried out using appropriate precautions. Having new users go through the institutional, SRL and ISAC biosafety guidelines [4, 6] and translate these to their own experimental set-up can provide an excellent starting point for users to understand the potential risks associated with cytometry and sorting. Additionally, as biosafety regulations vary by region and country, it is important that SRL plans are written with the appropriate regulations in mind and are re-evaluated periodically to reflect any changes in policies. Users coming from various areas or institutions may not be apprised of the specific biosafety plans and regulations of the facility. Training should be continuously updated and tailored to user needs, if possible, particularly in SRLs where customers carry out their own cell sorting experiments. SRL guidelines should be provided to users with periodic training updates and checks to ensure these guidelines are followed. Repercussions should these guidelines not be followed should also be established within the biosafety plan, including the workflow for reporting of any incident. When possible, it is recommended SRLs request access to the investigator biosafety protocols to enhance understanding and knowledge of the samples coming into the SRL. If given the opportunity, SRL Directors or Managers should serve on the biosafety committee to gather in-depth information about the samples that will be used in the SRL.

Similarly, protocols for training SRL personnel should be well-established and documented in accordance with institutional and facility guidelines. Details of training, including content, length and frequency, as well as, who will perform the training, should be included. For example, for sorting, the trainee may observe non biohazardous sorts for a period of time, followed by observation of biohazardous sorts for a period of time, and then perform a non-biohazardous sort under biohazardous containment conditions with observation of qualified personnel. When the trainee is deemed proficient by the trainer (i.e., SRL trained personnel, biosafety officer), and comfortable and adept with the procedure, they could then move to a biohazardous sort under observation of the trainer. Detailing these processes in the biosafety plan, as well as, including information regarding annual SRL personnel biosafety training requirements, helps to ensure consistent up-to-date training among SRL staff.

Use of an appointment form (Figure S1) can help SRL personnel appropriately prepare for samples and their associated risks while also reinforcing to users the biosafety considerations required for each experiment. Having this information required in writing for every sample provides a permanent record of the event, allows the SRL personnel to easily assess the biosafety risks associated, and presents the SRL personnel, user, and biosafety officer with a useful document for well-informed discussions of experiments if questions or concerns arise. If available, access to investigators' biosafety plans can allow SRL personnel to work with the investigator and the biosafety officer or committee to ascertain specific needs prior to scheduling an appointment.

Many SRLs track usage and training with a web-based booking system (either in-house or commercial) so authorized users may book an instrument or service in advance. These systems often have the ability to link appointment forms to each experiment and apply distinct instrument use policies that are dependent upon instrument preference and the level of user expertise. Such forms should be designed in collaboration or consultation with the institutional biosafety committee if possible and should gather at least a minimal level of sample type and preparation information, similar to the example shown in Figure S2. Having a decision tree like the example shown in Figure 1 may also help users assess biosafety containment needs for services or equipment provided by the SRL.

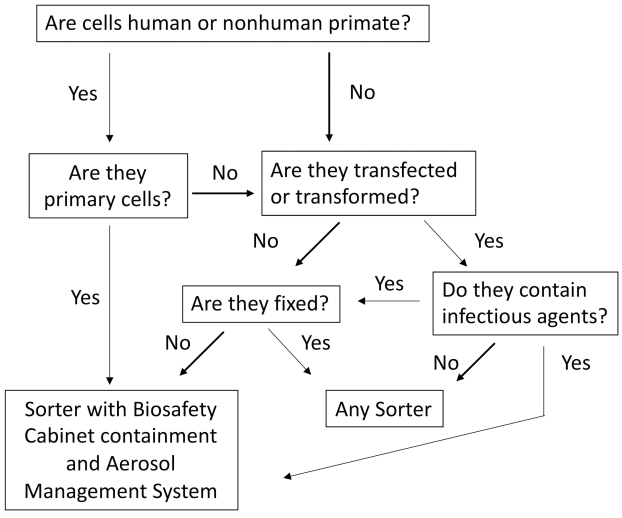

FIGURE 1 Example decision tree for cell sorting samples. Example of decision tree for determining cell sorter containment appropriate for specific samples. Posting a tree such as these can be useful for both shared resource laboratory (SRL) staff and users. (SPF, specific pathogen-free, to indicate that colony from which samples originated tested negative for certain pathogens and perhaps other adventitious agents that may interfere with research without causing disease)

2.4 | Interactions with biosafety officers and/or committees

Biosafety in an SRL is one component of overall laboratory safety. It is important to get to know all the institutional officials that preside over the safe use of potential hazards within the SRL. For many institutions, a biosafety officer and/or a committee that oversees all aspects of biosafety for the institution may be in place. As a biosafety plan is developed it is imperative to cultivate a good working relationship between the SRL and the Institutional Office of Biological Safety or the Biosafety Officer. Explaining the type of work being done in an

SRL will go a long way toward having a biosafety plan that is broad enough to include a wide variety of agents that could be encountered and specific enough to keep everyone safe. The responsibility to provide as much detail as possible for both the agents being used and any changes to the risk level that may occur as a result of flow cytometry or cell sorting lies with the individual research labs and their biosafety plans. SRL personnel will likely have a better understanding of the inherent biosafety risks associated with flow cytometry and cell sorting than their institutional biosafety officer; however, developing a collegial relationship with this expert will allow for effective two-way communication when questions arise. We recommend designating one member of the SRL to act as the primary liaison between the SRL and the biosafety officer and/or committee. For consistency in practices, this person should also be responsible for reviewing and updating the SRL biosafety plan at regular intervals.

Examining and becoming familiar with the available literature and recommendations of societies such as ISAC, the WHO, the European Biosafety Association, the American Biological Safety Association, or other regional or national guides, will provide important resources for establishing a biosafety plan. This information can be shared with the institutional biosafety officer and/or committee and provide a solid basis for establishing and maintaining a biosafety plan within the SRL.

3 | CONCLUSION

Establishing a biosafety plan is essential for a flow cytometry SRL. Here we outlined the necessary elements to be included in a biosafety plan, discussed methods for obtaining the relevant information from users to perform the relevant risk assessment, and provided guidelines for how to make this information accessible to SRL personnel and users. An example of a biosafety plan used by an established SRL is also provided (Figure S3).

Laboratory hazards are rarely limited to biological agents; chemical and radiological hazards are often present as well. Biosafety should not be approached separately from other laboratory hazards, but rather viewed as one component of a total laboratory safety program. We strongly encourage getting to know your institutional biosafety and other compliance officers, as they can become a critical part of your safety team. Actively seek the assistance of these experts as they will be able to provide up-to-date information on local, regional, and national regulations. While we focus in this manuscript on flow cytometry, microscopy cores also have similar biosafety concerns. A recent review provides information on addressing those specific challenges [7].

The effective SRL biosafety plan will provide necessary information for working with biological agents on the specialized equipment in the facility and should be an easily accessible resource for SRL personnel and users. A well thought out plan as well as processes for performing risk assessment on new agents will allow SRLs to be nimble in applying these measures should the need arise. As the biosafety plan is a living document, the information within this document will need review and revision at regular intervals, at least yearly, and will provide key information for training personnel and users within the SRL.

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AUTHOR CONTRIBUTIONS

Jessica Back: Conceptualization (equal); resources (equal); visualization (equal); writing – original draft (equal); writing – review and editing (equal). **Lola Martinez:** Conceptualization (equal); resources (equal); visualization (equal); writing – original draft (equal); writing – review and editing (equal). **Lauren Nettenstrom:** Conceptualization (equal); resources (equal); visualization (equal); writing – original draft (equal); writing – review and editing (equal). **Dagna Sheerar:** Conceptualization (equal); resources (equal); visualization (equal); writing – original draft (equal); writing – review and editing (equal). **Sherry Thornton:** Conceptualization (equal); resources (equal); visualization (equal); writing – original draft (equal); writing – review and editing (equal).

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ORCID

Jessica B. Back https://orcid.org/0000-0003-2424-9946

Sherry Thornton https://orcid.org/0000-0002-4271-236X

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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