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How to develop a Standard Operating Procedure for sorting unfixed cells

Ingrid Schmid¹

¹David Geffen School of Medicine at UCLA, Department of Hematology/Oncology, Los Angeles

Abstract

Written Standard Operating Procedures (SOPs) are an important tool to assure that recurring tasks in a laboratory are performed in a consistent manner. When the procedure covered in the SOP involves a high-risk activity such as sorting unfixed cells using a jet-in-air sorter, safety elements are critical components of the document. The details on sort sample handling, sorter set-up, validation, operation, troubleshooting, and maintenance, personal protective equipment (PPE), and operator training, outlined in the SOP are to be based on careful risk assessment of the procedure. This review provides background information on the hazards associated with sorting of unfixed cells and the process used to arrive at the appropriate combination of facility design, instrument placement, safety equipment, and practices to be followed.

Keywords

cell sorting; biosafety; occupational health; aerosol testing

1. Introduction and historical perspective

Fluorescence-activated cell sorters are instruments capable of separating cell populations based on their physical properties or by exploiting differences in cell surface receptors, intracellular structures, or molecular expression. While older commercial sorters were large and technically complex requiring dedicated and highly skilled operators, newer instruments are smaller, as well as easier to set up and run compared to historical flow sorters. Although sorting remains a complicated procedure demanding optimization by well-trained technologists, modern sorters opened up the possibility to bring cell sorting to a greater number of diverse laboratories for the separation of a multitude of differing sample types ranging from bacteria, to plant cells, to animal and human cells and tissues either freshly obtained or from cultures. The vast majority of sorting is performed on “live” cells which have not been treated with fixatives or reagents known to inactivate pathogens; thus, the hazard potential of sorting is higher than the exposure risk during analytic flow cytometry where samples are often fixed in formaldehyde-containing solutions for enhanced operator safety [1]. Furthermore, high instrument operating pressures and aerosol production, widely considered a major source for laboratory-acquired infections (LAI) [2], are inherent to jet-in-air cell sorters [3], but absent from flow analyzers. Thus, cell sorting is considered a high

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Ingrid Schmid, Mag. pharm. 12-236 Factor Building David Geffen School of Medicine, UCLA 650 Charles E. Young Drive South Los Angeles, CA, 90095 Tel. (310)-206-7289 FAX (310)-794-2145 schmid@mednet.ucla.edu.

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risk procedure compared to acquiring samples on an analytic cytometer. In fact, the term “Biohazard Sorting” has been created to encompass aspects of this process that are related to the protection of sort operators, to others involved in these experiments, and to the environment. Although “Biohazard Sorting” is mostly associated with the processing of samples known to contain infectious agents [4, 5], it also applies to cell sorting of unfixed human cell preparations or unfixed cells from other sources that may carry pathogenic organisms known to infect humans [6, 7].

Concerns for cell sorter operator safety emerged already in 1981 when J.T. Merrill tested various cell sorter modifications for their effect on aerosol production and escape [8]. However, it took the involvement of the International Society for Advancement of Cytometry (ISAC) to make progress leading to wide-spread attention to safety issues related to sorting. In 1997 ISAC published a Guideline document [9] providing for the first time written recommendations for the handling and sorting of unfixed cells, including known biohazardous samples, as well as for testing the efficiency of aerosol containment on cell sorters. In 2007, ISAC produced in conjunction with officials from the Centers of Disease Control and Prevention and the Food and Drug Administration, a new consensus standard for sorting of unfixed cells that was published in the journal Cytometry [7]. This standard, in combination with other publications related to laboratory safety, provides the basis for assessing the risk associated with sorting for a given laboratory. With thousands of flow sorters distributed over the world [10] that are performing cell isolation procedures considered essential for a wide variety of research and clinical applications, the challenge in generating practical safety protocols that offer protection, but do not hamper progress, becomes considerable. This review will address the various safety elements and current policies associated with the safe practice of sorting.

2. Safety considerations

2.1 Perform risk assessment

The general procedure for assessing the hazards associated with handling and processing samples in a laboratory was developed by safety professionals to determine the appropriate Biosafety Level (BSL) that can be expected to be effective in preventing laboratory-acquired infections (LAI). BSL are assigned in ascending order (BSL-1-4) by the degree of protection, accomplished through proper containment, which is provided to personnel, the environment, and the community. Risk assessment for performing cell sorting follows the same principles that apply to other laboratory procedures, and it involves the following steps to arrive at the appropriate containment level:

- Identify agent hazard and assign risk group (RG) classification
- Identify laboratory procedure hazards related to sample manipulation and equipment
- Determine appropriate BSL (1-4) that combine facility safeguards, practices, and safety equipment including the evaluation of their efficiency and the proficiency of staff in all the necessary safeguards
- Review risk assessment with biosafety professionals

The principal investigator or laboratory director is responsible for risk assessment and should work in close collaboration with the Institutional Biosafety Committee, if applicable, and/or Environmental Health and Safety professionals in order to ensure compliance with established guidelines and regulations.

2.1.1. Consider agent hazards—Agent hazards are directly associated with a specific pathogen and are linked to its capability to infect and cause disease in a susceptible host, its

virulence as measured by the severity of the disease it causes, and the availability of preventive measures or treatment. Various publications offer summary statements about various pathogens and their classification. On an international level, the World Health Organization recommends, for laboratory purposes, a classification into four risk groups (RGs) (Laboratory Biosafety Manual, 3rd ed., 2004, available at <http://www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf>) as does the Center for Disease Control and Prevention (CDC) publication “Biosafety in Microbiological and Biomedical Laboratories” (BMBL), 5th ed., published online in full text at <http://www.cdc.gov/od/ohs>:

- Risk Group 1: low individual and community risk, not associated with any disease in healthy adult humans
- Risk Group 2: moderate individual risk, low community risk, associated with human disease that is rarely serious and for which preventive or therapeutic interventions often exist
- Risk Group 3: high individual risk, low community risk, associated with serious or lethal human disease for which preventative or therapeutic interventions may exist
- Risk Group 4: high individual and community risk, associated with serious or lethal human disease for which preventive or therapeutic interventions usually are not available

RG2 agents are the ones most frequently encountered in the average laboratory as they comprise many bloodborne pathogens such as the Hepatitis viruses or cytomegalovirus [6, 11]; however, the human immunodeficiency viruses (HIV-1,-2) and the human T lymphotropic virus fall into RG3. Factors to consider in determining the level of containment include virulence, pathogenicity, infectious dose, environmental stability, route of spread, communicability, operations, quantity, availability of vaccine or treatment, and gene product effects. Containment levels relate to, but are not equivalent to RG levels, and may be raised or lowered from the initial risk group classification as a result of thorough consideration.

Valuable resources for RG classification of samples to be sorted include the web site of the Canadian Public Health Agency at www.publichealth.gc.ca, which features an extensive section on laboratory biosafety guidelines and safety data sheets on pathogen risk assessment, and the American Public Health Association “Control of Communicable Diseases Manual” [12] which provides information on laboratory-associated modes of disease transmission. Risk assessment for novel agents may involve utilization of recent scientific articles and textbooks and/or seeking advice from experts in the field. Technological advances have lead to the generation of modified viruses, bacteria, yeast, and other microorganisms. Guidelines from the National Institute of Health, available online at http://oba.od.nih.gov/rdna/nih_guidelines_oba.html, also provide RG classifications for pathogens and are a key resource on risk assessment for recombinant DNA experiments. The challenge in selecting the appropriate biosafety level for such work begins by establishing the classification of the non-modified organism and then proceeds to an evaluation for a possible increase in hazard potential associated with a given genetic alteration. If needed, advice from a virologist should be sought to determine the proper BSL for planned flow sorting experiments.

2.1.2. Determine procedure hazards—Procedure hazards are related to the manipulation of samples which are known to or potentially could contain pathogens and to the inherent risks associated with the operation of the instrument to be utilized for sorting. For laboratories processing human blood, general sample handling considerations in the US

involve following universal precautions as outlined in the Federal Code regulation “Occupational Exposure to Bloodborne Pathogens” formulated in 1991 [13], and additional local and institutional regulations. Other countries have developed their own regulations which contain regulatory elements similar to those for working with biological agents mandated in the US. The Clinical Laboratory Standards Institute offers a comprehensive Basic Laboratory Safety Manual for purchase that can be downloaded from their web site at <http://www.clsi.org>.

2.1.2.1 Consider risks associated with sample/sorter interaction: Selection of a nozzle with the appropriate size for the cells to be sorted is an essential factor for any successful sort experiment. It is recommended that the nozzle orifice be at least four times bigger than the cell diameter [14]. Sorting with a suitable nozzle is also a critical safety element because a mismatch between cell and nozzle size creates a potential for the partial or complete clogging of a sort nozzle. If during the partial clog a misdirected sort stream hits a hard surface, aerosol production in the sort chamber can become intense, generating an increased chance of exposing personnel to uncontained aerosols. If the nozzle opening is obstructed in its entirety, the sort stops. In both cases, the manipulations required to return to sorting in normal operational mode enhance the risk of operator exposure to pathogens contained in the sort sample due to accidental splashes and/or escape of sort aerosols.

Another element in preventing clogs is a well prepared sample with high viability and little debris which contains few aggregated cells. Certain cell types such as lymphocytes have a low tendency to aggregate, while monocytes, dissociated tissues, and cell suspensions obtained from adherent cells grown in plates are more problematic. Passing these samples through narrow gauge syringes and vortexing before sorting can help; however, mixing should be gentle and not done excessively as cells may break apart. Debris present in the sample or generated during these manipulations can heighten the tendency for aggregation as debris attached to cells leads to cell adhesion and can also produce spraying of the sort streams with an increase in aerosol production. Highly concentrated cell suspensions are more prone to clumping; therefore, cells should be diluted to the lowest possible density for the sort speed used. Frozen cell samples that are thawed for sorting frequently contain dead cells which promote aggregate formation leading to nozzle clogging problems. In these situations, adding 20 g/ml of DNase for 10 minutes at 37°C can help. Furthermore, spinning samples at 300 g for 5 to 10 minutes is sufficient to pellet cells. Higher centrifugation speeds can damage cells and compact them so densely that they are difficult to re-suspend. Sort samples are often chilled to preserve cellular structures and prevent capping of antibodies bound to cell surface receptors. However, the cold can aggravate clumping, thus, sorting at an intermediate temperature such as 15°C may be preferable over sorting at 4°C.

Options to remove cell aggregates include filtration through individually-cut nylon mesh filter sheets, e.g., available from Industrial Netting Inc. (Minneapolis, MN), or by using filter units such as 5ml tubes with 35 micron cell strainer caps (Becton Dickinson, Falcon), or individual 100, 70, and 40 micron cell strainers (Becton Dickinson, Falcon) or filter units with mesh diameters ranging from 10-150 micron from Partec (North America, Inc.) (Swedesboro, NJ). Filtering immediately before sorting gives cells less time to re-associate. If feasible, an in-line filter, e.g., from BD Biosciences (San Jose, CA), or Cytex Development Inc. (Fremont, CA), that is put on the up-take port can prevent cell aggregates from reaching the sort nozzle.

2.1.2.2 Assess instrumentation risks

2.1.2.2.1 Droplet and aerosol production during sorting and their hazard potential: Jet-in-air technology used for flow sorting involves a liquid stream carrying the cells to be selected

through a nozzle vibrating at high frequency. At a given distance from the nozzle orifice the stream is broken into droplets that pass by high voltage plates. These plates electrostatically charge the droplets containing the desired cells pre-selected by the operator and deflect them into the designated receptacles. The size of the sort droplets depends on the instrument operating pressure, the size of the nozzle orifice, and the vibration frequency [15]. Based on the instrument setting, sorters typically produce droplet ranges from 40-200 micron (Kevin Holmes, ISAC 2010 Biosafety Workshop, available on line at <http://www.isac-net.org>, Educational Resources, Tutorials from CYTO 2010, Special Workshop BioSafety) and small satellite droplets between 3-7 microns [7]. During sort failures, such as a partial nozzle block, the streams exiting the nozzle can strike a solid object leading to the production of secondary aerosols. These aerosols have various sizes down to the 5 to 3 micron range although their composition is variable as they can actually recombine to larger droplets. Large amounts of secondary aerosols are produced during high-speed sorting due to the high operating pressure [3]. The droplet size of an aerosol determines its movement and velocity in air as well as the speed of its settlement out of air due to gravitational forces [16]. Larger droplets settle quickly, while small aerosolized droplets can dry out and form droplet nuclei which can stay suspended in air virtually indefinitely [17], particularly if they are lifted by air currents. Aerosols are a serious laboratory hazard because they are hard to see. They not only expose the worker who is involved in a procedure, but also expose others in the vicinity, they can contaminate adjacent spaces, are pervasive, and they are the probable cause of many LAI. When safety professionals analyzed the causes for LAI, only a small proportion of all documented infections in the laboratory could be clearly associated with an accident such as a splash or needle stick. For the majority, the mode of transmission remained unclear, and many were thought to be caused by aerosol transmission [2].

The droplet size of an aerosol is directly related to its biohazard potential because it defines the location of the particle deposition during inhalation. When inhaled, larger $> 5 \mu\text{m}$ droplets remain in the upper respiratory tract while smaller ones penetrate into the lung of the exposed individual [18, 19]. When aerosols escape from the instrument into the environment, the infectious agents they may contain could be harmful, if inhaled [17, 20]. Thus, for safety reasons it is important to prevent aerosol escape through instrument design or attachment of optional safety devices and to verify the efficiency of aerosol containment using appropriate testing. Further critical aspects involve placing physical barriers between operator and hazard (personal protective equipment (PPE)) and mandatory operator training in the SOPs established in the laboratory (see below). Instruments, which sort cells by a fluidic switching mechanism, e.g., FACSCalibur™ with the sorting option (BD Biosciences), use an enclosed fluid system and do not produce aerosols, but cannot achieve the typical sort speeds required for most applications.

2.1.2.2.2 Consider instrument design features: From the early 1980's, when J.T. Merrill published his paper describing the generation of aerosols by flow sorters and a method to assess their production and escape during the application of various aerosol control measures [8], instrument manufacturers have modified safety aspects of cell sorter design. Since the publication of the ISAC guidelines in 1997, sorter manufacturers have made major strides in implementing features that place primary barriers between the operator and the potential hazard and remove aerosols from the sort chamber. Stream view and sort stream monitoring systems facilitate operator observation of sort stream stability without the need to come close to the area of the instrument that poses the greatest hazard. Furthermore, these systems allow the operator to monitor an increase in aerosol production due to a shift in stream positions and fanning. Current model sorters are typically equipped with completely enclosed sample ports and/or auxiliary vacuum pumps that remove aerosols from the sort area as optional attachments. Aerosol production is most intense during failure modes of

operation; thus, sorters that shut off the sample stream when the nozzle is partially clogged offer an additional safety margin.

With these advances in safety features, standard, free-standing sorters have become safer to use, but the most recent developments in sort systems involve enclosure of the instrument into a biological safety cabinet (BSC). Typically, aerosol-producing laboratory manipulations are to be performed in a BSC [2], a primary containment device, where directed airflow prevents potentially infectious aerosols from escaping into the room; but, previously, sorters were too large to fit. In the last decade, BSC enclosure has become an option with the introduction of benchtop sorters. For instance, the FACS Aria™ series of sorters (BD Biosciences), equipped with solid-state lasers, is small enough to fit into either a Walk-In Clean Air and Biocontainment Biological Safety Enclosure (BioPROtect^R II) or a BioPROtect^R IV, requiring less room, available from The Baker Co., (Sanford, ME). Beckman-Coulter (Fort Collins, CO) offers custom enclosure into a Biosafety Level II cabinet for their MoFlo^R Astrios™ and MoFlo^R XDP™ sorters. The Synergy^R benchtop sorter/analyzer and the Reflection^R parallel sorter from iCyt (Champaign, IL) can both be integrated into a Baker SterilGARD^R III Advance Biological safety cabinet. The JSAN™ desktop sorter (Bay bioscience, Kobe, Japan) with its small footprint can be readily enclosed in a BioPROtect^R Jr. BSC (The Baker Co.). BioSero, LLC (San Diego, CA) offers standard and custom biosafety enclosures that accommodate a variety of instruments and space limitations. It is critical for the effectiveness of the protection offered through a BSC that the operational needs of cell sorting are taken into account and that the BSC be maintained properly and certified at least annually according to the established NSF/ANSI Standard 49 to assure its integrity.

Other optional sorter enclosures provide barriers between instrument and operator, but do not have the design features of a BSC. The BD Influx™ sorter (BD Biosciences) can be equipped with an optional high-efficiency particulate air filtration (HEPA)-filtered enclosure. For older sorters such as the FACSVantage™ and FACSDiVa™ (BD Biosciences), a removable containment hood that is vented by a HEPA filter/fan unit and covers the sort area and the sample introduction port (Cytex Development Inc., Fremont, CA) is available to improve containment.

3. Arrive at the appropriate BSL for the planned sort experiments

Taking into account hazard risks for the putative samples to be subjected to sorting and the procedure hazards posed by the instrument, the appropriate BSL containment can then be selected with consists of combining facility requirements, containment devices, and practices.

3.1 Find initial BSL

BSL-1 containment is used for work with agents not known to cause disease in humans and no requirements specific to cell sorting are needed. Typical samples requiring BSL-1 containment include normal mouse and plant cells. BSL-2 practices, safety equipment and facilities are used for pathogens which are known to cause disease in humans, but can be easily contained and are not usually transmitted by aerosols. Frequently, sort laboratories will require this containment level as unfixed human samples, human cell lines, and genetically modified samples are typical samples to be separated by jet-in-air sorting. BSL-3 containment is applicable to biological agents causing serious and potentially lethal disease as a result of exposure by the inhalation route; thus, because of the aerosol production during sorting, it is advisable to consider alternate separation methods, e.g., magnetic bead separation, whenever possible. If a cell sorter is utilized, sorting must be performed in a BSL-3 facility following all associated practices. Examples of agents mandating BSL-3

containment include *Mycobacterium Tuberculosis* and *Monkey Pox*. Prions, the agents considered responsible for causing transmissible spongiform encephalopathies, derived from human tissues and cells are to be handled under BSL-2 containment while BSL-3 is required for manipulations involving bovine prions due to the high probability that these have been transmitted to humans. In addition, prions are characterized by their resistance to conventional inactivation and decontamination procedures [2] (see section 3.3.3), making them problematic agents to be run through a cell sorter. The ultimate level of containment, BSL-4, is reserved for work with exotic and highly dangerous organisms transmitted by aerosols for which no vaccination or therapies exist and which requires specialized high-containment facilities (Figure 1). Countries other than the US have developed their own containment level classifications, but they are based on the same principles as the ones applied in the US.

3.2 Consider placement of the flow sorter

After the initial assignment of a proper BSL, other elements of containment have to be taken into account. Appropriate sort facility design contributes to the protection of operators and provides a barrier to protect the environment from potentially released aerosols. Thus, a laboratory which has been classified as BSL-2 for other laboratory activities based on the samples it processes has to consider the enhanced hazard potential of a jet-in-air sorter whenever such an instrument is added. Consequently, a free-standing sorter used for sorting of pathogenic samples requiring BSL-2 containment should either be placed into a BSL-3 facility or into a separate, lockable BSL-2 laboratory, with negative air pressure equipped with easy to clean work surfaces and floors and a sink for hand washing [5, 7]. Room access must be limited and warning signs must be posted when experiments are in progress to prevent entry of persons not wearing the required personal protective equipment (PPE) (see below). Enclosure of a flow sorter into a BSC can abrogate the requirement to house the sorter in a separate room and to wear PPE, although PPE is strongly recommended during any manipulation on the instrument (Figure 2.).

3.3 Formulate laboratory practices

3.3.1 Select method for measuring the efficiency of aerosol containment—To prevent LAI, it is important to verify that the aerosol control measures on a free-standing or an enclosed sorter are effective in prevention of aerosol escape into the environment. The classic method for this assessment is based on the standard gravitational force method using lytic T4 bacteriophage and petri dishes with T4-susceptible *E. coli* lawns [4, 7, 8, 21]. This technique measures aerosols near the aerosol source and detects droplets that rapidly settle from air which generally constitute the majority of the aerosols produced during sorting. Sub-micrometer particles, i.e., droplet nuclei which may contain inorganic, organic material, or infectious agents from dehydrated small, < 5 µm droplets, can also be generated and may stay suspended in air for prolonged periods of time [17, 18]. These droplet nuclei can be captured with active air sampling methods as described by Andersen [16] where a sampling device is used to direct room air onto petri dishes containing T4-susceptible *E. coli* lawns [7]. Because it has been established that a single phage is sufficient to generate one plaque [8] the assay is considered sensitive, but the method is labor intensive, relies on the performance of biological materials, and results generally take overnight.

An easier, non-biological method that has become the most prevalent one for rapid visualization of aerosol production and escape in flow sorters uses highly fluorescent, melamine copolymer resin particles, trademarked GloGerm (Glo Germ Inc., Moab, UT) [22]. Peretto *et al.* [23] have increased the sensitivity and reproducibility of the original method by using a viable microbial particle sampler. The Glo Germ™ technique is suitable to be performed immediately before starting a sort. Glo Germ™ particles are easily detected

under a fluorescent microscope, but meticulous cleaning and handling of the air sampler and microscope slides are critical to avoid false positives, and diligent scanning of the entire slide is required to reliably detect escape of single particles.

Aerosol containment on any new instrument must be assessed before potentially pathogenic samples are sorted. If containment is incomplete, the sorter must be modified to achieve it. After the initial test, it is recommended, that for sorting of samples requiring BSL-2, re-assessment be performed monthly, and additionally after any sorter modification or any HEPA filter change in aerosol containment or management systems. For samples requiring BSL-3 or BSL-4 containment aerosol containment must be verified before each sort.

3.3.2 Select appropriate personal protective equipment (PPE)—Personal protection refers to the placement of physical, primary barriers between flow sorter operators and biohazard as a means to protect the operator from exposure in the event of a breakdown of mechanical barriers (engineering devices). While BSL-3 and BSL-4 practices require wearing of respiratory protective devices, BSL-2 does not. However, because of the potential release of aerosols during sorting, operators should use what has been termed “enhanced precautions”, raising the BSL-2 designation to BSL2+, and wear equipment that protects against aerosol exposure. Extent and types of PPE used differ between laboratories which routinely sort samples from individuals infected with HIV [4, 21, 23]. Nevertheless, at a minimum, operator's safety equipment must conform with BSL-3 recommendations [2] and consist of a disposable-wrap-around laboratory coat, gloves, safety glasses with side shields, and a face-piece respirator, e.g., N95, N99, N100 NIOSH-approved particulate respirators. A plastic shield may be worn over the respirator to provide an additional safety margin. Perfetto *et al.* [23] described the use of a complete DePuy Bio-Hazard Respiratory System (DePuy Chesapeake Surgical, Ltd., Sterling, VA) which consists of a body suit, a helmet, and its battery-powered respiratory system with electrostatic filter media, when performing biohazardous sorting. Any personal protective equipment must always be removed whenever the operator leaves the sort room or the adjacent anteroom.

Immunization against infectious agents should be offered to personnel, if available. Prophylactic HBV vaccination is highly recommended. Post-exposure prophylaxis should be available in any laboratory involved in sorting of HIV-positive specimens [24-26] and should always follow the latest recommendations from CDC, available online at <http://www.cdc.gov/mmwr>. Drawing a baseline serum sample from personnel before work is started, monitoring the health status of individuals, in particular those with compromised immunity, and periodic evaluation of serum samples may be appropriate for laboratories which routinely sort samples containing infectious agents.

3.3.3 Formulate instrument operation, troubleshooting, decontamination, and maintenance procedures—Instrument procedures for sorting will include start-up, alignment, calibration, sterilization, operation, and shutdown as described by the sorter manufacturer and established in the facility. In addition, it must detail the specific steps to be followed during sorting of potentially infectious specimens. Practices may involve turning on the auxiliary vacuum source at the proper setting to assure sufficient negative airflow. The setting needs to be high enough for the removal of aerosols from the sort chamber, yet low enough to not disrupt the sort streams. Depending on the instrument configuration, sort setup may include attaching the containment hood to the sorter, turning on the BSC, and/or performing aerosol containment testing either before the start of each sort [23] or at regular intervals as outlined in previous publications [7, 11] and stated above. Steps to be taken during an instrument malfunction such as a partial or complete nozzle blockage must be clearly defined. For example, the wait time required before opening the sort door after a clog has occurred will depend on the speed of aerosol clearance.

This time can be checked with canned smoke available for testing smoke detector function. Clearance time can also be measured by collecting Glo Germ™ particles with the sort chamber door open after the sort is stopped and the sample is no longer pressurized.

Rigorous cleaning after each sort, e.g. by running a bleach solution diluted to 10% followed by distilled water, clears the fluid lines and sort nozzles from residual cells and cellular debris. Utilization of a strong detergent, e.g., Contrad 70 (Decon Laboratories Inc., Bryn Mawr, PA) can provide effective removal of organic material improving the effectiveness of subsequent disinfection, an important measure to eliminate the potential for the spread of infectious agents. Appropriate disinfectants [13] for decontamination of equipment or work surfaces exposed to blood or other potentially infectious materials include diluted bleach, Environmental Protection Agency (EPA)-registered tuberculocides, EPA-registered sterilants or products registered to be effective against HIV or HBV as listed online at <http://www.epa.gov/oppad001/chemregindex.html>. Diluted bleach, 70% ethanol, and 3% hydrogen peroxide solutions are the most frequently used common disinfectants [27] for sorter decontamination [28]. Before creating a decontamination protocol for a specific sorter, it is important to check with its manufacturer to clarify that all instrument components exposed to the disinfectant can tolerate it.

Maintaining the flow sorter in excellent working condition is critical for processing hazardous samples. Nozzle tips must be cleaned frequently by sonication and should be replaced whenever they show wear and tear to minimize instrument trouble and lessen the chance for nozzle blockages, which are a major source for an increased risk of aerosol exposure. Another important measure is following a rigorous, regular preventive maintenance schedule as serious hazards can arise from sudden instrument malfunctions during a sort such as a broken fluid line, a stuck valve, a blocked waste line, or a damaged or clogged HEPA filter for air evacuation.

3.3.4 Determine training requirements—The time needed for novice sort operators to become proficient in sort operations varies, but on average ranges between six months to one year. Ideally, operators should also be experienced in handling potentially infectious samples [29]. Mandatory operator training in all the relevant safety aspects of sorting, including the procedures involved in testing the efficiency of aerosol containment on the cell sorter is essential for reducing hazard risks to sort personnel and others involved in these experiments. Only operators with considerable experience should start performing separations of samples known to carry human pathogens [6]. Training records need to be kept on file, and re-training must be performed whenever the sort procedure changes because of a new sorter or of sorting of samples with altered biohazard potential.

3.3.5 Write SOP—A SOP is a set of written instructions that document a routine or repetitive activity to be followed. The development and use of SOPs are an integral part of successful laboratory experiments as they provide personnel with information on how to perform a task properly. SOPs facilitate consistency among different individuals and also play an important role in laboratory safety because they detail the steps to be taken to prevent LAI. Sorting potentially infectious cells on a jet-in-air sorter is a hazardous procedure; thus, a SOP developed for it must contain not only step-by-step technical instructions on how to operate the flow sorter, but it must also detail how and when to use the safety elements to protect the operator and the environment based on the risk assessment process as outlined in this review. Review of the risk assessment with biosafety professionals will provide input from experts to assure that all relevant safety aspects have been taken into account.

There is no “correct” or “incorrect” format for writing a SOP; however, it should be concise, complete, easy-to-read, and state explicitly what is required. SOPs are a valuable training tool, and it is the responsibility of laboratory supervisors and directors to ensure that new operators are proficient in the procedure as described and to enforce its use because a SOP is of limited value if it is not followed. All SOPs must be reviewed and signed by the sort operator(s) and be easily accessible in the laboratory safety manual binder. SOPs need to be up-dated to reflect any changes in the hazard potential of the samples to be sorted, in the instrument features or attachments, or the PPE to be worn by the operators.

4. Conclusions

Cell sorting on jet-in-air sorters is an essential technique for isolation of cellular subpopulations, but it poses inherent risks of aerosol exposure. Thus, performing an accurate risk assessment of the hazards associated with sorting in a specific laboratory is an important aspect of sort laboratory management. With the increased variability in samples subjected to sorting and in instrument configurations and attachment options creating a SOP that contains the proper safety elements can be difficult. This review provides guidance in this process. Following the steps outlined here and using the resources provided, laboratory managers should be able to develop a SOP that includes safeguards to be applied during sorting of unfixed cells that are appropriate for the work conducted and the specific sort system set-up.

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Figure 1.
Decision chart to determine the appropriate containment level for planned sort experiments.

Figure 2.
Decision chart to determine the appropriate containment level for sort experiments requiring BSL2.