Methods 57 (2012) 392–397

Contents lists available at SciVerse ScienceDirect

Methods

journal homepage: www.elsevier.com/locate/ymeth

How to develop a Standard Operating Procedure for sorting unfixed cells

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ARTICLE INFO

Article history:
Available online 21 February 2012

Keywords:
Cell sorting
Biosafety
Occupational health
Aerosol testing

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.

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 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 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.

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doi:10.1016/j.ymeth.2012.02.002

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, third 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), fifth 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