Procedures for Flow Cytometry-Based Sorting of Unfixed Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Infected Cells and Other Infectious Agents

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Abstract
In response to the recent COVID-19 pandemic, many laboratories are involved in research supporting SARS-CoV-2 vaccine development and clinical trials. Flow cytometry laboratories will be responsible for a large part of this effort by sorting unfixed antigen-specific lymphocytes. Therefore, it is critical and timely that we have an understanding of risk assessment and established procedures of infectious cell sorting. Here we present procedures covering the biosafety aspects of sorting unfixed SARS-CoV-2-infected cells and other infectious agents of similar risk level. These procedures follow the ISAC Biosafety Committee guidelines and were recently approved by the National Institutes of Health Institutional Biosafety Committee for sorting SARS-CoV-2-infected cells. © 2020 International Society for Advancement of Cytometry

Key terms
biosafety and cell sorting; infectious cell sorting; ISAC Biosafety Committee; SARS-CoV-2 cell sorting procedure; COVID-19; coronavirus

Flow cytometry is a critical tool in clinical laboratories for diagnosis and monitoring of patients under various disease states, identification and characterization of infectious disease agents, and vaccine development (1,2). Recently, Cossarizza et al. (1) discussed specific approaches using flow cytometry for the study of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the novel human coronavirus responsible for the current coronavirus disease 2019 (COVID-19) pandemic. Flow cytometry facilities and laboratories, however, need to follow specific practices and procedures to prevent exposure of laboratory personnel to infectious agents. In a second recent paper, Cossarizza et al. (3) describe protocols and procedures for the safe handling, preparation, and flow cytometry analysis of fixed samples derived from blood (e.g. peripheral blood mononuclear cells) collected from COVID-19 patients. Here we describe an infectious cell sorting procedure for use with unfixed samples that follows the International Society for Advancement of Cytometry (ISAC) Biosafety Committee Guidelines and was recently approved by the National Institutes of Health (NIH) Institutional Biosafety Committee (IBC) specifically for sorting SARS-CoV-2-infected cells by flow cytometry. In this specific case, convalescent blood samples from COVID-19 patients, who were determined to be polymerase chain reaction (PCR) negative, was approved for these procedures. This protocol is also appropriate for blood samples containing other respiratory disease-causing agents such as pulmonary Mycobacterium tuberculosis.
Sorting flow cytometers have been shown to produce high concentrations of aerosols during partial nozzle obstructions or other malfunctions that disrupt the defined droplet pattern and stream trajectory and cause the stream to impact a hard surface (4,5). The size and concentration of aerosol particles depend on the sheath pressure, with a greater potential for release of high concentrations (up to 25,000 particles/cm³) of small (1–3 μm) aerosol particles at high (≥70 psi) pressures (4). Aerosol particles in this size range are problematic because they are more likely to deposit in lung alveoli, are associated with increased infectivity of some organisms, and can remain airborne almost indefinitely (6–11). Sorting flow cytometers generally pose more of a risk to operators as the stream is not fully enclosed, and the instrument must be opened to retrieve the sorted samples. Although no known laboratory-acquired infections (LAI s) have been linked to flow cytometry, the cause of many LAIs is unknown and presumed to be transmitted through aerosols (5). A recent study of SARS-CoV-2 suggests that measures to contain viral spread should focus on droplet (i.e., airborne) rather than fomite-based transmission (12). Another recent report suggests treating this virus as airborne, even though current evidence remains inconclusive and the infectious dose is unknown (13). Thus reducing or eliminating aerosolization of SARS-CoV-2 samples and providing sufficient containment for procedures at risk of generating aerosols is critical for preventing LAIs of this and other similar agents.

To reduce the biohazard exposure of instrument operators using high-speed sorting flow cytometers, the ISAC Biosafety Committee created safety guidelines for the sorting of unfixed samples (5,14,15). Recommendations for managing aerosol generation by sorting flow cytometers include operating instruments at lower sheath pressures (e.g., <70 psi); directly evacuating the sort chamber through a high-efficiency particulate air (HEPA) or ultraflow particulate air (ULPA) filter using an aerosol management system (AMS), aerosol management option (AMO), or other aerosol evacuation system; enclosing the flow cytometer within a primary containment device such as a Class II biosafety cabinet (BSC); and locating the flow cytometer in a dedicated room with restricted access and negative airflow. Specific engineering controls and procedures required for operator safety for particular institutions, workflows, or procedures should be determined using a risk assessment in collaboration with the IBC, Health and Safety Department, or other local biosafety office.

Several organizations, including the World Health Organization (WHO), the US Centers for Disease Control and Prevention (CDC), and the American Biological Safety Association (ABSA), have recently released general laboratory guidelines for working with SARS-CoV-2 samples which include some references to flow cytometry and cell sorting (16–18). These guidelines include:

1. Conduct an institutional or local risk assessment to ensure all procedures and analyses can be performed safely with appropriate risk control measures in place.
2. Follow good microbiological practices and procedures.
3. Perform nonpropagative laboratory work, including flow cytometry-based cell sorting of fixed or inactivated samples, at the biosafety level (BSL)-2 level. Procedures with a high likelihood of generating droplets or aerosols should be performed within a BSC or other primary containment device or should include additional barrier precautions for personnel such as surgical masks or face shields. Restrict access to the laboratory when work is being conducted.
4. Based on the risk assessment, perform flow cytometry-based cell sorting of unfixed samples at the BSL-2 level with enhanced precautions (also called BSL-2/3 or BSL-2 with BSL-3). All samples should be opened inside a BSC or other primary containment device. Additional personal protective equipment (PPE) should be used including respiratory protection (e.g. N95, N100, or powered air purifying respirator [PAPR]), double gloves, and eye protection.
5. Based on the risk assessment, perform high speed cell sorting, propagative work, and work with high concentrations of live virus at the BSL-3 level with inward airflow and a HEPA-filtered facility exhaust system. All sample manipulation should be done within a BSC or other primary containment device, and respiratory protection (N95 or greater) and face/eye protection is required.
6. Surface disinfect using appropriate disinfectants and contact times at every step. Appropriate methods and practices for management of all laboratory waste should be available in the facility.
7. Require training of laboratory personnel in handling pathogenic agents and for each specific procedure to be performed.

The above guidelines from WHO, CDC, and ABSA provide a general guide for handling SARS-CoV-2 samples in the laboratory. Here we expand on these guidelines and provide detailed procedures for all biosafety aspects of sorting unfixed SARS-CoV-2-infected cells and other respiratory disease agents. These procedures cover microbiological practices (laboratory setup, sample handling, decontamination, training of personnel), special practices for sorting flow cytometry (aerosol management and PPE), and infectious cell sorting. It is important to note that the protections and guidelines required to properly and safely carry out these procedures highlight the importance of the risk assessment and the involvement of each local biosafety office and/or IBC. At the NIH Vaccine Research Center (VRC), COVID-19 patient samples (PCR negative) can safely be sorted in a BSL-2 laboratory with certain BSL-3 practices in place during the sort (sometimes referred to as BSL-2/3). Three factors are critical among these practices: (1) the use of an AMS/AMO, (2) respiratory protection for the operator at times when the cell sorting chamber must be opened, and (3) a standard operating procedure (SOP) detailing the procedure in the event of a nozzle obstruction. See Holmes et al. (5) for guidance and details on performing a risk assessment, assigning a BSL, developing a SOP, and general recommendations for sample handling and processing for flow cytometry at each BSL.
MICROBIOLOGICAL PRACTICES FOR SORTING FLOW CYTOMETRY

General good microbiological practices required for the NIH VRC flow cytometry laboratory are outlined below. We recommend all work with SARS-CoV-2 and similar respiratory disease agents (BSL-2/3 and BSL-3) be performed in laboratories with negative airflow and filtered exhaust. The US Environmental Protection Agency List N includes all disinfectants approved for use against SARS-CoV-2 (https://www.epa.gov/pesticide-registration/list-n-disinfectants-use-against-sars-cov-2). Disinfectants that are on EPA lists traditionally achieve a minimum of a 3 log reduction of the targeted pathogen. For additional guidance on disinfectants, contact time, sharps, and hazardous waste management see Holmes et al. (5), Kampf et al. (19), and Biosafety in Microbiological and Biological Laboratories (BMBL) (20) and work with your local IBC or Health and Safety Department.

1. Good microbiological practices and procedures (16,20) should be followed including: never mouth pipette; never eat, drink, apply cosmetics, or handle contact lenses in the laboratory; appropriately handle and dispose of sharps; protect street clothing by wearing appropriate PPE or using dedicated laboratory clothes and shoes; perform all procedures in such a way as to minimize the creation of aerosols; clean work surfaces with an appropriate disinfectant after working with infectious materials; decontaminate or sterilize laboratory waste before disposal.

2. Verify all laboratory rooms are under negative airflow using an appropriate air flow indicator such as Ball-in-the-wall® (Airflow Direction Inc., Newbury, MA; Supporting Information Fig. S1).

3. Gloves should be worn at all times within the flow cytometry facility. When leaving a contaminated area, gloves can be wiped with a disinfectant (at least 30 s of contact time) or removed and replaced with new gloves.

4. Conduct all open manipulations involving infectious materials in BSCs or other primary containment devices.

5. Clean and decontaminate BSCs and other contaminated equipment and items used in the protocol with an appropriate disinfectant after each use, including removal and proper disposal of consumables and biohazardous waste.

6. Decontaminate all work surfaces after use, at least once per day, and after any spill of contaminated material. Work surfaces can also be decontaminated prior to starting work.

7. Hypochlorite of 10% (bleach) can be used as a primary disinfectant. Note that 10% bleach (10 ml hypochlorite in 90 ml water) stored in an opaque plastic container at room temperature will lose effectiveness upon exposure to light and air. It is recommended that 10% bleach solution be made fresh daily. Alternatively, hydrogen peroxide can be used as a primary disinfectant. Perxigard (Virox Technologies, Inc., Oakville, ON, Canada), which is an accelerated hydrogen peroxide formula, was found to kill SARS-CoV-2 within minutes after contact (21,22). 10% betadyne (10 ml concentrated betadyne in 90 ml water) and 70% ethanol (70 ml ethanol in 30 ml water) can be used as secondary disinfectants.

8. It is recommended that access to the flow cytometry laboratory is restricted when infectious materials are in use. Appropriate signage should be posted when access is restricted indicating any special precautions required before entering the room. Signage can also include the agent(s) in use and the name(s) and contact information of responsible individuals. See Supporting Information Figure S2 for example signage indicating whether a sort is in progress or “active.”

9. Biological spills can be decontaminated with 10% hypochlorite (bleach) or other appropriate disinfectant. If a spill occurs within a primary containment device, apply absorbent toweling to the area, and soak with an appropriate disinfectant. Allow 30 min of contact time before cleaning with additional disinfectant applications. Dispose of all contaminated materials as biohazardous waste. Spills of biohazardous materials outside of primary containment generally necessitate evacuation of the work area and/or laboratory until aerosols are cleared from the room.

10. Biological spills and accidents that result in overt or potential exposure to infectious materials must be reported. Appropriate medical evaluation, surveillance, and treatment must be provided and written records maintained.

11. Proper procedures must be followed for transport of infectious materials within and outside of the laboratory. These procedures generally include placing sealed samples within a labeled secondary leak-proof container, and disinfecting the outer container. Samples that have been inactivated using an approved method (e.g., fixation with formaldehyde solution) can be handled as non-infectious. Note that 0.7% and 1% formaldehyde were shown to effectively inactivate SARS CoV and render it non-infectious after 30 s of exposure (23).

12. Contaminated equipment must be decontaminated using a primary and secondary disinfectant before removal from the facility for repair or maintenance or packaging for transport, in accordance with the applicable local, state, or federal regulations. Service engineers entering the facility must be instructed on the appropriate protective clothing required by the facility.

13. All personnel should receive appropriate training on the potential hazards associated with various procedures, the necessary precautions to prevent exposures, and the exposure evaluation procedures. This training should be updated annually or on a regular basis, and additional training should be provided as necessary for procedural changes.

14. It is recommended that laboratory personnel receive appropriate immunizations or tests for the agents handled or potentially present in the laboratory, if available.
The AMS/AMO or other aerosol evacuation system must be operated with the BSC (for integrated systems) or to an external-filtered vacuum source such as a Buffalo Filter® (ConMed, Utica, NY). Negative airflow is created inside the sort chamber, and the air is evacuated through the hose(s) into HEPA or ULPA filters within the BSC or the external vacuum source. While sorting viable infectious material (infected cells) under high pressure, the following guidelines must be followed for proper aerosol containment. All operators should be trained and certified by the flow cytometry facility prior to performing any cell sorting procedures.

1. The BSC (for integrated AMS/AMO) or the HEPA filter within the AMS/AMO (for external units) should be certified after installation and at least annually thereafter. Factory HEPA filter testing may reflect the integrity of the filter paper before the housing was constructed and may not account for damage during packaging and shipment.

2. The AMS/AMO or other aerosol evacuation system must be on and functioning according to the manufacturer guidelines. Supporting Information Figure S3 shows the aerosol flow and the locations of the vacuum gauge and monitor. For the BD FACS Aria flow cytometer (BD Biosciences, San Jose, CA), the vacuum monitor should be set to 20%, and the vacuum gauge must read between 1.0 and 2.5 in. of water. The HEPA filter unit and tubing must be replaced after 6 months or if increased percentage is needed to achieve the required vacuum pressure.

3. The waste tank must contain enough hypochlorite to provide a final concentration of 10% when filled (e.g., 1 l of household bleach to a final 10 l of waste collected). If full, empty the waste tank before starting cell-sorting procedures. Allow at least 30 min of contact time before disposal.

4. The sort chamber camera system must be functioning according to the manufacturers guidelines. This camera system is used to monitor the sort stream and alerts the operator to potential increased aerosols. In this situation, the operator can correct the sort stream and reduce aerosol contamination. Some instruments, including the BD FACSAria, are equipped with a “Sweet spot” monitor, which should be used during all sorting operations. This device monitors drifts in the sort stream and corrects its position by automatically adjusting the wave amplitude. If a stream blockage is detected, the Sweet spot monitor will automatically shut down the stream and close the sort drawer.

**Special Practices for Sorting Flow Cytometry**

### Aerosol Management System

The AMS/AMO consists of a hose or series of hoses attached to openings in the sort chamber or sort collection area that are connected to the blowers of a BSC (for integrated systems) or to an external-filtered vacuum source such as a Buffalo Filter® (ConMed, Utica, NY). Negative airflow is created inside the sort chamber, and the air is evacuated through the hose(s) into HEPA or ULPA filters within the BSC or the external vacuum source. While sorting viable infectious material (infected cells) under high pressure, the following guidelines must be followed for proper aerosol containment. All operators should be trained and certified by the flow cytometry facility prior to performing any cell sorting procedures.

**Table 1.** PPE and additional protections and procedures required at each biosafety level at the NIH VRC; see infectious cell sorting procedure for more details about BSL-2/3

| TYPE OF PROTECTION                        | BIOSAFETY LEVEL BASED ON RISK ASSESSMENT |
|-------------------------------------------|------------------------------------------|
|                                           | BSL-2   | BSL-2 PLUS | BSL-2/3         |
| Aerosol containment testing               | Monthly | Weekly     | Each Sort       |
| Tyvek suit (full body)                    | Not required | Optional | Required        |
| Lab gown (closed front)                   | Required | Required   | (Tyvek only)    |
| Latex gloves                              | Single  | Single     | Double          |
| Respiratory protection (e.g., PAPR)       | Optional | Required   | Required        |

PPE and additional procedures recommended for flow cytometry laboratories at the NIH VRC are based on the BSL as determined through a risk assessment (Table 1).

**Measurement and Tolerances of Aerosol Containment**

The aerosol evacuation system must function properly to contain aerosols released during a partial nozzle obstruction or other instrument failure. Aerosols generated during a simulated partial nozzle obstruction were successfully contained when a BSC with an AMS was used, and the AMS was considered a critical component of these engineering controls (4,24). The ISAC guidelines recommend verifying containment of aerosols by the AMS/AMO on a regular basis (as determined by a risk assessment and assignment of BSL) and before working with potentially infectious or hazardous samples (5). Proper function of the AMS/AMO is generally not verified during annual BSC certification, and instruments that were not installed within a BSC can use an external aerosol evacuation system. Therefore, aerosol containment must be verified using an independent assay. Perfetto et al. (25) describes the method currently recommended by the ISAC Biosafety Committee to verify containment of aerosols by sorting flow cytometers. In this method, a “worst case scenario” is simulated by creating a large aerosol release inside the sort chamber, the aerosol particles are labeled with small (1 μm) fluorescent beads, and air samples are collected using a disposable air sampler designed to efficiently collect particles in the 1–3 μm size range in an attempt to detect the labeled aerosol particles. Aerosol containment is typically verified bi-monthly unless otherwise indicated by the risk assessment evaluation. Containment must also be verified after initial installation, removal of the instrument from the BSC, and any maintenance or repairs performed on the AMS/AMO or any of its components. Steps to verify aerosol containment of the BD FACS Aria are outlined below.
1. Aerosol containment testing should be performed at 70 psi or at the maximum sheath pressure used for all workflows. Analysis and sorting utilizing lower sheath pressures than that used during the containment test can be performed without additional containment testing. However, if higher sheath pressures will be used, the aerosol containment test must be repeated to verify that the additional aerosols released at these sheath pressures are contained.

2. Turn on the AMS (20%), and check for proper vacuum function (1.0–2.5 in. of water).

3. Prepare a sample of 1 μm Dragon Green beads (Bangs Laboratories, Fishers, IN; Supporting Information Fig. S4) such that an event rate of 40,000–50,000 events/s can be achieved. Set the trigger detector to green fluorescence (i.e., fluorescein isothiocyanate (FITC) channel). For the BD FACSAria set to 70 psi sheath pressure, add 20 μl of concentrated bead solution to 2 ml of buffer (phosphate buffered saline with 0.2% sodium azide and 0.5% Tween 20). Run the prepared bead sample at a flow rate of 5 or 6 to achieve the desired event rate. For other instruments and/or sheath pressures, adjust the sample flow rate and/or the bead concentration as needed to achieve the desired event rate. Supporting Information Figure S4 (top panels) shows an example of a scatter plot and histogram of the Dragon Green bead sample.

4. Create an aerosol release to simulate an instrument failure such as a partial nozzle obstruction. For the BD FACSAria, this is accomplished by covering the waste catcher with a small piece of rubber tubing forcing the stream to glance off the waste catcher shield (Supporting Information Fig. S5).

5. Attach a cyclex-d cassette (Environmental Monitoring Systems, Charleston, SC) to a vacuum pump, and verify the vacuum pump is set to 20 l/min. Place the cassette toward the front of the sort chamber approximately 5 cm (2–3 in.) from the sort block door. For the BD FACSAria, close the sort block door but do not install tube holders (Supporting Information Fig. S6). The main sort chamber should also be closed.

6. Click on sort drawer to retract, which will begin creating aerosols as the stream hits the rubber tubing covering the waste catcher (Supporting Information Fig. S5). Note: It is recommended that the operator wear respiratory protection (e.g., N95, N100, or PAPR) while aerosols are being generated.

7. Turn on the vacuum pump and collect an air sample for 10 min.

8. Turn off the vacuum pump and attach a fresh cyclex-d cassette. Turn the pump back on, and collect a positive control sample by sampling for 2 min with the AMS turned off. Both the sort block door and the sort chamber door should remain closed.

9. After all air samples have been collected, turn the AMS on and return the waste catcher to its normal position. Remove the rubber shield from the waste catcher.

10. Remove the cover slips from the cyclex-d cassettes (Supporting Information Fig. S7). Place each cover slip on a gridded microscope slide adhesive side down ensuring the beads and grid lines are in the same focal plane. In an extreme example of aerosol escape, a faint circle of dried PBS can be seen in the center of the slide (Supporting Information Fig. S7).

11. Examine the entire adhesive region for the presence of Dragon Green beads using a fluorescent microscope with a FITC filter (520–640 nm, see Supporting Information Figs. S4 and S7). Scan the slides using a 10x or 20x objective, and count all beads present.

12. The acceptance tolerances are zero Dragon Green beads detected after 10 min of active air sampling in front of the sort chamber door with no tube holder in place and the AMS turned on. The positive control slide must contain greater than 100 beads after 2 min of active air sampling with the AMS turned off and no tube holder in place.

   i. If beads are observed on the test slide, aerosol containment has NOT been verified. The operator should check all vacuum tubing and that the correct settings have been used on the instrument, and repeat the test.

   ii. If the test fails twice, infectious cell sorting must be aborted until aerosol containment can be verified. Contact the manufacturer, if needed, to perform corrective maintenance or repairs.

13. See Supporting Information Appendix S1 for an example checklist form for aerosol containment testing.

**Infectious Cell Sorting Procedure**

All laboratory practices using nonamplified specimens containing SARS-CoV-2, *M. tuberculosis*, and other agents within this risk group must be performed using the following guidelines in accordance with the CDC recommendations as outlined in BMBL (20). See Supporting Information Appendix S2 (BD FACSAria II), Supporting Information Appendix S3 (BD Influx, BD Biosciences, San Jose, CA), and/or the manufacturer’s instructions to properly start-up the flow cytometer.

1. Procedures involving samples containing live respiratory disease agents must be done at BSL-2/3 or higher.

   i. Respiratory protection (e.g., N95, N100, or PAPR) must be worn for all procedures involving live respiratory disease agents. See Supporting Information Appendix S4 for a description of the AirMAX HEPA filter PAPR (Bio-Medical Devices Intl., Irvine, CA) and an example checklist for starting work in the BSL-2/3 laboratory including inspection of the PAPR and laminar hood, verification of room pressure, and verification of an aerosol containment test.

   ii. Other recommended PPE includes double gloves, Tyvek suit (see image in Supporting Information Appendix S4) and/or dedicated laboratory clothing, shoe covers and/or dedicated laboratory shoes (not required if wearing Tyvek suit with integrated shoe covers), solid front disposable lab coat (not required if wearing Tyvek suit), disposable sleeves,
and eye protection such as safety glasses or face shields (not required if wearing a PAPR).

iii. Infectious disease agents must be handled inside a certified BSC or other primary containment device.

iv. It is recommended that the entire flow cytometer be placed in a BSC or other primary containment device with an integrated aerosol evacuation system.

v. Aerosol containment must be verified before every sort.

vi. Procedures with the potential to generate aerosols should be performed inside a BSC. See Supporting Information Appendix S5 for centrifugation procedures.

vii. When exiting the laboratory, all disposable PPE should be disposed as hazardous waste. All reusable PPE (e.g., safety glasses, PAPR hood) should be disinfected with 10% bleach or other approved disinfectant.

2. The flow cytometer must pass all tolerances of aerosol containment as described above. If these tolerances are not met, infectious cell sorting is not permitted.

3. The operator must wear PPE as outlined above. If the operator is not protected as described in this section, infectious cell sorting is not permitted.

4. A warning sign must be posted outside of the flow cytometer laboratory (see Supporting Information Fig. S2), and the room is limited to two individuals during the sort procedure.

5. Turn on and verify that the AMS is working correctly. For the BD FACS Aria, this device must have a vacuum pressure of 1.0–2.5 in. of water. If this tolerance is not met, infectious cell sorting is not permitted.

6. Close all barriers around the sort chamber. If this is not done, infectious cell sorting is not permitted.

7. All samples must be filtered through an appropriate sized filter (depending on the cell size and nozzle size) prior to sorting, and filtering must be done inside a BSC or primary containment device. For lymphocytes, a 40-μm mesh is generally recommended. This reduces the potential for clogging and decreases the risk of creating aerosols.

8. Monitor the sort performance using an internal camera such as the Accudrop camera. If during the sort the stream is deflected (due in part to a clogged flow cell tip), the BD FACS Aria is designed to stop automatically and block the sort tubes. The sort will not restart until the operator has cleared the clog. Use the following procedure to remove a clog from the cytometer.

i. Remove the sample from the sample chamber.

ii. Turn the stream off (unless turned off by the instrument in the automated shut-down mode) and then on again to see if drop delay and stream returns to normal pattern.

iii. If the obstruction is not removed by turning the stream off and on, wait 2 min and remove the sample collection tubes. Close the sample chamber, retract the sort drawer, and wait for an additional 2 min. This will allow any aerosols inside the sort block to be evacuated before the sort block door is opened. Remove the clogged nozzle and either replace with a new nozzle or decontaminate the nozzle in 10% bleach for 30 min before placing in a sonicator (2–5 min).

vi. Before reinserting the nozzle, check for a clear nozzle hole using a microscope. Dry the nozzle slot, stop-cock, and deflection plates using a Kimwipe or other lint-free toweling if necessary.

v. Sorting can be resumed after the clog is cleared from the original nozzle or it is replaced with a new nozzle. Repeat the procedure to verify droplet location and proper drop delay.

9. Do not remove any samples from the sort chamber until sample acquisition has been stopped, and wait 2 min before opening the sort chamber door. This procedure will clear aerosols from the sort chamber. After this time, sorted samples can safely be removed from the sort chamber.

10. When the sort is finished, proceed with the flow cell disinfection procedure and shutdown as listed in Supporting Information Appendix S2, Supporting Information Appendix S3, or following the manufacturer’s guidelines. Follow site-specific biosafety procedures for proper doffing of PPE.

**Summary**

Laboratories involved with vaccine development will be required to sort unfixed SARS-CoV-2-infected cells. Although sorting flow cytometers produce little to no aerosols under normal operation, the potential exists for release of high concentrations of aerosols if instrument failures occur (4). Containment of these aerosols is essential for operator safety when working with potentially infectious or otherwise hazardous samples, especially with sorting flow cytometers where the fluidics path is not entirely enclosed and which must be opened to retrieve sorted samples. Aerosol containment is accomplished through the use of primary containment devices, such as BSCs, and direct evacuation of the sort chamber or sort collection area using an AMS/AMO. Finally, when working with samples known to contain an infectious agent at a high concentration (e.g. bronchial lavages), a risk assessment must be performed to determine if a procedure should be done within a BSL-3 laboratory, or at a minimum with the sorter operational and certified within its own BSC or other primary containment device within a BSL-2 laboratory. This decision to increase the safety parameters (and likely the cost) is made to further lower the risk of potential exposure to personnel during the sort. Recommendations under the BSL-2/3 level as defined in the recent SARS-CoV-2 procedures are summarized below:

1. Review a risk assessment plan with your biosafety representative, such as the Institutional Biosafety Officer and/or the IBC.
2. Test aerosol containment prior to cell sorting using the cyclex-d procedure to validate instrument containment of aerosols while sorting.

3. Perform cell sorting with an instrument equipped with an operational and HEPA-filtered AMS/AMO.

4. Required use of PPE: Tyvek full body suit, gloves and shroud with HEPA-filtered PAPR to be used at particular times during the sorting procedure.

5. Measurement check for negative room air flow.

6. Clean surfaces before and after sort with 70% ethanol, 10% hypochlorite, or other approved disinfectant.

7. Maintain records of containment measurement and a safety checklist.

Implementation of these biosafety practices during the handling and sorting of risk agents such as SARS-CoV-2 will ensure that laboratories maintain a high level of public safety during vaccine development and deployment.

LITERATURE CITED

1. Cossarizza A, De Biasi S, Guaraldi G, Girardis M, Mussini C. For the Modena Covid-19 working group. SARS-CoV-2, the virus that causes COVID-19. Cytometry and the new challenge for global health. Cytometry A 2020;97A:340–343.

2. Alvarez-Barrientos A, Arroyo J, Cantón R, Nombela C, Sánchez-Pérez M. Application of flow cytometry to clinical microbiology. Clin Microbiol Rev 2000;13:167–195.

3. Cossarizza A, Gibellini L, De Biasi S, Lo Tartaro D, Mattioli M, Paolini A, Fidanza L, Bellinazzi C, Borella R, Castaniere I, et al. Handling and processing of blood specimens from patients with COVID-19 for safe studies on cell phenotype and cytokine storm. Cytometry A 2020. https://doi.org/10.1002/cyto.a.24009.

4. Holmes KL. Characterization of aerosols produced by cell sorters and evaluation of containment. Cytometry A 2011;79A:1000–1008.

5. Holmes KL, Fontes B, Hogarth P, Konz R, Monard S, Pletcher CH Jr, Wadley RB. Schmid I, Lambert C, Ambrozak D, Marti GE, Moos DM, Perfetto SP. International Society for Analytical Cytology biosafety standard for sorting of unixed cells. Cytometry A 2007;71A:414–437.

6. World Health Organization. Coronavirus Disease (COVID-19) Technical Guidance: Laboratory Testing for 2019-nCoV in Humans. Geneva, Switzerland: World Health Organization; 2020. https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/laboratory-guidance.

7. Centers for Disease Control and Prevention. Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19). Atlanta, GA: Centers for Disease Control and Prevention; 2020. https://www.cdc.gov/coronavirus/2019-ncov/lab/lab-biosafety-guidelines.html.

8. ABSA International Emerging Infectious Diseases Consortium. Considerations for Handling Potential SARS-CoV-2 Samples. Mundelein, IL: ABSA International; 2020. https://absa.org/wp-content/uploads/2020/03/ABSA2020_Covid-19-End-user.pdf.

9. Knight V. Viruses as agents of airborne contagion. Ann NY Acad Sci 1980;353:147–156.

10. Vincent JH. Health-related aerosol measurement: A review of existing sampling criteria and proposals for new ones. J Environ Monit 2005;7:1037–1053.

11. Thomas RJ. Particle size and pathogenicity in the respiratory tract. Virulence 2013;4:847–858.

12. Wölfel R, Corman VM, Guggemos W, Seilmaier M, Zange S, Müller MA, Niemeyer D, Jones TC, Vollmar P, Rothe C, et al. Virological assessment of hospitalized patients with COVID-2019. Nature 2020. https://doi.org/10.1038/s41586-020-2196-x.

13. Lewis D. Is the coronavirus airborne? Experts can’t agree. Nature 2020;580:175.

14. Schmid I, Nicholson JKA, Giorgi JV, Janossy G, Kunkl A, Lopez PA, Perfetto S, Seamer LC, Dean PN. Biosafety guidelines for sorting of unixed cells. Cytometry 1997;28:99–117.

15. Schmid I, Lambert C, Ambrozak D, Marti GE, Moos DM, Perfetto SP. International Society for Analytical Cytology biosafety standard for sorting of unixed cells. Cytometry A 2007;71A:414–437.

16. World Health Organization. Coronavirus Disease (COVID-19) Technical Guidance: Laboratory Testing for 2019-nCoV in Humans. Geneva, Switzerland: World Health Organization; 2020. https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/laboratory-guidance.

17. Centers for Disease Control and Prevention. Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19). Atlanta, GA: Centers for Disease Control and Prevention; 2020. https://www.cdc.gov/coronavirus/2019-ncov/lab/lab-biosafety-guidelines.html.

18. ABSA International Emerging Infectious Diseases Consortium. Considerations for Handling Potential SARS-CoV-2 Samples. Mundelein, IL: ABSA International; 2020. https://absa.org/wp-content/uploads/2020/03/ABSA2020_Covid-19-End-user.pdf.

19. Kampf G, Todt D, Pfaender S, Steinmann E. Persistence of coronaviruses on inanimate surfaces and their inactivation with biocidal agents. J Hosp Infect 2020;104:246–251.

20. US Department of Health and Human Services, Centers for Disease Control and Prevention, National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories, HHS Publication No. (CDC) 21-1112. Washington, DC: US Government Printing Office, 2009.

21. Omidbakht N, Sattar SA. Broad-spectrum microbicidal activity, toxicologic assessment, and materials compatibility of a new generation of accelerated hydrogen peroxide-based environmental surface disinfectant. Am J Infect Control 2006;34:251–257.

22. Rutowa WA, Gergen MF, Weber DJ. Efficacy of improved hydrogen peroxide against important healthcare-associated pathogens. Infect Control Hosp Epidemiol 2012;33:1159–1161.

23. Rahenias HF, Cinatl J, Morgenstern B, Bauer G, Preiser W, Doeter HW. Stability and inactivation of SARS coronavirus. Med Microbiol Immunol 2005;194:1–6.

24. Perfetto SP, Ambrozak DR, Koop RA, Roederer M. Measuring containment of viable infectious cell sorting in high-velocity cell sorters. Cytometry A 2003;52A:122–130.

25. Perfetto SP, Hogarth PJ, Monard S, Fontes B, Redef KM, Swan BK, Bajer J, Jellison ER, Lyon G, Lovelace 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;95A:173–182.