

Novel Rapid Method for Visualization of Extent and Location of Aerosol Contamination During High-Speed Sorting of Potentially Biohazardous Samples

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Background: Containment of potentially biohazardous aerosols that result from high-speed sorting of human cells has been an increasingly important problem in analytical cytometry. The current method for assessing the efficiency of aerosol containment involves detection of aerosols containing sorted T4 bacteriophage on lawns of T4-susceptible *Escherichia coli* on plates that are placed in and around the sort area. Although this method is sensitive, it is time consuming and involves maintenance and handling of bacteria and sorting of bacteriophage that may themselves serve as sources of contamination for sorted viable human cells.

Methods: Glo Germ™ (5- μ m melamine copolymer resin beads), which are fluorescent under black light illumination, were sorted on a Beckman-Coulter Elite ESP sorter in

order to visualize deposition of aerosols under normal and mock failure modes.

Results: Glo Germ was successfully used under both normal sorting conditions, as well as mock failure mode, to visualize aerosol formation.

Conclusions: We have developed a method to examine aerosol containment using modified Glo Germ, a product used for teaching aseptic technique in hospitals, industry, restaurants, and schools. Use of this technique represents a rapid, inexpensive, qualitative analysis of the extent and location of aerosol contamination from cell sorters. Cytometry 43:217–222, 2001. © 2001 Wiley-Liss, Inc.

Key terms: flow cytometry; biohazard; occupational health and safety; cell sorting; aerosol containment

One of the major advantages of flow cytometers is the ability to separate cell populations based on cell size, cell density, or fluorescence. With this advantage often comes the need for analyzing viable biological specimens. These can harbor unknown as well as known pathogens, such as the hepatitis viruses and human immunodeficiency virus (HIV). As part of the normal operation of the cell sorter, pressurized fluid exits a vibrating nozzle and forms droplets. The droplets containing the desired cells are electrostatically charged and are deflected into receptacles. The potential hazard to the operator occurs with the normal formation of aerosols of small and microdroplets (size range: 40–200 μ m and 3–7 μ m, respectively). Secondary aerosols can also be formed when the sorting streams splash into receptacles (1,2). In addition, aerosols can increase during failure modes of the cell sorter such as a clogged tip, air in the fluidics system, or other instrumental malfunction. Because aerosols have been shown to be of importance in the spread of infectious diseases (3–6), the safety of the operator and individuals around the sorter could be compromised. Because of the inherent risk involved, the International Society for Analytical Cy-

tology (ISAC) Biohazard Working Group has developed guidelines as precautions for cell sorter operators (7). Most current models of commercially available flow cytometers have designs that reduce the formation of aerosols and their subsequent release outside the sort collection chamber. In addition, methods have been developed to assess the efficiency of aerosol containment during the process of sorting biohazardous samples (1,8).

Current methods for testing aerosol containment during cell sorting include monitoring gravitational deposition of droplets (1,9) and the use of air samplers (8,9). The first method involves sorting lytic T4 bacteriophage in the presence of Petri dishes containing confluent lawns of T4-susceptible *Escherichia coli*. Following a 24-h incubation, the plates are examined for plaque formation. The

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amount of plaques formed correlate with lytic T4 bacteriophage deposition due to aerosols formed during sorting. The second method, air sampling, also involves sorting T4 bacteriophage. However, it uses an air sampler, which collects room air and deposits it on lawns of T4-susceptible *E. coli*.

Advantages of these methods are that they are quantitative and are accepted tests. In addition, the use of an air sampler does not require any special expertise other than those needed for the gravitational deposits method. Disadvantages of these methods include the need for intermediate knowledge of microbiology or advice from a microbiologist. Lack of this knowledge can lead to improper titrating of the T4 bacteriophage or mishandling of the *E. coli* plates, possibly giving erroneous results. Although not "hands-on" time consuming, preparation of T4 bacteriophage stocks should be performed at least a week prior to the aerosol test, which necessitates advanced planning and preparation. The need for reagent preparation (e.g., agar plates and nutrient broths) is also a disadvantage. In addition, if proper sterilization is not performed following sorting of the T4 bacteriophage, there is a potential for contamination of future viable sorts. Apart from the above-mentioned disadvantages, the second method involves the purchase of an air sampler.

We have developed a method to examine aerosol containment using a modified, commercially available product called Glo Germ™ (Glo Germ, Moab, UT). This product is used for teaching aseptic techniques in hospitals, industry, restaurants, and schools and is visualized with ultraviolet (UV) or black light. Glo Germ is available in two forms: a white powder and an orange oil-based suspension of a melamine copolymer resin. We did not use the white powder, which fluoresces blue under black light illumination because it was difficult to differentiate the copolymer resin from ever-present lint. Instead, we chose to use the oil suspension, which is bright orange under both visible light and black light illumination. The 5- μm size of the Glo Germ particles is similar in size to yeast, which makes it a comparable indicator.

There are several advantages to using Glo Germ. Because there is no need for knowledge of microbiology, there is little potential for error when compared with titration of T4 bacteriophage and agar plate handling. The preparation time is less than 30 min, does not need to be done in advance, and requires minimal reagents. Glo Germ is inert and, therefore, not biohazardous and will not cause contamination of future sorts. One of the major advantages is that results are immediate as opposed to the need for overnight incubation of *E. coli* plates. Glo Germ is also very inexpensive, even with the initial purchase of a black light. In conjunction with an image analysis system, it can potentially be quantitative as well.

MATERIALS AND METHODS

Because the resin in Glo Germ is 5% in mineral oil, it was modified into an aqueous base to better represent a real sort sample. This was achieved by spinning down 10

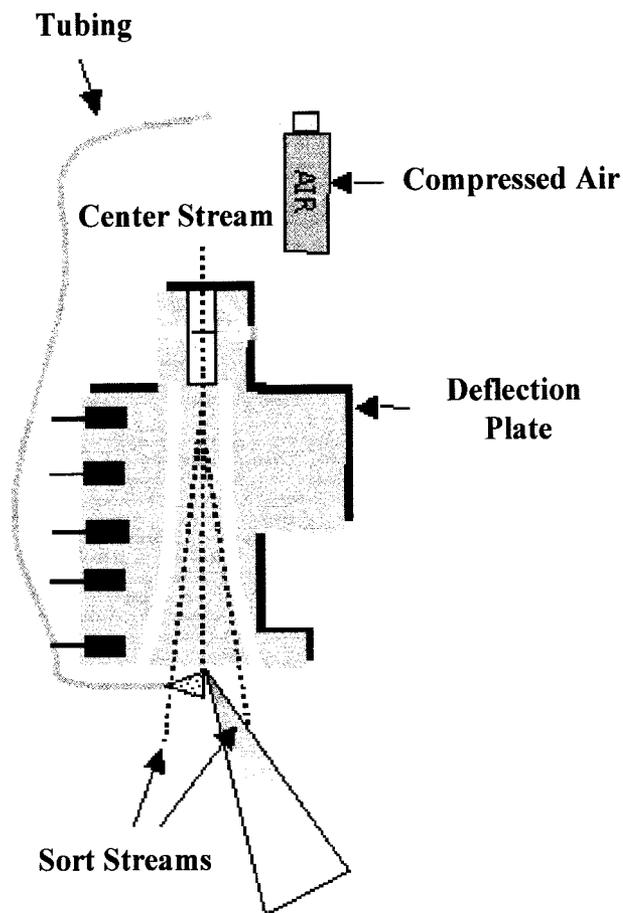
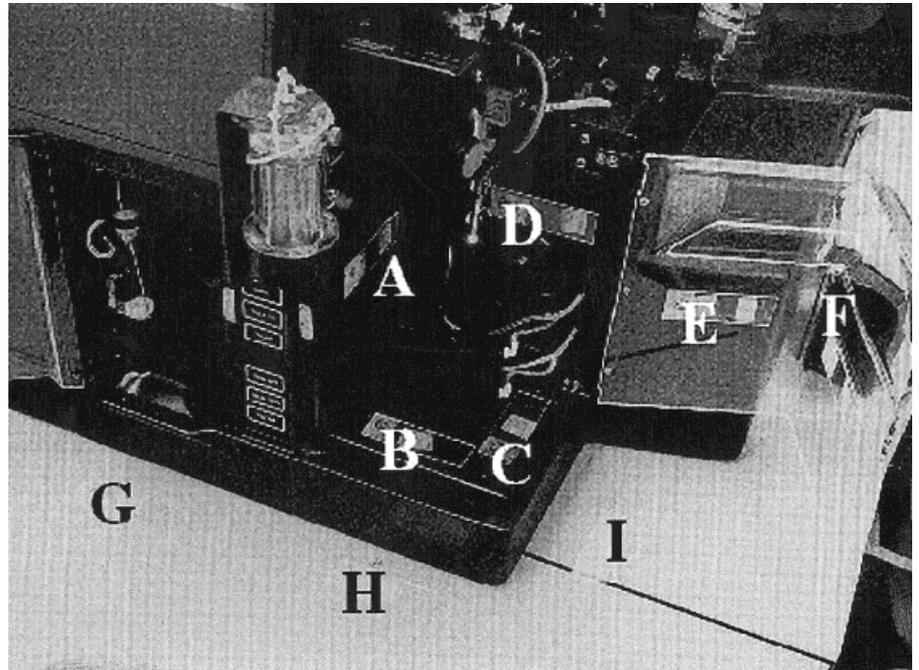


FIG. 1. Tubing attached to compressed air was positioned below the deflection plates, behind the center waste stream. A 10-s burst was used to simulate a failure mode.

ml of Glo Germ at 3,500 rpm for 5 min. The supernatant was removed and the pellet was resuspended in 95% EtOH. The tube was spun again and the remaining oil and EtOH were removed. The pellet was resuspended in 5 ml filtered phosphate-buffered saline (PBS (-))/10% fetal calf serum (FCS), which made the resin concentration approximately 10%. Without the EtOH step, the Glo Germ tended to clump when resuspended in the PBS (-)/10% FCS.

All analysis was performed on an Epics Elite ESP sorter (Beckman-Coulter, Miami, FL). Flow Check beads (Beckman-Coulter) were run to align the 488-nm air-cooled argon laser with resulting coefficients of variation (CV) of less than 2%. Normal sort setup was performed to establish frequency, drive, and delay. Two amorphous sort gates were set on the forward scatter (FSC) versus side scatter (SSC) log histogram for sorting to left and right collection tubes. Several sort rates were tested, ranging from 1,000 to 10,000 events per second. Samples were sorted for 2 h at each rate with the chamber door closed at all times, except for collection tube replacement as

FIG. 2. Positions of slides for examination of aerosol extent. Slides A-F are located inside the sort chamber. Slides G-I are located outside the sort chamber on the workstation.



needed. The biohazard filter fan was kept on at all times. A mock failure mode was constructed using a can of compressed air attached to tubing directed at the center waste stream (Fig. 1). A 10-s burst of compressed air was applied to simulate a clogged flow cell tip. To visualize the extent of aerosolization, slides were lettered and placed in and around the sort area (Fig. 2).

RESULTS

Under all sort rates and optimal conditions (proper frequency, drive and delay, closed chamber door, and

biohazard filter fan on), no fluorescence of Glo Germ was detected outside the chamber as seen as a lack of fluorescence on slides G, H, and I (data not shown). With all sort rates, Glo Germ was detected only on the inner sides of the collection tube holders (Fig. 3A) and under the grating within the sort chamber (Fig. 3B). Higher rates gave a more intense fluorescent glow. This demonstrated that the peak concentration of aerosol deposition remained localized to the center sort stream area.

Under mock failure mode, no aerosolization was detected outside the chamber with the chamber door

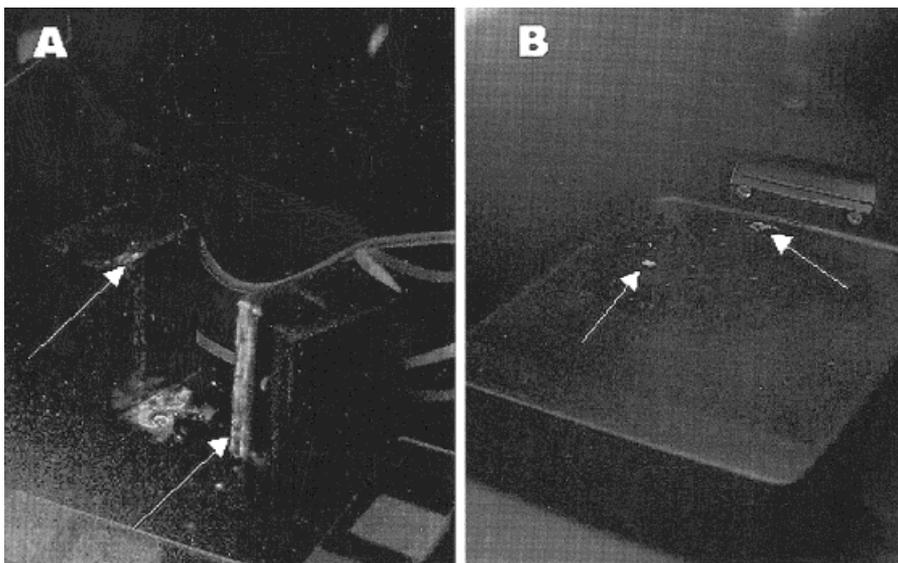


FIG. 3. **A:** Collection tube holders following Glo Germ sorting under normal, nonfailure, sorting conditions. **B:** Underneath sort chamber grating following Glo Germ sorting under normal, nonfailure, sorting conditions.

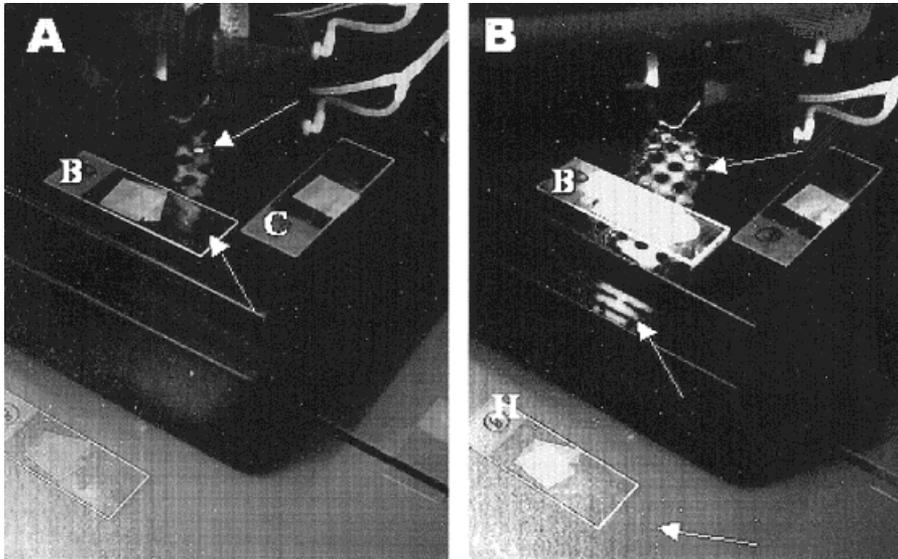


FIG. 4. **A:** Closed sort chamber door mock failure mode. Arrows show aerosolization only within the sort chamber. **B:** Open sort chamber door mock failure mode. Arrows show aerosolization within the sort chamber as well as on slide H located on the workstation outside the sort chamber.

closed. However, fluorescence was detected on the inside of the door, on slide B, and on the grate (Fig. 4A). With the chamber door open, considerable aerosolization was detected some distance from the chamber on the workstation, on slide H, as well as on the inner chamber grating and on slide B (Fig. 4B). Closer inspection of slide B, which was located inside the sort chamber (Fig. 2), shows many individual particles when viewed under $10\times$ magnification (Fig. 5B,C). The fluorescence is also clearly visible without magnification (Fig. 5A). Further examination of slide H, which was located outside the chamber (Fig. 2), shows fewer individual particles when viewed under $10\times$ magnification (Fig. 6B,C) but also is clearly

visible without magnification (Fig. 6A). The level of detection is apparent on slide G, which was located the furthest from the sort chamber (Fig. 2). On this slide, a small area of fluorescence is seen (Fig. 7A). When examined under $10\times$ magnification, the fluorescence is shown as emitting from a single although slightly larger Glo Germ particle (Fig. 7B,C).

Although the most time is spent focusing on aerosols in and around the sort collection chamber, the proximity of the sample stage to the operator makes it another important area that needs to be examined for contamination. The present study demonstrates that aerosols are also created in the sample stage area when a pressurized tube

FIG. 5. **A:** Slide B viewed without magnification following mock failure mode sorting. **B:** Bright field; $10\times$ magnification view of slide B. **C:** Black light; $10\times$ magnification view of slide B.

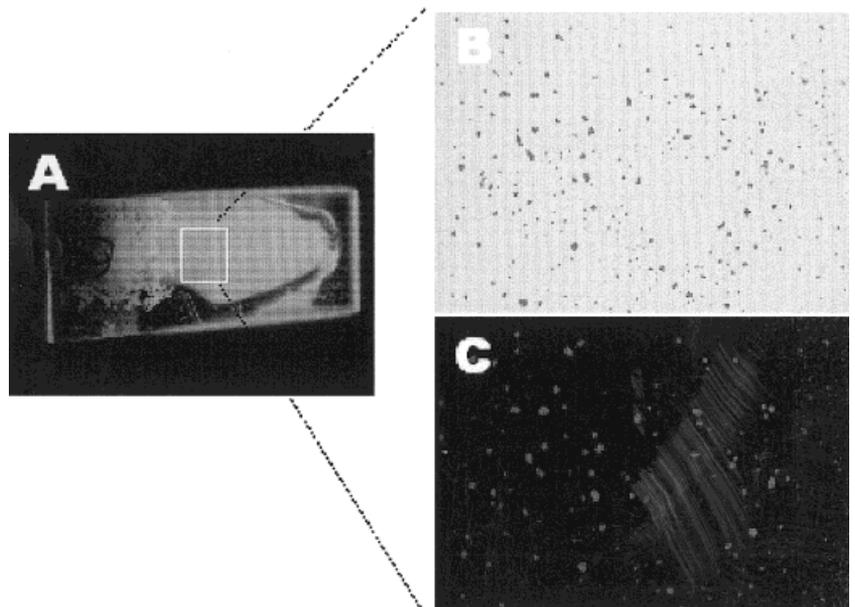
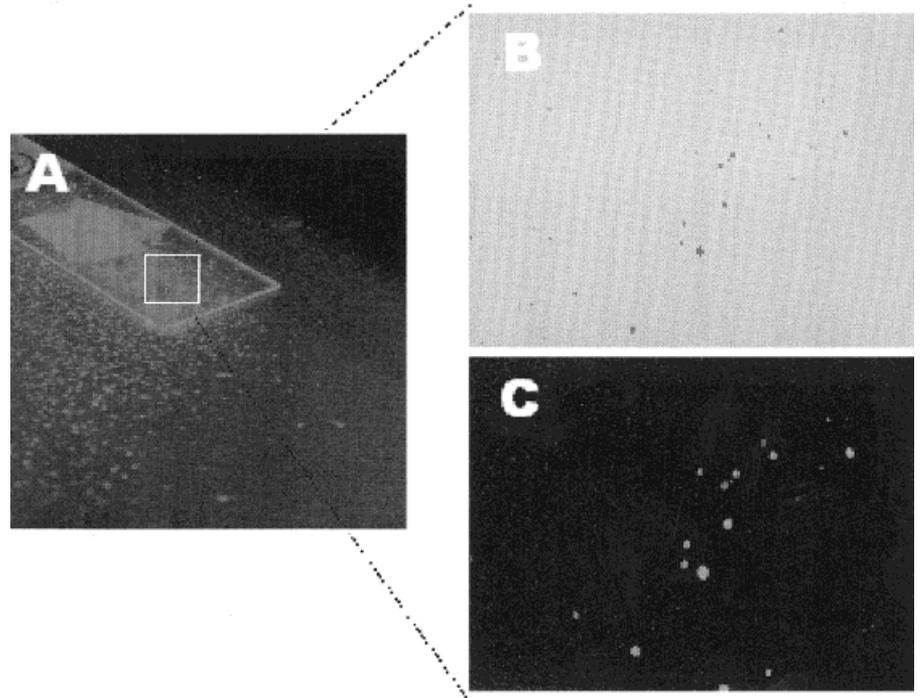


FIG. 6. **A:** Slide H viewed without magnification following mock failure mode sorting. **B:** Bright field; 10 \times magnification view of slide H. **C:** Black light; 10 \times magnification view of slide H.



is removed. Figure 8 shows the location of the deposition of these aerosols. Glo Germ particles were located on the back wall nearest the sample cap (Fig. 8, arrow), on the vacuum tubing attached to the sample cap (Fig. 8, arrow), and in the waste drain area (data not shown).

DISCUSSION

Aerosols can be a concern for flow cytometrists. They should be monitored and minimized for safety purposes. For the safety of the operator, the use of established guidelines for specimen handling (7) should be

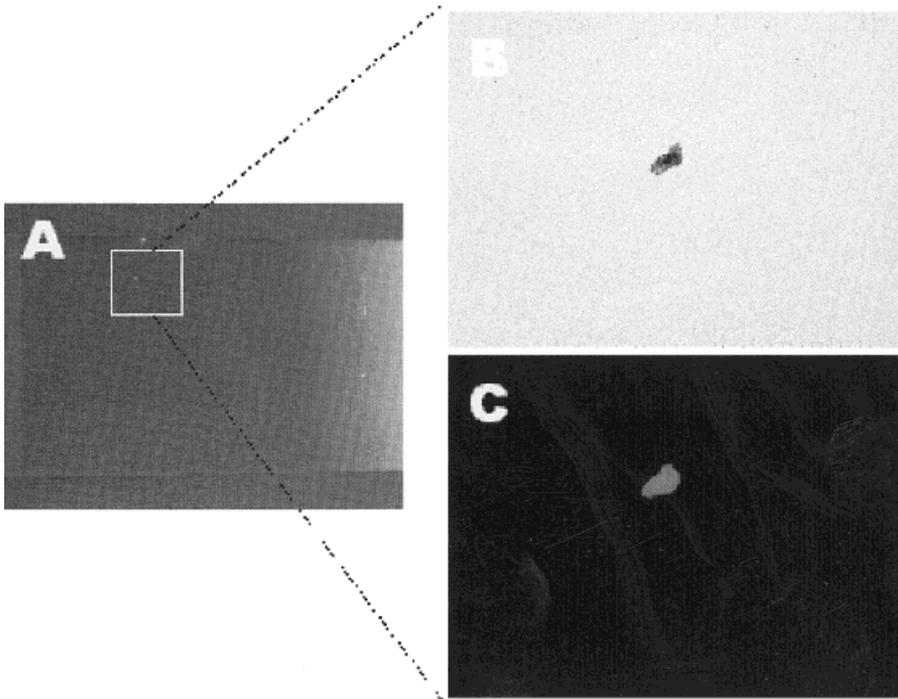


FIG. 7. **A:** Slide G viewed without magnification following mock failure mode sorting. **B:** Bright field; 10 \times magnification view of slide G. **C:** Black light; 10 \times magnification view of slide G.

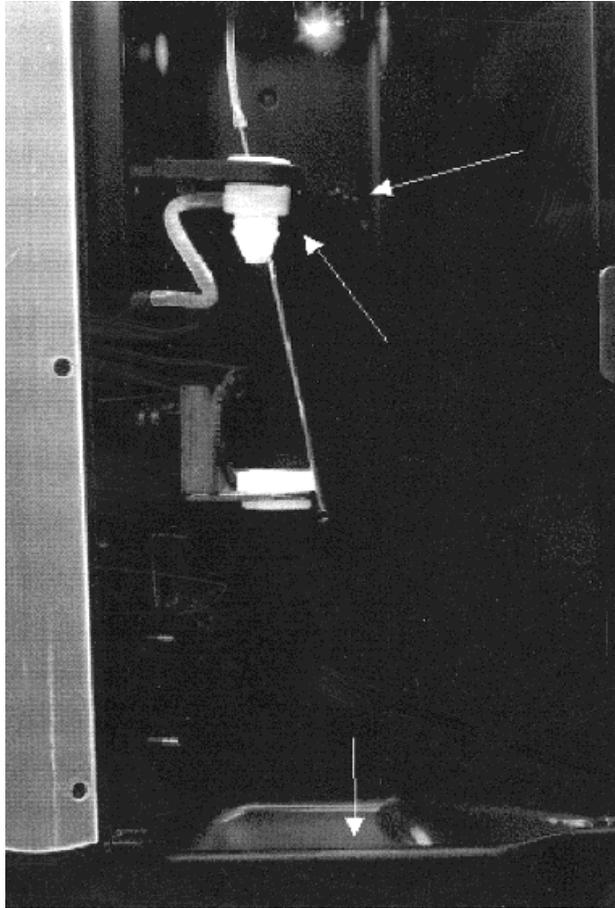


FIG. 8. Location of aerosolization in the sample chamber. Arrows indicate areas of aerosolization due to removal of sample tube under pressure.

in place and followed in every cell sorting facility. Current methods to assess aerosolization have advantages and disadvantages. Our new method for assessment of aerosols has the advantages of ease, speed, low cost, and the potential to be quantitative. Our method also confirms the observations reported by Ferbas et al. (10), using the T4 bacteriophage method, regarding the level of biohazard containment of the Beckman-Coulter Epics Elite sorter. There is clearly a need to further test this new method under various sort conditions; however, preliminary results show comparable results to those described by Merrill (1).

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