THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History®

CLEARING A SORTER CLOG

PURPOSE

The purpose of this procedure is to minimize exposure of the sort operator to potentially hazardous aerosols that occur when an instrument clogs during sorting.

SCOPE

Compliance with this procedure is the responsibility of all employees and users of the Advanced Cytometry and Sorting Facility at South Campus cell sorters.

DEFINITIONS

Aerosol: A suspension of particles 0.1 to 60 µm in size dispersed in air or gas. Aerosols occur during sorting when droplets hit a hard surface.

AMO System: Aerosol Management Option filter is used to evacuate aerosolized particles from the sort chamber.

Biosafety Level 2 (BSL-2): A classification of safety precautions required to work with biological agents that have a potential for mild infection and are not typically contagious as aerosols.

diH_2O: Deionized water.

HEPA Filter: Filter that captures 0.3 µm or larger particles.

Personal Protective Equipment (PPE): Devices worn by workers to protect against environmental hazards.

MATERIALS AND REAGENTS

- 5 ml polypropylene or polystyrene 40 µm filter cap round bottom tubes.
- Kimwipes.
- Contrad.
- diH_2O.
- 70% ethanol.
- 10% bleach.

- Disinfecting wipes.
- Buffalo Filter Whisper Aerosol Evacuation System.
- BD FACS Accudrop beads.

PROCEDURE

1.0 Preparation for Sorting

1.1. Ensure that the sorter has been prepared for the day by cleaning and running quality control. For information on instrument maintenance, please see the individual instrument SOP.

1.2. Sort operators should be trained in use and maintenance of the instruments, as well as ACSF biohazard containment procedures.

1.3. The ACSF treats all sorts as BSL-2 and uses enhanced precautions. ACSF safety measures include the sorters being in a separate room from the main lab and in an individual enclosure with a HEPA filter. Appropriate PPE include a front closing lab coat, gloves, and optional safety glasses and N-95 respirator.

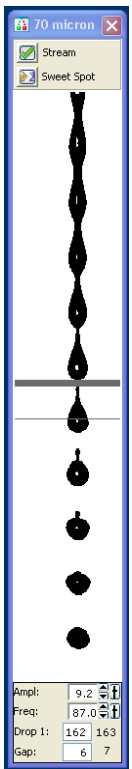
1.4. Sort samples should be resuspended in PBS, with less than 1.0% serum.

1.5. To reduce changes of clogging, filter all samples through 40 µm filter cap tubes prior to sorting.

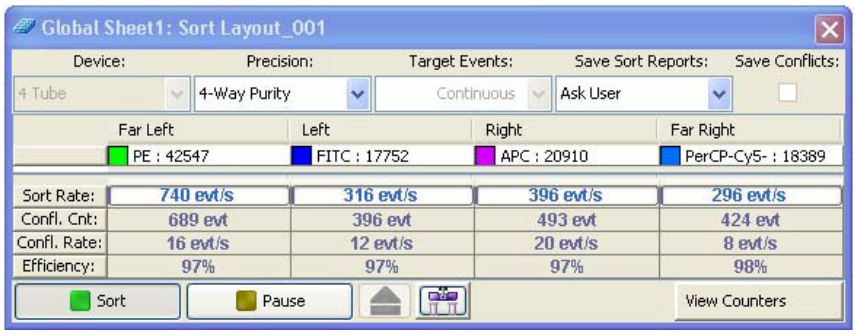
2.0 Clearing a Clog

2.1. When an instrument clog occurs during sorting, ask any additional personnel or users to leave the room.

2.2. Ensure that the instrument stream is off, and turn the sorter's AMO system to 100% or high. Open the sort drawer to evacuate all aerosols from the sort chamber for the Aria IIIu and Fusion sorters.

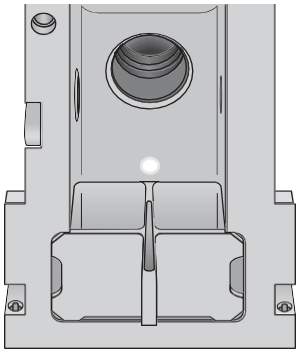


Turn off Stream

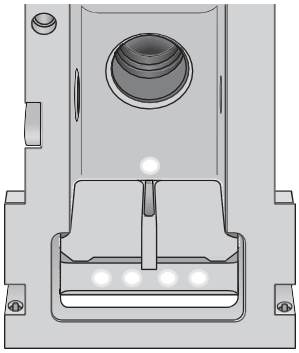


Open sort drawer

Sort Drawer Closed



Sort Drawer Open



Increase to 100%

2.3. Wait five minutes for any potential aerosols to clear from the sort chamber before opening or touching the sort chamber.

2.4. To clear a clog on the FACSAria IIIu or the FACSAria Fusion follow step A, and for the Influx follow step B.

A. FACSAria IIIu and FACSAria Fusion Clog Cleaning

- Remove the sample tube from the cytometer loading port and the sample collection tubes and cap them.
- Ensure that the deflection plate voltage is off and then clean and dry the plates with kim wipes. Use 70% ethanol or H_2O if necessary.

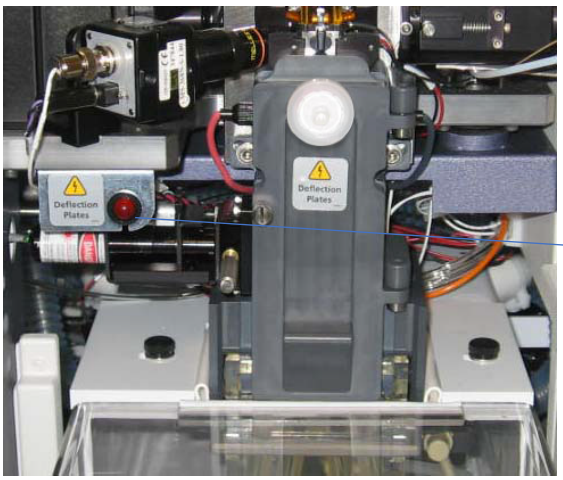
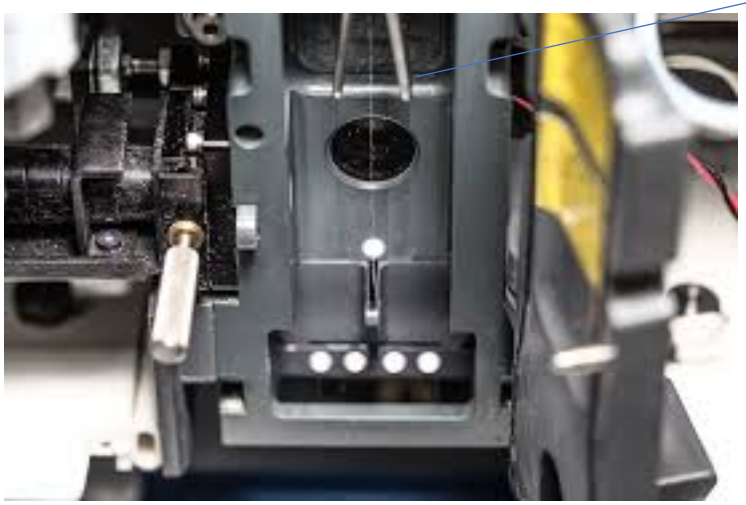


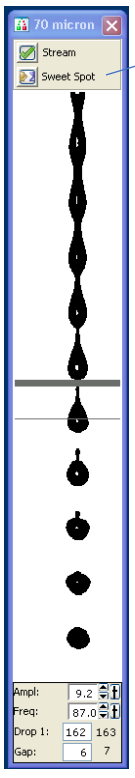
Plate Voltage Off – Red light is off

Clean and dry the sort plates



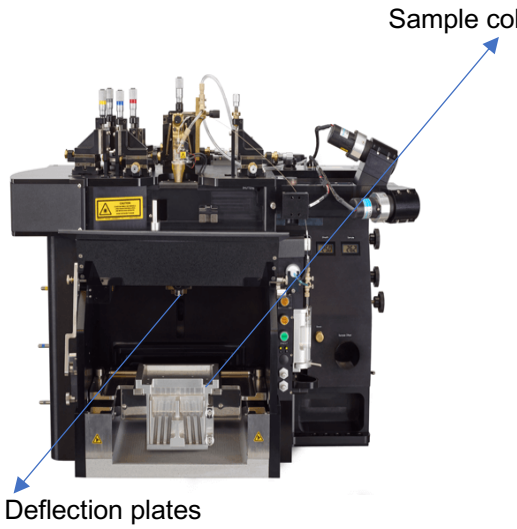
- Clean the flow cell three times by placing sterile diH_2O or Contrad in the cytometery loading port, then selecting Cytometer > Cleaning Modes > Clean Flow Cell, and click OK.
- Turn on the stream and verify that it is normal. Re-enable Sweet Spot.

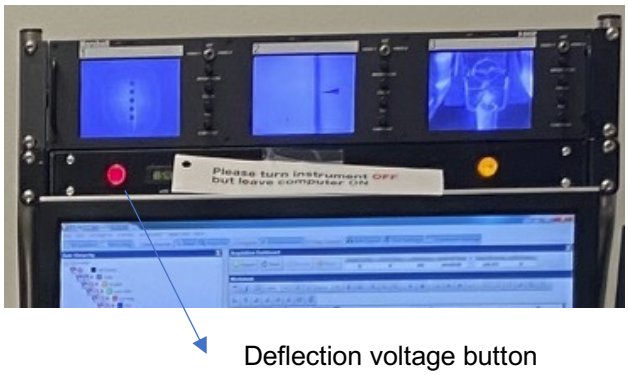
Turn on Sweet Spot



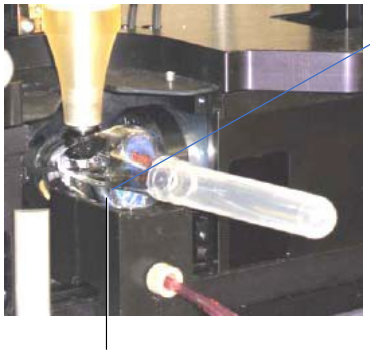
B. Influx Clog Cleaning

- Move the stage to the safe position.
- Remove the sample tube from the cytometer loading port and the sample collection tubes and cap them.

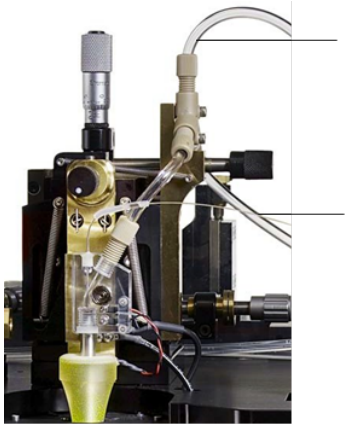




- Ensure that the deflection plate voltage is off and then clean and dry the plates
- Place the debubble reservoir beneath the nozzle. Press the green Run button on the instrument to fill the reservoir with sheath. Once the nozzle tip is submerged, press Run again to stop the flow of sheath.



- Press the yellow Purge button on the instrument to pull up sheath through the nozzle.
- Once the air bubbles have passed the Y shaped purge valve press the yellow Pulse button on the Influx to free any additional loose bubbles.



- Repeat the previous three steps until no more bubbles are released from the nozzle. If the bubbles persist, repeat the steps using 70% ethanol instead of PBS, or by briefly introducing air into the nozzle. Please see instrument specific SOP for details.

- Turn on the plate voltage and stream and verify that it is normal. Check stream alignment and drop deflection with Accudrop as necessary.

2.5. If the instrument is still clogged, turn off the stream, remove the nozzle, and sonicate until the obstruction has been cleared. Turn on the stream and verify that it is normal.

2.6. Check stream alignment and drop deflection with Accudrop as necessary.

2.7. Disinfect the sort collection device and sort chamber as necessary with 70% ethanol, 10% bleach, or disinfecting wipes

2.8. Return the AMO system to 10% or low.

2.9. Filter the sample again through a 40 µm filter cap tube, and resume sorting.

RELATED PROCEDURES

Personal Protective Equipment (PPE) Policy (UTMDACC Institutional Policy #ADM1118).

IN005: BD FACSAria IIIu Use and Maintenance.

IN006: BD FACSAria Fusion Use and Maintenance.

IN007: BD Influx Use and Maintenance.

TR001-HN06: Independent Sorter Training.

REFERENCES

Schmid, I., et al. “International Society for Analytical Cytology Biosafety Standard for Sorting of Unifxed Cells.” Cytometry Part A. (2007): 414-437.

National Institute of Health. “NIH Guidelines for Research Involving Recombinant of Synthetic Nucleic Acid Molecules (NIH Guidelines)”. 2016.

REVISION HISTORY

VERSION	ACTION	DATE	INITIALS
1.0	Initial release	11-29-2016	KMR
1.1	Version correction	08-21-2017	KCD
1.2	Version correction	04-08-2020	KMR
1.3	Version Update	04-29-2020	MSR/KMR
1.4	Version Update	9-15-2021	KC