

The following procedure has been modified from Perfetto, Stephen P. *FCC Cell Sorting Facility: Safety Practices and Procedures*. 2017 June 30. Vaccine Research Center, NIAID.

1. Unload the sample and turn off the stream if the instrument has not automatically gone into emergency stop mode
2. Turn the AMO up to 40% operation
3. Turn the stream on again to see if the break off returns to a normal pattern
4. If clog persists, turn off the stream and perform the clean flow cell option using Clenz or bleach. Turn the stream back on to check the break off
5. If clog still persists, wait 30 seconds before opening flow cell access door. Remove the nozzle and sonicate in distilled water for 30 seconds
6. Check the nozzle for a clean hole using the microscope. If debris persists, sonicate for additional time.
7. Install the nozzle, turn on the stream, and verify break off is stable. Repeat Accudrop drop delay calibration if necessary
8. Reduce AMO to 20% before re-starting the sort

Note: Collection tubes should not be removed from collection area chamber until sample acquisition and the stream have been stopped, and 30 seconds have passed with the AMO set to 20%