This procedure has been modified from the following:

Perfetto, Stephen P. *FCC Cell Sorting Facility: Safety Practices and Procedures*. 2017 June 30. Vaccine Research Center, NIAID.

Perfetto, Stephen P. et al. *Novel Impactor and Microsphere-Based Assay Used to Measure Containment of Aerosols Generated in a Flow Cytometer Cell Sorter*. 18 Dec 2018. Cytometry Part A; 95(2):173-182.

Materials:

Dragon Green beads, Bangs Laboratories, cat # FSDG004/FS03F

Cyclex-d impactor sampling cassettes, Environmental Monitoring Systems, cat # 120135

Pump with rotameter

Tween 20

Gridded microscope slides, VWR, cat # 63405-02

Procedure (performed every 6 months):

1. Perform fluidics startup on Aria and install 70 um tip
2. Turn Bio-bubble on and lower front flap so that instrument is enclosed in hood
3. Manually set Aria sheath pressure to 70 psi and allow stream/break-off to stabilize
    a. Frequency should be around 91.5
    b. Set trigger to B 530/30 fluorescence and FSC
    c. Create a SSC-A (log) vs B 530/30-A (log) plot and a single parameter B 530/30-A histogram in a global worksheet
4. Turn AMS on and set to 20% (vacuum gauge must read between 1.0 and 2.5 in. water (250-622 Pa)
5. Cover the waste catch with a small piece of rubber tubing so that when the collection tube area is uncovered, the waste stream is forced to glance off the waste catcher shield and yield a plume of aerosols
6. Set vacuum pump to 20 L/min and turn off. Place tubing attached to pump such that the end of the tubing is in front of the sort chamber approximately 2 in. from sort door and secure in place with tape.
7. Close door to deflection plates
8. Do not install tube holder but close the main sort chamber door
9. Attach cyclex-d cassette to tubing
10. Prepare Dragon green beads
    a. Buffer: add 10uL of Tween 20 to PBS (0.01% sodium azide) for a final Tween 20 concentration of 0.5%.
    b. Add 20uL of beads to 2mL of above buffer and vortex well
11. Load Dragon green beads as a sample and adjust flow rate to achieve 40,000 – 50,000 beads/sec
12. Remove covering from cyclex-d cassette attached to tubing, turn pump on and confirm vacuum is set to 20 L/min
13. Retract sort drawer and collect aerosols for 10 min.
14. Turn off vacuum, remove cyclex-d unit, and label as ‘AMS test’

15. Collect in failure mode (to generate positive control):
    a. Install fresh cyclex-d cassette
    b. Turn off AMS
    c. Collect for 2 min.
    d. Turn pump off, remove cyclex-d unit and label as positive control
16. Stop sample acquisition, return waste catch to normal position, turn AMS back on and remove rubber tubing from waste catch
17. Preferably in another room, remove the glass coverslip from the inside of the positive control cyclex-d cassette and place onto a gridded microscope slide
    a. Coverslip should be placed glue side down onto microscope slide
    b. Use FITC filter on microscope
    c. Using 10x or 20x objective, scan the slide and count all beads on the coverslip and record result (not necessary to count all the beads on the positive control)
    d. Positive control slip must contain greater than 50 particles after 2 min of active air sampling with AMS turned off and no tube holder in place
18. Repeat step 16 with the 'AMS test' cassette, count number of beads and record result
19. Acceptable tolerance is zero Dragon green beads detected after 10 min. of sampling in front of sort chamber door with no tube holder in place and AMS turned on to 20%
20. If AMS test coverslip is positive for beads, check all vacuum tubing, re-seat filter in AMS and repeat the test
21. Decontaminate Aria of Dragon green beads