Intelligent Biosafety: MoFlo Astrios fully integrated with the Biosafety Cabinet and Aerosol Evacuation System

Introduction

As with any sorter that requires jet-in-air sorting, aerosols are created both from the sort and waste streams. Depending on the virulence of the sample, there is a potential safety risk to the operator. The Astrios has been designed with an Aerosol Evacuation System (AES) either as a stand-alone unit or integrated into The Baker Company's SterilGARD III class II, type A2 Biological Safety Cabinet (BSC) to reduce operator user risks.

The Astrios software and hardware are fully integrated with the BSC with interlock controls for comprehensive operator safety. The MoFlo Astrios was designed and developed according to the National Institute of Health's biosafety guidelines for flow cytometry.

The MoFlo Astrios instrument is also designed to communicate with the BSC for increased operator safety by providing a comprehensive biosafety system with the BSC, Aerosol Evacuation System, and integrated software and system interlocks to contain biohazardous aerosols and reduce operator risk.

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Biosafety Interlock Safety Features

Astrios prevents or stops sorting in the following conditions:

Biosafety Cabinet airflow is inadequate: An interlock on the Astrios system ensures that if airflow on the Biosafety Cabinet is inadequate, the Astrios shuts off sheath and sample flow. This includes blocked airflow at the front of the instrument (e.g. placing lab notebooks or other lab equipment where they impair air flow).

Biosafety Cabinet does not have power or loses power: This interlock ensures that if the BSC is not connected to the Astrios, the Astrios will turn off sheath and sample flow.

Biosafety Cabinet alarms if unsafe conditions exist: The BSC has an audible alarm that will alert users if the view screen or other obstruction blocks airflow, reducing the risk of exposure to biohazardous materials.

Biosafety Design Features

Ergonomics: All of the required alignment micrometers are adjustable with the Astrios installed in the Biohood (Figure 1, 1).

Aerosol Evacuation System fully integrated into the Biosafety Cabinet: The aerosol evacuation system on the Astrios is connected to the BSC so that aerosol evacuation is always maintained when the BSC is active (Figure 3, not visible).

Specialized User Touch Panel mounting design: The touch panel arm of the BSC is mechanically designed so that airflow of the BSC is unlikely to be disrupted regardless of the position of the viewscreen (Figure 1, 2).

Seismic brackets availability: Seismic brackets are available to secure the BSC in earthquake susceptible locations (Figure 1, 3).

Fig. 1.

Methods

BSC and Astrios Integration: Astrios software and hardware as well as the integrated BSC were tested for BSC cabinet failure and potential operator usage failures.

AES Testing: Glo-Germ flourescent particles can be found in aerosols generated while sorting and were used here to simulate customer samples. This allows for testing of the AES.

BSC Testing: The Astrios with BSC was tested at Baker following NSF/ANSI-49 standards. These tests included the measurement of Colony Forming Units (CFU) from azB. subtilis while simulating daily operating procedures and events.

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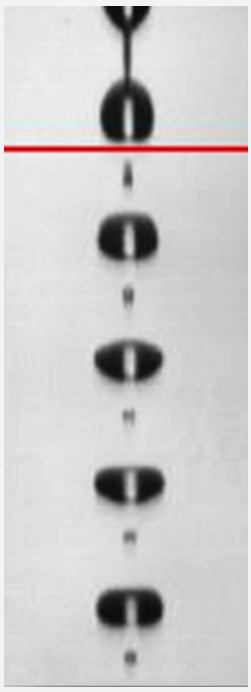

Fig. 2. Stream image from an Astrios 70 µm tip. The visible small drops are microsatellites that may contribute to aerosol generation.

Fig. 3. MoFlo Astrios with the Aerosol Evacuation System attached. The AES may also be integrated into the biosafety cabinet.

VHP Impact: The Astrios and BSC were decontaminated with non-condensing VHP using a Steris VHP 1000 instrument. Twenty-six total cycles of decontamination were conducted (built to withstand twenty cycles) with standard periodic instrument performance testing conducted in between cycles.

Aerosol Generation

What are aerosols? Aerosols are small droplets created by the release of the stream from the jet-in-air nozzle.

Microsatellite droplets: The microsatellite droplets found in between the sort drops may contain biohazardous particles (Figure 2).

Sort Streams: The deflection of the sort streams may be another location for aerosol generation.

Sort Rescue and Waste Catcher: The impact of the sort streams on the sort rescue and waste catcher may cause additional aerosols during sorting.

Aerosol Regulation

NIH adopted the NIH Biosafety Policy for Cell Sorters

- Provides guidance for the installation and use of cell sorters
- Defines biosafety levels as pertains to cell sorter operation
- Requires all cell sorters to have an Aerosol Evacuation System (AES)

MoFlo Astrios provides an Aerosol Evacuation System with or without the biosafety cabinet (Figure 3).

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AES Testing

Glo-Germ fluorescent particles were used to simulate customer sample aerosols while testing the AES effectiveness in removing potential safety hazards with stream misalignments in all sorting compartments.

Designed with consultation from the NIH, locations of the aerosol evacuation ports include (Figure 4):

- Interrogation Chamber
- Deflection Chamber
- Sort Chamber

Sort Purity with the AES System

- Tested six-way sorting with Spherotech Ultra-Rainbow eight-peak beads (Figure 6)
- Sorted 6 ways simultaneously, 1,000 events per second at different % AES
- Purity was greater than 99.00% at all streams, all AES vacuum strengths, suggested % AES for SOP is 20% (Figure 5)

Methods followed, "Measuring Containment of Viable Infectious Cell Sorting in High-Velocity Cell Sorters" by Perfetto, S. P., Ambrozak, D. R., Koup, R. A. and Roederer, M. (2003).

Sort Purity

%AES	Left Stream 3	Left Stream 2	Left Stream 1	Right Stream 1	Right Stream 2	Right Stream 3
Off	99.12%	99.22%	99.70%	99.81%	99.61%	99.53%
20%	99.81%	99.46%	99.86%	99.59%	99.86%	99.44%
40%	99.52%	99.62%	99.52%	99.81%	99.65%	99.44%
60%	99.63%	99.52%	100.00%	99.52%	99.43%	99.82%
80%	99.16%	99.81%	99.34%	99.72%	99.66%	99.36%
100%	99.14%	99.37%	99.35%	99.81%	99.36%	99.62%

Fig. 5. Aerotech concentrator, under vacuum, that captures the Glo-Germ aerosols on a slide.

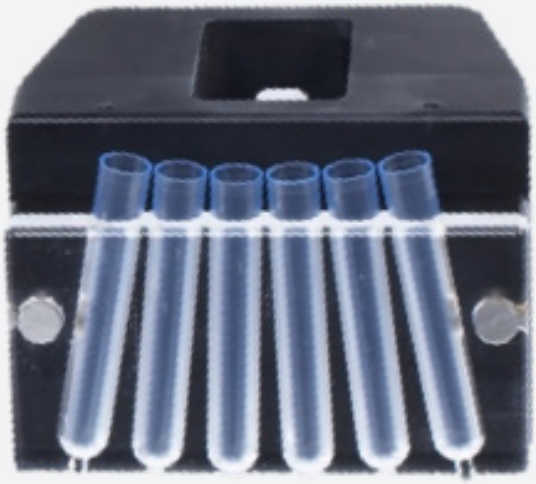

Fig. 6. Aerosol containment testing with the aerosol containment system and the biosafety cabinet. Both with and without BSC were tested.

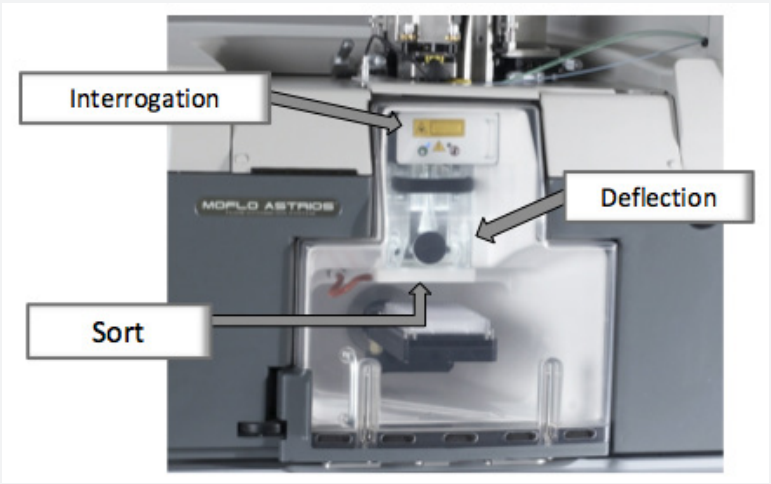

Fig. 4. Glo-Germ beads fluorescing under UV light.

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1. Glo-Germ particles (five µm melamine copolymer resin beads) in a suspension
2. Create both a "normal" and "aerosol-generating" sorting mode on the Astrios:
   a. Normal Sort Mode
   b. Aerosol-Generating
      i. Nozzle clog in the interrogation chamber
      ii. Unstable streams in the deflection chamber
      iii. Sort stream spray in the sort chamber
3. Set up AeroTech concentrator to capture aerosols at different distances from the aerosol-generating location of the Astrios.
4. Run sorts for at least 15 minutes at each normal and failure locations.
5. Test with both only the AES and the AES with the Biosafety Cabinet.

AES Results

The AES is effective in eliminating airborne aerosols. Glo-germ detection is only evident when the AES system is disengaged (Figure 7).

The fluorescence is not affected by AES.

- Average CV for 488-795/70 parameter was 3.18 and decreases to 2.70 with AES (100%)
- Average CV for 355-692/70 parameter was 3.26 and decreases to 2.49 with AES (100%)

The AES system does not cause dysfunction in Intellisort functionality, although there is a slight increase in drop delay adjustments at 100% AES vacuum (standard operating AES is 20%).

- The average amplitude change (for drop delay) is less than 10% between 0 and 100% AES for maintaining drop delay.

Glo-Germ Count

AES & Aerosol Location	20 mm	25 mm	30 mm	35 mm	40 mm	45 mm	50 mm	55 mm	60 mm	65 mm	70 mm	Total
20% Upper	0	0	0	0	0	0	0	0	0	0	0	0
100% Upper	0	0	0	0	0	0	0	0	0	0	0	0
20% Mid	0	0	0	0	0	0	0	0	0	0	0	0
100% Mid	0	0	0	0	0	0	0	0	0	0	0	0
20% Lower	0	0	0	0	0	0	0	0	0	0	0	0
100% Lower	0	0	0	0	0	0	0	0	0	0	0	0
0% Upper	0	1	5	1	0	3	0	2	3	0	0	15

Fig. 7. The Glo-Germ count per the % AES vacuum and the aerosol generation location. Upper=Interrogation Chamber, Mid=Deflection Chamber, Lower=Sort Chamber. The location of the Aerotech concentrator from the aerosol-generation is described by measuring the distance (mm). Glo-Germs are only detected with the AES system off (0%).

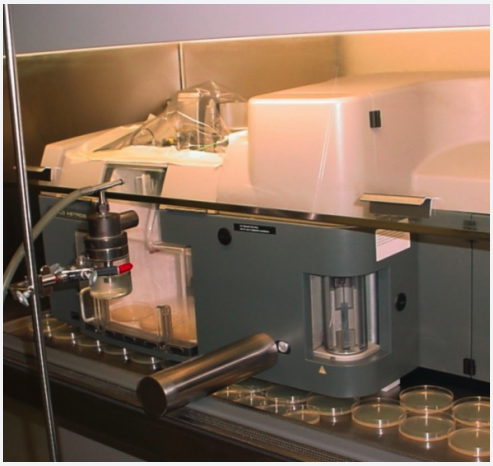

Fig. 8. Testing of the BSC with the MoFlo Astrios not operational. All tests passed.

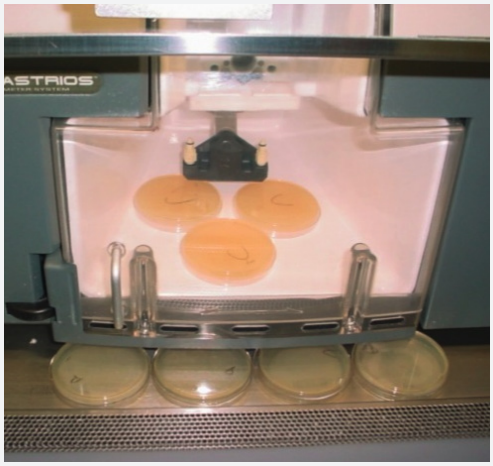

Fig. 9. The Astrios is operational during challenge testing and only showed spore growth when the vacuum is not operating. The Astrios passes NSF testing when operating correctly. This experiment provides evidence that spores are contained in the safety cabinet but not inside the stream sampling chamber when vacuum is not applied. Data provided by The Baker Company.

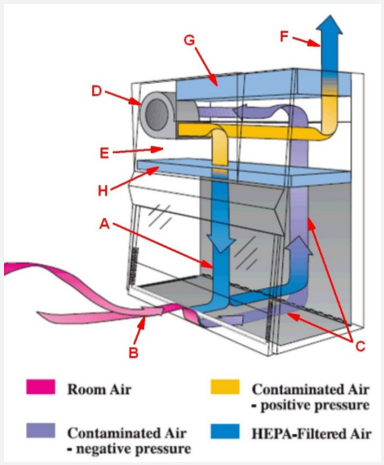

Fig. 10. Illustration of air flow on the SterilGARD Biosafety Cabinet. A: Filtered downflow air, B: Room air, C. Contaminated air, D. Blower motor, E: Plenum, F: Filtered exhaust air, G, H: HEPA filter. Image courtesy of The Baker Company.

- The standard deviation of the amplitude change increases slightly with AES 0.15 (Off), 0.17 (20%), (100%).

Conclusions of AES Testing

- The Astrios instrument performance maintains sort purity and drop delay while under AES.
- The presence of AES effectively removes airborne aerosols in the interrogation, deflection and sort chambers while activated at 20%.

NSF-49 Biohood Testing

NSF/ANSI-49 tests conducted by The Baker Company are used to determine the effect of aerosol containment and protection from external contaminants on a fully integrated MoFlo Astrios and BSC.

- All tests are performed to the NSF/ANSI Biosafety Standard 49-2011. These tests determine whether aerosols are contained within the cabinet, outside contaminants will not enter the cabinet work area, and aerosol contamination of other equipment in the cabinet is minimized (Figure 8).
- No more than 10 colony forming units (total) shall be counted for each test on the filter from six AGI impingers for passing results, as per the NSF standard and no more than five colony-forming units shall be counted on both slit sampler settling plates for passing results.

Conclusions of NSF-49 Biohood Testing

The Astrios and Biosafety Cabinet have individual and integrated safety features to achieve comprehensive user safety (Figure 9).

- The BSC integrated with a MoFlo Astrios exceeds the NSF-49 requirements for personnel and product protection.
- Testing with an operational Astrios shows that containment on a fully integrated Astrios and BSC system passes NSF-49 testing.

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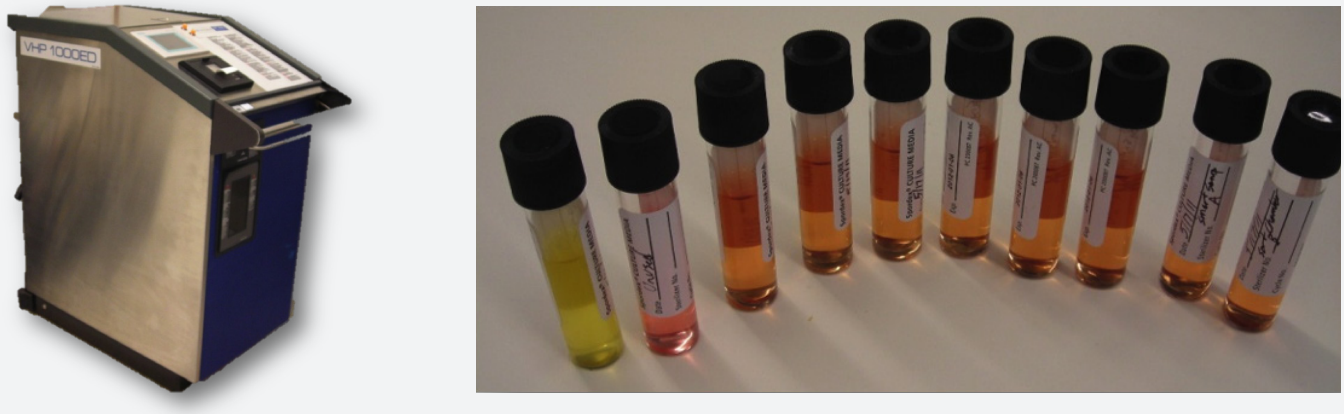


Fig. 11. Biological indicators demonstrate the effectiveness of the sterilization procedure. On the left, the sample is the control contaminated sample (yellow) and unused sample (pink). Each of the vials to the right was placed in different sections of the Astrios and the amber color indicates an effective sterilization.

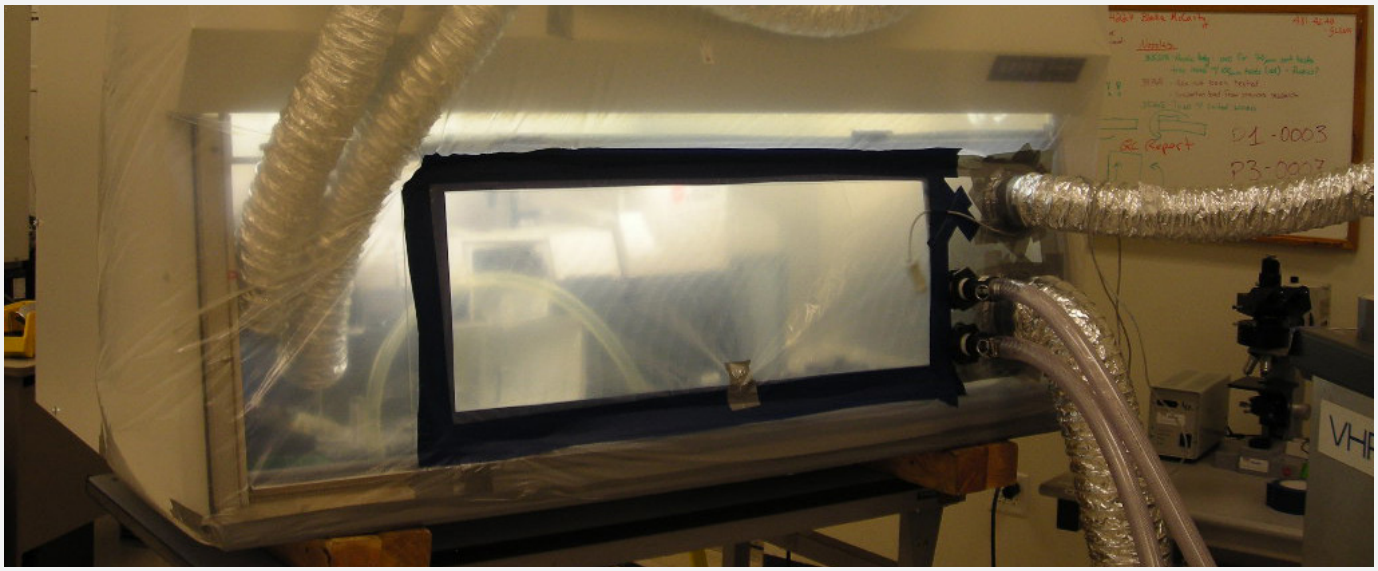


Fig. 12. MoFlo Astrios instrument undergoing VHP testing with the Steris 1000.

- The end user, if not utilizing the AES or BSC, may be exposed to airborne contaminants as seen with the BSC challenge testing.

Vaporous Hydrogen Peroxide Decontamination

The MoFlo Astrios instrument was tested with 26 cycles (specified to withstand twenty cycles) of vaporous hydrogen peroxide decontamination to determine the effect of decontamination on instrument performance (Figures 11 and 12).

- Vaporous hydrogen peroxide exposure at cold temperatures (less than 80° C) is an effective sterilization method.
- The precise mechanism is not known, but it is thought that the gaseous hydrogen peroxide molecules form free radicals and destroy organisms.

VHP Procedure and Astrios

- Seal the instrument with plastic sheeting and set up appropriate test strips and biological indicators.
- Run decontamination cycle.

- Run an instrument performance test:
    - o Laser power and noise
    - o Spherotech Ultra-Rainbow eight-peak calibration beads
    - o Six-peak Rainbow calibration beads
    - o Alignment particles
    - o Fluorescence stability (one hour)
- Run four cycles of VHP.
- Run an instrument performance test.
- Repeat until 26 cycles of VHP have been conducted.

Astrios Performance After VHP

Instrument CV performance

- CV instrument performance on all parameters was equivalent or better than the 21 systems that have been through manufacturing (Manufacturing Mean) (only 488 nm laser shown).

Instrument noise

- Measured by the separation of the top two peaks in the six-peak beads provided by Spherotech
- Indicator of instrument noise and linearity, lower value = better resolution
- Calculated

$$\frac{Median_{Peak\ 6} - Median_{Peak\ 5}}{Standard\ Deviation_{Peak\ 6} + Standard\ Deviation_{Peak\ 5}}$$

Results: Separation factor was lower or equivalent to the mean standard of the manufactured instruments.

Astrios Performance After VHP

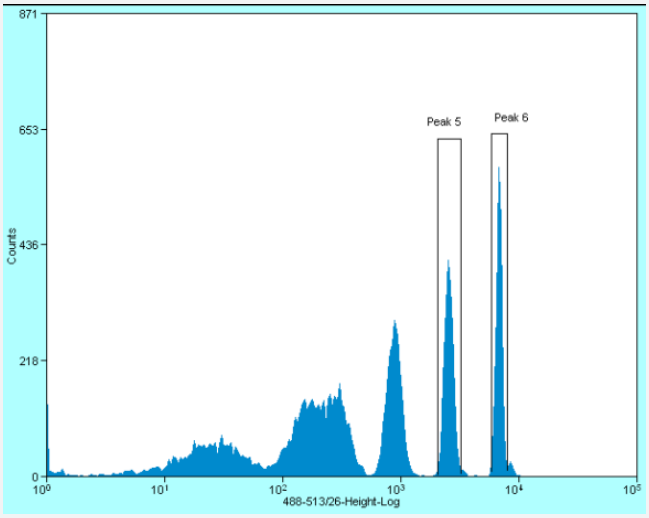
Instrument Sensitivity

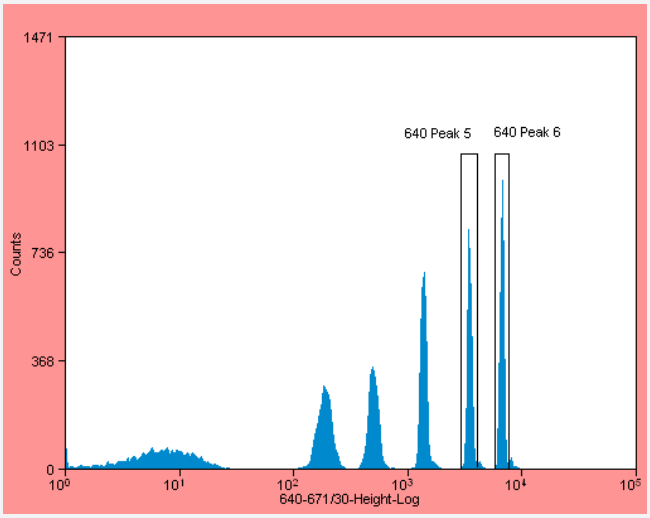
- Measured by using Spherotech's method: MEASURING MOLECULES OF EQUIVALENT FLUOROCHROME (MEF) USING SPHERO™ RAINBOW AND ULTRA RAINBOW CALIBRATION PARTICLES (Figure 16)
- Eight-peak bead performance

Calculations:

$$Relative\ channel\ number = \frac{Resolution}{4} log_{10}(Mean\ Channel\ \#)$$

- Eight-peak beads resolution was equivalent or better than initial performance testing (Figure 15).





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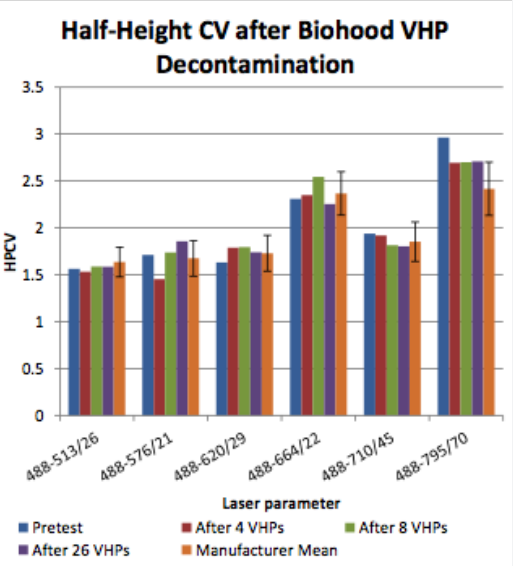

Fig. 13. Half-height CVs of the Biohood VHP Decontamination. The instrument overall performed equivalent or better than the Astrios tested in manufacturing.

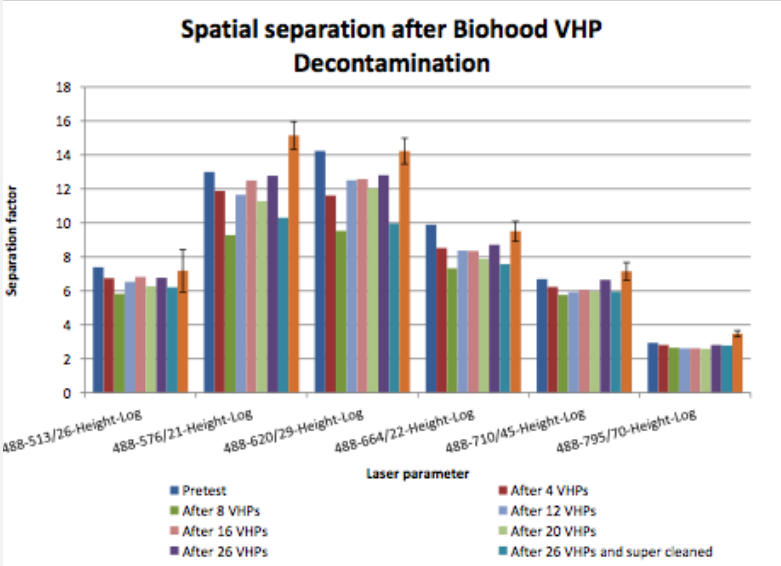

Fig. 14. Spatial separation (Six-peak bead performance) after Biohood VHP Decontamination.

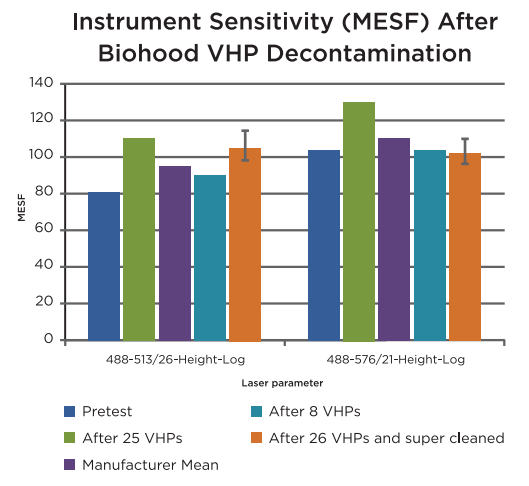

Fig. 15. Instrument sensitivity after VHP decontamination.

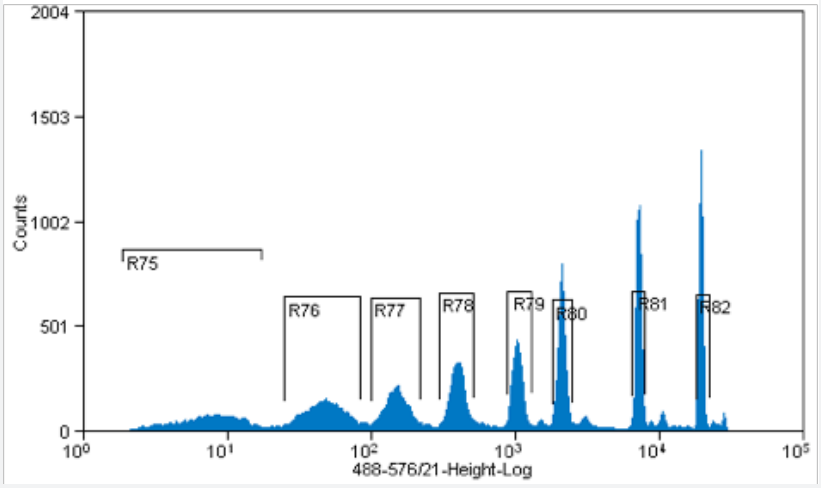

Fig. 16. Eight-peak bead performance on 488-576/21 during the VHP testing.

Conclusions of VHP Testing

- VHP does not degrade the Astrios performance as compared to manufacturing mean.
- There is more variation on day-to-day alignment than VHP performance degradation.
- Some moderate bleaching occurs with the anodized black components (new black coating developed).
- Some moderate loss of adhesive to stickers placed on the Astrios occurs (will be replaced as necessary).

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Results

BSC and Astrios Integration: System and software interlocks prevented hazardous situations from occurring.

AES Testing: Glo-Germ fluorescent particles were contained by the Aerosol Evacuation system regardless of the location of aerosol generation, with the AFS set to at least 20%.

BSC Testing: The Astrios passed all NSF/ANSI-49 tests.

VHP Impact: VHP decontamination testing showed equivalent system performance before and after decontamination and equivalent performance to newly manufactured instruments.

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