



**US Environmental Protection Agency  
Office of Pesticide Programs**

**Office of Pesticide Programs  
Microbiology Laboratory  
Environmental Science Center, Ft. Meade, MD**

**Standard Operating Procedure for  
Use and Maintenance of Biological Safety Cabinets**

**SOP Number: EQ-11-00**

**Date Revised: 04-18-14**

SOP Number	EQ-11-00
Title	Use and Maintenance of Biological Safety Cabinets
Scope	The purpose of this SOP is to describe the procedures used to monitor the use and maintenance of the Biological Safety Cabinets (BSC).
Application	Proper use and cleaning of the BSCs are the responsibilities of laboratory personnel. Performing maintenance and repairs on the BSCs and maintaining annual certification of the BSCs are the responsibilities of the Facility Manager or his designees (e.g., operations, maintenance and repair contractors).

	Approval	Date
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Date SOP issued:	
Controlled copy number:	
Date SOP withdrawn:	

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<b>1. Definitions</b>	Abbreviations/definitions are provided in the text.
<b>2. Health and Safety</b>	Follow procedures specified in SOP MB-01, Laboratory Biosafety. The Study Director and/or lead analyst should consult the Safety Data Sheet for specific hazards associated with products.
<b>3. Personnel Qualifications and Training</b>	Refer to SOP ADM-04, OPP Microbiology Laboratory Training.
<b>4. Instrument Calibration</b>	<ol style="list-style-type: none"> <li>1. BSC working parameters are controlled by the Facilities Manager or his designee.</li> <li>2. Work zone air downflow is uniform and held to an average velocity of 60 LFPM (0.30 m/s) measured in the plane of the bottom edge of the viewing window. The BSC will alarm if the average velocity falls below 54 LFPM or above 64 LFPM. The significance of this airflow control is that the work zone air is flowing down like a huge piston carrying contaminants rapidly out of the work zone with 24 air changes per minute. The work zone airflow is ducted such that the quantity of air leaving through the rear perforated area is half of the downflow air quantity. The remainder, that is the other half of the downflow air, and the air entering through the work access opening flow into the front perforated area. Room air flowing into the 8 inch (203 mm) work access opening forms the air barrier protecting personnel at 105 ±5 FPM (0.53 ±0.25 m/s). The exhaust volume is displayed on the front panel. The BSC alarms if the exhaust volume falls below 980 CFM or rises to above 1250 CFM.</li> </ol>
<b>5. Sample Handling and Storage</b>	Not applicable
<b>6. Quality Control</b>	<ol style="list-style-type: none"> <li>1. For quality control purposes, the required information is documented on the appropriate form(s) (see section 14).</li> <li>2. Certification: The BSCs are recertified annually by a qualified technician. A series of tests are performed on the BSC during recertification including the HEPA filter leak test and a measurement of the downflow blower speed (designated “Blower” or “Blower Speed” on the certificate and report).</li> <li>3. Scheduling for recertification: Scheduling recertification is the responsibility of the Facility Manager or his designee. Certificates are maintained by the Facility Manager. The OPP Microbiology Laboratory requests a copy of the Test and Certification Reports each year for review.</li> </ol>

	<p>4. HEPA filter usage and replacement:</p> <ol style="list-style-type: none"> <li>a. HEPA filters on each BSC do not need to be replaced under normal usage, and barring an accident (puncture), until the exhaust volume or the access flow velocity cannot be maintained. Making this determination and scheduling HEPA filter replacement is the responsibility of the Facility Manager or his designee.</li> <li>b. The Facility Manager takes into consideration the downflow blower speed (if <math>\geq 90\%</math>) and other indicators (e.g., age – 8 to 10 years) to determine the need for replacement of the HEPA filter.</li> <li>c. Once the HEPA filter is replaced, the BSC is recertified and the certification is annotated by a Performance Certification sticker.</li> </ol>
<p><b>7. Interferences</b></p>	<ol style="list-style-type: none"> <li>1. Appropriately clean and ensure certification of all BSCs when in use (see section 12.1).</li> <li>2. Laboratory staff performing work in the BSC must wear a long-sleeved lab coat and are encouraged to wear gloves even when not manipulating culture. This minimizes the shedding of skin flora into the work area and concurrently protects the hands and arms from viable agent contamination (see section 15).</li> <li>3. Gas burners contribute to heat build-up in the cabinet and disrupt the laminar air flow. Applying a flame to tube and flask necks is a conventional method of minimizing airborne contamination when the tubes or flasks are opened. However, because the laminar flow BSCs create an environment that has little to no airborne particulates, it is not necessary to flame tube and flask necks.</li> <li>4. If a procedure requires the use of a flame (e.g., to flame sterilize hooks, etc.), use a burner with a pilot light so that the flame can be ignited only when required. Do not use constant flame gas burners.</li> <li>5. Gas burners should only be used from the center of the work surface to the right rear, where resulting air turbulence will have a minimal effect. Do not use gas burners on the left of the work surface due to its influence on the electronic airflow control system (see section 15).</li> </ol>
<p><b>8. Non-conforming Data</b></p>	<ol style="list-style-type: none"> <li>1. Management of non-conforming data will be consistent with SOP ADM-07, Non-Conformance Reports.</li> <li>2. When BSCs need service, (e.g., alarms are sounding, the BSC fails to advance past the warm-up mode, the BSC is not functioning as it usually does, etc.), staff may notify the Facilities Helpline at (410) 305-4357. The Facility Manager or his designees investigate and make a service call if</li> </ol>

	necessary. Laboratory staff notes the event on the BSC Monitoring Record Form (see section 14) and documents any corrective action taken.
<b>9. Data Management</b>	1. Data will be archived consistent with SOP ADM-03, Records and Archives.
<b>10. Cautions</b>	<ol style="list-style-type: none"> <li>1. Clean the ultraviolet lamps in the BSC with a lint-free cloth dampened with alcohol on a quarterly basis. Failure to clean the ultraviolet lamps in the BSC will reduce the lamps' effectiveness.</li> <li>2. Check grills and diffuser grids periodically for spilled or splashed nutrients which may support microbial growth and result in the liberation of contaminants into the protected work environment. Quarterly cleaning of the area under the BSC work surface and grill is required (see section 12.2 a-g).</li> <li>3. A permanent paper catch is installed behind the rear divider panel of the work zone. This area forms the return air path to the motor/blower. If the air flow is blocked, it could seriously affect the performance of the cabinet. The paper catch should be checked and cleaned if procedures require the use of paper products. Any paper removed must be properly disposed of as contaminated hazardous waste.</li> </ol>
<b>11. Special Apparatus and Materials</b>	<ol style="list-style-type: none"> <li>1. BSC #1 NuAire Model No. NU-430-600 Serial No. 78536AFU located in room B204</li> <li>2. BSC #2 NuAire Model No. NU-430-600 Serial No. 78538AFU located in room B204</li> <li>3. BSC #3 NuAire Model No. NU-430-600 Serial No. 78537AFU located in room B204</li> <li>4. BSC #4 NuAire Model No. NU-430-600 Serial No. 78539AFU located in room B205</li> <li>5. BSC #5 NuAire Model No. NU-430-600 Serial No. 76788AFP located in room B207</li> <li>6. BSC #6 NuAire Model No. NU-430-600 Serial No. 76786AFP located in room B207</li> <li>7. BSC #7 NuAire Model No. NU-430-600 Serial No. 85756AGY located in room B202</li> <li>8. BSC #8 NuAire Model No. NU-430-600 Serial No. 85755AGY located in room B202</li> <li>9. BSC #11 NuAire Model No NU 430-600, Serial No. 90496042204 located in room B201</li> </ol>

	10. BSC # 12 NuAire Model No. NU 430-600, Serial No. 90474042104 located in room D122
<b>12. Procedure and Analysis</b>	Follow the instructions below for operation/use of the BSC as well as required quarterly maintenance.
12.1 Use and Operation	<ul style="list-style-type: none"> <li>a. Turn on the blowers, lights, and outlets, and allow equipment to operate for a minimum of 15 minutes before initiating aseptic manipulations.</li> <li>b. Disinfect the interior surface of the BSC work space thoroughly by spraying/wetting with an appropriate amount of an EPA registered hospital disinfectant; allow to stand for the label-specified contact time before wiping.</li> <li>c. Do not use halogenated materials (e.g., bleach). These materials are not recommended for use on stainless steel surfaces of the BSC.</li> <li>d. Place the apparatus and materials into the cabinet. Exercise care to ensure that no items are placed over the front intake grills. Arrange the work area so that clean and dirty (used) materials are well separated.</li> <li>e. Avoid passing contaminated materials over uninoculated cultures or clean glassware to avoid contamination.</li> <li>f. Transfer viable materials (such as test cultures) as deeply into the cabinet (away from open face) as possible.</li> <li>g. Allow purging of the work space without any user activity should for 2-3 minutes after materials and apparatus have been placed in the unit(s). This helps the area get rid of all contamination that may have been introduced with the items.</li> <li>h. Record the Downflow (FPM) and Exhaust (CFM) rates on the BSC Monitoring Record Form (see section 14) immediately prior to use.</li> <li>i. Perform steps 12.1 a-h prior to commencing work.</li> <li>j. After completion of work, allow the BSC to run for 2-3 minutes without activity to purge the unit.</li> <li>k. Remove all materials, cultures, and equipment and decontaminate BSC as in section 12.2.</li> <li>l. When working with bacteria in spore form (e.g., <i>Bacillus subtilis</i> spore suspensions, <i>Clostridium difficile</i> spore suspension, spore strips, etc.), as an additional step, turn on the ultraviolet light and leave it on overnight.</li> <li>m. Refer to the laboratory's Biosafety Plan for <i>Bacillus anthracis</i> for BSC clean up procedures following work with select agents.</li> <li>n. Turn off lights and outlets. Turn off blowers as per facility</li> </ul>

	<p>recommendations.</p> <p>o. Do not use cabinet as a repository for excess lab equipment during periods of non-operation.</p> <p>p. Record maintenance on the BSC Monitoring Record Form (see section 14).</p>
12.2 Quarterly Maintenance:	<p>a. Thoroughly clean and disinfect all BSCs on a quarterly basis.</p> <p>b. Remove panel and grill from the BSC and set aside.</p> <p>c. Disinfect all surfaces of the BSC thoroughly by spraying/wetting with an appropriate amount of a hospital disinfectant and allow to stand for the label-specified contact time before wiping.</p> <p>d. Disinfect panel and grill thoroughly by spraying/wetting with an appropriate amount of a hospital disinfectant and allow to stand for the label-specified contact time before wiping.</p> <p>e. Replace the panel and grill, and properly secure.</p> <p>f. Clean the ultraviolet lamps in the BSC with a lint-free cloth dampened with alcohol on a quarterly basis.</p> <p>g. Record quarterly maintenance on the BSC Monitoring Record Form (section 14).</p>
13. Data Analysis/ Calculations	None
14. Forms and Data Sheets	<p>Forms are stored separately from the SOP under the following file names:</p> <p>BSC Monitoring Record Form EQ-11-00_F1.docx</p> <p>BSC Quarterly Maintenance Record Form EQ-11-00_F2.docx</p>
15. References	<p>1. Bordner, R.H., Winter, J.A. &amp;, Scarpino, P.V., eds. 1978. Microbiological Methods for Monitoring the Environment, Water and Wastes. EPA 600/8-78-017, Environmental Monitoring &amp; Support Lab., U.S. Environmental Protection Agency, Cincinnati, Ohio.</p> <p>2. NuAire Labguard Class II, Type B2 Laminar Flow Biological Safety Cabinet Operation and Maintenance Manual.</p>