

**Frederick National Laboratory
for Cancer Research**

sponsored by the National Cancer Institute

Vaccine, Immunity and Cancer Directorate
Standard Operating Procedure

SOP Title: Biosafety Cabinet (BSC) Use and Maintenance

Document ID: 26000

Version

2.0

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Released by / Effective Date:

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1. PURPOSE

- 1.1. The purpose of this procedure is to describe the use and maintenance of the Biosafety Cabinets (BSC).

2. SCOPE

- 2.1. This procedure applies to all BSCs.

3. REFERENCES

- 3.1. EHS-WM-1: Disposal and Minimization of Chemical Waste
- 3.2. EHS-WM-2: Biological Waste Handling and Disposal
- 3.3. ISM144: Effective Use of Biological Safety Cabinets Safetygram
- 3.4. HSL_GL_001: Waste Disposal at the Advanced Technology Research Facility
- 3.5. HSL_LAB_005: Plasmid DNA Transfection in HEK293TT for VLP Production and Purification
- 3.6. 10007: Non-Routine Equipment Maintenance
- 3.7. 10009: General Record Review

4. RESPONSIBILITIES

- 4.1. The Research Associate, hereafter referred to as Analyst, is responsible for reviewing and following this procedure, and documenting performance of equipment maintenance.
- 4.2. The Scientific Manager or designee is responsible for training personnel in this procedure and reviewing associated documentation.
- 4.3. The Quality Assurance Specialist is responsible for quality oversight and approval of this procedure.
- 4.4. Trained personnel perform equipment maintenance record review per "10009: General Record Review."

5. DEFINITIONS

- 5.1. As Needed Maintenance – maintenance that is performed outside of routine maintenance but is not performed in response to equipment malfunction.
- 5.2. Routine Maintenance – maintenance that is performed at planned intervals to identify and prevent problems before they result in equipment failure.

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- 5.3. Non-routine Maintenance – maintenance that is performed in response to equipment malfunction or failure.

6. REAGENTS, MATERIALS AND EQUIPMENT

- 6.1. Biosafety Cabinet (BSC), Class II
- 6.2. Bleach, Clorox, Concentrated (FNLCR Warehouse, Cat # 68100251 or equivalent)
- 6.3. Cavicide (FNLCR Warehouse, Cat # 79300360 or equivalent)
- 6.4. Filter, Vacushield, Vent Device PTFE (PALL, Cat # 4402 or equivalent)
- 6.5. 1 L Flask with Optional Ring Weight
- 6.6. Hood Cleaning Wand (Swiffer, Cat # PGC09060 or equivalent)
- 6.7. Ster-ahol (VWR, Cat # 14003-358 or equivalent)
- 6.8. Wipe, Low-Lint, Wypalls (FNLCR Warehouse, Cat # 79300335 or equivalent)
- 6.9. Wipe, Ultra Low-Lint, TechniCloth (VWR, Cat# TWTX1112 or equivalent)

7. HEALTH AND SAFETY CONSIDERATIONS

- 7.1. Proper safety precautions should be taken while working in a laboratory setting. This includes, but is not limited to, proper protective equipment such as lab coats, safety glasses, closed-toe shoes, and non-latex gloves.
- 7.2. Refer to the respective Safety Data Sheet (SDS) when working with any chemicals.
- 7.3. Refer to “HSL_GL_001: Waste Disposal at the Advanced Technology Research Facility,” “EHS-WM-001: Disposal and Minimization of Chemical Waste,” and “EHS-WM-002: Biological Waste Handling and Disposal” regarding waste disposal processes at the respective location.
- 7.4. If the BSC or its blower was turned off, or if the sash was lowered until closed, turn on the BSC and blower, and allow the fan to run for at least 30 minutes before using the BSC to ensure a clean, filtered working environment.
- 7.5. UV lights will not be used in BSCs.
- 7.6. Any item placed inside the BSC (such as pipettes, pens, vortex mixers, microcentrifuges, spray bottles), should be considered “dirty” and must be decontaminated before removing.
- 7.7. Whenever possible, unplug equipment inside BSC when not in use.

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- 7.8. When outlets inside BSC are not in use, ensure the electrical plug covers are in place. Do not spray disinfectant near an uncovered electrical plug.
- 7.9. Use a Hood Cleaning Rod to clean difficult to reach spaces inside BSC. Limit how far personnel reach into BSC to no more than arm's length.

8. EQUIPMENT USE

8.1. General Information

- 8.1.1. When working in a BSC, do not block airflow by placing objects or arms on the front air grill or the rear panel. This will disrupt the airflow and could contaminate both the experiment and the air outside of the hood. See Attachment 1 for BSC airflow details.
- 8.1.2. The number of items stored in the BSC must be kept at a minimum and placed away from air vents so they do not disrupt proper airflow.
- 8.1.3. No more than 3 boxes of pipette tips and 1 of the following can be stored in the BSC when not in use: vacuum aspirator, vortex mixer, or semiautomated pipetting device (epMotion 96, Hydra 96, or Liquidator 96).
- 8.1.4. No other items and no more than 4 items can be stored in the BSC when not in use. See Attachment 2 for an example of general layout.
- 8.1.5. Pipette tip waste boxes are removed from the BSC at the end of the workday.
- 8.1.6. The BSC is cleaned daily prior to use, after each use, and as needed during use. If the hood is not used, no daily cleaning needs to be performed.
- 8.1.7. When cleaning, wipe the hood from top to bottom and back to front, finishing with the work surface and front grill. Use the hood cleaning rod to reach the rear wall when cleaning.
- 8.1.8. Cavicide, if available, is used as the primary disinfectant cleaner. When cleaning with Cavicide, allow a minimum 3-minute contact time before wiping with a low-lint wipe. If Cavicide is not available for use, the Scientific Manager provides a substitute and guidance for use.
- 8.1.9. Ster-ahol is never used as a primary disinfectant cleaner. Ster-ahol is used as a secondary cleaning agent to remove precipitate or residue left behind from Cavicide or other primary disinfectant cleaner. Ster-ahol must be sprayed on low-lint wipe outside of the BSC, never sprayed directly on the surface inside a BSC.

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- 8.1.10. The vacuum aspirator must always have a vacushield filter linked between the vacuum source and aspirator's in-line filter system attached to tubing. See Attachment 2 for example of set up. If vacuum flow is slower than expected, change vacushield filter.
- 8.1.11. Proper precautions are taken to ensure there is no cross contamination.
- 8.1.12. Maintain Clean to Dirty workflows.
- 8.1.13. Clean the interior work surface if a spill occurs, when an experiment is finished, and between Analysts.
- 8.1.14. Additional precautions are decided on a case by case basis, or when recommended by the Scientific Manager.
- 8.1.15. The plenum is defined as the air-filled space inside the BSC.
- 8.1.16. If an outside contractor performs maintenance on the BSC, it is the responsibility of the Analyst to enter the information on "26000-02: BSC Maintenance Form."
- 8.1.17. See Attachment 3 for common process codes used on Form "26000-01: BSC Daily Use Form."
- 8.1.18. If a BSC is equipped with a UV light, the UV light may not be used as a method to disinfect (germicidal) the cabinet.
- 8.1.19. Any externally exhausted BSC must have the blower motor active with the glass panel sash raised to the appropriate level all the time. Ensure the sash level monitor is turned on before using BSC.
- 8.1.20. BSCs which are not Type B2 Units may have the glass panel sash closed after the blower motor has been turned off. Type B2 Units are externally vented and should never have their glass panel sash closed.
- 8.1.21. If glass panel sash is closed, Analyst must raise the sash to the appropriate level, turn on the blower motor, and allow the blower to run for at least 30 minutes prior to use.
- 8.1.22. All electrical devices stored in the BSC must be unplugged from the electrical outlet when not in use, unless a weatherproof cover completely surrounding the electrical plug is in place.

8.2. Daily Use

- 8.2.1. Record daily use on 26000-01.

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- 8.2.2. Prior to beginning work in BSC, spray inside with primary disinfectant. Allow disinfectant to sit for recommended time. Wipe down BSC using a clean, low-lint wipe.
- 8.2.3. Ster-ahol may be used as a secondary disinfectant to remove residue following primary disinfectant cleaning. If using Ster-ahol, spray on clean, low-lint wipe outside of BSC then wipe down hood.
- 8.2.4. The aspirator may be used as a vacuum source for filtering reagents and media without adding any disinfectant.
- 8.2.5. When aspirating biological fluids, add bleach (at 10% final concentration) to the aspirator's 1L flask before use.
- 8.2.5.1. Allow the bleach and biological material to be in contact for 30 minutes before disposal. The contents in the flask may be safely poured down the laboratory sink with copious water.
- 8.2.5.2. Rinse the aspirator (flask and tubing) with tap water and allow to dry before use. Do not get vacushield filter wet.
- 8.2.6. Clean hood with ster-ahol between experiments, and between Analysts to prevent cross contamination.
- 8.2.7. If multiple Analysts performs subsequent processes (Process #2, Process #3) in BSC, each Analyst records information in the column of form 26000-01 which is initiated by the first analyst.
- 8.2.7.1. First Analyst to use BSC for the day documents: date, airflow, disinfectant information, process #1 code, and initials.
- 8.2.7.1.1. When BSC has a digital or mechanical numerical display for airflow, record numerical display.
- 8.2.7.1.2. See Attachment 3 for process codes.
- 8.2.7.2. Document Process #2 information; N/A if not Applicable.
- 8.2.7.3. Analyst who performs Process #2 initials under Process #2 code.
- 8.2.7.4. Document Process #3 information; N/A if not Applicable.
- 8.2.7.5. Analyst who performs Process #3 initials under Process #3 code.
- 8.2.7.6. Indicate which Disinfectant(s) was (were) used for Shutdown.

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8.2.7.7. Analyst who performs Shutdown initials in Comments.

8.2.8. If performing separate Transfections ("HSL_LAB_005: Plasmid DNA Transfection in HEK293TT for VLP Production and Purification") within the same day in the same BSC, record the second Transfection under Process #2.

8.2.9. After use at the end of the day, clean and remove all items from the BSC with primary disinfectant, except items listed in section 7.1.2.

8.2.10. Clean BSC with primary disinfectant per step 7.2.2. Ster-ahol may be used following the Cavicide cleaning per step 7.2.3.

8.3. Quarterly Maintenance

Note: Quarterly Maintenance is performed once a quarter when BSC is in use. When BSC is not in use, a note is recorded, and quarterly cleaning must be performed prior to returning BSC to routine use and maintenance.

8.3.1. Disinfect and remove all items from inside BSC.

8.3.2. Soak aspirator (flask and tubing,) if used, with 10% bleach, then rinse three times with tap water. Do not clean/soak in-line filter. Allow aspirator to air dry overnight prior to use.

8.3.3. Turn off hood fan prior to cleaning the BSC.

8.3.4. Spray low-lint wipes with primary disinfectant and wipe all stationary internal components of the BSC, such as the screens, work surface, side and rear panels. See Attachment 4 for surface cleaning guidance.

8.3.5. Remove work surface panel by the handles from BSC and support beams.

Note: It is recommended to have two Analysts lift work surface panel.

8.3.6. Clean negative pressure tray (BSC surface below work surface panel), the underside of work surface panel, and support beams with primary disinfectant.

8.3.7. Allow primary disinfectant to sit for recommended time, and wipe using a clean, low-lint wipe; a hood cleaning rod may be used for hard to reach places in BSC.

8.3.8. Spray secondary disinfectant (Ster-ahol) on clean, low-lint wipe, and wipe hood surfaces and work surface panel.

8.3.9. Reassemble hood and turn on hood fan.

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8.3.10. Allow air to circulate in BSC for at least 30 minutes prior to use.

8.3.11. Change in-line vacuum filter quarterly.

8.3.12. Document maintenance on 26000-02.

8.4. As Needed Maintenance

Note: Document as needed maintenance in its respective section on form 26000-02.

8.4.1. Change in-line vacuum filter when noticeably contaminated.

8.4.2. In the case of a biological spill within the BSC follow the quarterly maintenance steps outlined in section 8.3.

8.5. Non-routine Maintenance

8.5.1. In the case that the BSC is not operating correctly, transition processes being performed to another unit (when applicable), post a sign stating the equipment is out of service and initiate non-routine maintenance documentation per "10007: Non-Routine Equipment Maintenance."

8.5.2. Document the nature of any failures or malfunctions, how and when it was discovered, and the personnel involved on "10007-01: Non-Routine Equipment Maintenance Form."

8.5.3. Initiate a service request and complete the non-routine maintenance process following 10007.

9. ATTACHMENTS

9.1. Attachment 1: Airflow in a BSC

9.2. Attachment 2: BSC Layout

9.3. Attachment 3: Common Codes

9.4. Attachment 4: Quarterly BSC Cleaning

9.5. Attachment 5: 26000-01, BSC Daily Use Form

9.6. Attachment 6: 26000-02, BSC Maintenance Form

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10. REVISION HISTORY

Version	Change	Reason
1.0	New document number assigned. As needed and non-routine maintenance section added. Forms reformatted.	Reflect current practice.
2.0	Update quarterly maintenance procedure Daily Use form is updated to reflect, BSC flow rate Maintenance form is updated to reflect, as needed maintenance	Clarify quarterly maintenance and reflect current practices

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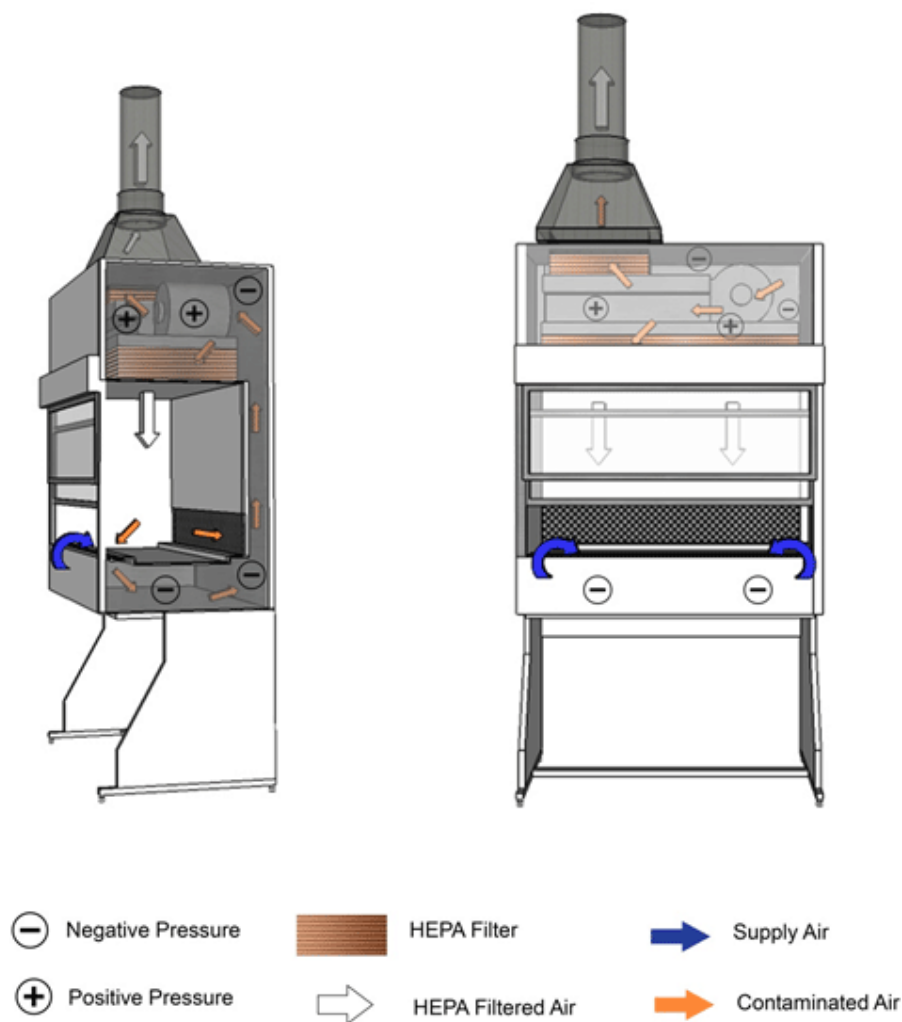
2.0

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Attachment 1: Airflow in a BSC



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Attachment 2: BSC Layout

1L Flask secured with green ring weight
connected to in-line vacushield filter and



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Attachment 3: Common Codes

Process Code	Process Description
AVIDITY	HPV or SARS-CoV Avidity Assay
BCA	BCA Protein Assay
CELLS	Cell Maintenance
ELISA	HPV Type Specificity Assay, HPV ELISA, or SARS-CoV ELISA
GEL	Coomassie Blue Gel Set Up
LF	Lateral Flow or Point of Care Assay
LUM	Luminex Assay
PBNA	Pseudovirion Based Neutralization Assay
PLASMID-HPV TYPE	Plasmid Purification
REAGENT	Reagent Preparation
SERUM	Serum Isolation and Aliquots
TSFXN-HPV TYPE	HPV Type-specific Transfection Process

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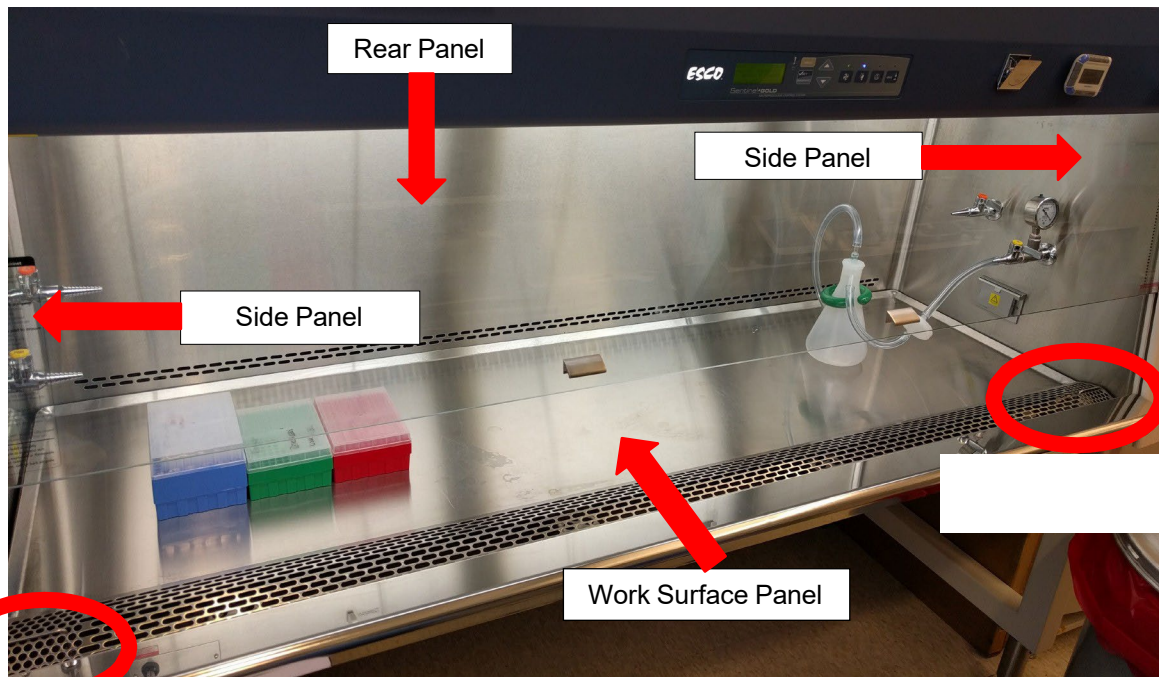
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Attachment 4: Quarterly BSC Cleaning



Work Surface Panel
Handle

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Attachment 5: 26000-01, BSC Daily Use Form

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Form Title: BSC Daily Use Form			
Document ID: 26000-01		Version:	DRAFT
Associated SOP: 26000		Effective Date:	TBD
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Equipment ID:		Year:			
Date:					
Air flow ok?	Value?	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
Primary Disinfectant Lot:					
Primary Disinfectant Exp:					
Secondary Disinfectant Lot:	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A
Secondary Disinfectant Exp:	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A
Start Up:	<input type="checkbox"/> Primary Disinfectant <input type="checkbox"/> Secondary Disinfectant	<input type="checkbox"/> Primary Disinfectant <input type="checkbox"/> Secondary Disinfectant	<input type="checkbox"/> Primary Disinfectant <input type="checkbox"/> Secondary Disinfectant	<input type="checkbox"/> Primary Disinfectant <input type="checkbox"/> Secondary Disinfectant	<input type="checkbox"/> Primary Disinfectant <input type="checkbox"/> Secondary Disinfectant
Process 1:					
Initials:					
Clean:	<input type="checkbox"/> N/A <input type="checkbox"/> Primary Disinfectant <input type="checkbox"/> Secondary Disinfectant	<input type="checkbox"/> N/A <input type="checkbox"/> Primary Disinfectant <input type="checkbox"/> Secondary Disinfectant	<input type="checkbox"/> N/A <input type="checkbox"/> Primary Disinfectant <input type="checkbox"/> Secondary Disinfectant	<input type="checkbox"/> N/A <input type="checkbox"/> Primary Disinfectant <input type="checkbox"/> Secondary Disinfectant	<input type="checkbox"/> N/A <input type="checkbox"/> Primary Disinfectant <input type="checkbox"/> Secondary Disinfectant
Process 2:	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A
Initials:	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A
Clean:	<input type="checkbox"/> N/A <input type="checkbox"/> Primary Disinfectant <input type="checkbox"/> Secondary Disinfectant	<input type="checkbox"/> N/A <input type="checkbox"/> Primary Disinfectant <input type="checkbox"/> Secondary Disinfectant	<input type="checkbox"/> N/A <input type="checkbox"/> Primary Disinfectant <input type="checkbox"/> Secondary Disinfectant	<input type="checkbox"/> N/A <input type="checkbox"/> Primary Disinfectant <input type="checkbox"/> Secondary Disinfectant	<input type="checkbox"/> N/A <input type="checkbox"/> Primary Disinfectant <input type="checkbox"/> Secondary Disinfectant
Process 3:	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A
Initials:	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A
Shutdown:	<input type="checkbox"/> Primary Disinfectant <input type="checkbox"/> Secondary Disinfectant	<input type="checkbox"/> Primary Disinfectant <input type="checkbox"/> Secondary Disinfectant	<input type="checkbox"/> Primary Disinfectant <input type="checkbox"/> Secondary Disinfectant	<input type="checkbox"/> Primary Disinfectant <input type="checkbox"/> Secondary Disinfectant	<input type="checkbox"/> Primary Disinfectant <input type="checkbox"/> Secondary Disinfectant
Comments:	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A
	Initials:	Initials:	Initials:	Initials:	
Reviewed by/date:					

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Attachment 6: 26000-02, BSC Maintenance Form

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Maintenance Year:

Equipment ID:

Quarterly Maintenance

Quarter	Q1	Q2	Q3	Q4
Primary Disinfectant Lot Number				
Primary Disinfectant Expiration Date				
Secondary Disinfectant Lot Number				
Secondary Disinfectant Expiration date				
Bleach Lot Number	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A
Bleach Expiration Date	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A
Performed by/date:				
Reviewed by/date:				

As Needed Maintenance

Date	Activity Performed	Performed by/date	Reviewed by/date

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