BIOSAFETY CABINET USE AND SAFETY

INTRODUCTION

Biological Safety Cabinets, (BSCs), also known as tissue culture hoods, are designed to provide personnel, environmental and product protection when appropriate practices and procedures are followed. Videos demonstrating the proper use of BSCs are available for Stanford personnel at Research & Laboratory Safety - BioSafety. Information provided in this fact sheet supplements the videos.

THE CLASS II BSCs

Class II BSCs rely on directional movement of air to provide containment. Airflow is drawn into the front grille of the cabinet, providing personnel protection. The most commonly used BSC is a Class II A2, shown in Figure 1. The A2 recirculates 70% of air and is not suitable for volatile solvents or isotope usage. For chemical use within a BSC contact EH&S (723.0448).

Figure 1. Class II A2 BSC

INSTALLATION, MAINTENANCE AND CERTIFICATION

BSC installation, required annual certification, decontamination and maintenance **must** be done by certified (accredited by the National Sanitation Foundation) professionals. Arrangements and payment for any of the above work is to be scheduled by the PI or the Department.

TSS. Inc. is Stanford University's designated vendor for Biosafety cabinet service; they can be reached at 1.800.877.7742.

WORK PRACTICES

Personal Protective Equipment

- Appropriate personal protective equipment (PPE) must be worn. Lab coats must be buttoned. Gloves should be pulled over the wrists of lab coat, not worn inside coat. Additional PPE to be used as recommended.

Preparing BSC for work

- Confirm BSC annual (within 12 months) certification is current; information found on sticker on front of BSC.
- Operate cabinet blowers at least 3-5 minutes before beginning work to allow the BSC to "purge" particulates.
- Use alcohol to clean work surface of BSC and to sterilize any glass, etc. that is being used; amount of alcohol in BSC must be for only one day's work.

Working in the BSC

- When working in the cabinet, move arms in and out slowly, perpendicular to the face opening to reduce disruption of air curtain.
- Perform all operations at least 4 inches from the front grille on the work surface.
- For BSC clean-up, apply alcohol using wipes vs. spray bottles to minimize solvent vapor concentrations being re-circulated in the hood. Cabinet sash to remain open to allow for alcohol evaporation; sash can be lowered after sufficient time. The recommended minimum time for sash opening is 10 minutes.
- Do not bring potentially contaminated materials out of the cabinet until they have been surface decontaminated. Alternatively, contaminated materials can be placed into a closable container for transfer to an incubator, autoclave, or another part of the laboratory.

MATERIAL PLACEMENT WITHIN THE BSC

Disposable underpads can be placed on the work surface but must not cover the front or rear grille openings. The use of toweling facilitates routine cleanup and reduces splatter and aerosol generation during an overt spill.

- Place all material as far back in the cabinet as practical, toward the rear edge of the work surface and away from the front grille of the cabinet.
- Place aerosol-generating equipment (e.g. vortex mixers, tabletop centrifuges) toward the rear of the cabinet.
- The workflow should be from "clean to dirty" Materials and supplies should be placed in the cabinet in such a way as to limit the movement of "dirty" items over "clean" ones.

LIQUID WASTE

A vacuum flask system is required to provide protection to the central building vacuum system or vacuum pump and to personnel who service the equipment. Figure 2 illustrates a proper set-up for handling liquid waste.

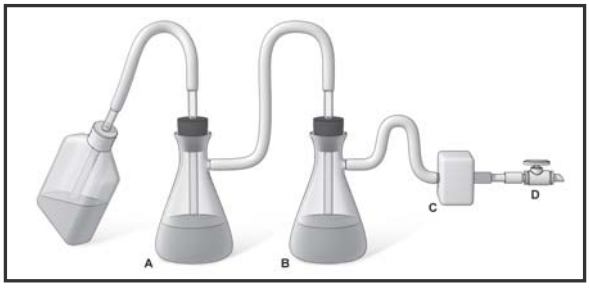

Figure 2. Liquid Waste. The left suction flask (A) is used to collect the contaminated fluids into a suitable decontamination solution; the right flask (B) serves as a fluid overflow collection vessel. An in-line HEPA filter (C) is used to protect the vacuum system (D) from microorganisms.

- Connect the primary flask to an overflow collection flask and to an in-line HEPA filter.
- Both flasks shall contain an appropriate disinfectant for the material used.
- The vacuum flasks may be set up within the cabinet; however, to save room, the system can be placed on the floor beneath or next to the BSC, using a secondary container to contain the flasks and a longer hose connection to the vacuum system.
- Once inactivation occurs, liquid materials can be disposed of as noninfectious waste. Empty the waste from the flask when it reaches no higher than ¾ full. Replace the flask with fresh disinfectant.

OPEN FLAMES IN BIOSAFETY CABINETS

- Per Stanford University policy (Open Flames in BSCs) **open flames in BSCs are not to be used**. Remove Bunsen burners and/or replace with alternative technology (discussed below).
- If a researcher requests to use open flames, Biosafety personnel, working with SoM Health and Safety, if appropriate, will meet with the researcher and discuss issues and solutions.
- If it is deemed absolutely necessary for the work being done, use a pilotless burner or safety touch-plate microburners to provide a flame on demand. It must be emphasized that this practice should only be used as a last resort for resistant researchers. The cost of these burners is quite high ($600 +).

Stanford University has taken a strong stance **against the use** of gas burners or alcohol flames in Biosafety cabinets. This decision has been made in accordance with recommendations from numerous agencies. The Centers for Disease Control and Prevention (CDC) reports that "open-flames are not required in the near microbe-free environment of a biological safety cabinet" and create "turbulence which disrupts the pattern of air supplied to the work surface", jeopardizing the sterility of the work area. This is also the recommendation of the World Health Organization (WHO) as well as the major Biosafety cabinet manufacturers.

Early microbiologists had to rely on open flames to ensure sterility. With the advancement of modern technology, including the introduction of the Biosafety cabinet, the use of an open flame is no longer necessary.

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The use of open flames in a BSC:

- Disrupts the air flow, compromising protection of both the worker and the work.
- Causes excessive heat buildup, may damage HEPA filters or melt the adhesive holding the filter together, thus compromising the cabinet's integrity (Figure 3).
- Presents a potential fire or explosion hazard. Electrical components such as the fan motor, lights and electrical outlets are not designed to operate in flammable atmospheres, where a flash fire could be ignited by a spark.
- Inactivates manufacturers' warranties on the cabinet; cabinet manufacturers will assume no liability in the event of fire, explosion or worker exposure due to the use of a flammable gas in the cabinet. Additionally, the UL approval will automatically be void.

Figure 3. Don't let this be your cabinet! Fire damage resulting from the use of an open flame and alcohol inside a BSC.

UV LIGHTS

Numerous factors affect the activity of the germicidal effect of UV light, which require regular cleaning, maintenance and monitoring to ensure germicidal activity.

The Center for Disease Control (CDC) and the National Institute of Health (NIH) agree that UV lamps are not recommended nor required in Biological Safety Cabinets (BSCs).

UV lamps must be turned off when the room is occupied to protect eyes and skin from UV exposure, which can burn the cornea and cause skin cancer. Proper use and cleaning of BSCs negates any need for the use of UV lamps.

REFERENCES

1. NSF International (NSF) Standard 49; Class II (Laminar Flow) Biohazard Cabinetry, The NSF Joint Committee on Biohazard Cabinetry, May 1992.
2. Ultraviolet Radiation Exposures in Biomedical Research Laboratories, Mark L. Noll. Appl. Occup. Environ. Hyg. 10(12) December, 1995, pp. 969-972.
3. Centers for Disease Control and Prevention; The National Institutes of Health. Biosafety in microbiological and biomedical laboratories. 4th ed. Washington, DC. 1999.
4. America Biological Safety Association (ABSA); Position Paper on the Use of Ultraviolet Lights in Biological Safety Cabinets: http://www.ehs.umass.edu/ABSA%20UV%20light%20paper.pdf

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