

**SOP: SP041.2**

**Edited by: DRM 3/28/2023**

**General Use of Biosafety Class II Hood**

**Materials:**

1. 70% Ethanol
2. 2.5% Vesphene
3. Wypall
4. Swiffer
5. Pipette Basket and Pipette Jar.

**Protocol:** (note 1)

1. \_\_\_ Ensure that adequate time has been pre-booked on the sign-up calendar in llll to perform the work in question. UV light should be turned on for at least 30 minutes prior to use.
2. \_\_\_ Wash hands and forearms thoroughly (~2minutes).
3. \_\_\_ A clean lab gown (dedicated to tissue culture usage only) and new gloves must be worn at all times while working in the Biosafety cabinets (note 2). Spray gloves with 70% EtOH prior to entering biosafety hood.
4. \_\_\_ Before commencing work, ensure that all supplies to be used are present, and that an adequate number of pipettes and materials such as 70% ethanol and 2.5% Vesphene are available (note 3).
5. \_\_\_ Turn the UV-light off.
6. \_\_\_ Open the window of the Bio-safety cabinet to the correct height (marked on the cabinet) and immediately turn the fan blower on.
7. \_\_\_ Wrap a Wypall around a Swiffer (note 4). Generously spray the Wypall with 2.5% Vesphene and wipe down all areas of the BSC. (note 5)
8. \_\_\_ Wrap a new Wypall around the Swiffer. Generously spray the Wypall with 70% Ethanol and wipe down all areas of the BSC.
9. \_\_\_ All materials introduced into the hood should be sprayed down with 2.5% Vesphene and then 70% Ethanol prior to entry to prevent contamination of the work.
10. \_\_\_ To maximize sterility in the biosafety hood, keep items in the center of the hood and at least 6 inches behind the glass. Ensure that nothing is laying against the intake vents.
11. \_\_\_ Good Sterile Technique must be observed at all times regardless of the work to be performed (note 6).
12. \_\_\_ All spills should be cleaned immediately with 2.5% Vesphene and then 70% Ethanol.
13. \_\_\_ When completed, remove all materials used from the Biosafety cabinet.
14. \_\_\_ Wrap a Wypall around a Swiffer. Generously spray the Wypall with 2.5% Vesphene and wipe down all areas of the BSC.
15. \_\_\_ Wrap a new Wypall around the Swiffer. Generously spray the Wypall with 70% Ethanol and wipe down all areas of the BSC.
16. \_\_\_ Turn off the blower fan.
17. \_\_\_ Close the Biosafety cabinet window and turn the UV light on.

18. \_\_\_ Dispose of all waste materials generated in the appropriate location: biohazardous material in the biohazard waste, pipettes in the pipet canister.
19. \_\_\_ Ensure that the area around the hood is clean and that general use material (e.g. Pipettes) are well stocked.
20. \_\_\_ Check the general waste bin and Pipette canister and if full please dispose of the waste in an appropriate fashion (note 7).

**Notes:**

1. This protocol refers to the use of the C210 Biosafety Class II hood for routine work, i.e. non-hazardous, to maintain the sterility of reagents and prepared buffers etc. Further guidance must be sought when using biological samples such as bacterial strains or for tissue culture purposes. Such work must not be performed until adequate training has been received by senior lab personnel and their approval has been given for such work. Consult the CSU Environmental Health Services office for appropriate biosafety certifications.
2. A new pair of gloves must be worn each time the user works in the hood.
3. When working with any Microorganisms for the first time, seek guidance from senior lab personnel before beginning any work.
4. The Swiffer should be on top of the BSC.
5. Make sure to clean all three walls and floor of the BSC. Also wipe down the grate and inner glass.
6. Laboratory personnel unfamiliar with such practices must seek guidance and where appropriate training from other lab members before commencing work.
7. For biohazardous trash, seal the bag with autoclave tape, write the lab name, date and individuals name and mark the bag "Autoclave and dispose". Replace the autoclave bag in the biohazard bin. For used pipettes, remove the pipette basket from the 1% Lysol, drain the pipettes of excess Lysol, and place in a bio-hazard autoclave bag and mark as above. Return the inner holder to the Lysol reservoir and return to the hood area.