

## PROTOCOL REQUEST INSTRUCTIONS

These protocol submission guidelines are required to facilitate the protocol submission and approval process, and for maintaining accurate inventory of multiple versions of all BSL-3 protocols.

The following template provides a standardized format for the information required, however content may be modified as necessary to meet the specific aims of the study and development of the protocol.

Each Protocol Submission consists of two parts:

- a. [Protocol Submission Form](#): This form must be completed and submitted along with each protocol. This form contains prompts for required administrative information.
- b. [Main Body and Appendices of the protocol](#): This document template includes instructions, as well as the standard format and language for submitting your protocol.

Both the Protocol Submission Form and the Main Body of the Protocol with Appendices (along with any supporting documents) must be submitted to the BSL3 RA for review by the BSL3 Advisory Committee. Submissions are due 14 days before the next scheduled Advisory meeting.

2. For protocol amendments, a “Summary of Changes” should be provided as the first page (page i) of the document, as indicated in the template. The Summary of Changes must provide hyperlinks or specific reference to all changes in the protocol. If there are no changes or if this is the first submission, please delete and do not submit that page.
3. All Protocol Template instructions and prompts are in *blue italics*. *Please delete the italicized text* and replace it with your entry in your formatted text.
4. Emphasized instructions or information will have **red highlighting**.
5. All sections in the Protocol Template should be retained to facilitate rapid review. If a section is not appropriate or needed for your protocol, please *delete the text in blue italics* and insert “Not Applicable” after the section number.
6. Depending on the on the type of submission, format your information as follows:
  - No highlighting – for original text
  - **Yellow highlighting** – for changes from the last submitted protocol
  - **Green highlighting** – for additions from the last submitted protocol
5. For reviews, changes or corrections, please retain the ORIGINAL TITLE FOR ALL VERSIONS OF THE SAME PROTOCOL for ease of identification and review. If you wish to change the protocol title, please request this change separately.
  - a. Each protocol submission must have a corresponding version number and version date. As reviews or changes are submitted and approved (Summary of Changes page required), the version number and version dates must be updated.
6. A Letter of Approval will be filed when the protocol is approved

7. Note that we cannot accept MS Word files that:
  - i. are read-only
  - ii. are password protected or contain macros
  - iii. are saved with a file extension other than .doc or docx
8. For problems or questions encountered with any submission documents, please contact the BSL-3 RA by e-mail ([mm39@case.edu](mailto:mm39@case.edu)).

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NEW	REVIEW	AMENDMENT		PERSONNEL CHANGE	OTHER

## SUMMARY OF CHANGES

*Please provide a list of changes from the previous approved version of the protocol. All changes must be described in a point-by-point format with specific reference to page and section numbers OR with hyperlink to the location of the change (i.e., Page 3, section 1.2, replace 'xyz' and insert 'abc'). When appropriate, a brief justification for the change should be included.*

***If there are no changes or if this is the first submission, please delete and do not submit this page!***

#	Page/ Section	Change
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		

For easy identification, please highlight the changes in the body of your protocol as follows:

- No highlighting – for original text
- **Yellow highlighting** – for changes from the last submitted protocol
- **Green highlighting** – for additions from the last submitted protocol

*(Please retain the section break below, so that the protocol begins on page “1” of the document.)*

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***Remove this text and insert title here - Title:(Format: Arial size 16 bold)***

## INTRODUCTION

*Remove this text and insert Introduction here (optional)*

### 1. PATHOGEN(S)

*Remove this text and insert the full name of your pathogen here*

### 2. EQUIPMENT OR MATERIALS REQUIRED

*Remove this text and insert equipment and materials here*

### 3. METHODS

*Remove this text and insert your protocol methods here*

### 4. REFERENCES

*Remove this text and insert references here*

### 5. BSL-3 STANDARD OPERATIONS

*Remove this text and edit this section below to include all SOPs relevant to your protocol*

#### A. DISINFECTION:

- a. Disinfection with 15% bleach made fresh daily.
- b. Carboy: A 20% bleach solution is freshly made daily when needed for decontamination of plasticware.

B. Sterile, filter barrier tips will be used for all procedures.

C. All items in the incubator will be labeled by lab/user/date. Items will be checked at least once weekly and documented. If there is evidence of contamination, the affected items will be immediately bagged, sprayed with 15% bleach, double bagged and then autoclaved. The RA will then be

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notified.

- D. Transportation to and from the incubator will be done in a sealed Tupperware container. Flasks will remain in Tupperware container within the incubator, with only a crack opened.
- E. Transportation to and from the centrifuge will be within the sealed centrifuge bucket(s) or rotors.. Buckets will be loaded and unloaded inside the BSC only. The outer surface of the vials will be decontaminated upon removal from the safety buckets or rotors. The centrifuge bucket/rotor will be decontaminated before being placed inside the centrifuge. Decontamination will be done with **15% bleach.**
- F. All pipet tips will be ejected into a trough filled with after filling the tip first with bleach solution. Beginning concentration of bleach is 20% (200ml in 1L) to allow for residual fluids not to exceed 200ml for a final concentration between **15% and 20% bleach**
- G. PREPARATION: Before starting work in the BSC in BSL3:  
**All work will be done inside the BSC. Make sure to have inside of the cabinet:**
  - a. Beaker containing 100 - 150 ml bleach for a final volume up to 1 L of waste. All liquid waste generated including culture media, sample, reagent solutions, and washes will be disinfected following use with a final concentration of **15% fresh bleach solution for at least 20 minutes.**
  - b. Trough with bleach solution to decontaminate tips and pipettes and residual (under 200ml total) liquid waste only.  
Decontaminate all tips, needles, and pipettes by placing them in provided troughs filled  $\frac{1}{3}$  with **20% starting solution of bleach (200 ml bleach per 1L).** Make sure to draw up bleach into the pipette/tip before ejecting it into the tip collection tray. Allow trough contents to soak in bleach solution for **at least 20 minutes.**
  - c. A biohazard bag lining the small bucket in the hood to collect all other waste. This bucket is for any garbage generated in the hood such as test tubes, flasks, gloves, plates, and materials used to wipe the hood's work surface.
  - d. Spray bottles with **15% bleach for decontamination**
  - e. Safety buckets for spinning samples in the centrifuge.
- H. WORKING INSIDE THE BSC:
  - a. All work requiring the opening and closing of primary containers of biohazardous materials will be done in a biosafety cabinet. No primary container of a biohazardous material will be opened outside of a biosafety cabinet.

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- b. Anytime hands need to be removed from the BSC the outer layer of gloves must be removed, disposed of within the BSC, and replaced with new gloves.
- c. Appropriate personal protective equipment will be worn at all times.
- d. All fluids, other than those labeled as disinfectants or sterile buffers, are to be treated as biohazards. All cultures, whether they are wet, gel or solid, are to be treated as biohazards. All items within a Biosafety cabinet are to be treated as potentially infectious.

#### I. BSL3 BSC Cleanup Protocol:

- a. Cleaning centrifuge and buckets
  - i. Safety buckets are to remain in the hoods until decontaminated completely. Decontaminate the safety buckets at the end of use for the day or when the safety buckets become visually contaminated in the middle of the protocol. The inside and outside of the centrifuge safety buckets and rotors will be sprayed with 15% bleach and allowed a full minute of contact time before spraying with 70% ethanol wiping to remove residue and returning it to the centrifuge. Upon the completion of the use of the centrifuge for the work session the interior of the centrifuge will be cleaned with 15% bleach and 70% ethanol.
- b. Beaker containing bleach for liquid waste.
  - i. Liquid waste
    - a. All liquid waste generated will be disinfected following use with a final concentration of 15% fresh bleach solution for at least 20 minutes prior to bringing the waste out of the BSC and disposing of down the sink with copious amounts of water. All reusable containers from the hood should be immersed completely in a carboy containing a freshly-made disinfectant solution. The items must soak for at least 20 minutes. They are then to be emptied, rinsed, and hand dried with paper towels before storage.
- B. Trough with bleach solution to decontaminate tips, sharps, and pipettes.
  - a. Allow trough contents to soak in bleach solution for at least 20 minutes. Drain the bleach solution while flushing with running water. Add water to the trough to rinse residual bleach before autoclaving on the biohazard (DRY) cycle, 121 degrees for 1hr. Pipette troughs must be emptied immediately after autoclaving into a sharps bin to prevent a sharps hazard when they have cooled.
- C. Cleaning the BSC
  - a. Spray the entire work surface of the BSC with 15% bleach, followed by

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70% ETOH and wipe.