TITLE:	**IBC40-Removal of Materials from the ABSL-3 or BSL-3 Laboratory**
OVERVIEW:	The *CDC/NIH's Biosafety in Biomedical and Microbiological Laboratories*, 5th Edition provides guidance on the removal of some materials from the high containment laboratory This policy provides an overview of recommendations contained in this document with additional suggestions for those materials not specifically described.
APPLIES TO:	All individuals working within the ABSL-3 and BSL-3 laboratories.
DEFINITION(S):	Not applicable
PROCEDURES:	**Note:** Generally, all cultures, stocks, and other potentially infectious materials are decontaminated by autoclaving before removal from the ABSL-3/BSL-3 laboratory. In some instances however, materials can be removed from these laboratories without autoclaving by using procedures as described below. **Removal of Equipment** 1. Equipment must be wiped down with an appropriate disinfectant (e.g. fresh 10% bleach) before repair, maintenance, or removal from the containment laboratory. 2. In some cases where decontamination cannot be properly addressed by using the wipe-down procedure, an assessment will be conducted by the biosafety officer in consultation with the PI/Lab Director to determine the best approach for decontamination (e.g., gaseous decontamination may be considered in some circumstances). **Removal of DNA** 1. For bacteria and fungi, subculture 10% of the DNA sample volume for each individual extraction process to test for sterility. The appropriate medium, incubation conditions, and time of incubation are determined on an individual basis as described in each specific project IBC protocol that has been approved. For viral agents, sterility testing will be evaluated on a case-by-case basis and the specific procedure outlined in the IBC protocol that has been approved. 2. The DNA sample can only be removed from the containment laboratory when sterility has been documented. 3. Once sterility has been documented, the container containing the DNA sample is sealed, disinfected and then placed into a biohazard

	bag or other container. This container is surface decontaminated using an appropriate disinfectant (e.g. fresh 10% bleach) prior to removal from the containment laboratory. **Removal of Cell and/or Protein Extracts** 1. For bacteria and fungi, subculture 10% of the cell or protein extract sample volume for each individual extraction process to test for sterility. The appropriate medium, incubation conditions, and time of incubation are determined on an individual basis as described in each specific project IBC protocol that has been approved. For viral agents, sterility testing will be evaluated on a case-by-case basis and the specific procedure outlined in the IBC protocol that has been approved. 2. The cell or protein extract sample can only be removed from the containment laboratory when sterility has been documented. 3. Once sterility has been documented, the container containing the cell or protein extract sample is sealed, disinfected and then placed into a biohazard bag or other container. This container is surface decontaminated using an appropriate disinfectant (e.g. fresh 10% bleach) prior to removal from the containment laboratory. **Removal of live cultures** 1. Live cultures can be removed from the containment laboratory for transfer to another containment laboratory on campus or for transfer to an off-site facility. 2. The container (e.g. tube, Petri dish, or vial) is sealed, disinfected, and placed into a secondary container for transport. This secondary container is decontaminated using an appropriate disinfectant. 3. The secondary container is subsequently placed into an outer container prior to removal from the containment laboratory. This outer container is also decontaminated using an appropriate disinfectant.
RECORD KEEPING:	The Principal Investigator is responsible to verify and ensure documentation that sterility testing has been successfully completed prior to removal of the DNA and/or cell or protein extract sample from the containment laboratory. Documentation should include: 1. Date sample tested 2. Total volume of sample 3. Total volume tested 4. Testing conditions

	(incubation temperature, medium used, etc.) 5. Date samples testing read 6. Results of testing 7. Initials of individual evaluating the test Where formal documentation of sterility is requested, refer to the template described in **Appendix #1** as an example. Where formal documentation of responsibility is required, refer to the template described in **Appendix #2** as an example.
OTHER INFORMATION:	Avirulent stocks of risk group 3 organisms (see **Appendix 3** for examples) can be manipulated in the BSL-2 laboratory using a biosafety cabinet for containment IF the stock has never been used in the BSL-3 laboratory. Once an avirulent stock has been placed into the BSL-3 laboratory, this stock and subcultures from this stock CAN NO LONGER be manipulated in the BSL-2 laboratory. Removal of a select agent risk group 3 agent from the containment laboratory requires special handling and documentation. Refer to Select Agent Policy #SA25, Intra-Facility Transfer of a Select Agent or Toxin, for additional information. For materials to be shipped off-site, refer to Policy #SA-22, Receiving and Sending Select Agents and Policy #IBC-30, Shipping and Receiving of Category A High Consequence Pathogens for additional handling and packaging requirements.
REFERENCES:	U.S. Department of Health and Human Services, CDC/NIH. 2007. Biosafety in Microbiological and Biomedical Laboratories, 5th Edition.
STATUS:	Updated: July 7, 2015

Appendix #1

(Put on laboratory letterhead)

CERTIFICATE OF ANALYSIS

Date:

1. Method of Purification and Extraction

a. Name of Organism

b. Method of Extraction and Purification

c. Place of Purification (Building where lab is located)

d. Date of Purification

2. DNA Sterilization Verification

a. Procedure

(Example….Detection of viable organism was done by seeding 10% of a given lot of nucleic acid material on solid media plates and incubating sample appropriate for cultivation of the organism. Sterility of nucleic acid was determined by assessing growth after three days of culturing appropriate for the organism.)

b. Results

(Example…After 3 days of incubation, the chromosomal DNA sample on sheep blood agar at 37°C in ambient air was negative for growth. Thus, the DNA listed in paragraph 1a above is considered sterile.)

c. Date of Sterility Verification

(Example….The chromosomal DNA stock samples listed in paragraph 1a that was tested for sterility as described in paragraph 2a was confirmed to be sterile on [date].)

3. Place of Confirmation

(List laboratory facility where sterility testing was done.)

4. DNA Quantitation

(Example…The preparation of chromosomal DNA was quantified using the Nanodrop 100 Spectrophotometer. Following quantitation, the sample was determined to be at a concentration of [list ng/µl]. After sterility was confirmed [list volume of sample in µl] or a total of [total amount of sample in µg] was sent to [list location where sample was sent]).

PI Signature _____ Participant Signature _____
Title _____ Title _____
Address _____ Address _____

Addendum #2

(Put on Letterhead)

UNMC Sender: Date:
Sender's Fax#:
(Recipient will FAX a signed copy to the UNMC Sender prior to shipment)

Recipients
ACCEPTANCE OF RESPONSIBILITY

It is recognized that the material requested from the University of Nebraska Medical Center could potentially be pathogenic and may represent a potential hazard to the public health. Accordingly, the Requesting Facility agrees to the following:

(For Culture Isolates)

- the Recipient will take full responsibility for the security of the culture isolates received which includes limiting access to only personnel trained to perform the approved research and for select agents, perform the necessary background check as needed to eliminate access by individuals defined as "restricted persons",

(For Other Biological Material such as DNA)

- although the material has been tested in-house by the Sender and deemed to be sterile, the Recipient is ultimately responsible for the materials submitted since the Sender cannot guarantee sterility once the material has been offered for shipment,

(For all Materials)

- investigators who work with the material at the Requesting Facility are qualified through education and training to work with such material in accordance with accepted safety standards,

- the Requesting Facility acknowledges that University of Nebraska Medical Center disclaims all liability concerning the receipt, handling, storage, and use of the material, and

- the Requesting Facility will not hold the Board of Regents of the University of Nebraska, the University of Nebraska Medical Center, or any other agencies within the University system responsible for the content or use of the requested material.

REQUESTING FACILITY SIGNATURE
(I hereby certify that I am authorized to sign for the Requesting Facility.)

_____ _____
Type or print name/Title Signature
Date_____ _____

Appendix 3

Examples of Avirulent Select Agents

Yersinia pestis	Tjiwide S and CDC A1122
Bacillus anthracis	Sterne
Brucella abortus	Strain 19 and RB51
Coxiella burnetii	Phase II, Nine Mile Strain
Francisella tularensis subspecies *novicida*	Utah 112 (ATCC 15482)
Francisella tularensis subspecies *holartica*	LVS
Francisella tularensis biovar *tularensis*	ATCC 6223