

SOP for cultivation of biohazardous organisms from samples for labs already approved as BSL-3

Isolation of RG2 organisms

1. In the event that an RG-2 organism is obtained from environmental samples and will **NOT** be used for further analysis:
 - a. Dispose of the solid samples (e.g. plates) into an orange autoclave bag with a biohazard symbol, and autoclave the waste.
 - b. Dispose of the autoclaved waste as Regulated Medical Waste (RMW). For supplies and to request RMW pickup, follow instructions on the EHS website (http://www.ehss.vt.edu/programs/waste_regulated_medical.php).

2. In the event that an RG-2 organism is obtained from environmental samples and **WILL** be cultured for further analysis:
 - a. Contact the IBC (ibc@vt.edu). You will need to amend your IBC protocol to include the list of the RG-2 organisms that will be cultured and a description of the experiments/analysis that will be performed with the organism(s).
 - i. If you do not currently have an IBC protocol, you will need to submit a full protocol to the IBC for review and approval, prior to starting experiments with the organism(s). The samples can be stored in the lab during processing of the IBC protocol.
 - b. After receiving IBC approval, handle the cultures using BSL-2 containment practices and discard all waste as RMW.

Isolation of RG3 organisms

1. In the event that an RG-3 organism is unintentionally isolated from samples and will **NOT** be used for further analysis:
 - a. Immediately seal the samples in orange autoclave bag(s) and place the sealed bag(s) in a secure location in the BSL-3 lab.
 - b. Immediately contact the Responsible Official and University Biosafety Officer, Charlotte Waggoner (ren@vt.edu), the Alternate Responsible Official and Associate Biosafety Officer Anna Kroner (akroner@vt.edu), for details on how the organism should be destroyed and disposed.
 - c. Dispose of the samples following specific instructions from the BSO.

2. In the event that an RG-3 organism is obtained from environmental samples and **WILL** be cultured for further analysis:

- a. Contact the IBC (ibc@vt.edu). You will need to amend your IBC protocol to include the list of the RG-3 organisms that will be cultured and a description of the experiments/analysis that will be performed with the organism(s). The samples can be stored in the BSL-3 lab during processing of the IBC protocol.
- b. After receiving IBC approval, handle the cultures using BSL-3 containment practices and discard all waste as described in the BSL-3 manual.

Isolation of Select Agents

1. In the event that an organism designated as a Select Agent is unintentionally isolated from samples:
 - a. Immediately seal the samples in orange autoclave bags and place the sealed bag in a secure location in the lab.
 - b. Immediately contact the Responsible Official and University Biosafety Officer, Charlotte Waggoner (ren@vt.edu), the Alternate Responsible Official and Associate Biosafety Officer Anna Kroner (akroner@vt.edu), for details on how the organism should be destroyed and disposed.
 - c. Dispose of the samples following specific instructions from the Responsible Official.

A list of agents designated as Select Agents can be found on the Federal Select Agent Program (FSAP) website (<https://www.selectagents.gov/>).

Isolation of RG4 organisms

1. In the event that an RG-4 organism is unintentionally isolated from samples:
 - a. Immediately seal the samples in orange autoclave bag(s) and place the sealed bag(s) in a secure location in the **BSL-3** lab.
 - b. Immediately contact the Responsible Official and University Biosafety Officer, Charlotte Waggoner (ren@vt.edu), the Alternate Responsible Official and Associate Biosafety Officer Anna Kroner (akroner@vt.edu), for details on how the organism should be destroyed and disposed.
 - c. Dispose of the samples following specific instructions from the BSO.