



BIOSAFETY LEVEL 2

Laboratory Manual

Meridian Research Institute

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This manual must be read and acknowledged by every individual prior to independent access to a BSL-2 designated laboratory. Retain the signed acknowledgement (Section 6) on file with your Principal Investigator.

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1 Purpose, Scope, and Institutional Context



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This **Biosafety Level 2 (BSL-2) Laboratory Manual** establishes the policies, procedures, and containment requirements governing all work with biological agents classified at Risk Group 2 within the research facilities of **Meridian Research Institute (MRI)**, Cambridge, MA.

The manual applies to all laboratory personnel—principal investigators, postdoctoral researchers, graduate students, laboratory technicians, and visiting scholars—who access BSL-2 designated spaces in Buildings 7, 11, and 14 of the MRI main campus. Compliance is mandatory and forms part of each employee's and student's terms of engagement with the Institute.

1.1 Regulatory Framework

This manual is issued in conformance with the following regulatory and guidance documents:

- *Biosafety in Microbiological and Biomedical Laboratories*, 6th edition (BMBL-6), Centers for Disease Control and Prevention / National Institutes of Health [1].
- *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* (NIH Guidelines), current revision [3].
- Massachusetts Department of Public Health (MDPH) regulations, 105 CMR 480.000 — Storage and Disposal of Infectious or Physically Dangerous Medical or Biological Waste [2].
- Occupational Safety and Health Administration (OSHA) Bloodborne Pathogens Standard, 29 CFR §1910.1030 [4].

1.2 Biosafety Committee

The Meridian Research Institute Institutional Biosafety Committee (IBC) oversees all biological research activities on campus. The IBC meets quarterly and reviews all new BSL-2 protocols submitted via the MRI BioSafe electronic submission portal.

Institutional Biosafety Officer:	Dr. Elena Vasquez
Deputy Biosafety Officer:	Dr. Marcus Holloway
Emergency Hotline (24/7):	+1 (617) 555-0194
Email:	biosafety@meridian-ri.edu
Office Location:	Building 3, Room 102

1.3 Risk Group 2 Agents

Biological agents assigned to Risk Group 2 (RG-2) are associated with human disease that is rarely serious and for which preventive or therapeutic interventions are often available. Examples of agent categories studied at MRI BSL-2 facilities include:

- *Adeno-associated virus* (AAV) serotypes 1–9 in non-replication-competent constructs used for gene-delivery research.
- *Staphylococcus aureus* (including methicillin-sensitive clinical isolates) studied under the NexaTox antimicrobial resistance program.
- *Hepatitis B virus* (HBV) surface antigen constructs — non-infectious, sub-genomic fragments only.
- Human cell lines (HEK-293, HeLa, Jurkat) treated as RG-2 per BMBL-6 guidance on human-derived materials.

Work with Select Agents, Toxins, or Risk Group 3 / 4 organisms is **not covered by this manual**. Such research requires a separate protocol approved by the IBC and the CDC/USDA Select Agent Program. Contact the Biosafety Office before initiating any work that may involve agents at higher risk classifications.

2 Containment Requirements

BSL-2 containment is achieved through a layered system of physical barriers, safe work practices, and administrative controls. The following tables summarise the minimum requirements for all BSL-2 designated laboratories at Meridian Research Institute.

2.1 Facility Requirements

Table 1. BSL-2 Facility Physical Requirements

Requirement	Status	Implementation Notes
Self-closing, lockable doors	REQUIRED	Keyed-access RFID locks; doors must remain closed during work with open specimens.
Hand-washing sink (near exit)	REQUIRED	Sensor-activated; soap and paper towels provided.
Bench surfaces impervious to water	REQUIRED	Epoxy-resin benches throughout; inspect for cracks quarterly.
Biological Safety Cabinet (BSC) Class II A2	REQUIRED	Certified annually; blower run 5 min before use; re-certified after relocation.
Eye-wash station	REQUIRED	Weekly flushing log maintained; within 10 s travel from any work area.
Autoclave (on-site or in building)	REQUIRED	Spore-strip testing monthly; records retained 3 years.
Windows non-opening or fitted with insect screens	REQUIRED	Screen mesh ≤ 16 lines per inch where applicable.
Sharps containers (UN-approved)	REQUIRED	Replace at three-quarter capacity; never recap needles.
HEPA vacuum available	RECOMMENDED	Use for any dry-powder aerosol risk; do not use standard vacuum cleaners.
Negative air pressure relative to corridor	RECOMMENDED	Required for rooms with known aerosol-generating procedures; verify with smoke test.

2.2 Personal Protective Equipment

Table 2. Minimum PPE Requirements by Procedure Type

PPE Item	Procedure Category			
	Routine Culture	Aerosol Risk	Animal Work	Sharps Use
Laboratory coat (solid-front)	REQUIRED	REQUIRED	REQUIRED	REQUIRED
Nitrile gloves (single)	REQUIRED	REQUIRED	REQUIRED	REQUIRED
Double-glove	—	REQUIRED	REQUIRED	RECOMMENDED
Safety glasses / goggles	RECOMMENDED	REQUIRED	REQUIRED	RECOMMENDED
Face shield	—	REQUIRED	REQUIRED	—
Respiratory protection (N95)	—	REQUIRED	REQUIRED	—
Dedicated lab footwear	RECOMMENDED	REQUIRED	REQUIRED	RECOMMENDED
Impervious apron	—	RECOMMENDED	REQUIRED	—

— = not required. All PPE removed before leaving the laboratory.

Glove Removal Protocol

Gloves must be removed using the **beak technique** before touching any surface outside the immediate work area. Removed gloves are placed directly into the biohazard waste bag. Hand hygiene (soap and water or 70% ethanol gel) is performed immediately after glove removal, and always before leaving the BSL-2 space.

2.3 Decontamination Standards

Table 3. Approved Decontaminants for BSL-2 Surfaces and Materials

Agent	Concentration	Contact Time	Use Cases & Limitations
Sodium hypochlorite (bleach)	10% v/v	20 min	Bench surfaces, spills, non-porous materials. Corrosive to metals; prepare fresh daily.
70% ethanol	70% v/v	1 min	BSC work surface before and after use. Flammable; not for large-area spills.
Quatern. ammonium compound (Nexaclean™)	As supplied	10 min	Broad spectrum; effective against enveloped viruses. Not sporicidal.
Autoclave (steam)	121 °C / 15 psi	30–60 min	Liquid and solid waste; validated monthly.
Formaldehyde (fumigation)	37% (formalin)	12 h	Room decontamination only; IBO authorisation required.

2.4 Waste Segregation and Disposal

All biologically contaminated waste generated in BSL-2 areas must be segregated at the point of generation into one of the following categories:

- 1. Regulated Medical / Biological Waste (RMBW):** Items contaminated with RG-2 agents — pipette tips, culture tubes, cell culture flasks, gloves. Collected in *red biohazard bags* inside rigid secondary containers. Autoclaved on-site before transfer to the campus waste contractor (NexaWaste Solutions).
- 2. Sharps Waste:** Needles, scalpels, broken glass. Collected in UN-approved, puncture-resistant containers. Never filled above the marked fill-line.
- 3. Liquid Biological Waste:** Culture supernatants, media, rinse water. Treated with 10% bleach (final concentration) for a minimum of 20 minutes before drain disposal, *or* autoclaved.
- 4. Chemical-Biological Mixed Waste:** Contact the MRI Environmental Health & Safety office (ehs@meridian-ri.edu) before disposal.

3 Spill Response and Emergency Procedures

Biological spills at BSL-2 must be managed calmly and systematically. The response differs depending on whether aerosolisation is likely (e.g., centrifuge failure, pressurised container breach) or unlikely (e.g., overturned culture flask on bench). The following flowchart provides decision logic for both scenarios.

3.1 Spill Response Flowchart

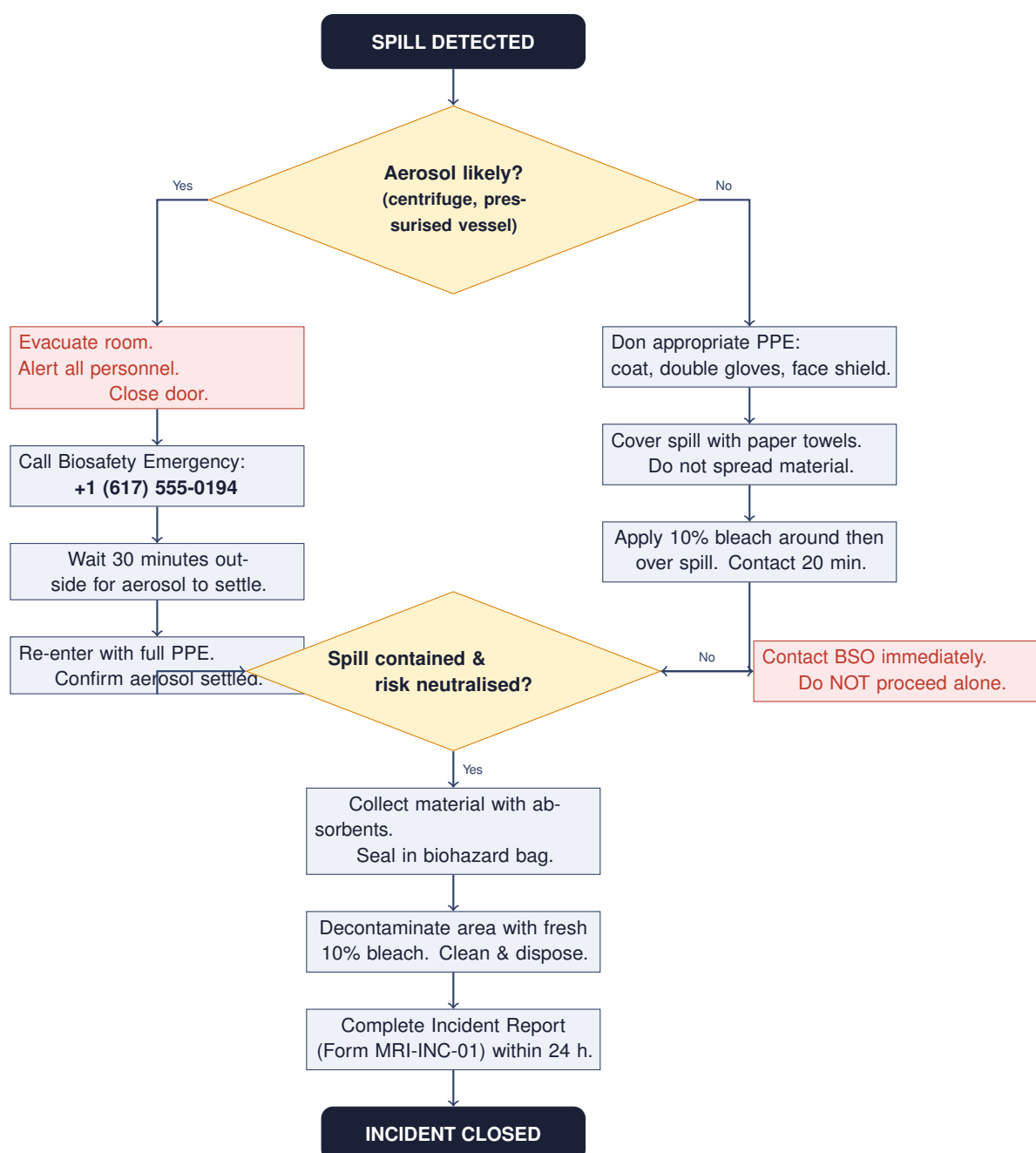


Figure 1. BSL-2 Biological Spill Response Decision Flowchart

3.2 Step-by-Step Spill Response Procedures

3.2.1 Minor Bench Spill (No Aerosol Risk)

Minor Bench Spill Protocol

1. **Alert:** Notify others in the room. If the spill involves a known pathogen, verbally announce “biological spill” so colleagues can take appropriate precautions.
2. **PPE:** Don double gloves, safety glasses, and lab coat if not already wearing them. Add a face shield if splashing is possible.
3. **Contain:** Place dry paper towels over the spill without pressing down. Work from the outer edges toward the centre.
4. **Disinfect:** Carefully pour 10% bleach around the perimeter of the towels, then over the top. Allow **20 minutes** contact time.
5. **Collect:** Using forceps or a dust pan (never bare hands), collect the soaked towels and place in a biohazard bag.
6. **Re-clean:** Wipe the area with fresh 10% bleach, followed by 70% ethanol to remove bleach residue from equipment surfaces.
7. **Report:** Document the spill using Form MRI-INC-01. Minor spills not involving personnel exposure may be reported to the PI within 24 h.

3.2.2 Major Spill or Centrifuge Accident (Aerosol Risk)

Do not attempt cleanup until the room has been evacuated for at least 30 minutes to allow aerosols to settle and/or be removed by the ventilation system. Failure to observe this wait time may result in inhalation exposure.

Aerosol Spill Protocol

1. **Stop work. Evacuate immediately.** Hold breath and exit. Post “Do Not Enter – Biological Spill” sign on the door.
2. **Remove contaminated clothing** at the door threshold; bag and seal immediately. Wash exposed skin and eyes for 15 minutes at the nearest eyewash / safety shower.
3. **Call the Biosafety Emergency Line** (+1 617-555-0194) and your Principal Investigator within 15 minutes.
4. **Wait 30 minutes** before re-entry (aerosol settling time).
5. **Re-enter with full PPE:** double gloves, N95 respirator, face shield, solid-front gown, boot covers.
6. **Proceed as Minor Spill steps 3–7** for cleanup.

3.3 Medical Emergencies and Exposure Incidents

Needlestick / cut:	Remove gloves; wash thoroughly with soap and water for 5–10 min. Do not squeeze or suction the wound. Report immediately to Employee Health (Building 1, Room 22).
Eye splash:	Irrigate with eyewash for minimum 15 min. Report to Employee Health and Biosafety Office immediately.
Ingestion:	Do not induce vomiting. Contact Poison Control (1-800-222-1222) and Employee Health.
All exposures:	Must be reported within 4 hours using Form MRI-INC-01. Post-exposure prophylaxis (PEP) availability is agent-specific; Employee Health will advise.

 **Never delay seeking medical attention to complete paperwork.**

Form MRI-INC-01 can be completed after receiving initial medical evaluation. The priority is your health and safety.

4 Training, Access Control, and Recordkeeping

4.1 Mandatory Training Requirements

No individual may independently access a BSL-2 laboratory at Meridian Research Institute without first completing the training modules listed in Table 4. Training records are maintained in the MRI Learning Management System (ApexLearn) and are audited annually by the IBC.

Table 4. Mandatory BSL-2 Training Curriculum

Training Module	Format	Renewal	Prerequisite / Notes
BSL-2 Laboratory Safety Fundamentals	Online	Annual	Must be completed prior to lab access.
Bloodborne Pathogen Training (OSHA)	Online	Annual	Required for all personnel handling human-derived materials.
Biosafety Cabinet Operation & Certification	Hands-on	Biennial	Delivered by Biosafety Office staff; 2 h workshop.
Autoclave Operation & Validation	Hands-on	Biennial	PI-level sign-off required before unsupervised use.
Chemical Hazard Communication (HazCom)	Online	As updated	Covers SDS interpretation and labelling requirements.
Incident Reporting and Emergency Response	Online	Annual	Includes Form MRI-INC-01 walkthrough.
Select Agent Awareness (if applicable)	Online	Annual	Required only if laboratory is adjacent to BSL-3 operations.

4.2 Access Control

BSL-2 spaces are secured with RFID card-access control managed by MRI Facilities. Access is granted upon verification of:

- Completed and current mandatory training record in ApexLearn.
- Active IBC-approved research protocol (**must cover the agents and procedures to be performed**).
- PI or lab manager authorisation submitted via the Biosafety Portal.

Tailgating and Propped Doors

Allowing un-badged individuals to “tailgate” through access-controlled doors is a **Level-2 Safety Violation** and may result in immediate suspension of laboratory access pending review. BSL-2 doors must never be propped open.

4.3 Working Alone and After-Hours Policy

Working alone with viable BSL-2 agents outside of normal staffed hours (08:00–18:00, Monday–Friday) is **prohibited** unless:

1. A written *Lone-Worker Risk Assessment* (Form MRI-LW-02) has been approved by the IBC and is on file.
2. The researcher has registered their after-hours work via the ApexLearn after-hours log **before** entering the facility.
3. A check-in call with MRI Security (+1 617-555-0220) is made at the start and end of the session.

4.4 Visitors and Collaborators

All visitors to BSL-2 spaces must be escorted by an authorised MRI personnel member at all times. External collaborators conducting experimental work (not merely observing) must additionally:

- Complete MRI-equivalent biosafety training or provide documentation of equivalent institutional training for IBC review.
- Be listed on an active MRI IBC protocol.
- Sign the MRI Visitor Biosafety Acknowledgement (Form MRI-VBA-01).

4.5 Recordkeeping Requirements

Table 5. Minimum Recordkeeping Requirements

Record Type	Retention	Location / Owner
Training completion certificates	5 years	ApexLearn (auto-retained)
Autoclave validation logs	3 years	Equipment logbook (lab)
Biosafety cabinet certification records	Life of cabinet + 1 year	Biosafety Office
Incident reports (MRI-INC-01)	10 years	EHS Office (paper + digital)
Inventory of RG-2 agents	Current + 1 year	Biosafety Portal
Visitor access logs	3 years	Security Office
After-hours work logs	1 year	ApexLearn / Security Office

5 Standard Microbiological Practices

The following standard microbiological practices are the foundation of BSL-2 containment and **apply at all times** in designated BSL-2 spaces, regardless of whether active experimental work is in progress.

5.1 General Laboratory Conduct

- **No eating, drinking, handling contact lenses, applying cosmetics, or storing food/beverages** in BSL-2 areas. This includes items kept in refrigerators used for biological materials.
- **Mouth pipetting is strictly prohibited.** Mechanical pipetting devices must be used at all times.
- **Minimise aerosol generation.** Avoid vigorous pipetting, open-container centrifugation, and flaming loop techniques that generate splatter.
- **Work surfaces are decontaminated** with an appropriate agent (see Table 3) before work begins and after any spill or at the end of each work session.
- **All procedures that pose an aerosol or splash risk** (e.g., vortexing open tubes, sonication of infectious material) must be conducted in a certified Class II A2 BSC.

5.2 Biosafety Cabinet (BSC) Operation

Class II A2 BSC Operating Procedure

1. Confirm annual certification label is current and cabinet is not overdue.
2. Turn blower on; allow to run for **5 minutes** before starting work. This purges the cabinet of any contaminants from the prior session.
3. Wipe work surface with 70% ethanol. Allow to evaporate before placing materials inside.
4. Place materials in the BSC: large items at the back, active workspace in the centre, waste container to one side. **Do not block the front or rear grilles.**
5. Perform all work with slow, deliberate movements to avoid disrupting airflow. Minimise in-out arm movements.
6. After work: wipe all items with 70% ethanol before removing. Discard waste into biohazard bag inside the BSC.
7. Run blower for an additional **3 minutes** after work is complete.
8. Wipe work surface with 70% ethanol or 10% bleach; allow contact time.
9. Log BSC use in the cabinet logbook (date, PI, duration, agent used).

UV Lights in BSCs

UV lamps in BSCs **do not replace chemical decontamination** and must never be used as the sole decontamination method. UV penetration is limited and will not decontaminate shadowed areas. UV lamps should be run only when the BSC is unoccupied and the sash is down. Exposure to UV light can cause serious eye and skin injury.

5.3 Transport and Transfer of Biological Materials

Movement of RG-2 agents between rooms, buildings, or off-campus must be managed according to the following tiers:

Table 6. Transport Requirements for RG-2 Biological Materials

Transport Tier	Distance	Packaging	Requirements
Within same room	N/A	Closed, labelled tube	Gloves worn; tube in secondary container if possible.
Between rooms / same building	<200 m	Primary + secondary container	Secondary must be leak-proof; biohazard label on outer container. Notify BSO.
Between buildings on campus	Any	Triple packaging	Notify BSO; use designated transport cart; never carry in pockets or open bags.
Off-campus / shipping	Any	IATA / DOT compliant	Requires Biosafety Office pre-approval, shipping documentation, and dry ice/UN-approved coolant if applicable.

5.4 Sharps Safety

- Hypodermic needles, syringes with needles, and scalpel blades are used only when no safer alternative exists (e.g., blunt-end needles, needleless systems).
- **Recapping is prohibited.** Used needles are placed directly into an approved sharps container without bending, breaking, or removing from the syringe.
- Scalpel blades are removed with a forceps-style remover, never by hand.
- Glass Pasteur pipettes are strongly discouraged; plastic alternatives are preferred. When glass pipettes are used, they are decontaminated then disposed of in a dedicated *broken-glass* container lined with a biohazard bag.

5.5 Labelling of Biological Materials

All containers holding RG-2 agents or materials must be labelled with:

1. **Biohazard symbol** and the word “BIOHAZARD” in orange or red.
2. Common and scientific name of the agent or cell line.
3. Date of preparation and concentration/titre (where applicable).
4. Investigator name and contact number.
5. Special handling instructions (e.g., “Keep frozen”, “Inactivated”).

Labels must remain legible under storage conditions (cryogenic labels for -80°C material; waterproof labels for wet-ice usage).

6 Acknowledgement and Compliance Declaration

I, the undersigned, acknowledge that I have read, understood, and agree to comply with all requirements set forth in the **Meridian Research Institute BSL-2 Laboratory Manual (Document No. MRI-BSL2-004, Revision 4.1)**.

I understand that:

- Non-compliance may result in **immediate suspension of laboratory access** and referral to the Institutional Biosafety Committee for disciplinary review.
- I am responsible for ensuring that **all personnel under my supervision** (if applicable) also comply with this manual.
- I must **report any suspected or actual breach** of biosafety containment to the Biosafety Office within 4 hours of discovery, even if no injury or illness has occurred.
- This acknowledgement does not supersede any additional requirements imposed by my specific IBC-approved research protocol.

Printed Full Name:

MRI Employee / Student ID:

Signature:

Date:

Department / Laboratory:

Principal Investigator:

IBC Protocol Number(s):

Expected Duration of Access:

Return the completed, **original signed** copy of this page to:
Biosafety Office, Building 3, Room 102

Meridian Research Institute, 480 Discovery Drive, Cambridge MA 02141
A digital scan may be submitted to biosafety@meridian-ri.edu with the subject line
BSL2-ACKNOWLEDGEMENT-[Your LastName]-[Date], but the **original must follow by campus mail within 5 business days**.
Retain a photocopy for your personal records.



"Safety is not a procedure. It is a culture."

— Dr. Elena Vasquez, Institutional Biosafety Officer, MRI

References

- [1] Centers for Disease Control and Prevention and National Institutes of Health. *Biosafety in Microbiological and Biomedical Laboratories*. U.S. Department of Health and Human Services, Washington, DC, 6th edition, 2020.
- [2] Massachusetts Department of Public Health. Regulations governing the management of infectious and physically dangerous medical or biological waste. 105 CMR 480.000, 2022.
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