Appendix M—Large-Scale Biosafety

Introduction

When working with biological agents in large-scale quantities, there are unique considerations that must be addressed in order to ensure worker and environmental protection. Large-scale biological production facilities should use the laboratory scale principles of risk assessment set forth in BMBL Section II, and by ISO 35001, Biorisk Management for Laboratories and Other Related Organizations.

In addition to laboratory scale risk assessment requirements, the utilization of larger equipment and volumes of chemicals or raw materials requires risk management strategies beyond biological safety alone. The following sections apply risk management steps to give readers the most pertinent information for managing risk in large-scale production. The recommendations assume that those performing risk assessments for large-scale work will involve industrial hygienists and other process safety specialists when implementing risk assessment and control measures for large-scale operations.

Appendix K of *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)* prescribes safety practices and containment procedures for large-scale (i.e., >10 liters per container) facilities. These guidelines can be applied to all large-scale work with biological materials (e.g., genetically modified organisms [GMO] and non-GMO, human, and animal/zoonotic pathogens). Please ensure familiarity with local regulations as these may differ from recommendations in this text.

Risk Assessment

Integrate the steps and processes utilized in laboratory biological risk assessment for any large-scale project. Risk assessment should be done during planning, when elements of the process change, and during periodic reviews of existing biological production processes, particularly after incidents or process failures. Risk control measures must be installed to mitigate unacceptable risk. Systems must be evaluated to determine their contribution to risk. The Good Practice quality guidelines and regulations (GxP) include three commonly used GxPs: Good Clinical Practices (GCP), Good Laboratory Practices (GLP),[1] and Good Manufacturing Practices (GMP);[2] GxP product Impact Assessment (IA) analysis can be extended to evaluate biosafety and laboratory biosecurity-related systems that govern exposure control, process room and environmental protection, decontamination, access control and accountability. Risk assessments should focus on the biological, chemical, physical, product, and equipment biosafety and laboratory biosecurity risk points. Production technologies and equipment with the potential for misuse (laboratory biosecurity/dual-use/export control) may also be

included in the risk assessment. Subject matter experts in engineering; Heating, Ventilation, and Air Conditioning (HVAC); quality control; occupational health; security; and health, safety, and environment (HSE) should always be consulted when making risk-based determinations.

Hazard Identification

The first step of risk assessment is hazard identification. Review additional factors that are unique to large-scale biological processes. Additional factors include but are not limited to:

1. Unique strains utilized primarily for research or manufacturing processes (e.g., producing high titers of a toxin);
2. High volumes (>10 liters) and high concentrations of product;
3. Specialized equipment and processes with unique risk points require a Hazard Analysis of Critical Control Points and/or Hazard and Operability studies;
4. Pressurized vessels and lines for biological and chemical reactions pose a risk for aerosol generation (e.g., bioreactors, fermenters, thermal inactivation tanks); and
5. Atypical routes of transmission (e.g., inhalation of biological agents or toxins not normally transmitted via the aerosol route).

Non-biological hazards to consider when performing a risk assessment may include, but are not limited to:

1. Hazardous chemicals: formaldehyde or similar for inactivation, large quantities of detergents, disinfectants and caustics, adjuvants, preservatives, solvents for down-stream processing, allergens or toxins, and asphyxiants;
2. Physical hazards: noise, steam, heat, cold, and radiation including UV and lasers;
3. Life-safety hazards: confined space, working at heights, line breaking, and pressurized systems;
4. Ergonomics;
5. Process safety-relevant controls (e.g., fire/explosions; pressurized systems);
6. Preventative maintenance (PM): solid and process effluent waste streams and control measures employed, including PM of relevant equipment;
7. Processes to control release of material (i.e., human and environmental risks), including corresponding emergency procedures; and
8. Risk points associated with equipment.

Hazard Evaluation

As with laboratory risk assessment, the hazards associated with the biological agent/material and process equipment must be evaluated. In addition, the operational integrity of containment equipment and facility safeguards and the capability of area staff to effectively control potential hazards must be considered. Staff capability will depend on the training, technical proficiency, and good habits of all team members.

Large-scale research and production pose additional risks that require evaluation. Increased growth, vessel size, and enhanced aeration magnify the aerosol generation risk. By design, the biological agent concentration is greatly increased. Therefore, protection from aerosol transmission must be considered for agents normally transmitted by insect bite or injection.

Chemical risks are also increased due to handling of dry powders for media preparation, pumping of acid or base for pH control, and preparation/addition of inactivation chemicals for vaccine preparation. Closed system transfer technology may be foreign to those with experience limited to the laboratory.

Risks due to hazardous energy (i.e., electrical, steam, pressurized gases) are also magnified. Hazardous energy control procedures such as removing the power cord or closing a supply valve become complex and may be poorly understood by those with experience limited to the laboratory.

Risk Control

Risk mitigation strategies identified in large-scale research and production follow the same principles (i.e., hierarchy of controls) established to control HSE risks.[3] Those performing risk assessments for large-scale work may be able to eliminate a hazard or substitute to reduce risk. When this is not possible, engineering, administrative and/or work practice controls, and PPE are utilized.

Engineering Controls

Selecting the proper engineering solution is an iterative process.[4,5] The design provisions for a large-scale biological production facility will differ greatly depending on whether the work is dealing with an exotic, indigenous, eradicated, novel, or emerging disease-causing agent; a highly allergenic compound; a GMO, carcinogenic or highly toxic product; or a well-characterized and attenuated childhood vaccine.

Many controls must be considered in the process, including HSE-risk, biosafety, and laboratory biosecurity. In addition, large-scale GxP facilities must evaluate quality design controls for product as well as personnel and environmental protection. Consider state and local regulations when implementing the design of

a large-scale biological production facility. A large-scale facility balancing GxP and biosafety requirements will need to evaluate the following basic facility principles:

Clean to Dirty The process design must include controls to prevent contamination spread within the facility and to the environment. If applicable, an assessment of conflicts between GxP and biosafety requirements must also occur to achieve two different definitions of clean. If there are two competing requirements, implement controls that address the highest consequence events and identify alternate methods to meet the intent of the competing requirement. For example, if an operation requires positive-pressure environment to achieve product protection, you can create an air pressure sink in an anteroom to ensure containment of the biological agent.

Change Rooms and Barriers Establish donning and doffing needs by creating an operational flow diagram. This will help clarify how many actions an operator must take for a given procedure or process step when passing through a personnel barrier or door. The review should cover normal operations, planned and unplanned maintenance, and emergencies. This process should identify the potential demand in PPE for the facility, the number and locations of room(s), and room size(s) necessary for storing PPE and changing. Facilities covered by GxP requirements must consider PPE and workflow requirements to achieve product protection in addition to personnel and environmental protection.

Airlocks and high/low-risk rooms (i.e., biologicals vs. cleanrooms) The design must address biosafety concerns as well as applicable GxP requirements to achieve personnel, environment, and product protection, if required.

Surfaces Floor, wall and ceiling, door and window, and other exposed component surfaces must be impervious and easy to clean. The materials must be resistant to a host of chemicals including liquid and gaseous disinfectants, if needed, for decontamination or prevention of cross-contamination. Construction attributes of floor strength, ceiling height, segregation need, piping (i.e., materials, product, and waste) and energy lines must support and promote large-scale processes.

HVAC system, room pressure, and airflow The design of the airflow must provide personnel and environmental protection. In the event a process area must be positive-pressure, consider designing the room airlock or changing area as a pressure sink. Exhaust air filtering systems may be required, as in the case of vaccine plants producing live attenuated vaccines, to prevent ductwork contamination. GxP requirements may also require product protection design considerations.

Gaseous Decontamination The HVAC system, walls, and wall penetrations must be made such that the room can be decontaminated without a negative impact to adjacent spaces. The decontaminant employed must be appropriate for the

process and biological agents handled. Use the same principles for gaseous decontamination of a laboratory, but the quantities used and the clearing times will differ substantially.

Spill Containment When designing for spill containment, consider the biological, chemical, and physical processes in an area. Always review spill scenarios while designing a facility. Identify what and how much can be released, where spilled materials will flow (e.g., are there drains leading to an effluent decontamination system (EDS) or will materials released be captured within a containment dike), if manual inactivation will be required, and what emergency response activities will encompass.

Kill Tanks/EDS Systems Ensure EDS systems can inactivate effluent from production waste and spills. It is particularly beneficial to have a facility designed with secondary failsafe systems when large amounts of material are processed. The exact method used will depend on local regulations and the materials in question. Numerous options exist, including chemical inactivation using acids or caustics, and heat inactivation (batch or continuous). Ensure holding tanks have stirrers when volumes are large. Most facilities employ hard piping, and a process to clean and decontaminate these lines between production areas and the EDS must be integrated into the plan.

Those performing risk assessments for large-scale work will also determine the type of equipment to be used by considering production needs and risk assessment results.[6] Historically, the standard has been fixed equipment (i.e., stainless steel bioreactors) with a combination of hard and flexible hose piping for upstream (i.e., biological agent propagation) and downstream (i.e., biological agent purification, concentration, and potentially inactivation) processes. Increasingly, single-use (SU) equipment is replacing fixed equipment for upstream processes. The “ballroom” concept, where both upstream and downstream processes are in one large production facility, is now accepted for select biological processes.[7] The ballroom concept relies on maintaining closed systems at all times.

1. Ballroom Layout Advantages
    a. More flexibility to accommodate different process trains;
    b. Improved operational efficiency and oversight (e.g., avoids having to move equipment between rooms); and
    c. Reduction of footprints and cost.
2. Ballroom Layout Disadvantages
    a. Increased risk of contamination spread in upset conditions to downstream processes;
    b. Need for typically open operations (e.g., cell expansion, column packing or powder addition) to be handled in closed systems;

c. Need for enhanced environmental monitoring to be conducted to detect a breach in any closed system and need to ensure contamination or cross-contamination has not occurred; and
d. Challenging area and equipment decontamination when production areas are shared.

A non-comprehensive list of containment requirements and associated risk points is provided below to assist in the assessment of risks associated with SU equipment.

Containment Requirements and Example Risk Points[7–10]

1. Viable organisms should be handled in a closed system or other primary containment.
    a. Ensure the bioreactor bag is compatible with maximum output temperature of heating control circuit;
    b. Ensure the tubing is compatible with process media, including pH control solutions and stability testing has been performed; and
    c. Implement procedures to ensure that probes are not removed during operation.
2. Culture fluids are not removed from a system until organisms are inactivated.
    a. Implement procedures for removing bioreactor bag(s) containing infectious agent(s).
3. Inactivation of waste solutions and materials with respect to their biohazard potential.
    a. Implement procedures for processing used bioreactor bags containing infectious agents;
    b. Ensure presence of biosafety cabinet for removing reusable components before destruction;
    c. Ensure the waste disposal procedure compatible with bioreactor bags;
    d. Implement a procedure for safely autoclaving used bag;
    e. Implement a procedure for safe packing and transport to incinerator if the used bag will be directly incinerated; and
    f. Ensure the incinerator facility can burn large quantities of silicone tubing and bag film.
4. Control of aerosols by engineering or procedural controls to prevent or minimize release of organisms.
    a. Implement controls to prevent bioreactor bag overfilling during additions;
    b. Ensure proper procedure for tubing welding;
    c. Ensure proper procedure for tube weld integrity test;

d. Ensure regular PM of tubing welders to prevent misalignment; and
e. Ensure that plastic quick connectors (non-steamable) release viable organism(s) when released.

5. Treatment of exhaust gases from a closed system to minimize or prevent release of viable organisms.
   a. Consider exhaust gas filtration;
   b. Consider controls of exhaust filter clogging with foam and humidity; and
   c. Ensure there is an exhaust filter holder positioned to encourage condensate drainage.

6. Closed system that has contained viable organisms not opened until sterilized by a validated procedure.
   a. Ensure the bioreactor bag is compatible with inactivation chemical.

7. Closed system to be maintained at as low a pressure as possible to maintain integrity of containment features.
   a. Implement a process safety management study of gas overlay and sparging system to determine susceptibility to overpressure, including post-power failure;
   b. Ensure bag installation procedures to prevent damage;
   c. Ensure pressure control to limit aeration and overlay pressure;
   d. Ensure the pressure alarms are interlocked to the gas supply;
   e. Ensure pressure relief devices are installed on gas supplies and properly sized;
   f. Consider installing in-line pressure relief before the bioreactor to protect against gas regulator failure; and
   g. Ensure the gas supply valves fail closed upon power interruption.

8. Rotating seals and other penetrations into closed system designed to prevent or minimize leakage.
   a. Consider magnetic couplings to eliminate rotary seals;
   b. Implement procedures to ensure stirrer operates during pre-use integrity test;
   c. Ensure rotary seals engineered to prevent infectious agent release; and
   d. Consider that over-speed may result in decoupling and in-bag rupture.

9. Closed system shall incorporate monitoring or sensing devices to monitor the integrity of containment.
   a. Consider bioreactor bag pressure logging;
   b. Ensure that loss of pressure (low-pressure alarm) results in sparge/overlay shutdown; and

    c. Ensure that the sensors respond quickly enough to pressure changes.

10. Validated integrity testing of the closed containment system.
    a. Consider integrity test procedures pre-inoculation.

11. Emergency plans required for handling large losses of cultures.
    a. Implement a leak detection system for bottom- or side-mounted probes;
    b. Consider bottom- or side-mounted sensors guarded to prevent impact damage;
    c. Consider respiratory PPE as part of operating PPE or ensure respiratory PPE availability for emergency cleanup;
    d. Ensure a contaminated worker emergency procedure available;
    e. Ensure a large spill clean-up procedure available, including a spill kit;
    f. Ensure personnel trained in large-scale clean-up of infectious organisms; and
    g. Consider gas decontamination of production suite post-incident.

12. Requirements for controlled access area.
    a. Ensure aerosol-containment within skid (i.e., process module);
    b. Consider a spill containment pan to contain or divert entire bioreactor contents for inactivation;
    c. Ensure the pan will divert a worst-case leak scenario to biowaste without spill to the floor;
    d. Consider spill containment within the suite (dike, bund, raised door threshold) to contain entire bioreactor contents for inactivation;
    e. Ensure the suite exhaust HEPA filtration for fluid transfers outside bioreactor containment; and
    f. Ensure the suite is designed to prevent the release of infectious aerosols using differential pressure and sealing of room penetrations.

Those performing risk assessments for large-scale work will also need to review equipment types and assist in the evaluation of the choice that will best balance the needs of GxP and biosafety. These equipment types include:

Pumps and Pipes The type of piping used will depend on how the process is laid out. Hard piping will need clean-in-place (CIP) and sterilization-in-place (SIP) for both GxP and biosafety reasons. Soft hoses allow for quick changes and cleaning. The type of pump will have to meet the volume demands of production. Peristaltic pumps are often used in combination with soft hoses. The risk assessment must show what type of piping and pump to use to meet

GxP (if applicable), biosafety, and general HSE demands. Make sure that points where pipes penetrate walls are correctly sealed to promote safe gaseous decontamination. Additionally, pump operation should be evaluated for hearing protection implementation.

Compressed Air and Gases Compressed air is one means of transferring fluids between vessels. The safety review will identify elevated pressure points, type of relief valve protection required, and rupture disc failure scenarios. Some processes require asphyxiants, such as CO_2 or N_2, and safety measures are to be established to mitigate associated risk.

Electrical Power Power should be installed in a manner that prevents water ingress in all production and failure modes. Planning and construction must follow local electrical codes and the Occupational Safety and Health Administration electrical standards. Large fixed equipment fermenters and equipment often require high voltage power, which creates the need for additional safety measures including emergency stop buttons to shut down equipment and installation of water and dustproof electrical enclosures.[11,12] Special care must be taken when solvents are used in production; follow applicable national codes, such as NFPA, UL, and OSHA. UPS needs must be evaluated based on the equipment and facility needs. An emergency generator may be essential to maintain biocontainment.

Production equipment including bioreactors, fermentors, filtration units and centrifuges In all upstream and some downstream processes, equipment is used while the product is still infectious. These units must be set up to eliminate the risk of aerosol release. Prior to charging process equipment with live biological material, the integrity of the closed system should be verified. Before opening a closed system for maintenance or cleaning, in situ decontamination of the vessel is required. To prevent an aerosol release occurring as a result of an upset condition, small equipment can be placed inside a containment device such as a biological safety cabinet. Larger equipment containing infectious agents should reside in rooms under negative pressure. If negative pressure can't be achieved, room entry and exit airlocks may be used as negative air pressure "sinks" to prevent the escape of aerosols into adjacent areas.

Work Practice and Administrative Controls

Good microbiological practices are vital and apply in the same way as they do in biological research laboratories. Chemical hygiene, hearing protection evaluations in equipment areas, ergonomic, and safety principles apply to large-scale biological production areas as they do in other research laboratories and production areas. Access should be restricted to trained personnel only. Other administrative controls include:

Occupational Health Employers should offer workers appropriate medical surveillance programs to identify immune suppression and other underlying medical conditions, which could be risk factors that necessitate adaptations or accommodations. Occupational physicians should advise on, from a medical point of view, protection measures and procedures (e.g., fitness for duty to wear respirators or perform specific tasks). Where appropriate, the physician will offer vaccination, or provide vaccines, with follow up on titers. In addition to surveillance, clinical treatment procedures for accidental exposure should be developed. For biological agents susceptible to antibiotics, antimicrobial susceptibility testing results should be obtained before large-scale operations begin.

Emergency Response Plans for different emergency situations should be established, including spill protocols. Where appropriate, post-exposure prophylaxis and policies for isolation of potentially infected people should be established. One differentiating factor between small and large spill clean-up is that, unless there is an immediately dangerous for life and health (IDLH) situation, the operator in a large-scale facility must remain in the room long enough to stop and contain the release to minimize HSE consequences. Further information on emergency preparedness and response can be found in Biological Safety: Principles and Practices.[13]

Laboratory Biosecurity The risk management strategy for a large-scale risk assessment should define both a biosafety containment strategy (refer to BMBL Section II, NIH Guidelines' Appendix K, and the area-specific risk assessment) and a laboratory biosecurity strategy. The biosafety containment strategy defines controls that mitigate risk from an unintentional release, and the laboratory biosecurity strategy defines controls that prevent theft of biological agents that are associated with human health and/or agricultural industry impact. Likewise, materials, equipment, technology, and knowledge of dual-use potential needs to be addressed and a strategy developed to address misuse.[14–18]

Training Biosafety, laboratory biosecurity, and GxP training (if applicable) are essential in large-scale biological production. For large-scale processes, training should review the epidemiology, signs/symptoms of infection, mode of transmission, risk-mitigating controls including donning and doffing of PPE, and emergency response procedures, area-specific SOPs, including spill response protocols, required for the biological agent/material handled. Workers should understand when PPE is required for product protection vs. personnel protection. An understanding of the handling requirements for inactivated vs. unconfirmed inactivated materials is critical. Training should include a knowledge check.

Ergonomics The ergonomic issues associated with large-scale operations differ from those encountered in the laboratory. Material handling in large-scale operations will present a larger risk of ergonomic injury. To address the ergonomic issues associated with material handling, include the nature of the load in the risk

assessment (i.e., the weight distribution and shape of the load), the capabilities of the individual performing the task, the duration and frequency of the task, and the environment in which the material handling task is performed (e.g., space limited or extreme temperature environments). Mitigate ergonomic risks by mechanical means (e.g., lifts, hand trucks, pushcarts), redesign of the work area (e.g., ramps to replace stairs, automated transfer of materials to replace manual transfer), redesign of the work task (e.g., pushing rather than pulling), and training of personnel (e.g., proper lifting technique).

Waste Handling The processes of waste handling are the same as for research laboratories but larger amounts require different logistics. For guidance on validation of decontamination agents and procedures, refer to Appendix B. Key considerations include inactivation of organisms in situ vs. external to process vessel or container. Consider inactivation methodologies for solid infectious waste streams as well as wastewater from production effluent (i.e., determine if there will be an impact to the site wastewater treatment permit due to the presence of organics including preservatives such as thimerosal or adjuvants).

Review and Checking of Risk Control Measures Risk control measures need to be evaluated for efficacy in order to protect people and the environment. The organization should maintain a risk control register, which should be periodically reviewed. The strategy should address the major risk streams (e.g., chemical, physical, biological, and ergonomic).

Preventative Maintenance Preventative maintenance is vital to avoiding process contamination and to ensuring biocontainment. Safety and security-related equipment and infrastructure should be incorporated into a preventive maintenance program that incorporates a change control process. For example, rotary seals in fermenters must be monitored for increased loss of seal water or steam pressure and should be replaced before failure; high-pressure piston seals of homogenizers must be replaced regularly to prevent aerosol release; autoclave temperature and pressure sensors require regular calibration, and steam traps must be maintained. Depending on design, autoclave bioseal or air differential seals should be tested (e.g., smoke, pressure hold, soap bubble, and helium leak testing) to determine whether they have deteriorated. When required, HEPA filters (i.e., HVAC and equipment) should be integrity tested annually and critical barrier HEPAs should be monitored for pressure differential. Thermal or chemical inactivation systems should undergo regular inspection for corrosion and preventative maintenance of gaskets, seals, and sensors, as well as addition pumps, to ensure proper operation. Validation of inactivation parameters is also required by using spore-based indicators or the actual production organisms. Continuous flow thermal inactivation systems should undergo regular chemical clean-in-place cycles to remove coagulated protein residues, which can reduce system efficiency.

PPE/Gowning

PPE and gowning are used for both personnel and product protection. When PPE is utilized for product protection, it is designed to prevent shedding of foreign material into the production process and final product and to contain skin and respiratory shedding from the worker. Standard cotton or synthetic materials are not acceptable because they are prone to shedding. When PPE is utilized for worker protection, it should be assessed against physical, chemical, and biological hazards. Cotton laboratory coats or jumpsuits are easily saturated with chemical and biological liquids during a large release or spill and do not provide adequate protection. Man-made, water-resistant polymers are a better choice; they are less apt to become saturated. Refer to the material permeation rate or breakthrough detection time. The most protective options for personnel protection are gowns made of microporous laminated materials or jumpsuits with covered zippers.

Depending on the chemicals and/or biological materials handled, large volumes at high concentration plus the inherent increased risk of aerosol generation may require respiratory protection. Common disposable, half-face respirators (e.g., N95) may be sufficient for biological material protection, but they are not designed for chemical protection and may not be sufficient to protect against large volumes of a concentrated high-risk pathogen. Therefore, a risk assessment should be performed to identify the appropriate respirator required for the operation (i.e., filtering facepiece, tight-fitting facepiece, PAPR or SCBA).

Conclusion

Large-scale growth of biological agents is necessary in a variety of settings and requires an evaluation of both the GxP and biosafety requirements. With careful planning and a robust risk assessment of the unique requirements of a large-scale facility, it is possible to design and operate a facility that protects the product, workers, and the environment.

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