

**UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
PLANT PROTECTION AND QUARANTINE**

**CONTAINMENT GUIDELINES
FOR
PLANT PATHOGENIC BACTERIA
(Includes mycoplasmas, spiroplasmas)**

[(Revised Feb. 2010) - Plant Pathogenic Bacteria]

CONTAINMENT GUIDELINES FOR BACTERIAL PLANT PATHOGENS

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CONTAINMENT GUIDELINES FOR BACTERIAL PLANT PATHOGENS

I. PURPOSE OF THIS DOCUMENT:

These guidelines are a reference to help you (a scientist of a state, federal, or commercial entity) design, build, maintain, and operate a containment facility for receipt of high risk non- indigenous bacterial plant pathogens (including mycoplasmas, spiroplasmas). These guidelines are not intended for the containment of imported high risk nematode plant pathogens, airborne fungal plant pathogens, viruses, nematodes, or arthropods. Widely distributed indigenous plant pathogenic bacteria may not require a containment facility. These guidelines are not intended for pathogens and vectors of medical and veterinary importance. If there is doubt as to the applicability of guidelines to specific organisms, contact Containment Facility (CF) scientific personnel for appropriate guidance.

During inspections or reinspections of your facility, USDA, APHIS, PPQ personnel will review these guidelines and any risk mitigation instructions that may accompany your permit. When your facility meets containment standards and risk mitigation instructions by USDA, APHIS, PPQ it will be considered "adequate" from a containment standpoint; then permits may be issued to you.

The inspection and permitting procedures of the USDA, APHIS, PPQ are intended to prevent the release of nonindigenous plant pests to the environment of the United States. Accidental or purposeful release of these organisms is a violation of the [PLANT PROTECTION ACT](#) and is subject to civil and/or criminal penalties, loss of permits, and destruction of valuable cultures.

Components of this Document:

Your containment facility must meet the "**Standards**" listed in the grey, shaded boxes. To help you meet these standards, ask biotechnology industries, university biosafety committees, and/or contractors for research hospitals and other research institutions to recommend specialized professional contractors. Professionals know state, local and federal laws that regulate construction, including the installation of emergency doors, incinerators, air intake and exhaust ducts, emergency lighting, plumbing, and many other features. PPQ's permit specialists have little or no knowledge of these laws.

N.B. Notify PPQ of any structural changes prior to implementation, the development of blueprints, signing of construction contracts, start of construction, etc.

[Other useful containment references (non-USDA-APHIS) are: 1) "Containment Facilities and Safeguards for Exotic Plant Pathogens and Pests" 1999. R. P. Kahn and S. B. Mathur. (eds.) APS Press, St. Paul, MN; 2) "A Practical Guide to Containment (Plant Biosafety in Research Greenhouses)". Adair, D. and R. Irwin 2008 (revised edit.) Information Systems for Biotechnology, Virginia Tech, Blacksburg (<http://www.isb.vt.edu>); 3) "Containment Standards for Facilities Handling Plant Pests" 2009 (1st edition) Canadian Food Inspection Agency, Office of Biohazard Containment and Safety, (<http://www.inspection.gc.ca/english/sci/bio/plaveg/placone.shtml>)].

The **“Suggestions”** listed under each Standard are methods or equipment that are commonly used at this time to accomplish each containment standard. The design, construction and operation of your containment facility may vary, depending on the organisms you wish to contain, your research objectives, the functionality of your equipment and structural components, and your location. **Again, we recommend professional advice on all design issues.**

Safety of facility personnel should not be compromised by containment requirements. Emergency exits should not be blocked by equipment; their operation should not be obstructed with tape or caulk.

We encourage Good Laboratory Practices (GLP) in all facilities; however the standards and suggestions listed here **only** cover containment. Cross contamination of cultures within the containment may or may not signify poor containment.

USDA, APHIS, PPQ welcomes alternatives that are proven to meet or exceed the standards. To insure timely permitting, please review this document, and research design alternatives. Once design options are narrowed, call or fax PPQ's Containment Facility staff at (301) 734-5304 or FX (301) 734-5392 and continue discussions as the facility is planned and built.

II. CONSTRUCTION STANDARDS FOR THE ENTIRE STRUCTURE

CONSTRUCTION STANDARD A. Locate the facility in areas with minimal human, agricultural and environmental risk. Identify the facility as dedicated and secure.

SUGGESTIONS:

1. Locate the facility in areas relatively free of agricultural zones, high-risk microclimates (e.g. known flood zones) or other high-risk areas.
2. If possible design the containment facility as a separate, dedicated building. If this is not possible, design and build to prevent pest escape.
3. Install a 15 foot- wide buffer strip of gravel and/or pavement from the foundation and around the containment building(s).
4. Install a fence 6 ft or higher around the facility.
5. At the main entry to containment, post:
 - Containment director/ containment officer name and contact numbers.
 - A sign stating, "ACCESS IS BY AUTHORIZED PERSONNEL ONLY",
 - Emergency telephone numbers.

CONSTRUCTION STANDARD B. Design the floor plan to prevent escape of the enclosed organism(s)

SUGGESTIONS:

1. Install one primary entry/exit.
2. Install a shower and restroom at the primary entrance. This is may be required to contain certain species of bacteria which adhere to clothing or skin. Most species of bacteria do not require a shower for containment.
3. If local building code allows, install a vestibules for emergency exits. Design the facility with laboratories and rearing rooms connected to a main laboratory.
4. Build restrooms outside of containment areas. However, if restrooms must be built inside a containment area, use the same construction standards used throughout the facility and place the restroom next to low risk areas.
5. Build offices outside of containment areas.
6. Install self-closing doors throughout the containment structure(s). Install exterior doors that lock.
7. Install a central closet for cleaning supplies.
8. Insure negative air pressure throughout the facility. This may be required for bacteria, which have aerielly dispersed spores (i.e. *Streptomyces* spp.)
9. Insure air moves from the least to most hazardous rooms.

CONSTRUCTION STANDARD C. Construct walls, ceilings and floors that are impenetrable to the enclosed organisms, and withstand repeated cleaning and decontamination

SUGGESTIONS:

1. Construct the walls and ceilings with building materials that resist moisture and withstand repeated decontaminations with bleach or other caustic solutions.
2. Install floors that are impenetrable to the organism and withstand repeated cleanings. Monolithic (in one-piece) floors, e.g. poured concrete, asphalt tile, chemically resistant paint etc. are desirable. Wood floors are **not** acceptable.
3. Install floor drains to collect liquid wastes for sterilization.
4. Seal junctions, holes or penetrations of walls, ceilings, and floors with plaster, caulk, or equivalent materials.
5. Suspended or dropped ceilings are **not** acceptable.

CONSTRUCTION STANDARD D. If windows are necessary, install Windows impenetrable to the enclosed organisms

SUGGESTIONS:

1. Install breakage resistant glazing (Plexiglas, Lexan, etc).
2. Install windows that do not open.
3. Seal joints between the windowsills, frames, etc. and walls with appropriate materials.
4. Store extra transparent panels nearby for emergency use.

CONSTRUCTION STANDARD E. Install Doors that contribute to the security of the facility.

SUGGESTIONS:

1. Install self-closing, steel doors with magnetic gaskets throughout the containment structure(s).
2. Install exterior doors that lock.
3. Install thresholds and gaskets that seal the exterior and interior doors with their frames. The space between seal and doorframe should not exceed 6.3mm (0.250 inch).
4. Emergency doors
 - Post signs on the exterior and interior of emergency exits stating USDA, APHIS Containment Facility - Emergency Exit Only.
 - Insure emergency doors are not commonly used as an entrance (remove exterior handles, use doors with interior or internal hinges, etc.).
 - Install audible alarms that activate when emergency exit doors are opened

CONSTRUCTION STANDARD F. Design and install an **HVAC System** (Heating, Ventilation and Air Conditioning) that prevents escape of the contained organisms.

SUGGESTIONS:

1. If possible, install an HVAC system dedicated to the containment areas. If not possible, then install filters to prevent organism escape from containment areas to areas or buildings outside of containment.
2. Install filters in the HVAC exhaust, which will prevent escape of bacterial spores (i.e. *Streptomyces* spp., etc.). This may include HEPA or other filters.
3. Insure air moves from outside to inside when exterior doors are opened.
4. Insure air moves from the least to most hazardous rooms. Install devices to measure the airflow direction.
5. Seal connections in air ducts, plenums, etc. with caulk or an equivalent material.
6. Seal vents to interior surfaces with caulk or an equivalent material.
7. Install filters and screens of the HVAC system so they are easy to clean, decontaminate and replace.
8. Install tandem filters so one side of the ventilation ductwork and its filter can be decontaminated while the other side supplies air.

CONSTRUCTION STANDARD G. Design and install an **Electrical System** that maintains containment features under normal and emergency situations and is impenetrable to the contained organisms.

SUGGESTIONS:

1. If containment requires negative air pressure, install an alarm to indicate power failure.
2. Install an alternative power source (generator, battery bank, etc.) for use when normal power is lost or interrupted if negative air pressure is used in the facility to contain aerielly dispersed bacterial spores (*Streptomyces* spp., etc.)
3. Install weatherproof electrical boxes, receptacles, light fixtures, switches, etc.
4. Seal electrical boxes, lighting, switches, wiring, conduit, etc, with appropriate materials (caulk, foam, etc,) that are impenetrable to the contained organisms and withstand repeated decontaminations with bleach or other caustic solutions.

CONSTRUCTION STANDARD H. Design and install a Plumbing System to contain the organisms and remove liquid wastes.

SUGGESTIONS:

1. Seal sewer or drains (sink, floor, shower, etc.) and collect effluent for sterilization.
2. Sterilize effluents from sinks, floor drains, etc with steam or its equivalent before releasing them into the sewer system.

3. Sewer vents must be sealed with filters, which prevent the escape of bacterial spores.

CONSTRUCTION STANDARD I. Install Vacuum Cleaning Systems that prevent the escape of the contained organisms. A vacuum system is not required, however if one is installed it should prevent the escape of contained organisms.

SUGGESTIONS:

1. Use vacuum appliance only in facility.
2. Autoclave or sterilize vacuum filters and waste before disposal.

CONSTRUCTION STANDARD J. Install Communication System that allows communication between the interior and exterior of the facility and prevents organism escape.

SUGGESTIONS:

1. Install a telephone(s) or intercom system.
2. Install a computer (LAN, modem, etc.) or Fax machine to allow for communication and data transfer to and from the facility.

III. CONSTRUCTION STANDARDS FOR SPECIALIZED ROOMS.

CONSTRUCTION STANDARDS FOR SPECIALIZED ROOMS A. Build Greenhouses with security and containment features.

SUGGESTIONS:

1. Construct the foundation of concrete, concrete block, brick, or similar material.
2. Extend the foundation below the soil line to insure a permanent and stable structure. Build the foundation at least 3 ft. above the soil line.
3. Construct greenhouse floors of materials that are impervious to the contained organisms and can withstand repeated disinfecting with caustic liquids.
4. Install a frame strong enough to support the translucent walls and ceilings.
 - Install translucent wall and ceiling materials strong enough to guarantee the security of the facility. Plexiglas, Lucite, lexon, safety glass, and wire-reinforced glass are acceptable. Polyethylene, vinyl and/or plastic sheeting are NOT acceptable.
 - Seal the translucent panels to the frame with caulk or appropriate materials on the inside and outside surfaces.
 - Consider the installation of screens over the roof to protect it from hail.
 - Consider the installation of an alarm system to detect broken glazing panels or other breaches of containment.

5. Seal joints between the greenhouse and other containment rooms with caulk or other suitable material.
6. If containment greenhouse is detached from the primary containment facility, install a vestibule at each door. (See specialized room section on **Vestibules**.)
7. Install doors between the greenhouse and the rest of the facility that close completely, and seal to their frames.
8. Insure the HVAC system can be turned off to allow greenhouse fumigation. The HVAC system must be the equivalent in design and operation to the system used in the primary containment facilities.
9. Cover ventilation ducts with filters to contain aerially dispersed spores.(if present)

CONSTRUCTION STANDARDS FOR SPECIALIZED ROOMS B. Vestibules

SUGGESTIONS:

1. Install a vestibule in each entrance and /or exit. (Have contractor check local construction codes on vestibules at emergency exits, as they may be prohibited.)
2. Shower rooms can count as a vestibule for the main entrance (see restroom construction).
3. Design vestibule to be at least 6 feet long from door threshold to door threshold.
4. Insure vestibule doors interlock, so that only one door can be opened at a time.
5. Insure thresholds and gaskets of doors seal to their frames.

CONSTRUCTION STANDARDS FOR SPECIALIZED ROOMS C. Install Showers and Restrooms to prevent organism escape.

SUGGESTIONS:

1. Place showers/ restrooms near lowest risk rooms.
2. Insure thresholds and gaskets of restroom doors seal to their frames.

IV. EQUIPMENT STANDARDS

EQUIPMENT STANDARD A. Use Benches, Tables and Other Furniture that are easy to inspect and clean.

SUGGESTIONS:

1. Install work surfaces and laboratory furniture (bench tops, etc.) impervious to water, and resistant to chemicals and heat.
2. Insure spaces between benches, wall cabinets, and equipment are easy to clean and inspect.
3. Dedicate cleaning equipment (mops, brooms, buckets, etc.) for use only in the containment area, and store it in the containment area.

EQUIPMENT STANDARD B. Use equipment to Sterilize contained organisms, soil, plant material, solid waste **or Decontaminate** solid waste and contaminated or infested articles before removing it from the facility.

SUGGESTIONS:

1. Install an autoclave. Double-door pass-through model is recommended. Conduct tests to evaluate effectiveness of autoclave on an annual basis.
2. Effluent from greenhouses, sinks, drains, and other sources must be collected and treated with heat (121°C) for at least an hour. The efficacy of this system must be tested on an annual basis.
3. Install a gas sterilizer for articles that would be damaged by steam. Double-door pass-through model is recommended. This system must be tested on an annual basis.
4. If you are considering an incinerator within the facility, consult your contractor about state, local, and federal laws and ordinances.

EQUIPMENT STANDARD C. Install a Biosafety cabinet to work with organisms.

SUGGESTIONS:

1. Install a Biosafety cabinet, Class II, type A/B3, in which to open foreign source packages. A licensed professional must certify these cabinets annually.

V. OPERATIONAL STANDARDS

OPERATIONAL STANDARD A. A Containment Director is responsible for the daily operation of the facility and its physical integrity.

SUGGESTIONS:

A **Containment Officer** is responsible for the organisms contained in the facility. He/she also maintains a copy of the Standard Operating Procedures (SOP) Manual for the facility. SOPs contain directions for normal use, maintenance, and disinfection of the facility and its equipment.

SOPs also describe how to:

- Respond to a typical emergency event (power outage, fire, glass breaks in containment area (flood, hurricane, earthquake, tornadoes, etc.). Biosecurity for unauthorized removal of materials from containment area.
- Replace translucent panels in greenhouse.
- Monitor visitors.

Copies of SOPs should be available to workers within the containment areas. Each revision must be dated. The SOPs should be revised when changes to procedures are implemented; SOPs should be regularly checked to see if revision is necessary. Revisions of the SOPs should be sent to Containment Facilities staff at the address below.

Containment Officer;

- Implements the SOPs and conditions listed in permits for organisms held by the facility.
- Trains employees and/or authorized personnel in the SOPs.
- Updates copies of construction records (blueprints) for the facility.
- Maintains daily, weekly and monthly maintenance records of the facility.

The Containment Officer updates these lists:

- The names and phone numbers of those to call during emergencies as changes occur.
- The plants species in facility, as changes occur.
- Authorized personnel, as changes occur.
- Incoming and outgoing shipments of permitted organisms, including dead or destroyed incoming organisms, and submits list to USDA, APHIS, PPQ, PPE (Permits & Pest Evaluation) by January 31 of each year (see address on last page.)

SOPs also describe procedures related to all operating standards listed below:

OPERATIONAL STANDARD B. Only Authorized Personnel have routine access to the facility.

Once your facility is adequate to receive plant pests, the behaviors of people who have access to your facility will have far more impact on the containment of the organism than any containment feature. Your selection of individuals to work in this facility is critical to maintenance of plant pest containment. In addition to picking good personnel, please consider the suggestions below.

SUGGESTIONS:

1. Lock exterior doors at all times. Insure only authorized personnel have keys.
2. Train authorized personnel in the SOPs.
3. List the personnel authorized to enter the facility.
4. Require that visitors sign a logbook. Authorized personnel must accompany visitors at all times to insure they do not bring in or carry out pests.
5. Insure emergency exit doors are not used routinely as an entrance.

OPERATIONAL STANDARD C. Wear, sterilize, and handle personal Apparel to minimize the risk of organism escape.

SUGGESTIONS:

1. Insure visitors and employees wear laboratory coat in the containment area and remove it prior to leaving the containment area. Depending on the nature of the plant pathogen to be contained head and foot covers, gloves, overalls and showers may be appropriate.
2. Prohibit entry of overcoats, hats, backpacks, purses, etc. into the containment areas, as these articles may allow organisms to hide and escape.

OPERATIONAL STANDARD D. Use Personal Cleanliness to contain organisms.

SUGGESTIONS:

1. If aerially dispersed spores of contained plant pathogens are present in the facility, insure authorized personnel and visitors completely shower before exiting.

OPERATIONAL STANDARD E. Clean and Disinfect the interior of the facility and its equipment regularly.

SUGGESTIONS:

1. Clean and disinfect the facility, its furniture, and its equipment regularly with bleach or a similar disinfectant.
2. Describe how to change air filters without contamination of containment area.
3. Eliminate undesired pests and pathogens (e.g., any arthropods which might vector contained plant pathogens) from the facility.
4. Autoclave or sterilize solid wastes (cultures, plant materials, soil, trash, etc.) prior to disposal. Wastes should be sterilized in a timely manner, and should not be allowed to accumulate and decay.

OPERATIONAL STANDARD F. Open and Handle packages of permitted organisms to prevent organism release.

SUGGESTIONS:

1. Establish one enclosed area that is easy to disinfect, to open packages received from foreign sources.
2. Place foreign source packages in the biosafety cabinet before opening.
3. Autoclave or incinerate packing materials immediately after the removal of specimens and cultures.
4. Establish procedures to eliminate hitchhiking pests (insects and plant pathogens, fungus, bacteria, etc.)

OPERATIONAL STANDARD G. Start, grow, and store cultures with as few exotic contaminants as possible. Cross contamination indicates poor laboratory practice. However, it may not indicate containment problems.

SUGGESTIONS:

1. List all nonindigenous plant materials used to culture plant pathogens.
2. Sterilize/destroy all packing materials from shipments and contaminants shortly after receipt.
3. Autoclave or sterilize media used to rear organisms before removing it from the facility.
4. Destroy contaminated organisms as soon as detected. This may mean destroying beneficial cultures, if pathogens are found in the culture.
5. Store tissue cultures in screw top vials or equivalent.

OPERATIONAL STANDARD H. Follow all PPQ Regulatory Requirements for organisms received, reared in, or released from the containment facility.

SUGGESTIONS:

1. Meet all PPQ requirements or conditions as listed in permits for organisms kept in the facility. Permits from other Federal and State Agencies may also be required for certain plant pests. Receipt of USDA Plant Pest Permits does not relieve applicants from the responsibility of obtaining other permits. USDA Permits may be withheld or revoked if other Federal and State requirements are not satisfied
2. Send SOP and blueprints to:
Containment Facilities
USDA, APHIS, PPQ, PPB, CF, Unit 133
4700 River Road, Riverdale, MD 20737
Phone (301) 734-5592
FAX (301) 734-5392
3. Obtain permission from PPQ prior to shipping regulated organisms outside of the facility.
4. Maintain a list of all organisms described in PPQ permits that enter and leave the facility. Submit the above list to USDA APHIS PPQ (at address below) by January 31 of every year.
USDA, APHIS, PPQ, PPB, Unit 133
4700 River Road, Riverdale, MD 20737
Phone (301) 734-8896
FAX (301) 734-4300
5. If the facility stops operating as a containment facility, either temporarily or permanently, notify PPQ immediately in writing.

Subject: Containment of Nonindigenous Plant Pathogens

To: Prospective Permittee

Enclosed are DRAFT CONTAINMENT GUIDELINES FOR BACTERIAL PLANT PATHOGENS. Please review these documents and discuss specifications for your containment facility with the PPQ Containment Facility group, phone (301) 851-2046, or fax (301) 734-5392 before you contract or start construction.

Thank you,

Containment Facility Staff