UNIVERSITY OF RHODE ISLAND
USDA APHIS INSPECTED QUARANTINE FACILITY
KINGSTON, RI

STANDARD OPERATING PROCEDURES MANUAL
May 18, 2017
TABLE OF CONTENTS

I. Construction standards for the entire structure
   A. Location and Identification
   B. Floor Plan
   C. Walls, ceilings, and floors
   D. Windows
   E. Doors
   F. HVAC system
   G. Electrical system
   H. Plumbing system
   I. Vacuum Cleaning systems
   J. Vacuum Aspiration systems
   K. Communication system

Construction standards for specialized rooms
   A. Glasshouse
   B. Vestibules
   C. Showers and Restrooms

III. Equipment standards for specialized rooms
   A. Benches, Tables and other Furniture
   B. Sterilization or Decontamination Equipment
   C. Cages and Containers
   D. Biosafety Cabinet

Operational standards
   A. Containment Director
   B. Authorized Personnel
   C. Apparel
   D. Personal Cleanliness
   E. Facility Cleaning & Disinfecting
   F. Chemical Safety
   G. Opening & Handling Incoming Packages
   H. Growing & Storing Organisms and/or Cultures
   I. PPQ Regulatory Requirements

Facility Maintenance and Repairs

Contingency Plans for Emergencies
   A. Accidental Release of an insect under quarantine.
   B. Damage to Windows, Doors, Walls
   C. Medical Emergencies
   D. Fire
   E. Storms
I. Construction standards for the entire structure

A. Location and Identification

The University of Rhode Island Quarantine Facility is located at the end of the University's greenhouse complex at the corner of Upper College Rd. and Flagg Rd. The facility was funded primarily by a 1990 Champlin Foundations grant. Richard Casagrande, Professor of Entomology at the University of Rhode Island, and Lisa Tewksbury, Research Associate at URI use the laboratory as a quarantine facility for newly imported exotic biological control agents, and also as a rearing and research facility for biological control agents that have already cleared USDA quarantine requirements.

Description of Facility:

The facility consists of two parts: an outer office/general lab, and an inner quarantine/rearing lab. There are two entrances to the facility (doors #1 and #2). Both doors are labeled on the outside: "Insect Biological Control Laboratory, Limited Entry". Both doors enter into the office/general lab, which is exterior to the quarantine facility. One entrance is from outdoors, and the second is from the Plant Science and Entomology Dept.’ growth chamber facility.

Office/General Lab:
The quarantine lab entrance, (door #3) is accessed from the main laboratory, and opens into the vestibule. This door has a sign on the outside that reads "USDA APHIS Inspected Quarantine Facility - Only Authorized Personnel May Enter."

The visitor's logbook is kept next to the quarantine lab entrance, and the standard operating procedure manual is kept in the office. The quarantine officer and assistant quarantine officer use this room as their office. All permits and documentation regarding the quarantine facility will be located in this room.

There is an intercom system which has a doorbell button to alert anyone in quarantine to push the button on their receiver intercom. There are three receivers, one in each rearing room which can be used to speak with the person using the doorbell intercom outside of quarantine.

Vestibule:
The vestibule has the provision of a double door entry. The door to the vestibule (door #3) has a Simplex Unicon L1000 Series Combination Lock. After entering the vestibule, the first door must be closed before the second (door #4) is opened. If both doors are opened simultaneously a buzzer will sound.

The vestibule has a blacklight trap operating at all times. It is a Sears Bugwacher electronic insect killer.
The vestibule walls are painted dark green to adhere to the specification that vestibule walls are painted a dark color.

**Quarantine Prep. Room**

The quarantine preparation room is the room immediately after the vestibule. There is a coat rack on the right with lab coats to be put on when entering the facility, and taken off just before leaving.

There is a SANYO laboratory (MLS 3751L) autoclave in this room, which is used to sterilize soil, plant materials, trash, and instruments leaving the quarantine facility.

There is also a dishwasher, which is used to clean cages, plastic bins, petri dishes, and other materials that are used in the quarantine rearing rooms. The dishwasher reaches a temperature over 54° C, which we have found to kill 100% of all insects we have exposed to a normal wash cycle. Tested insects include *Harmonia axyridis*, *Lilioceris lilii*, and *Galerucella calamiensis*.

There is a double basin sink with two drains. Each drain is covered with a 30 mesh screen, sealed with 3M 5200 Marine Adhesive Sealant.

Drainwater from the sink drains and the dishwasher drains into the URI sewer system which is pumped to a treatment facility in Narragansett before being pumped about 1 mile offshore into Narragansett Bay.

There is an emergency exit light in front of the door to the vestibule.

From the interior preparation room there is access to two interior rearing rooms (rooms #1 and #2) through doors 5 and 6. Door #7 is locked (from the inside) and posted with signs on both sides. In the Quarantine Rearing room the sign reads, "**Emergency Exit Only.**"

**Quarantine Room #1**

Room #1 will be used as an isolation room for opening packages of biological control agents, and as a rearing room. There is a hood to be used for opening packages of shipped insects. There is also an aspirator available to catch small flying insects that would most likely be found on the window (described later). Each room in quarantine has a window. There is a refrigerator/freezer within this room that will be used to hold insects, freeze specimens. There is also a Percival I-36LL growth chamber in this room.

**Quarantine Room #2**

This is a rearing room. There is a refrigerator/freezer within this room that will be used to hold insects, freeze specimens, and freeze lab coats before they are taken out of quarantine to be laundered. There is also a Percival I-36LL growth chamber in this room.

**Quarantine Room #3**
This room is currently within quarantine, and the door to the main lab (door #8) is locked and labeled, "USDA APHIS Inspected Quarantine Facility - Do Not Enter", and all three rearing rooms are accessed only through the quarantine vestibule. There is a Percival I-36LL growth chamber in this room.

**Objectives:**
1. Receive shipments of foreign arthropods for biological control of insect and weed pests.
2. Rear foreign arthropods in facility, and conduct necessary testing prior to release under strict quarantine conditions.
3. Rear biological control agents in facility for distribution in Rhode Island and other northeast states.

**B. Floor Plan**

A copy of the blueprints of the building, and a detailed building operation manual are located in the main laboratory of the quarantine facility. A floor plan is appended to this description.

There is only one entry/exit into the quarantine facility itself, and this entry door has a combination lock. There is a vestibule at the primary entry. The office is outside of the containment facility.

There are manometers recording negative pressure in all of the quarantine rooms, including the vestibule. The location of each manometer is marked on the floor plan. Negative pressure is maintained by adjusting the dampers in the exhaust ducts in the attic above each of the quarantine rooms. It is also fine-tuned by adjusting the louvers in both the intake and exhaust vents in each room. The vestibule, quarantine prep room, and quarantine room #3 are all maintained at (0.05 to 0.1 inches water) negative pressure relative to the general lab (which is slightly above ambient air pressure). The innermost labs (quarantine rooms #1 and #2) are maintained with similar negative air pressure relative to the quarantine prep room that they open into. In other words, the air pressure gets more negative as you get farther into the quarantine lab.

**C. Walls, ceilings, and floors:**

**Walls:** All exterior walls of the facility are double concrete block, with insulation between the block layers. Inner walls are of single thickness concrete block.

**Ceilings:** Ceilings are of gypsum wallboard. All seams are sealed, outlets and doors are gasketted. Walls and ceilings are painted with high-gloss white epoxy paint for easy detection of insects.

**Floors:** The entire facility has a poured epoxy floor that extends 6” up all walls. The floor is a light gray-green in color. It is seamless and maintained to a high gloss finish through occasional applications of Butcher’s Wax “High noon” floor finish.
D. Windows: All windows in quarantine lab are triple glazed with an outer shatterproof glass layer, sealed in frames and permanently closed. They are carefully sealed around the perimeter with a silicone-based sealant.

E. Doors: All interior doors are self-closing, steel mounted in steel frames. They are Fenestra Presidential "W" Seamless doors. They are sealed on the top and sides by a magnetized rubber gasket. Every doorway has an oak threshold that is sealed to the floor with silicone. Every door has a vinyl strip (door sweep) on the bottom that has 6 bottom strips to provide a complete seal. The exterior doors from the office/general lab are also steel.

There is a red sign on the entry door (door #3) to the quarantine rooms "USDA APHIS Inspected Quarantine Facility - Only Authorized Personnel May Enter", with the names of the authorized personnel on the sign. There are red signs which say "Insect Biological Control Facility - Limited Entry" on both doors into the exterior lab (doors #1 and #2). There are exit signs at each of the exit doors. The certificate of inspection for the facility is prominently displayed. The entry door is kept locked at all times.

F. HVAC System:
The containment facility has an HVAC system dedicated to it. Except for the ducts in the ceilings, the entire HVAC system is confined to the attic above the quarantine lab. Vents and HVAC ducts into the quarantine laboratory are covered with screens of 100 mesh. All of the intake and outflow vents are marked on the floor plan. There are essentially two separate systems for inflow and exhaust. The inflow system begins with a large screened duct at the north end of the A-frame roof. Air is drawn in by a large squirrel cage fan, which immediately pulls it through a bank of standard paper filters. Following the filters, the air passes through a chamber where steam is pumped into the airstream from a DriSteam humidifier. This air which is now either heated or cooled and humidified is then passed through another set of filters before entering ducts which lead to the ceiling of each room. At the ceiling of each room the air passes through 1 last filter (3/4" polyester) before passing through a 100-mesh nylon screen and entering the room. In the exhaust system air is filtered by 3/4" polyester before passing through a 100-mesh nylon screen and then entering the exhaust ducts located in the ceiling. Exhausted air is pumped through a dedicated squirrel cage fan and through another series of paper filters before being pumped outdoors at the south side of the attic.

G. Electrical system: Outlets and switches have spring-loaded covers and fluorescent light fixtures are sealed to be arthropod and mite-proof. All electrical conduits have an explosion-proof fitting before it leaves each room. These fittings are sealed internally with caulking to prevent possible movement of insects between rooms through the conduit. The two main electrical panels are located in the office/general lab. In the event of power failure, there is a back-up generator available.

H: Plumbing system:
There is a sink in the containment area for cleaning. Drainwater from the sink drains and the dishwasher drains into the URI sewer system that is pumped to a treatment facility in Narragansett before being pumped about 1 mile offshore into Narragansett Bay.
I: **Vacuum Cleaning system:** There is no vacuum cleaning system in the facility.

J: **Vacuum Aspiration system:** There is no vacuum aspiration system in the facility.

K: **Communication system:** Communication between exterior lab and quarantine facility is by telephone and intercom

II. **Construction standards for specialized rooms**

A: **Glasshouse:** There is not a glasshouse attached to the containment facility. There is a University greenhouse accessed through the containment facility office that we use to grow plants needed for insect rearing.

B: **Vestibules:** The vestibule has the provision of a double door entry. The door to the vestibule (door #3) from the office has a Simplex Unicon L1000 Series Combination Lock. After entering the vestibule, the first door must be closed before the second (door #4) is opened. If both doors are opened simultaneously a buzzer will sound.

The vestibule has a blacklight trap operating at all times. It is a Sears Bugwacker electronic insect killer. The vestibule walls are painted dark green to adhere to the specification that vestibule walls are painted a dark color.

C: **Showers and Restrooms:** There are no showers or restrooms in the containment facility. Restrooms are available in the University greenhouse facility, accessed through the office.

III: **Equipment standards for specialized rooms:**

A: **Benches, Tables, and other Furniture:** Within the rearing rooms tabletops and counters are all covered with white plastic laminate.

B: **Sterilization or Decontamination Equipment:**

A SANYO laboratory (MLS 3751L) autoclave is used to sterilize soil, plant materials, trash, and instruments leaving the quarantine facility. All materials are double-bagged and autoclaved at 123° C for 30 minutes. Deionized or distilled water is used inside the stainless steel tank, and tap water is used in the steam exhaust tank. The water is drained, poured down the sink and replaced regularly. Trash is then discarded, and instruments are washed in the dishwasher.

To be sure of the correct functioning of the autoclave, indicator strips will be used with each bag to indicate that the appropriate sterilization temperature was reached. A biological indicator test kit will also be used once every 6 months to confirm that the sterilization within the autoclave is successful. An autoclave log will be kept to record routine operation of the autoclave (Appendix C). If there are problems with either the indicator strips, or the biological indicator test kit this will be recorded in the log. The load will then be re-autoclaved after placing new indicator strips with the material. If
minimum time and temperature is not attained on the second cycle, we will contact the person responsible for maintaining the unit to initiate repairs. Waste will then be treated at the alternate autoclave facility. The thermometer on the autoclave will be calibrated annually, and a written record will be maintained. This will be done as part of our SANYO routine servicing. Cages, plastic bins, and petri dishes are sterilized in a dishwasher, or sterilized with a 10% bleach solution.

C: Cages and Containers:
All adult insects kept in the rearing rooms are kept in 18" x18"x18" or 30” x 30” x 18” insect cages, or plastic containers The cages were obtained from Ward's Biological Supply or Bioquip. They are screened aluminum cages with front and back Plexiglas door panels with tight closing spring latches. Larvae are reared in plastic bins.

To enable us to keep small parasitic wasps in the above cages we will cover cages in Nytex 102-mesh nylon screen from Tetko, Inc.

D: Biosafety Cabinet: There is no biosafety cabinet in this containment facility.

IV. Operational standards

A. Containment Director: Lisa Tewksbury
   Assistant Containment Director: Heather Faubert
   Address: Plant Science Dept.
            University of Rhode Island
            9 East Alumni Avenue, Suite 7
            Kingston, RI 02881
   Phone: 401-874-2750
   FAX: 401-874-2494

   Responsibilities of the Containment Director:

   1. Receipt of Incoming Organisms
      Packages will be taken into Quarantine Room #1. Packages will be inspected for any damages or openings, before being opened inside of a double sleeve cage. Any insects and packing materials will be autoclaved before being discarded. If packaging can be used again it will be put in a freezer for two days before being used again to kill any insects that may be in the packaging.

   2. Maintaining records of all insects received in shipments, reared in the facility, and released from facility. Quarantine Officer will keep a copy of PPQ Form 526 for each organism brought into the facility. Maintains a copy of the Standard Operating Procedures (SOP) Manual, updating as necessary.

   3. Annual reporting on organisms received into facility.

   4. Proper containment of all organisms in the quarantine areas, and handling in a manner to prevent escape of organisms.
5. Obtaining authoritative identification of organisms, and maintenance of voucher specimens.

6. Authorizing the release of organisms from quarantine after screening.

7. Packaging and shipment of organisms in such a manner as to prevent their escape during transport.

8. Creation of permanent records on a database for all organisms received into facility.

9. Regular cleaning and maintenance of facility.

10. Responds to emergency events at containment facility.

11. Monitors visitors.

12. Trains authorized personnel in the SOPs.

13. Maintains daily, weekly, and monthly maintenance records of the facility (Appendix D).

14. Updates lists of names and phone numbers of people to call during emergencies, and authorized personnel.

**B: Authorized personnel:** Only authorized personnel have routine access to the facility. Exterior doors are locked at all times. Only authorized personnel will be given the combination for the main entry door to the quarantine facility. Before entering the facility all visitors will fill out the Quarantine Lab Entry/Exit Log. Authorized personnel must escort all visitors. Authorized personnel do not need to complete the log, but will enter the facility through the vestibule. The first door of the vestibule must be closed before the second is opened. If it isn't, a warning buzzer will sound.

**Authorized Personnel:**
Richard Casagrande
Lisa Tewksbury
Heather Faubert
Alana Russell
Taras Pleskun
Shayna Krasnoff
Amelia May
Tania Santos
Courtney Graham
Rob Healey
Julia Rushton

**Procedures for visitors:**

Before entering the vestibule visitors will be instructed in the function and operation of the quarantine facility. They will be made aware of the importance of preventing release of insects from the facility.
They will be told to leave personal belongings in the general lab, and to refrain from touching anything inside the rearing rooms. Authorized personnel must escort all visitors. In the preparation room everyone, authorized personnel and visitors, must put on lab coats before entering one of the rearing rooms. Before entering the vestibule everyone must remove their lab coats, and check themselves for the presence of insects. When authorized personnel are satisfied that everyone has been checked, visitors will leave through the vestibule.

C: Apparel: Authorized personnel and visitors will wear laboratory coats in the containment area. Coats will be put on in the preparation room before entering laboratory-rearing rooms, and they will be removed before exiting through the vestibule. Overcoats, hats, purses, etc. will be prohibited from the containment facility.

D. Personal Cleanliness: All authorized personnel and visitors will wash their hands before exiting the containment facility.

E. Facility Cleaning and Disinfecting:

1. A SANYO laboratory (MLS 3751L) autoclave is used to sterilize soil, plant materials, trash, and instruments leaving the quarantine facility. All materials are autoclaved at 123° C for 30 minutes.

2. Benches are sanitized using a 10% bleach solution after every use.

3. Cages, plastic bins, and petri dishes are sterilized in a dishwasher, or are washed in the sink, first with a 10% bleach solution and then with dish detergent.

4. Laboratory coats will be kept in the freezer for 48 hours before being laundered outside the facility.

5. The Quarantine Officer will regularly clean the quarantine facility. All necessary cleaning materials are kept inside the preparation room. Trash collected during regular cleaning within the facility will be autoclaved and then discarded.

6. Air filters are changed three times a year within the containment area.

F. Chemical Safety

The following chemicals are stored outside of quarantine in room 120C (Appendix A): Ethyl alcohol and ethyl acetate. MSDS forms are available in a labeled black notebook in the Quarantine Lab office. When either of these chemicals needs to be disposed of the hazardous waste will be collected in appropriate containers, labeled with URI Hazardous Waste labels, sorted by hazard class and stored in the lab's Hazardous Waste Accumulation Area for pickup and proper disposal by Safety and Risk Management.

In the event of a spill or accident, the University Police will be called at: 42121. If an eyewash or a shower are needed they are available in the laboratory next door, room 121. If a trip to the hospital is necessary the book of MSDS forms will be brought as well.
G: Handle and Open Packages:
1. Rearing room #2 has been designated as the room to use to open packages received from foreign sources.
2. Packages are placed in a sleeve cage before opening.
3. Packing materials are immediately autoclaved after the removal of specimens.

H: Growing and Storing Organisms and Cultures:
1. All arthropods are confined in cages that prevent escape.
2. All packing materials from shipments are autoclaved immediately after receipt.
3. All materials used for rearing permitted organisms are autoclaved before removing from the containment facility.
4. Any contaminated organisms will be destroyed as soon as detected.

I: PPQ Regulatory Requirements:
1. Met all PPQ requirements or conditions as listed in permits for organisms kept in the containment facility.
2. Send SOP and blueprints to Plant Pest Containment Program Assistant
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 Plant Pest Containment Program Assistant by January 31 of every year.
5. Maintain voucher specimens for each organism shipped from the facility.
   Use taxonomists recognized by the SEL to identify voucher specimens.
   Notify APHIS-PPQ semi-annually of organisms released into the environment— their identification, the identifier’s name, the number of organisms shipped, location that received the shipment, organization that received the shipment, and where voucher specimens are located.
   House and maintain voucher specimens on site.
   Allow access to voucher specimens.
6. If the facility stops operating as a containment facility notify PPQ.
7. Notify PPQ of any structural or containment changes prior to implementation, the development of blueprints, signing of construction contracts, start of construction, etc.

V: Facility maintenance and repairs:
The quarantine officer on a monthly basis will inspect the facility, and a record will be kept of any repairs that are required.

Routine cleaning and minor repairs will be performed by authorized personnel whenever possible. All important utilities (heating, air conditioning, hot water, electrical boxes, etc.) are located outside of quarantine. If it is necessary for unauthorized university employees to perform a repair within the quarantine facility, they will follow all of the guidelines for entering the facility, and will be escorted at all times by an authorized person.
The building operation manual has detailed maintenance checklists for the Trane Modular Climate Changer Central Station Air Handler (pp. 78-82) and the Trane Air-Cooled Cold Generator (pp.26-27). These include monthly and annual procedures to keep the equipment operating efficiently.

VI: Contingency plans for emergencies:

The quarantine facility was built to be able to withstand most types of emergencies. There are windows to allow ambient light for daylight operating in the case of a power failure. All plumbing is on the interior of the building to prevent freezing of pipes in the event of power outage/heat failure. The concrete block construction with a trussed A-frame roof is designed to withstand any hurricanes or winter storms that we might encounter. Dr. Casagrande lives less than 1 mile from this facility and he is called by the University police in the event of any emergencies at this facility.

The local fire and police departments have been informed of the nature of our facility, and given phone numbers for Dr. Casagrande and Lisa Tewksbury to contact in case of an emergency.

A: Accidental release of an insect under quarantine.
Any suitable host plants of the insect released will be inspected for insects. If any are discovered those insects will be collected or killed using a broad-spectrum insecticide, such as a synthetic pyrethroid.

The following people will be notified in the event of the accidental release of insects under quarantine:

David Hirsch
USDA APHIS PPQ
Room G7 53 North 6th St.
New Bedford, MA 02740
508-997-5955

Kate Aitkenhead
USDA APHIS PPQ
900 Northrop Rd., Suite 6
Wallingford, CT 06492
203-741-5644

Matthew Green
DEM Division of Agriculture
235 Promenade St., Rm. 370
Providence, RI 02908-5767
401-222-2781 ext. 4516

B: Damage to Windows, Doors, and Walls:
Dr. Casagrande will be responsible for making immediate repairs to any damaged structures to prevent the release of insects. He lives within a mile from the facility, and has a shop well equipped with repair materials, plywood, etc. Once this is done the necessary provisions will be made for permanent repairs.
C: Medical Emergencies:  
There is one phone within the prep room, and one phone within the general lab. In the event of medical emergencies the appropriate emergency assistance will be summoned. The ill or injured person's lab coat will be removed and they will be inspected for insects before leaving the quarantine rooms. Local emergency personnel have been notified of the nature of our facility.

D: Fire:  
The local fire department has been informed of the purpose of our facility. The interior quarantine prep room, and the exterior general lab/office, are both equipped with a fire alarm. The prep room is also equipped with an ABC fire extinguisher.

E: Storms:  
There has never been a tornado in Rhode Island. In the event of damage caused to the facility by a hurricane or other major storm, Dr. Casagrande will make immediate repairs.

Appendix A: Greenhouse diagram (quarantine lab attached)
Appendix B: Quarantine Lab
Appendix C: Autoclave Log Template
# AUTOCLAVE LOG

<table>
<thead>
<tr>
<th>Date &amp; Time</th>
<th>Initials</th>
<th>Description of Waste</th>
<th>Cycle Time (minutes)</th>
<th>Max. Temp. (°C)</th>
<th>Indicator Change (+/-)</th>
<th>Spore test Date</th>
<th>Spore Test Results (+/-)</th>
<th>Comments/Repairs</th>
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**MAINTENANCE:** (Indicate date, vendor name, service, etc.)

**Appendix D: Repair Log Template**
# REPAIR/MAINTENANCE LOG

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<th>Date &amp; Time</th>
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<th>Description of Repair</th>
<th>Person conducting repair</th>
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