

## CDC Import Permit Inspection Checklist for Arthropod Containment Level 2 (ACL-2)

Entity Name: \_\_\_\_\_  
 Street Address: \_\_\_\_\_  
 City, State, Zip: \_\_\_\_\_  
 Lead Inspector: \_\_\_\_\_  
 Other Inspectors: \_\_\_\_\_  
 Building/Room(s): \_\_\_\_\_  
 PI(s): \_\_\_\_\_

Inspection Date: \_\_\_\_\_

Entity Name:		Inspection Date:			
Reference	Statement	Yes	No	N/A	Comments
CFR: 71.54 (b)	<b>Unless excluded pursuant to paragraph (f) of this section, a person may not import into the United States any infectious biological agent, infectious substance or vector unless:</b>				
CFR: 71.54 (b)(1)	It is accompanied by a permit issued by CDC. The possession of a permit issued by CDC does not satisfy permitting requirements placed on materials by the U.S. Department of Agriculture that may pose hazards to agriculture or agricultural production in addition to hazards to human health.				
CFR: 71.54 (b)(2)	The importer takes measures to help ensure the shipper complies with all permit requirements and conditions.				
CFR: 71.54 (b)(3)	The importer has implemented biosafety measures commensurate with the hazard posed by the infectious biological agent, infectious substance, and/or vector to be imported, and the level of risk given its intended use.				
CFR: 71.54 (b)(4)	The importer is in compliance with all applicable legal requirements concerning the packaging and shipment of infectious substances.				
CFR: 71.54 (c)	If noted as a condition of the issued permit, subsequent transfers of any infectious biological agent, infectious substance or vector within the United States will require an additional permit issued by the CDC.				
<b>A</b>					
Location	Furniture and incubators containing arthropods are located in such a way that accidental contact and release by laboratorians, custodians, and service persons is unlikely. This may be achieved by locating arthropods in dedicated rooms, closets, incubators located outside the traffic flow or similar measures.				

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<b>Supply Storage</b>	The area is designed and maintained to enhance detection of escaped arthropods. Equipment and supplies not required for operation of the insectary should not be located in the insectary. All supplies for insect maintenance that must be kept within the insectary are located in a designated area and not on open shelves. It is recommended that a closed storage room, cabinets with tight fitting doors or drawers be used. Doors and drawers are opened only for access. Insect diet should be kept in sealed containers.				
<b>General Arthropod Elimination</b>	Accidental sources of arthropods from within the insectary are eliminated. This may be accomplished by cleaning work surfaces after a spill of materials, including soil or water that might contain viable eggs. Pools of water are mopped up immediately.				
<b>Primary Container Cleaning and Disinfestation</b>	Practices should be in place such that arthropods do not escape by inadvertent disposal in primary containers. Cages and other culture containers are appropriately cleaned to prevent arthropod survival and escape (e.g. heated to over the lethal temperature or killed by freezing). Containers are disinfected chemically and/or autoclaved if used for infected material. Autoclaving or incineration of primary containers is recommended for containers holding uninfected material.				
<b>Primary Container Construction</b>	Cages used to hold arthropods are non-breakable and screened with mesh of a size to prevent escape. Containers are preferably autoclavable or disposable. Openings designed to prevent escape during removal and introduction of arthropods are recommended.				
<b>Disposal of Arthropods</b>	Living arthropods are not to be disposed of. All wastes from the insectary (including arthropod carcasses, and rearing medium) are transported from the insectary in leak-proof, sealed containers for appropriate disposal in compliance with applicable institutional or local requirements. All stages of arthropods are killed before disposal. Autoclaving or incineration of arthropod materials is recommended. Material may be killed with hot water or freezing before flushing down drains. Infected arthropods are autoclaved or incinerated.				
<b>Isolation of Uninfected Arthropods</b>	Spread of agents to uninfected arthropods is prevented. Generally, this is accomplished by isolating infected material in a separate room.				
<b>Primary Container Identification and Labeling</b>	Arthropods are identified adequately. Labels giving species, strain/origin, date of collection, responsible investigator, and so on are firmly attached to the container (and cover if removable). Vessels containing stages with limited mobility (e.g. eggs, pupae, hibernating adults) are securely stored.				
<b>Prevention of Accidental Dispersal on Persons or via Sewer</b>	Before leaving the insectary and after handling cultures and infected arthropods, personnel wash their hands, taking care not to disperse viable life stages into the drainage system. No infected material is disposed of via the sewer, all material is destroyed by heat or freezing and preferably by autoclaving or incineration. Air curtains are recommended as appropriate.				

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<b>Pest Exclusion Program</b>	A program to prevent the entrance of wild arthropods (e.g. houseflies, cockroaches, spiders) and rodents effectively precludes predation, contamination, and possible inadvertent infection.				
<b>Escaped Arthropod Monitoring</b>	Investigators assess whether escapes are occurring by instituting an effective arthropod trapping program to monitor the escape prevention program. Oviposition traps, ground level flea traps, oil-filled channels surrounding tick colonies, light traps for mosquitoes and so on are recommended. Records of exterior captures are maintained.				
<b>Source and Harborage Reduction</b>	Harborage and breeding areas are eliminated. Furniture and racks are minimized and can easily be moved to permit cleaning and location of escaped arthropods. Equipment in which water is stored or might accumulate (e.g. humidifiers) is screened to prevent arthropod access, or chemicals to prevent arthropod survival.				
<b>Microbiological and Medical Sharps</b>	Syringes that re-sheath the needle, needle-less systems, and other safe devices are used when appropriate. Plastic-ware is substituted for glassware whenever possible.				
<b>Arthropod Sharps</b>	In addition to minimizing arthropod sharps, these are restricted for use in the insectary if infected materials are used.				
<b>Routine Decontamination</b>	Equipment and work surfaces are routinely decontaminated with an effective chemical or by radiation (e.g. heat) after actual or potential contact with an infectious agent, and especially after overt spills and splashes of viable materials (including soil or water that might contain infectious agents or eggs.)				
<b>Notification and Signage</b>	Persons entering the area are aware of the presence of arthropod vectors. If infected material is present, a BSL-2 biohazard sign is posted on the entrance to the insectary listing all species handles within and is updated whenever new species are introduced or pathogenic infectious agents known or suspected to be present, lists the name and telephone number of the responsible person(s) and indicates any special requirements for entering the laboratory (e.g. the need for immunizations or respirators).				
<b>Procedure Design</b>	All procedures are carefully designed and performed to prevent arthropod escape.				
<b>Safety Manual</b>	A safety manual is prepared, approved by the IBC, and adopted. The manual contains emergency procedures, standard operating procedures, waste disposal and other information necessary to inform personnel of the methods for safe maintenance and operation of the insectary.				

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<b>Training</b>	Laboratory personnel are advised of special hazards are required to follow instructions on practices and procedures contained in the safety manual. Adherence to established safety procedures and policies is made a condition of employment and is part of the annual performance review of every employee. Personnel receive annual updates and additional training as necessary for procedural or policy changes. Records of all training are maintained.				
<b>Medical Surveillance</b>	An appropriate medical surveillance program is in place. All personnel receive appropriate immunizations or tests for the agents handled or likely to be present. When appropriate, a serum surveillance system is implemented (see BMBL for guidance). Personnel are aware of the symptoms of infection and the procedure to follow in reporting these. In general, persons who may be at increased risk of acquiring infection, or for whom infection may be unusually hazardous (e.g. immunocompromised), are not allowed in the insectary unless special personal protection procedures are in place to eliminate extra risk.				
<b>Access Restrictions</b>	Routine access is limited to trained persons and accompanied guests. Service persons are made aware of the hazards present and the consequences of arthropod release and contact with agents that may be present.				
<b>Special Arthropod Handling Containers and Areas</b>	Infected arthropods are prevented from release into the laboratory area. This may be accomplished by secure glove boxes, biosafety cabinets, custom handling trays etc. These may vary from BSL recommendations insofar as necessary to safely contain both the arthropod and any agent. Such modifications should be made only in consultation with experts in handling the specific types of infected arthropods and biosafety experts. A dedicated area for handling infected material is recommended. This is preferably a separate cubicle, walk-in incubator, or screen room.				
<b>Safe Transport in the Laboratory</b>	All infectious and potentially infectious samples are collected, labeled, transported, and processed in a manner that contains and prevents transmission of the agent(s). Transfer of arthropods between manipulation and holding areas is in non-breakable secure containers.				
<b>B</b>					
<b>IACUC and IBC Approval</b>	IBC approval is required and IACUC if vertebrates are used as hosts				
<b>Housing of Non-Arthropod Animals</b>	Other animals are not accessible to the arthropods. Animals used as hosts or blood sources generally are not housed with arthropods. If present, they are adequately protected from access by escaped arthropods, and protocols are approved by the IBC and IACUC.				

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<b>Containment During Blood-Feeding</b>	Arthropods fed on host animals are prevented from accidental transfer to host cages. When handling/removing animals after exposure to arthropods, precautions must be taken to prevent arthropod escape through screens, covers, and by flying. Host animals are inspected closely (e.g. concealment in fur, ears, crevices), and primary container is sufficiently robust to prevent escape during feeding. ACL-2 containment of arthropods during blood-feeding are more stringently assured by special practices and container design.				
<b>Blood Source</b>	The blood source is considered as a source of inadvertent arthropod infection and transmission. Measures are implemented to prevent such an event. Use of sterile blood or blood from sources known to be pathogen-free is recommended. In contrast, use of blood from animals or humans whose disease status is uncertain is to be avoided.				
<b>Escaped Arthropod Handling</b>	Loose arthropods must be killed and disposed of, or recaptured and returned to the container from which they escaped. Infected arthropods must not be killed with bare hands, and must be transferred using filtered mechanical or vacuum aspirators				
<b>Accidental Release reporting</b>	A release procedure is developed and posted. This includes contacts and immediate mitigating actions. Accidents that result in release of infected arthropods from primary containment vessels, or that result in overt exposure to infectious material must be reported immediately to the insectary director who is responsible for ensuring that appropriate and documented action is taken to mitigate the release. Location, number, and type of material are prominently posted until the source is eliminated. Follow-up medical evaluation, surveillance, and treatment are provided as appropriate, and written records are maintained.				
<b>Movement of Equipment</b>	All equipment must be appropriately decontaminated and disinfested before transfer between rooms within the insectary, and before removal from the insectary.				
<b>C</b>					
<b>Eye and Face Protection</b>	Appropriate face/eye and respiratory protection are worn by all personnel entering the insectary.				
<b>Gloves</b>	Gloves are worn when handling potentially infected arthropods, blood, and associated equipment and when contact with potentially infectious material is unavoidable.				
<b>Torso Apparel</b>	White laboratory coats, gowns, and/or uniforms are worn at all times in the insectary when handling blood, vertebrate animals, and infected materials.				
<b>Personal Clothing</b>	Clothing should minimize the area of exposed skin (e.g. skirts, shorts, open-toed shoes, sandals, tee shirts are inadvisable), since this can increase the risk of attracting and being bitten by a loose arthropod.				

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<b>Arthropod-Specific Personal Protective Equipment</b>	Personal protective equipment is worn as appropriate e.g. respirators for arthropod-associated allergies, particle masks, head covers. Personal protective equipment is used for all activities involving manipulations of infected or potentially infected arthropods.				
<b>D</b>					
<b>Location of Insectary</b>	The insectary is separated from the areas that are open to unrestricted personnel traffic within the building. These are opened, for example, by key lock, proximity reader, or card key.				
<b>Insectary Doors</b>	Recommended entrance to the insectary is via a double-door vestibule that prevents flying and crawling arthropod escape. For example, the two contiguous doors must not be opened simultaneously. Internal doors may open outwards or be sliding, but are self-closing, and are kept closed when arthropods are present. Additional barriers (e.g. screened partitions, hanging curtains) are highly recommended.				
<b>Insectary Windows</b>	Windows are not recommended. Any windows present are resistant to breakage (e.g. double paned or wire reinforced) and well sealed. If present, fixed light windows are recommended.				
<b>Vacuum Systems</b>	If a central vacuum system is installed, each service outlet is fitted with suitable barriers/filters to prevent arthropod escape. Filters are installed to permit decontamination and servicing. Other vacuum devices are appropriately filtered to prevent transfer and exhausting of arthropods.				
<b>Interior Surfaces</b>	The insectary is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior walls are light-colored so that a loose arthropod can be easily located, recaptured, or killed. Gloss finishes, ideally resistant to chemical disinfectants and fumigants, are recommended. Floors are light colored, smooth and uncovered. Ceilings are low as possible to simplify detection and capture of flying insects.				
<b>Floor Drains</b>	Floor drains are modified to prevent accidental release of arthropods and agents. If present, traps must be filled with an appropriate chemical treatment to prevent survival of all arthropod stages (e.g. mosquito larvae).				
<b>Plumbing and Electrical Fixtures</b>	Internal facility appurtenances (e.g., light fixtures, pipes and ducting) are minimal since these provide hiding places for loose arthropods. Penetrations of walls, floors, and ceilings are minimal and sealed/caulked. Ideally, light fixtures are flush with the ceiling, sealed, and accessed from above.				

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<b>HVAC</b>	Ventilation is appropriate for arthropod maintenance, but does not compromise containment of the agent or arthropod. Examples include, exhaust air is discharged to the outside without being recirculated to other rooms; appropriate filter/barriers are installed to prevent escape of arthropods; the direction of airflow in the insectary is inward; a progressively negative pressure gradient is maintained as distance from the main entrance increases; fans located in the vestibule and internal corridor can be used to help prevent escape of flying arthropods; air curtains are located in vestibules and doorways.				
<b>Sterilization Equipment</b>	An autoclave is available conveniently located to rooms containing arthropods within the insectary building.				
<b>Sink and Shower</b>	The facility has a hand-washing sink with hot water and with suitable plumbing to prevent arthropod escape.				
<b>Illumination</b>	Illumination is appropriate for arthropod maintenance but does not compromise arthropod containment, impede vision, or adversely influence the safety of procedures within the insectary. Lighted (or dark) openings that attract escaped arthropods are avoided.				
<b>Biosafety Cabinets</b>	HEPA filtered exhaust air from Class II biological safety cabinets can be recirculated into the insectary provided that it is certified annually. If exhausting to the outside, the cabinet must be installed appropriately.				
<b>Facility Compliance Monitoring</b>	The facility is evaluated annually for compliance to the ACL-2 level. The principle investigator or insectary director inspects the facility annually to ensure that alterations and maintenance have not compromised the containment characteristics. Adequacy of the practices and facility in view of changes in research protocols, agents, or arthropods are considered.				

**Inspector summary and comments:**

Recommendations:

Inspector completing checklist:

Date:

Other inspectors present:

Date: