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ACL1	Arthropod Containment Guidelines (ACG) American Society of Tropical Medicine and Hygiene (ASTMH). Uninfected Arthropod non- pathogenic, present in local geography, or exotic non viable	N/A	Conform	Non- Conform	Comments
	Furniture and incubators containing arthropods are located in such a way that accidental contact and release is minimized. This may be achieved by locating arthropods out of the flow of general traffic, avoiding hallways, or placing them in closets.				
	The area is maintained to allow detection of escaped arthropods. For example, materials unrelated to arthropod rearing and experimentation (e.g., plants, unused containers, clutter) that provide breeding sites and harborages are minimized.				
	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				
	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).				
	Cages used to hold arthropods effectively prevent escape of all stages. Screened mesh, if used, is durable and of a size appropriate to prevent escape. Non-breakable cages are recommended. Bags, rearing trays and so on effectively prevent leakage and escape.				
	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 material infected with a non-pathogen is recommended. Material may be killed with hot water or freezing before flushing down drains.				
	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.				
	Personnel take appropriate precautions to prevent transport or dissemination of arthropods from the insectary on their persons or via the sewer.				
	A program to prevent the entrance of wild arthropods (e.g., houseflies, cockroaches, spiders) and rodents effectively precludes predation, contamination, and possible inadvertent infection.				
	Investigators assess whether escapes are occurring. An effective arthropod trapping program is recommended to monitor the escape prevention program.				
	Harborage and breeding areas are reduced as appropriate. Furniture and racks are minimized and can be easily moved to permit cleaning and location of escaped arthropods.				
	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.				
	Persons entering the area are aware of the presence of arthropod vectors.				

	Special Practices				
	IACUC approval is required for use of vertebrate animals used as hosts. IBC approval is required for non-exempt recombinant DNA protocols.				
	Animals not necessary for culture of the arthropods are not accessible to the arthropods. Animals used as hosts or blood sources may be housed within the insectary but are adequately protected from access by escaped arthropods. Protocols for vertebrate animal use are approved by the local IACUC.				
	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 the primary container is sufficiently robust to prevent escape during feeding.				
	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.				
	Escaped arthropods are killed or collected and properly disposed of.				
	The insectary director is notified promptly of accidental release of vectors.				
	Safety Equipment (Primary Barriers)				
	Gloves are worn when handling host animals or blood used to feed the arthropods.				
	White laboratory coats, gowns, and/or uniforms are worn at all times in the insectary when handling blood and vertebrate animals.				
	Personal protective equipment is worn as appropriate e.g., respirators for arthropod-associated allergies, particle masks, head covers.				
	Facilities (Secondary Barriers)				
	The insectary area is separated from areas that are used for general traffic within the building.				
	Doors openings, whether covered by rigid panels, glass, screens, plastic sheets or cloth, minimize escape and entrance of arthropods.				
	Windows, if present, effectively prevent escape of the smallest arthropods contained within.				
ACL1	Arthropod Containment Level -1				
	A. Standard Practices				
	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 out of the traffic flow or similar measures.				
	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.				
	General Arthropod Elimination. ACL-1				

In addition to cleaning cages and culture containers to prevent arthropod escape as in ACL-1, 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.				
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.				
In addition to standard ACL-1 disposal practices, autoclaving or incineration of arthropod materials is recommended. Infected arthropods are autoclaved or incinerated.				
Spread of agents to uninfected arthropods is prevented. Generally this is accomplished by isolating infected material in a separate room.				
Primary Container Identification and labeling . ACL-1				
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 through the sewer. If uninfected materials are 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.				
Pest Exclusion Program. ACL-1				
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. Particularly in the case when exotic arthropods are used, exterior monitoring is recommended. Records of exterior captures are maintained.				
Harborage and breeding areas are eliminated. Furniture and racks are minimized and can be easily 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 contains chemicals to prevent arthropod survival.				
Microbiological and Medical Sharps. ACL-1				
In addition to minimizing arthropod sharps, these are restricted for use in the insectary if infected materials are used.				
Equipment and work surfaces in the insectary 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).				
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 handled within and is updated whenever new species are introduced or pathogenic infectious agents are present. The hazard warning sign identifies the arthropod species, agent(s) 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 insectary (e.g., the need for immunizations or respirators).				
All procedures are carefully designed and performed to prevent arthropod escape				

<p>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.</p>				
<p>Laboratory personnel are advised of special hazards and 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.</p>				
<p>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.</p>				
<p>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.</p>				
<p>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, walking incubator, or screen room.</p>				
<p>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.</p>				
<i>Special practices</i>				
<p>IBC approval is required and IACUC if vertebrates are used as hosts.</p>				
<p>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 IAUCUC.</p>				
<p>Recommendations for ACL-1 containment of arthropods during blood-feeding are more stringently assured by special practices and container design.</p>				
<p>Blood Source. ACL-1</p>				
<p>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.</p>				

<p>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.</p>				
<p>All equipment must be appropriately decontaminated and disinfested before transfer between rooms within the insectary, and before removal from the insectary.</p>				
<p>Safety Equipment (Primary Barriers)</p>				
<p>Appropriate face/eye and respiratory protection are worn by all personnel entering the insectary.</p>				
<p>Gloves are worn when handling potentially infected arthropods, blood, and associated equipment and when contact with potentially infectious material is unavoidable.</p>				
<p>White laboratory coats, gowns, and/or uniforms are worn at all times in the insectary when handling blood, vertebrate animals, and infected materials.</p>				
<p>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.</p>				
<p>In addition to ACL-1 measures, personal protection equipment is used for all activities involving manipulations of infected or potentially infected arthropods.</p>				
<p>Facilities (Secondary Barriers)</p>				
<p>The insectary is separated from areas that are open to unrestricted personnel traffic within the building. It is recommended that this be accomplished by at least two self-closing doors that prevent passage of the arthropods. Increased levels of physical isolation are recommended, e.g., separate buildings, wings, suites.</p>				
<p>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.</p>				
<p>Windows are not recommended, but if present cannot be opened and are well sealed. Windows must be resistant to breakage (e.g., double paned or wire-reinforced).</p>				
<p>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.</p>				
<p>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 as low as possible to simplify detection and capture of flying insects.</p>				
<p>Floor drains are modified to prevent accidental release of arthropods and agents. If present, traps must be filled with an appropriate larvae chemical treatment to prevent survival of all arthropod stages (e.g., mosquito larvae).</p>				

	Internal facility appurtenances (e.g., light fixtures, pipes, 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.				
	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.				
	An autoclave is available conveniently located to rooms containing arthropods within the insectary building.				
	The facility has a hand-washing sink with hot water and with suitable plumbing to prevent arthropod escape.				
	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.				
	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.				