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Arthropod Containment Levels **(ACLs)**

WHEN ARTHROPODS are used, facilities, trained staff and established practices must be in place to ensure appropriate safety, and the protection of health and well-being of workers and the environment. This publication provides guidelines for laboratory work with arthropod vectors of pathogenic agents, and has been prepared in response to concerns related to the consequences of an accidental release of arthropods. These consequences (risk factors) are basically answering the question "What happens if the arthropod escapes?" and the suggested containment levels address the question "How do we prevent escape?" If working with a vector in a particular set of circumstances (see Table 1), a certain containment level may be recommended. The IBC is an essential component in establishing the appropriate ACL. It is responsible for reviewing a research protocol and decides at what level of containment the experiments must be performed.

Where an arthropod is infected with an agent, the containment level required is automatically increased to at least that required for the agent, regardless of factors such as the competence of that arthropod for the particular pathogen. An example is the use of male mosquitoes to propagate dengue viruses. Although they cannot transmit by bite, the presence of the agent requires that they be held at BSL-2 level. Furthermore, in recognition of the fact that escape of uninfected exotic arthropods is to be prevented by all reasonable means, unless unusual measures are taken to reduce risks, these are also handled at the ACL-2 level or higher.

One advantage of working with certain arthropods is that the risk of release can be effectively manipulated by, e.g., performing relatively high-risk experiments during the winter when any escaped arthropods would quickly be killed by adverse environmental conditions. For example, the IBC might use such biological considerations to "downgrade" a particular protocol from ACL-3 to ACL-2, providing that experiments are performed during a particular period. Documentation of the justification for this decision-making process should be prepared to ensure careful consideration of the risks.

It is impossible to prescribe universal levels of containment for a particular species since the risks associated with its accidental release from a laboratory are determined by several factors e.g., the climate at the facility and history of transmission in that location. The accidental release of an uninfected anthropophilic tropical vector species during the winter in Wisconsin, might be considered as significantly less of a "risk" than the release of the same species in a tropical area in which it could become permanently established and act as a bridging vector of an established zoonotic pathogen to humans. Furthermore, the existence of zoonoses means that we have to consider certain pathogens that are predominantly an animal health issue. USDA guidelines must therefore be considered when assigning a containment level to a particular vector species.

Although specific details are not covered here, it is important to develop a response procedure that is appropriate in case of an accidental release. The ideal response would be one in which all released arthropods are killed almost immediately after the escape. This may be impossible if the escaped arthropods get outside of the laboratory, hence the use of several barrier levels are recommended to maximize the opportunities for location and destruction of the escapees.

TABLE 1. SUMMARY OF ARTHROPOD CONTAINMENT LEVELS

Arthropod containment level:	1		2	3	4
Arthropod distribution, escaped arthropod fate	Exotic, inviable or transient	Indigenous	Exotic with establishment, indigenous, and transgenic		
Infection status	Uninfected or infected with non-pathogen		Up to BSL-2	Up to BSL-3	BSL-4
Active VBD cycling	No	Irrelevant			
Practices	ACL-1 Standard Arthropod-Handling Practices		ACL-1 plus more rigorous disposal, signage, and limited access	ACL-2 with more highly restricted access, training and record-keeping	ACL-3 with high access restriction, extensive training, full isolation
Primary Barriers	Species-appropriate containers		Species-appropriate containers	Escape-proof arthropod containers, glove boxes, BSC	Escape-proof arthropod containers handled in cabinet or suit laboratory
Secondary Barriers			Separated from laboratories, double doors sealed electrical/ plumbing openings. Breeding containers and harborages minimized	BSL-3	BSL-4

Three fates of arthropods upon accidental escape are classified here: (1) Inviable; conditions are sufficiently unfavorable to the arthropod that reproduction does not occur. (2) Transient; conditions vary either seasonally or annually such that the arthropod could reproduce upon escape but would be eliminated during a typical climatic year. (3) Establishment; the conditions found in the range of the arthopod are sufficiently similar to those of the laboratory location that escaped arthropods could reasonably be expected to persist through a typical climatic year. Active Local VBD Cycling means that transmission of vector-borne diseases of public health importance that are known to be or probably transmitted by the arthropod are cycling in the locale. Indigenous species are those biological species whose current range includes the research location. All others are considered exotic.

ARTHROPOD CONTAINMENT LEVEL 1 (ACL-1)

Arthropod Containment Level 1 (ACL-1) is suitable for work with uninfected arthropod vectors or those infected with a non-pathogen including: 1) arthropods that are already present in the local geographic region regardless of whether there is active vector borne disease transmission in the locale, and 2) exotic arthropods that upon escape would be inviable or become only temporarily established in areas not having active vector-borne disease transmission. This category would include most educational use of arthropods. A summary of the containment levels is provided in Table 1.

A. Standard practices

Location of arthropods. 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.

Supply storage. 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.

General arthropod elimination. 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.

Primary container cleaning and disinfestation. 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, or chilled below, the lethal temperature).

Primary container construction. 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.

Disposal of arthropods. 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.

Primary container identification and labeling. 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.

Prevention of accidental dispersal on persons or via sewer. Personnel take appropriate precautions to prevent transport or dissemination of arthropods from the insectary on their persons or via the sewer.

Pest exclusion program. A program to prevent the entrance of wild arthropods (e.g., houseflies, cockroaches, spiders) and rodents effectively precludes predation, contamination, and possible inadvertent infection.

Escaped arthropod monitoring. Investigators assess whether escapes are occurring. An effective arthropod trapping program is recommended to monitor the escape prevention program.

Source and harborage reduction. 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.

Microbiological and medical sharps. 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.

Notification and signage. Persons entering the area are aware of the presence of arthropod vectors.

B. Special practices

IACUC and IBC approval. IACUC approval is required for use of vertebrate animals used as hosts. IBC approval is required for non-exempt recombinant DNA protocols.

Housing of non-arthropod animals. 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.

Containment during blood-feeding. 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.

Blood source. 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 arthropod handling. Escaped arthropods are killed or collected and properly disposed of.

Accidental release reporting. The insectary director is notified promptly of accidental release of vectors.

C. Safety equipment (primary barriers)

Gloves. Gloves are worn when handling host animals or blood used to feed the arthropods.

Torso apparel. White laboratory coats, gowns, and/or uniforms are worn at all times in the insectary when handling blood and vertebrate animals.

Arthropod-specific personal protective equipment. Personal protective equipment is worn as appropriate e.g., respirators for arthropod-associated allergies, particle masks, head covers.

D. Facilities (secondary barriers)

Location of insectary. The insectary area is separated from areas that are used for general traffic within the building.

Insectary doors. Door openings, whether covered by rigid panels, glass, screens, plastic sheets or cloth, minimize escape and entrance of arthropods.

Insectary windows. Windows, if present, effectively prevent escape of the smallest arthropods contained within.

ARTHROPOD CONTAINMENT LEVEL 2 (ACL-2)

Arthropod Containment Level 2 (ACL-2) must be practiced if working with exotic and indigenous arthropods infected with BSL-2 agents associated with animal and/or human disease, or that are suspected of being infected with such agents. Uninfected genetically modified arthropod vectors also fall under this level provided the modification has no, or only negative effects on viability, survivorship, host range, or vector capacity (see Risk Assessment). ACL-2 builds upon the practices, procedures, containment equipment, and facility requirements of ACL-1. It is more stringent in the physical containment, disposal, and facilities design. Moreover, access is more restricted than ACL-1. The decision to cultivate infected exotic arthropods under ACL-2 conditions in active transmission areas or in cases in which establishment is a possibility requires that measures that otherwise would only be recommended or preferred must be met.

A. Standard practices

Location of arthropods. 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.

Supply storage. 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. As per ACL-1

Primary Container Cleaning and Disinfestation: 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.

Primary Container Construction: 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.

Disposal of Arthropods: In addition to standard ACL-1 disposal practices, autoclaving or incineration of arthropod materials is recommended. Infected arthropods are autoclaved or incinerated.

Isolation of Uninfected Arthropods: 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. As per ACL-1

Prevention of Accidental Dispersal on Persons or via Sewer: 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. As per ACL-1

Escaped Arthropod Monitoring: 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, etc., on are recommended. Particularly in the case when exotic arthropods are used, exterior monitoring is recommended. Records of exterior captures are maintained.

Source and Harborage Reduction: 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. As per ACL-1

Arthropod Sharps: In addition to minimizing arthropod sharps, these are restricted for use in the insectary if infected materials are used.

Routine Decontamination: 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).

Notification and Signage: 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). An example of a sign is provided with the Editorial.

Procedure Design: All procedures are carefully designed and performed to prevent arthropod escape

Safety Manual: 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.

Training: 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.

Medical Surveillance: 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.

Access Restrictions: 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.

Special Arthropod Handling Containers and Areas: 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.

Safe Transport in the Laboratory: 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. Special practices

IACUC and IBC approval. IBC approval is required and IACUC if vertebrates are used as hosts.

Housing of non-arthropod animals: 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.

Containment during blood-feeding. Recommendations for ACL-1 containment of arthropods during blood-feeding are more stringently assured by special practices and container design.

Blood source. As per ACL-1

Escaped arthropod handling. 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.

Accidental release reporting. 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.

Movement of equipment. All equipment must be appropriately decontaminated and disinfested before transfer between rooms within the insectary, and before removal from the insectary.

C. Safety equipment (primary barriers)

Eye and face protection. Appropriate face/eye and respiratory protection are worn by all personnel entering the insectary.

Gloves. Gloves are worn when handling potentially infected arthropods, blood, and associated equipment and when contact with potentially infectious material is unavoidable.

Torso apparel. White laboratory coats, gowns, and/or uniforms are worn at all times in the insectary when handling blood, vertebrate animals, and infected materials.

Personal clothing. 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.

Arthropod-specific personal protective equipment. In addition to ACL-1 measures, personal protection equipment is used for all activities involving manipulations of infected or potentially infected arthropods.

D. Facilities (secondary barriers)

Location of insectary. 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.

Insectary doors. 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.

Insectary windows. 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).

Vacuum systems. 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.

Interior surfaces. 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.

Floor drains. 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).

Plumbing and electrical fixtures. 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.

HVAC. 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.

Sterilization equipment. An autoclave is available conveniently located to rooms containing arthropods within the insectary building.

Sink and shower. The facility has a hand-washing sink with hot water and with suitable plumbing to prevent arthropod escape.

Illumination. 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.

Facility compliance monitoring. 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.

ARTHROPOD CONTAINMENT LEVEL 3 (ACL-3)

Arthropod containment level 3 (ACL-3) involves practices suitable for work with potential or known vectors that are, or may be infected with, BSL-3 agents associated with human disease. Arthropods that are infected or potentially infected with BSL-3 pathogens may pose an additional hazard if the insectary is located in an area where the species is indigenous, or if alternative suitable vectors are present, as an escaped arthropod may introduce the pathogen into the local population. ACL-3 builds upon the practices, procedures, containment equipment, and facility requirements of ACL-2. It differs in that access is more restricted, and the microbiological containment takes a more prominent role in determining the practices and facilities.

An aspect of working with BSL-3 pathogens that needs to be addressed is the use of biological safety cabinets. The BMBL states that "All procedures involving the manipulation of infectious materials are conducted within biological safety cabinets or other physical containment devices, or by personnel wearing appropriate protective clothing and equipment." Most workers with BSL-3 agents utilize biosafety cabinets, rather than more cumbersome protective equipment, and seem to regard this approach as the standard. Many medical entomologists and vector biologists have therefore been introduced into BSL-3 research under the impression that they must perform all work involving BSL-3 agents within a biosafety cabinet.

Manipulating small arthropods in a biosafety cabinet can be extremely difficult. The airflow can blow small arthropods around the cabinet, into the filters, and into inaccessible locations. If working with cold-anesthetized mosquitoes on a chill table for example, the arthropods can be blown from the table, recover, and then fly around. The airflow may also disrupt the presentation of cues such as body temperature that stimulate host seeking and feeding. The use of a biological cabinet can thus increase the risks associated with working with arthropod vectors. Whereas a cabinet might safely be used to prepare infectious material, the best option may be to perform infectious procedures in a secure area not exposed to strong air currents. Hunt and Tabachnick (1996) recommend that "insects are never manipulated on an open bench." These workers provide plans to construct a purpose-designed glove box for such work. SALS (1980) stated "infection, anesthetization with carbon dioxide, and transfer of arthropods are done in such a manner that risk of infection of workers by aerosols is minimized. This can be accomplished by use of (a) protective clothing and respirator masks, (b) a BSC, or (c) a plastic isolator with sleeve openings with or without an air exhaust." The researcher should wear appropriate personal protective clothing and equipment and carefully follow BSL-3 procedures. The necessary clothing may impair dexterity that is essential for performing procedures such as the dissection of small arthropods. In such cases, the researcher should carefully weigh the risks presented and reduced by the use of such by protective gear. However, safety cannot be compromised. A researcher might rehearse these procedures using the required clothing for BSL-3 work, but working with uninfected arthropods.

To prevent arthropod escape, arthropod work is performed in a designated area, preferably small and self-contained within the laboratory for example, a cage-like room constructed of fine mesh (see Facilities). In the event of escape, the search area is therefore small, and the chances of locating the escaped arthropod are correspondingly high. When maintaining arthropods that require ACL-3, biosafety cabinets may be inappropriate because of the airflow and reduced humidity. Safe containment of the arthropods is thus

achieved through the use of several levels of containment (cages within incubators, and designated insectary areas) within the BSL-3 laboratory, and appropriate procedures (traps, etc.) including those described below. It is recommended that where possible, the researcher take advantage of the safety provided by working within a biological safety cabinet. Procedures such as virus isolation from frozen mosquito pools can be easily performed in a cabinet. Glove boxes may also be useful for manipulating small infected arthropods.

A. Standard practices

Location of arthropods. Furniture and incubators containing arthropods are located in such a way that accidental contact and release by laboratorians, custodians, and service persons does not occur. This is usually achieved by locating arthropods in dedicated rooms, wings or suites in incubators located out of the traffic flow in areas of the building dedicated to BSL-3 activities.

Supply storage. Equipment and supplies not absolutely required for ongoing ACL-3 work are removed from the insectary after appropriate decontamination. Those present 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 open only during access.

General arthropod elimination. In addition to measures for general arthropod elimination within the insectary, materials used to wipe or mop are autoclaved before disposal. Only persons trained and equipped to work with arthropods and BSL-3 agents clean up spills.

Primary container cleaning and disinfestation. Care is taken to disinfest primary containers in a manner that does not create aerosols. All primary containers are autoclaved or incinerated.

Primary container construction. Cages used to hold arthropods are non-breakable and screened with mesh of a size to prevent escape. Containers are autoclavable or disposable. Openings are designed to prevent escape during removal and introduction of arthropods. Disposable containers are recommended.

Disposal of arthropods. In addition to ACL-2 disposal practices, the outer surfaces of containers are decontaminated before moving the material. All arthropod waste materials are autoclaved or incinerated.

Isolation of uninfected arthropods. Where possible, only arthropods requiring ACL-3 procedures are housed in the ACL-3 insectary. If it is necessary to house ACL-2 or lower arthropods in the ACL-3 insectary, all procedures and practices must meet the ACL-3 standards.

Primary container identification and labeling. As per ACL-1

Prevention of accidental dispersal on persons or via sewer. Before leaving the insectary and after handling cultures and arthropods, personnel wash their hands, taking care not to disperse viable life stages into the drainage system. No material is disposed of through the sewer. Non-infected material may be destroyed by heat or freezing if followed by autoclaving or incineration.

Pest exclusion program. As per ACL-1

Escaped arthropod monitoring. Additional measures are taken to measure the effectiveness of the arthropod trapping program and these are documented. As part of the IBC review and commissioning process of a new facility, the physical integrity and security practices might be tested by a simple release-recapture study. A known number of non-infected arthropods would be released and then these would be recaptured to assess the physical integrity of security barriers. Such an experiment is described by Hunt and Tabachnick (1996). Exterior and within-building monitoring is considered. Records of exterior captures are maintained.

Source and harborage reduction. As per ACL-2

Microbiological and medical sharps. Sharps are stringently limited and use is justified only when alternatives are not available.

Arthropod sharps. In addition to minimizing arthropod handling sharps, these are restricted for use in the insectary regardless of infection status of material handled.

Routine decontamination. As per ACL-2

Notification and signage. ACL-2 measures are implemented with BSL-3 signage.

Procedure design. All procedures are carefully performed to prevent arthropod escape and the creation of aerosols or splatters. Protocols are practiced with non-infected arthropods/animals and modified before implementation.

Safety manual. As per ACL-2

Training. The training required for laboratory personnel under ACL-3 is more detailed and extensive, and BSL-3 certification is required if infected materials are handled.

Medical surveillance. In addition to the measures required for medical surveillance under ACL-2, assessment is made by the occupational health physician for persons who may be at unusual risk.

Access restrictions. The insectary director limits access to the insectary to the fewest number of persons possible. Personnel who must enter the insectary for program or service purposes when work is in progress are accompanied by trained laboratorians and are advised of the potential hazard to themselves, co-workers, and the potential consequences of arthropod release. Because of the increased risk to non-trained personnel, laboratory staff should perform general cleaning activities that would otherwise be performed by custodial staff.

Special arthropod handling containers and areas. All work is done within a primary barrier. Appropriate biological safety cabinets, other physical containment devices, and/or personal protective equipment are used whenever conducting procedures to infect arthropods with BSL-3 agents, or when handling arthropods. Appropriate designs will consider the life history and behavior of the arthropod and may differ from that required by the agent alone. Such modifications should be made in consultation with biosafety experts. Manipulation of arthropods and, for example, rearing of transovarially infected

immature stages, are performed in a designated area. SALS (U.S. Dept. Health and Human Services, 1999) suggests "a separate room or double screened area that is separated from the main insectary by rooms having two screened or solid doors that open inward and closing automatically."

Safe transport in the laboratory. As per ACL-2

B. Special practices

IACUC and IBC approval. As per ACL-2

Housing of non-arthropod animals. As per ACL-2

Containment during blood-feeding. Recommendations for ACL-1 containment of arthropods during blood-feeding are strictly assured by special practices and container designs that prevent escape of arthropods.

Blood source. As per ACL-1

Escaped arthropod handling. Loose arthropods must be killed and disposed of, or recaptured and returned to the container from which they escaped. Infected arthropods are not killed with hands, and must be transferred using filtered mechanical or vacuum aspirators. Only personnel properly trained and equipped to work with designated arthropods and BSL-3 infectious agents are to recover and/or kill escaped arthropods.

Accidental release reporting. As per ACL-2

Movement of equipment. As per ACL-2

Inventory of arthropods. In addition to appropriate primary containment cages, when possible, the number of arthropods must be included on the label, and records are maintained to account for all arthropods from the time of transfer to the ACL-3 insectary to the time of termination. Vessels containing low mobility stages (e.g., eggs, pupae, hibernating adults) should not be stored within the ACL-3 insectary unless they meet the ACL-3 criteria.

C. Safety equipment (primary barriers)

Eye and face protection. As per ACL-2

Gloves. Personnel wear gloves when handling infected arthropods or host animals and associated equipment. Gloves are removed aseptically.

Torso apparel. White laboratory coats, gowns, and/or uniforms in the insectary are worn at all times by all personnel entering the insectary. Wrap-around or solid-front gowns are worn over this clothing. Front-button laboratory coats alone are unsuitable. The gowns are removed and left in the insectary. Before leaving the insectary, scrub suits and uniforms are removed and appropriately contained and decontaminated before laundering or disposal.

Foot apparel. Boot, shoe covers, or other protective footwear, and disinfectant foot baths (with appropriate anti-arthropod measures) are available and used where indicated.

Personal clothing. As per ACL-2

Arthropod-specific personal protective equipment. As per ACL-2

Pesticide. Pesticide for emergency use is available in areas in which escape of arthropods is likely.

D. Facilities (secondary barriers)

Location of insectary. The insectary is strictly separated from areas that are open to unauthorized, untrained personnel within the building by locked doors. These are opened, for example, by key lock, proximity reader, or card key.

Insectary doors. Access to the facility is limited to trained, approved personnel by a self-closing and self-locking door. The external insectary entry doors are controlled by a key lock, card key, or proximity reader. Entry into the insectary is via a double-door entry that includes a change room and shower(s). Showers are plumbed to prevent arthropod escape. An additional double-door access (air lock) or double-door autoclave may be provided for movement of supplies and wastes into and out of the facility respectively. The two contiguous doors must never 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., hanging curtains) are recommended.

Insectary windows. 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.

Vacuum systems. As per ACL-2

Interior surfaces. In addition to the recommendations for ACL-2, spaces around doors are sealed to facilitate decontamination or troughs surrounding door frames can be installed and filled with sticky or greasy material that will trap crawling arthropods.

Floor drains. Floor drains are not recommended. If present, traps must be filled with an appropriate treatment to prevent survival of any arthropod stage (e.g., mosquito larvae). Ideally, all drains are plumbed to a holding tank to facilitate heat or chemical treatment to kill all stages of arthropod prior to disposal into the waste system.

Plumbing and electrical fixtures. As per ACL-2

HVAC. Ventilation is appropriate for arthropod maintenance, but does not compromise containment. Exhaust air is discharged to the outside without being re-circulated to other rooms. Exhaust must be dispersed away from occupied areas and air intakes, or the exhaust must be HEPA-filtered. 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. Personnel must verify that the direction of the airflow is proper (a visual monitoring device/meter is recommended to confirm directional inward airflow). Audible alarms alert personnel to system failure.

Sterilization equipment. An autoclave is available within the suite of rooms containing arthropods.

Sink and shower. In addition to the ACL-2 recommendation, an appropriately plumbed shower is available within the insectary suite.

Illumination. As per ACL-2

Biosafety cabinets. HEPA-fitted exhaust air from Class II biological safety cabinets can be re-circulated into the insectary provided that it is certified annually. If exhausting to the outside, the cabinet must be installed appropriately. If Class III cabinets are used they must be installed appropriately.

Facility compliance monitoring. The completed ACL-3 insectary design and operational procedures must be documented by the PI and reviewed by the IBC. The insectary must be tested for verification that the design and operational parameters have been met prior to operation. ACL-3 insectaries are re-verified at least annually against these procedures as modified by operational experience.

ARTHROPOD CONTAINMENT LEVEL 4 (ACL-4)

ACL-4 safety guidelines are for the most dangerous pathogen-infected arthropods. No compromise is acceptable at this level of work. BSL-4 agents are associated with a high risk of infection from aerosol exposure, and cause life-threatening disease. Certain other pathogens such as those listed as "restricted animal pathogens" may also necessitate BSL-4 containment if used in vectors. For vector work, production of aerosols is a potential risk when preparing infectious meals or inocula, and can also result from analytical practices involved in virus isolation. If work with vectors must be performed in a BSL-4 facility, then BSL-4 requirements must be strictly followed. As described below, vectors must be safely contained at all times possibly by use of specially designed apparatus that is tested and approved prior to use.

Of the twelve viruses requiring BSL-4 containment in the USA, five are transmitted by arthropods: Central European encephalitis, Congo-Crimean hemorrhagic fever, Kyasanur Forest disease, Omsk hemorrhagic fever, and Russian Spring-Summer encephalitis. Only ticks have been implicated in their natural transmission cycles, although other arthropods have been experimentally infected with BSL-4 agents (e.g., *Aedes aegypti* with Marburg, and *Mesostigmata* mites with Junin). With this information one might at present only consider measures and protocols that safely contain species of ticks as relevant to BSL-4 research with arthropods. However, with the recent emergence of new diseases, it is perhaps necessary to consider other arthropods as potential vectors, particularly flying insects. Furthermore, research on newly discovered pathogens often requires experimental attempts to infect arthropods in an attempt to determine the life cycle. Species of arthropods—principally ticks that have been collected from areas in which infections with a BSL-4 agent are actively being or suspected of being transmitted—are processed as though they were infected with a BSL-4 agent.

As the number of BSL-4 laboratories is quite limited, the reader should refer to the appropriate sections (e.g. pages 36–51 and 69–74) of the BMBL. For arthropod work, a simple, minimalist approach is adopted. An area designated for arthropod research is small, light-colored and contains only items required for the study. There are two types of

BSL-4 laboratories: A) the cabinet laboratory where the agent is handled in a Class III Biological safety cabinet, and B) the suit laboratory. Personnel working in a BSL-4 suit facility shower in and then don one-piece positive pressure personnel suits ventilated by a life support system. Arthropods requiring ACL-4 would typically be adults for use in pathogen transmission studies. However, there may be circumstances in which immature stages such as nymphal ticks might be maintained to be able to stimulate pathogen reactivation to facilitate isolation. Construction of a BSL-4 facility, and required operating procedures, are sufficient to guarantee that no early life stage could survive, since, for example, all liquid waste is decontaminated.

When used in a BSL-4 facility, an arthropod must never be handled outside of a primary containment barrier e.g., cages are opened only in an arthropod secure glove box (Hunt and Tabachnik, 1996). As required for ACL-3, every arthropod is counted and accounted for throughout the experiment. No one enters or leaves the room until all arthropods are accounted for and secured in double taped cages and placed in secondary sealed holding trays. If one is missing and cannot be found, the facility is shut down and treated with a pesticide.

The nature of this research and the protective equipment required dictates that staff must be trained to the very highest level. Since working with arthropods often requires the use of small instruments and hence considerable dexterity, it is recommended that a specific person be designated for this work and be trained extensively using a space suit so that they are well rehearsed before actual ACL-4 work. Equipment that is used for ACL-3 work will be specially adapted for ACL-4 research, and such work would require extensive practice.

This article has been cited by:

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