

Arthropod Containment Guidelines

(Draft 2.1)

The American Committee of Medical Entomology

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Introduction

Laboratories in which living arthropods are reared and maintained for research purposes have been in existence for decades with few reports of harm to their workers or to the communities in which they are located. Many of these organisms are associated with potential risks when they escape since some are vectors of infectious human diseases. When they are experimentally infected with a human pathogen, the arthropods represent an immediate risk to those who come into contact with them. Even when they are uninfected, they can represent a risk to the community if, by escaping, they become the crucial link completing the transmission cycle for the disease they vector.

There are two prominent examples of initially small exotic vector releases that resulted in significant disease increases. In the early 1900s, anopheline mosquitoes were drastically reduced in northeastern South America due to eradication campaigns. The concomitant drop in incidence of malaria and other human infectious diseases was reversed after *Anopheles gambiae* was discovered in the port city of Natal, Brazil in 1930 (1). The African malaria vector appears to have been accidentally introduced into the area by rapid marine mail service. Although the release was not from a laboratory, the introduction of a highly efficient vector is widely thought to be responsible for the resurgence of malaria in Brazil. Fortunately, an aggressive effort to eradicate *An. gambiae* by conventional means was successful. A second example concerns the current distribution of the Chagas disease vector, *Rhodnius prolixus*, throughout rural Central America. While this species is considered indigenous to northern South America, it is thought to have been introduced into Central America through a laboratory escape that occurred in El Salvador in 1915 (2). While there are a number of other triatomine species throughout Central America that transmit Chagas disease to humans, the establishment of *R. prolixus* was especially important because of its close association with humans and domestic dwellings. For this reason, it is now considered the most important vector of Chagas disease in Central America and the species targeted for elimination by the Central American Chagas Disease Control Initiative. The introduction to the latest edition of Biosafety in Microbiological and Biomedical Laboratories (BMBL) notes that despite the long history of accidental laboratory infections of workers and their immediate contacts, "laboratories working with infectious agents have not been shown to represent a threat to the community." Nevertheless, as

we have illustrated, there have been serious community impacts of escaped uninfected exotic arthropod vectors.

These two examples, and the advent of transgenic technology, illustrate the need to revisit the issue of arthropod containment with a particular view to preventing inadvertent escape and establishment of the organisms and providing guidelines for protection of laboratory workers. These guidelines are intended to do that. It has been drafted by a subcommittee of the American Committee on Medical Entomology (ACME), and circulated widely among medical entomology professionals. The Committee membership and drafting procedures are summarized in Appendix I (not written as of draft 2.1).

Transgenic arthropods and those containing microbes modified by recombinant DNA technology are addressed here solely in the context of public health significance. The emphasis is therefore on the phenotypic changes resulting from the modification rather than ecological and environmental issues.

Background

Several documents particularly influenced the effort to create these guidelines and shaped their content:

1980. The Subcommittee on Arboviral Laboratory Safety, a sister organization of ACME, published guidelines in the American Journal of Tropical Medicine and Hygiene (3). These were developed on the basis of a survey of almost 600 laboratories and covered a wide range of pathogenic viruses. They addressed issues relevant to the safety of laboratory workers and assigned each virus to one of four "levels of containment and practice" based on the severity of the associated human disease, the method of transmission and the amount of experience handling the organism. The document addresses containment of the arthropod vector for each level, but only when it is infected by the viral pathogen. It did not address non-viral (e.g. eukaryotic or bacterial) pathogens or their vectors, transgenic animals, nor did it take into account biological containment that may be provided by the location in which the research is conducted.

1984. Biosafety in Microbiological and Biomedical Laboratories (BMBL) was published in response to a series of surveys, beginning for example with (4), of laboratory acquired

infections. Now in its fourth edition, this Public Health Service document describes the practices, facilities, and equipment needed to safely work with potentially dangerous pathogens in a laboratory (5).

1995. The American Mosquito Control Association adopted a position that containment of genetically manipulated arthropods be addressed by funding and regulatory agencies. A letter sent to the Directors of the National Science Foundation, the Centers for Disease Control and Prevention, the National Institute of Allergy and Infectious Diseases, the Pan American Health Organization, and the Administrator of the USDA urged that "guidelines for research be developed to ensure that no exotic pathogens are accidentally released, and to ascertain the potential for the released organisms to alter vector-borne disease transmission patterns."

1996. Hunt and Tabachnick prepared a forum article on containment of very small arthropod vectors (6). This addressed the peculiar containment needs of laboratories working with arthropods too small to be restricted by conventional insectary precautions.

Higgs and Beaty authored a chapter entitled "Rearing and Containment of Mosquito Vectors" (7). The chapter gives practical advice and illustrations for the design, construction and operation of insectaries.

1997. *The Molecular Biology of Disease Vectors* was published (8). It contains several chapters describing methods for the experimental infection of a wide variety of insects. Although most of the emphasis of this textbook is methodological, safety aspects are also addressed.

1998. The US Department of Agriculture, through its Animal and Plant Health Inspection Service, developed draft guidelines for the containment of non-indigenous, phytophagous arthropods and their parasitoids. These guidelines include standards for construction, equipment and operations when handling arthropod pests of plants. The general containment principles for this class of arthropods are relevant to hematophagous arthropods.

1999. The Department of Health and Human Services, through the National Institutes of Health, issued revised guidelines for the safe handling of organisms that contain recombinant DNA, including arthropods. Paralleling the BMBL, these guidelines also

specify that an IBC review all research protocols involving recombinant DNA and approve the level and implementation of appropriate containment (9).

The American Society of Tropical Medicine and Hygiene, American Committee of Medical Entomology adopted a resolution to develop these Arthropod Containment Guidelines for adoption by the ASTMH in coordination with the CDC Office of Health and Safety and the National Institutes of Health.

Intent

This document describes the arthropod handling practices, safety equipment, and facilities constituting Arthropod Containment Levels 1-4 (ACL 1-4). These are recommended by the American Society of Tropical Medicine and Hygiene / American Committee of Medical Entomology for work with a variety of uninfected arthropods and those carrying infectious agents and transgenic vector arthropods in laboratory settings. The principles of risk assessment and specific practices and equipment will also be useful in non-traditional arthropod research settings such as tents, greenhouses, and outdoor cages. While plant and animal biologists may find this document useful, the focus of this document is on arthropods that transmit diseases of public health importance. More details specifying the arthropods that are generally not covered in these guidelines can be found under 'Arthropod Containment Levels.'

This document is strictly concerned with research that involves arthropods of public health importance. Arthropods to be considered include among others: Insects (Diptera – mosquitoes, tsetse flies, black flies, sand flies, midges; Hemiptera – reduvids; Anoplura – lice; Siphonaptera – fleas), and Arachnids (Acari – ticks, mites). All life-cycle stages, eggs, larvae, nymphs, adults must be considered under the term arthropod. The small size, highly motile characteristics of some arthropods (especially flying and jumping), and relative long life and resistance of some stages, makes the containment of arthropods a unique problem. The diversity of these organisms and complex life cycle often means that procedures and practices to safely contain the animal are species-specific. Conversely, the specific culture requirements of some species make maintenance difficult but containment relatively straightforward since they cannot survive outside of the preferred habitat. Although non-insects are considered here, the

designated area in which these organisms are maintained and cultured, will hereafter be referred to as an insectary.

From the above, it follows that many arthropods (e.g., fruit flies, cockroaches, and various Lepidoptera, Coleoptera etc.) that have been collected locally, or have been purchased from pet stores or commercial vendors for study or educational purposes are usually exempt from these guidelines. These guidelines specifically do not cover *Drosophila* spp. unless modified in such a manner that they would be of public health concern. Also, experiments planned with *non-vector* arthropods that are infected with an agent, symbiont, or manipulated with nucleic-acid vectors would fall under these guidelines if the unique combination of the arthropod and agent create novel public health risks not covered in BMBL. Arthropods are an important educational tool in many schools and colleges, and are collected and cultured for many different purposes. These guidelines are not intended to discourage the development of an interest in entomology or impact upon interests that involve the use of arthropods. In these settings, maintenance and rearing techniques are at the discretion of the individual, instructor etc, and do not fall within the containment criteria described in these guidelines although the principles may be useful.

Readers unfamiliar with general biosafety principles should refer to BMBL Section II, 'Principles of Biosafety,' (www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4s2.htm) for an introduction to the topic.

This document supplements 'Biosafety in Microbiological and Biomedical Laboratories' by providing safety principles that include the arthropod vector. In many cases, we have borrowed language and form directly from BMBL. Readers should refer to BMBL for standard and special microbiological practices appropriate for the agents with which they work. These have been repeated here when they would apply to the vector alone as well as the agents. Throughout, the authors have attempted to formulate guidelines that are consistent with those of BMBL yet recognize the fact that biological containment (i.e. location- and season-specific fate of escaped arthropods in the environment) significantly confounds risk assessment and therefore appropriate safety practices. Furthermore, the flying, crawling, burrowing, and reclusive habits of arthropods combined with the agents they may carry introduce an element of risk-increasing behavior not covered by BMBL.

These recommendations are advisory. They are intended to provide a voluntary guide or code of practice as well as principles for upgrading operations. They also offer a guide and reference in the construction of new laboratory facilities and in the renovation of existing facilities. However, the application of these recommendations to a particular laboratory operation is to be based on a risk assessment of the special agents, activities, and geographic location rather than used as a universal and generic code applicable to all situations.

Authorities

Importation, possession, transport, and use of arthropods of public health importance falls under the purview of the Public Health Service / Centers for Disease Control and Prevention, Office of Health and Safety. Many arthropods transmit both human and animal disease and may therefore also be regulated by the United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS, (301)-734-7834. Export of some agents and vectors containing them is regulated by the U.S. Department of Commerce, Bureau of Export Administration (www.bxa.doc.gov). Arthropods modified by recombinant DNA methods or containing similarly modified microbes are addressed in 'Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines) available from the NIH Office of Biotechnology Activities (www4.od.nih.gov/oba/guidelines.html).

Risk Assessment for Arthropod Vectors

The intent of this section is to provide guidance and to establish a framework for selecting the appropriate arthropod containment level (facilities, equipment, and practices) that reduce risk of exposure to a vector and associated agents to an absolute minimum.

"Risk" implies the probability that harm, injury, or disease will occur among laboratorians or the general public due to accidental release of a competent disease vector and/or associated pathogens. In the context of vector research laboratories, risk assessment considers two kinds of effects: direct effects such as biting, infestations and myiasis, and indirect morbidity and mortality due to the diseases transmitted. The latter is by far of highest concern, and direct effects will not be considered here. Therefore, in this document, arthropod containment levels are directly correlated with the appropriate BSL levels of the agents with which they are naturally or experimentally infected or may transmit in the event of accidental release (see BMBL Section VI).

While the focus of this document is public health risk, effects on animals due to arthropods known to transmit animal disease are to be considered. Researchers are encouraged to consult with the U.S. Fish and Wildlife Service and USDA-Animal Plant Health Inspection Service regarding risks and regulation before completing a risk assessment.

The laboratory director or principal investigator has primary responsibility for assessing risks in order to set the appropriate biosafety level for the work. This is done in close collaboration with the Institutional Biosafety Committee (IBC) to ensure compliance with established guidelines and regulations. Development and review of the risk assessment and the planned safety precautions by consultation with researchers knowledgeable about the biology and public health significance of the arthropod is highly recommended.

In performing a qualitative risk assessment, all the risk factors are first identified and explored considering related information available such as BMBL, the NIH Recombinant DNA Guidelines, the Canadian Laboratory Biosafety Guidelines, the WHO Biosafety Guidelines, and the American Committee on Arboviruses Catalogue of Arboviruses, and the American Committee of Medical Entomology. In many cases, one must rely on other

sources of information such as field data, the literature concerning aspects of vector competence, and environmental requirements through consultation with recognized experts in arthropod and pathogen relationships.

The greatest challenge of risk assessment lies in those cases where complete information on many of these factors is unavailable. A conservative approach is advisable when insufficient information forces subjective judgment.

Principles of risk assessment:

Arthropod risk assessment is primarily a qualitative judgment that cannot be based on a prescribed algorithm. Several factors must be considered in combination: the agents transmitted, whether the arthropod is or may be infected, the mobility and longevity of the arthropod, its reproductive potential, biological containment, and epidemiological factors influencing transmission in the proposed location or region at risk.

Vector arthropods of infectious agents fall into the following discrete categories. Each category has a range of risks that need to be assessed.

Arthropods not containing known infectious agents:

Risk from these materials to laboratorians is similar to that experienced by the general public; nuisance due to consequences of escape and temporary or permanent establishment. Consequently the public health risk is likely to be low unless epidemiological conditions exist that could reasonably be expected to result in an increase in transmission of a regionally endemic disease, or establishment of the released vector leads to future transmission potential for an exotic pathogen. In the event that establishment is likely, the arthropod must be handled under more stringent containment conditions.

In the event of accidental release and even transient establishment of an uninfected arthropod, pathogenicity of the agents that could be transmitted must be considered in the context of the location in which the work will be performed or in regions to which escaped arthropods could likely migrate. For example, escape of an exotic malaria vector in a malarious region has significantly higher probability of increasing transmission and therefore higher risk than escape in a non-malarious region.

Answers to the following questions will affect the level of risk due to accidental escape of uninfected arthropods:

- Is the arthropod species already established in the locale?
- If the species is extant in the locale, is the stock or sub-strain known to differ phenotypically from the local form in such a way that it is likely to affect disease transmission?
- If the arthropod is exotic, is it likely that the arthropod would become temporarily or permanently established in the event of accidental escape?
- Are the agents that the arthropod is known to transmit cycling in the locale, or has the agent been present in the past?
- Are agents that the arthropod could reasonably be expected to transmit to animals present in the locale?
- Would accidental release of the arthropod increase the risk to humans and animals above that already in existence in the event of introduction of exotic pathogens in the area?
- Could the arthropod be controlled or locally eradicated by traditional methods (e.g. spraying, trapping) in the event of escape?

Arthropods containing known infectious agents

Arthropods that are known to be, or suspected of being, infected with infectious agents always have risks that must be identified, and appropriate precautions must be taken for worker and public health safety. The characteristics of most known infectious agents have been well defined and are the starting point for determining risk from these arthropods. Information useful to risk assessment can be obtained from laboratory investigations, disease surveillance, and epidemiological studies. Infectious agents known to have caused laboratory-associated infections are included in the BMBL's agent summary statements (Section VII). Other sources include the American Public Health Association's manual, *Control of Communicable Diseases*(10). Literature reviews on laboratory acquired infections also may be helpful (11, 12, 13, 14).

The pathogenicity of the infectious or suspected infectious agent, including disease incidence and severity (i.e., mild morbidity versus high mortality, acute versus chronic disease) is the most important consideration in assessing the risk due to accidental exposure to an infected arthropod vector. As the initial criterion, it is clear that the more severe the potentially acquired disease, the higher the risk.

Readers will observe that the ACL-2 level that we describe here has broad latitude in the specific practices. This reflects, in part, the widely differing degrees of effects of arthropod-borne agents, many of which fall within the BSL2 level. Considerable variation in morbidity and mortality exists within the level 2 classification. For example level 2 arboviruses range from La Crosse virus with a 1% or less mortality rate and limited, mild neurological sequelae to Eastern Equine Encephalitis (EEE) with a mortality rate that approaches 50% in clinical cases and survivors that frequently suffer long term or permanent neurological deficits. Higher containment levels are recommended for agents that cause disease in humans that are considered potentially severe, life threatening or cause residual damage. Our general approach in formulating these guidelines, has been to include a wide range of ACL-2 features that reflect this broad range of agent potency. Moreover, the possible natural and artificial modes of infection (e.g. parenteral, airborne, ingestion) of the agent are considered. This is essential to prevent infections in laboratorians.

The established availability of an effective prophylaxis or therapeutic intervention is another essential factor to be considered. The most common form of prophylaxis is immunization with an effective vaccine. Considering the example above, while EEE carries intrinsically higher risk than LaCrosse virus to laboratory workers who become infected, a vaccine is available for the former. In some instances therefore, immunization may affect the biosafety level or ACL. However important, the availability of therapeutics and vaccines only serves as an additional layer of protection beyond engineering controls, proper practices and procedures, and the use of personal protective equipment. Occasionally, immunization or therapeutic intervention (antibiotic or antiviral therapy) may be particularly important in field conditions. The offer of immunizations is part of risk management to protect laboratory workers. For example, vaccination may be demanded, as a condition of employment, for any laboratory worker working with yellow fever virus, or any pathogens for which a suitable, efficacious vaccine is available.

Medical surveillance is encouraged to ensure that the instituted safeguards provide the expected health outcomes. Surveillance may include serum banking, monitoring employee health status, and participating in post-exposure management. In the arthropod vector laboratory, this must be combined with regular monitoring for escaped arthropods for example through direct counting of infected arthropods, an effective arthropod trapping program and regular inspection of the facilities for disrepair that could result in escape.

Risk assessment must also include an evaluation of the experience and skill level of at-risk personnel such as laboratorians and maintenance, housekeeping, and animal care personnel. Additional education may be necessary to ensure the safety of persons working at each biosafety level.

Arthropods containing unknown infectious agents or of which the status is uncertain

The challenge here is to establish the most appropriate containment level with the limited information available. Some questions that may help in this risk assessment include:

- Why is an infectious agent suspected?
- What route of transmission is indicated?
- Are agents that the arthropod transmits transferred horizontally?
- Are there reasons to believe that a novel or unknown agent is present?
- What epidemiologic data are available?
- What is the morbidity or mortality rate associated with the agent?
- What medical data are available?

The responses to these questions may identify the agent or a surrogate agent whose existing agent summary statement can be used to determine a biosafety level. In the absence of hard data, a conservative approach is advisable, and stringent precautions

are indicated. For example, collections of vectors, particularly adults, from endemic regions must always be treated with suspicion that they may contain infected individuals.

Vector Arthropods containing recombinant DNA molecules

The purpose of this section is to present principles of risk assessment of vector arthropods that have been genetically modified, typically via recombinant DNA technology. This includes both vector arthropods that contain modified microbes or which themselves are genetically modified. These principles primarily address the public health significance of the modified organisms rather than environmental concerns. These technologies continue to evolve rapidly, and experimental procedures designed to derive novel modified symbionts and recombinant arthropods are becoming commonplace. The National Institutes of Health publication, Guidelines for Research Involving Recombinant DNA Molecules (9) is a key reference in establishing an appropriate biosafety level for work involving recombinant microorganisms intended for use in arthropods.

In selecting an appropriate arthropod vector containment level for such work, the greatest challenge is to evaluate the potential biohazard *change* resulting from a particular genetic modification relative to the unmodified arthropod. In the context of public health, the selection of an appropriate level begins by establishing the phenotypic change in the arthropod and/or microorganism due to the DNA manipulation, and potential impact of escaped arthropods containing the modification. Among the points to consider in work with recombinant arthropod vectors and those containing recombinant microbes are:

- Does the inserted gene encode a product known or likely to alter the vector capacity or competence for pathogens it is known to transmit?
- Does the inserted gene cause phenotypic changes that could significantly affect ones' ability to control the arthropod in the event of accidental escape e.g. an insecticide resistance marker?
- Does the modification have the potential to alter the range or seasonal abundance of the arthropod?

- If so, would the new range increase the likelihood that the vector could transmit new pathogens?
- Does the modification have the potential to increase the reproductive capacity of the arthropod that carries it?
- Is the phenotype conferred by the modification, including its marker and other expressed genes if any, consistently expressed after numerous generations of propagation?
- Is the modification undergoing rearrangement or other mutation at a measurable rate?
- Can the DNA transgene vector be mobilized in natural populations?
- Is the host range of the symbiont known?
- Would the modified symbiont pose an increase in risk to immunocompromised persons relative to the native symbiont?
- Is the entire sequence of the DNA insertion known, and are the coding sequences defined?
- Is the original insertion site known so that stability can be assessed later?

This list of questions is not meant to be exhaustive. Rather, it is provided to illustrate the information needed to provide an accurate and conservative assessment of risk to judge the appropriate containment level. Since in many cases the answers to the above questions will not be definitive, it is important that the organization have a properly constituted and informed IBC, as outlined in the NIH guidelines, to evaluate the risk assessment and provide prudent adherence to the appropriate safety guidelines for the assigned risk.

Arthropod Containment Levels

When arthropods are used, facilities, trained staff and established practices must be in place that ensure appropriate safety, protection of health and well-being of workers and the environment. This publication provides guidelines for laboratory work with arthropod vectors of disease-causing pathogens, and have 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”. We emphasize that these are guidelines and not rules per se. If working with a vector in a particular set of circumstances (see Table 1), a certain containment level may be recommended. However the IBC that reviews a research protocol will decide 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 by 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, for example, 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 “down-grade” from ACL-3 to ACL-2 providing that experiments are performed during a particular period. Documentation and justification for this decision making process may be necessary in the event that an accidental release occurs.

It is difficult 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, for example, the location of the facility. 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 an area where it could become permanently established and act as a bridging vector of an established zoonotic pathogen to man. 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 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 the event of an accidental release occurring. The ideal response would be one in which all released vectors are killed almost immediately after the escape. If the escaped arthropods get outside of the laboratory this may be impossible, hence the use of several barrier levels.

A summary of the containment levels is provided in Table 1 and greater detail of the specific practices in Table 2. The arthropod containment levels generally parallel biosafety levels. For example, arthropods infected with a BSL-2 agent are usually handled as the ACL-2 recommends.

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. 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 refugia 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. 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).

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 etc. effectively prevent leakage and escape.

Disposal of Arthropods. Living arthropods are not disposed of inadvertently. 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, etc. is 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 Refugia Reduction. Refugia 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.

Housing of Non-Arthropod Animals. Other animals not necessary for culture of the arthropods are not accessible to the arthropods. Animals used as a 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 IBC and 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 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. Doors 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 present.

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 only recommended or preferred for lower risk ACL-2 situations be stringently 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 etc.

Supply Storage. The area is maintained to allow detection of escaped arthropods. Equipment and supplies not required for operation of the insectary is minimized to allow detection of escaped arthropods. If supplies must be kept within the insectary, they 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.

General Arthropod Elimination. 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 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 Un-Infected 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. 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. 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. are considered. Particularly in the case when exotic arthropods are used, exterior monitoring is considered. Records of exterior captures are maintained.

Source and Refugia Reduction. Refugia 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 contain chemicals to prevent arthropod survival.

Microbiological and Medical Sharps. 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).

Procedure Design. All procedures are carefully designed and performed to minimize the risk of arthropod escape

Safety Manual. A safety manual is prepared 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 potentially present. When appropriate, a serum surveillance system is implemented

(see BMBL for guidance). Personnel are aware of the symptoms of infection and are aware of 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 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 IAUCUC.

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. 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 if infected.

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 prior to transfer between rooms within the insectary, and prior to 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 etc.

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., 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 pose an additional hazard if the insectary is located in an area where the species is indigenous, or if alternative suitable vectors are present, since 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 highly 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 says “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. The airflow may also disrupt the presentation of cues such as body temperature that stimulate host seeking and feeding. 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 use of a biological cabinet can thus increase the risks associated with working with arthropod vectors. We recommend that while a cabinet might safely be used to prepare infectious material, the best option is to perform infectious procedures in a secure area not exposed to strong air currents. Hunt and Tabachnick (6) 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 (3) 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 approved BSL-3 procedures. The necessary clothing may impair dexterity that is essential for performing procedures such as the dissection of small arthropods. 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 minimize the risk of 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 only opened for access.

General Arthropod Elimination. In addition to measures for general arthropod elimination within the insectary, materials wiped or mopped up 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 prior to moving the material. All arthropod waste materials are autoclaved or incinerated.

Isolation of Un-infected 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. 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. Uninfected material may be destroyed by heat or freezing if followed by autoclaving or incineration.

Pest Exclusion Program. 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 commissioning process of a new facility the physical integrity and security practices could be tested by a simple release-recapture study. A known number of uninfected arthropods would be released and then attempts made to account for all of them. Such an experiment is described by Hunt and Tabachnick (6). Exterior and within-building monitoring is considered. Records of exterior captures are maintained.

Source and Refugia Reduction. 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. 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 un-infected arthropods / animals and modified before implementation.

Safety Manual. 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 individuals 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.

Special Arthropod Handling Containers and Areas. All work is done in 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 (5) 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. ACL-2

B. Special practices

IACUC and IBC Approval. ACL-2

Housing of Non-arthropod Animals. 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. 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. ACL-2

Movement of Equipment. 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. 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 prior to 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. ACL-2

Arthropod-Specific Personal Protective Equipment. 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 by key lock, proximity reader, card key, etc.

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. 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. 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. 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. 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 infections 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 tick 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 arthropod 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.

Since 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, white 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, one can envisage circumstances where immature stages such as nymphal ticks might be maintained in order 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 (6). 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 individual be designated for this work and be trained extensively using a space suit so that they are well rehearsed prior to 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.

Transportation and Transfer of Biological Agents and Arthropod Vectors

Transportation refers to the packaging and shipping of these materials by air, land, or sea, generally by a commercial conveyance. Transfer refers to the formal process of exchanging these materials between facilities.

Biological agents include infectious agents of humans, plants, and animals as well as the toxins that may be produced by microbes and by genetic material potentially hazardous by itself or when introduced into a suitable gene delivery agent. Etiologic agents and infectious substances are closely related terms that are found in the transfer and transportation regulations. Biological agents may exist as purified and concentrated cultures but may also be present in a variety of materials such as body fluids, tissues, soil samples, etc. Arthropod vectors are organisms such as mosquitoes, ticks, and fleas that may transmit infectious agents to animals or humans. Biological agents and materials and vectors that are known or suspected to contain them are recognized by federal and state governments as hazardous materials, and their transportation and transfer is subject to regulatory control. Transport and transfer of live, uninfected vectors may also be subject to federal and state regulatory control.

Transportation

Regulations on the transportation of biological agents and live vectors are aimed at ensuring that the public and the workers in the transportation chain are protected from exposure to any agent that might be in the package, and that the package prevent escape of the agent or live vector. Protection is achieved through (a) the requirements for rigorous packaging that will withstand rough handling and contain all liquid material within the package without leakage to the outside, (b) appropriate labeling of the package with the biohazard symbol and other labels to alert the workers in the transportation chain to the hazardous contents of the package, (c) documentation of the hazardous contents of the package should such information be necessary in an emergency situation, and (d) training of workers in the transportation chain to familiarize them with the hazardous contents so as to be able to respond to emergency situations.

Regulations

Public Health Service 42 CFR Part 72. Interstate Transportation of Etiologic Agents.

This regulation is in revision to harmonize it with the other U.S. and international regulations (see Federal Register 64(208) p. 58022). A copy of the current regulation may be obtained from the Internet at (<http://>). The revisions are expected to have little if any effect on the recommendations below.

Department of Transportation. 49 CFR Parts 171-178. Hazardous Materials Regulations.

Applies to the shipment of both biological agents and clinical specimens. Information may be obtained from the Internet at: (<http://>).

United States Postal Service. 39 CFR Part 111. Mailability of Etiologic Agents.

Codified in the Domestic Mail Manual 124.38: Etiologic Agents Preparations. A copy of the Domestic Mail Manual may be obtained from the Government Printing Office by calling 1-202-512-1800 or from the Internet at: (<http://>).

Occupational Health and Safety Administration (OSHA). 29 CFR Part 1910.1030.

Occupational Exposure to Blood-borne Pathogens.

Provides minimal packaging and labeling requirements for transport of blood and body fluids within the laboratory and outside of it. Information may be obtained from your local OSHA office or from the Internet: (<http://>).

Dangerous Goods Regulations (DGR). International Air Transport Association (IATA).

These regulations provide packaging and labeling requirements for infectious substances, materials, clinical specimens that have a low probability of containing an infectious substance, and live vectors. These are the regulations followed by the airlines. These regulations are derived from the Committee of Experts on the Transport of Dangerous Goods, United Nations Secretariat, and the Technical Instructions for the Transport of Dangerous Goods by air that is provided by the International Civil Aviation Organization (ICAO). A copy of the DGR may be obtained by calling 1-800-716-6326 or through the Internet at: <http://www.iata.org/cargo/dg/index.htm>, or (<http://>).

General Packaging Requirements for Transport of Live Arthropod Vectors

Transport of live arthropod vectors requires packaging that prevents the escape of the arthropods and agents, maintains their viability, and protects personnel in the transportation chain from exposure to the contents. This is true regardless of whether the arthropods are infected or not. Fortunately, unlike many animals, most arthropod vectors require neither large containers, ventilation, feeding, nor added water during their transport. Most are shipped without free water so the possibility of leaking is rare, and the container temperatures normally maintained during shipments is adequate. This means that appropriate physical packaging of vector arthropods is fairly simple and, for infected arthropods, can be similar to that which is appropriate for the agents they contain.

The following section is intended to provide specific instructions for determining the type of container and labeling required for shipment of vector arthropods.

IATA Live Animal Regulations 26th Edition (LARs) describes containers that are appropriate for the shipment of arthropods including insects and arachnids. The design of these, while not as demanding, is consistent with containers used to ship etiologic agents (See Container Requirement 62 of LARs). It is therefore possible to select containers that satisfy the requirements of LARs, DOT 49 CFR Part 173.196 - Transportation of Etiologic Agents, and USPHS 42 CFR Part 72 - Interstate Shipment of Etiologic Agents. (NOTE TO READER: At this time, IATA Dangerous Goods Regulations prohibit air shipment of 'infected live animals' that prevents air transport of any of the infected classes below. We believe this is a prohibition that should be exempted for vector arthropods packaged according to the recommendations below. The regulations allow shipping of select agents. Although additional hazard is presented by infected arthropods, the increased hazard does not exceed that of agents presently allowed.)

Three cases will be considered:

- Uninfected exotic and domestic arthropods
- Domestic and exotic arthropods containing a non-select agent

- Domestic and exotic arthropods containing a select agent

Definitions:

Domestic arthropods: Those that are extant in the 49 continental United States. Note that this differs from the definition used in Risk Assessment and Containment Levels.

Exotic arthropods: All others

Select agent: Etiological agents listed in 42 CFR Part 72

Non-select agent: Agents other than those above that are known to cause disease in humans.

- Uninfected exotic and domestic arthropods that vector disease are packaged consistent with the minimum packaging requirements of 42 CFR 72.2. This requires that the container must prevent 'leakage (i.e. escape, note added) of the contents, shocks, pressure changes, and other conditions incident to ordinary handling in transportation.' We recommend that this consist of three levels of containment including: a primary receptacle consisting of a sealed plastic bag or tube surrounded by padding, a secondary container such as an insulated chest whose lid is sealed with tape, and a durable fiberboard, wood, plastic or wooden outer container. The container may bear the 'live animal' label naming the species within. If aquatic stages are shipped, the container should also contain sufficient absorptive material to absorb and contain all of the water.
- Domestic and exotic arthropods containing a non-select agent are packaged as above. The outer container bears a 'biohazard' label as described in CFR 72.3. An itemized description of the contents is placed between the outer and inner containers.
- Domestic and exotic arthropods containing a select agent are packaged, labeled and tracked as required for the agent they are suspected of containing. This includes all

attendant regulations required for the agent alone including notice of delivery and failure to receive, laboratory registration etc.

Transfer

Regulations on the transfer of biological agents and live vectors are aimed at ensuring that the change in possession of biological materials is within the best interests of the public and the nation. These regulations require documentation of the personnel, facilities, and justification of need for the biological agent in the transfer process and subsequent approval of the transfer process by a federal authority. The following regulations fit in this category:

Importation of Etiologic Agents of Human Disease and Live Vectors

42 CFR Part 71 Foreign Quarantine. Part 71.54 Etiologic Agents, Hosts and Vectors.

This regulation requires an import permit from the Centers for Disease Control and Prevention (CDC) for importing etiologic agents of human disease, any materials that may contain etiologic agents including live animals and live vectors. This regulation also requires that an import permit be obtained by the recipient for transfer from the original permit-holder of an imported etiologic agent or live vector within the United States. An application and information on importation permits may be obtained by calling 1-888-CDC-FAXX and enter document number 101000 or on the Internet at: <http://www.cdc.gov/od/ohs/biosfty/imprtper.htm>. Interstate transfer of biological agents and live vectors may also be restricted by state regulations. Shippers and recipients of these materials may obtain additional information directly from state health or agriculture departments.

Importation of Etiologic Agents of Livestock, Poultry and Other Animal Diseases

9 CFR Parts 92, 94, 95 96, 122 and 130.

These regulations require an import permit from the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Veterinary Services to import or domestically transfer etiologic agents of livestock, poultry, other animals, and any materials that might contain these etiologic agents. Information may be obtained at (301) 734-3277, or from the Internet at: <http://aphisweb.aphis.usda.gov/ncie>.

Importation of Plant Pests

7 CFR Part 330. Federal Plant Pest Regulations. General; Plant Pests; Soil; Stone and Quarry Products; Garbage.

This regulation requires a permit to import or domestically transfer a plant pest, plant biological agent, or any material that might contain them. Information can be obtained by calling 301-734-3277 or through the Internet at:

<http://www.aphis.usda.gov/ppq/ppqpermits.html>.

Transfer of Select Biological Agents of Human Disease

42 CFR Part 72.6 Additional Requirements for Facilities Transferring or Receiving Select Agents.

Facilities transferring or receiving select agents must be registered with the CDC and each transfer of a select agent must be documented. Information may be obtained on the Internet at:

<http://www.cdc.gov/od/ohs/lrsat.htm>.

Export of Etiologic Agents of Humans, Animals, Plants and Related Materials

Department of Commerce. 15 CFR Parts 730 to 799.

This regulation requires that exporters of a wide variety of etiologic agents of human, plant and animal diseases, including genetic material, live vectors, and products that might be used for culture of large amounts of agents, must obtain an export license. Information can be obtained by calling the DoC Bureau of Export Administration at 202-482-4811 or through the Internet at: <http://bxa.fedworld.gov>, or <http://www.bxa.doc.gov>.

Arthropod Containment Level	1		2	3	4
Arthropod distribution, escaped arthropod fate	exotic, inviable or transient	indigenous	exotic, 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	not applicable			
Practices	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 (2), sealed electrical/plumbing openings. Breeding containers and refugia minimized.	BSL-3	BSL-4

Table 1. Summary of Arthropod Containment Levels. Three fates of arthropods upon accidental escape are classified here:(1) Inviabile; 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 arthropod 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

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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. However, subpopulations (strains, geographically distinct forms etc.) whose phenotype is known or suspected to vary significantly in ways that could reasonably be expected to increase the vector competence should be handled under the more stringent conditions within ACL-2 even if uninfected.

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