Safe working with arthropods

Containment and control for work with uninfected, infected and transgenic animals in research

Note: Edition 1 covers containment of flying species only.

Institute of Safety in Technology and Research

Introduction

1. Appropriate containment of arthropod vectors of pathogens capable of causing human and animal disease used in research is necessary to control the risks:

- to those working with the pathogens
- of escape of the pathogens to the external environment
- of escape of infected arthropods to the external environment
- of establishment of new vectors in the environment

2. With the emergence (and re-emergence) of a substantial number of arthropod-borne pathogens in recent years, research into the diseases caused and their vectors has increased and is both a national and international priority as regards protection of public health, animal health and welfare and also impact on the economy.

3. The primary purpose of this guidance is to provide advice on meeting the minimum requirements and offering advice on good practice for work with arthropods infected with human or animal pathogens (including genetically modified vectors and/or pathogens), as defined in:

- The Control of Substances Hazardous to Health Regulations (COSHH) – covers work with unmodified biological agents (micro-organisms that are hazardous/infectious for humans)
- The Genetically Modified Organisms (Contained Use) Regulations (GM (CU)) – covers work with genetically modified micro-organisms including those that can infect humans and animals and larger modified organisms such as transgenic animals
- The Specified Animal Pathogens Order (SAPO) – covers work with pathogens that infect animals, including those that have been genetically modified and zoonotic agents (those that infect humans and animals)

Infobox1: Guidance on legislative requirements

Guidance on the application of COSHH to work with infected animals:

1. ACDP guidance *Working safely with research animals: management of infection risks*: Appendix 5 – Containment of invertebrates
2. ACDP guidance *Biological agents: managing the risks*: Appendix 3.2 – Work with Hazard Group 3 parasites (NB: information relating to containment of invertebrates is the same as that in 1).

Guidance on the application of the GM (CU) Regulations to work with animals infected with GM micro-organisms and GM arthropods:

3. Scientific Advisory Committee on Genetic Modification (SACGM) *Compendium of guidance: Part 3 – containment and control of activities involving genetically modified micro-organisms and Part 5 – genetic modification of animals*

Guidance on the application of SAPO to work with animals infected with animal pathogens:

4. Department of Environment, Food and Rural Affairs (DEFRA) guidance *Animal Pathogens – guidance on controls*
5. HSE Guidance for licence holders on the containment and control of specified animal pathogens

4. Although some guidance exists regarding the requirements of the above legislation, there is only limited information that addresses the specific needs of work with infected arthropods (Infobox 1).

5. Work with arthropods, particularly in containment, presents many challenges because of their:

- Small size – can make detection difficult
- Motility – they can fly, swim, climb, jump and crawl
- Complex life cycles – these can be relatively long and include stages that are able to withstand harsh environments; for example, the eggs of some mosquito species can withstand periods of desiccation and remain viable
- Environmental conditions – necessary for survival and behaviour such as feeding, which may require control of temperature, humidity, light levels and nutrition
- Sensitivity – special conditions commonly encountered in high containment laboratory environments, such as air handling, certain chemicals (e.g. disinfectants, anticoagulants) and vibration

6. Although this guidance is aimed at those working with arthropods that can transmit human and/or animal pathogens, the principles of containment described will have application for those working with arthropods that transmit plant pathogens (see Appendix 1 for an overview of plant health legislative controls).

7. The Institute of Safety in Technology and Research (ISTR) Biosafety Steering Group (BSG), in consultation with the Health and Safety Executive (HSE), the Advisory Committee on Dangerous Pathogens (ACDP) and with input from the UK biosafety community and those who work with infected arthropods have prepared the following guidance to supplement and complement the official guidance that is available. There is no legal requirement to follow this guidance, although it may be of use when considering the design, operation and management of these specialist containment facilities.

8. ISTR serves safety professionals in technology and research including education, industry, government agencies and consultancy. The aim of the Institute is to enhance the knowledge, competence and professional development of its members through networking, accreditation, knowledge exchange, workshops and symposia, communication with regulators and partnerships with other safety organisations. The ISTR BSG represents the interests of UK biosafety nationally and internationally on behalf of ISTR.

Scope

9. This guidance covers research work with exotic and native (to the UK) species of arthropods that are vectors of human and/or animal disease. All life-cycle stages (eggs, larvae, nymphs, adults) must be considered.

10. Arthropods to be considered include among others:

- Insects
  - *Diptera* – mosquitoes, tsetse flies, black flies, sand flies, midges
  - *Hemiptera* – reduviids
  - *Anoplura* – lice
  - *Siphonaptera* – fleas
- Arachnids

- *Acari* – ticks, mites

11. This first edition focuses on those arthropod species that can fly as these present the greatest challenge as regards containment. Containment of other species will be prepared and published in subsequent editions.

12. Although there are other important vectors of human and animal disease such as helminths they are not covered by this guidance.

13. Work with uninfected, infected and transgenic forms of these vectors is covered. Infected means those arthropods that have been deliberately/intentionally infected as well as wild-caught arthropods known or suspected of being infected. Work with non-vector arthropods e.g. Drosophila spp that have either been modified such that they create a risk to human/animal health or the environment or used as a model for infection and deliberately infected with a pathogen is also covered .

14. This guidance supplements that given on containment requirements as set out in Infobox1. Guidance is provided on the:

- General principles of design and operation of facilities including work with uninfected arthropods
- Additional controls needed for work with infected arthropods

Principles of containment

15. Infected arthropods must be housed and handled at a level of containment appropriate to the level of risk posed by the infecting agent (including those that are genetically modified). The requirements of the legislation are similar for both human and animal pathogens, in that they specify levels of increasingly stricter controls; Containment Levels 1-4, with level 4 being the highest level of control. The need to work at a particular level is dictated in the first instance by a defined classification of the infecting agent (COSHH1 and SAPO2) and, where appropriate, assessment of additional measures needed to control the risks of work with a GM agent. As a starting point, any area (room/facility) used to work with agents and/or arthropods infected with such agents must meet the minimum relevant legislative requirements e.g. work with a Hazard Group (HG) 2 agent that infects humans must be carried out in a Containment Level (CL) 2 facility (see Appendix 2 for minimum requirements of legislation).

16. The containment and control measures required by the different legislation are broadly similar. However, it is important to note that there are differences; primarily because of the differences in approach as regards protection of worker health vs controlling the risk of laboratory escape into the wider environment.

Assessing the risks (human health and the environment)

17. Although work may be carried out at a particular containment level as required by the classification of the pathogen, all activities must be subject to specific risk assessment to justify those measures beyond the minimum specified in legislation, or else make the case as to why a particular measure is not needed, for example because of the means of transmission of the infection.

18. As the risk increases, for example the greater the chance of escapees becoming established and being able to act as a vector of infection in the outside environment, the robustness of the

[1] Approved List of Biological Agents

[2] Animal Pathogens – guidance on controls

controls selected must increase, for instance there should be greater emphasis on the use of physical rather than management or procedural measures.

19. Assessment of work with infected arthropods should consider:

Table 1: issues to consider for assessment of work with infected and/or transgenic arthropods

	Considerations
Life cycle of arthropod and/or infecting agent(s)	Working with certain stages of the life may present less of a risk than others – so could the work be carried out using these stages?
Life cycle of infecting agent	Working with certain stages of the life cycle may present less of a risk than others – so could the work be carried out using these stages?
Means of transmission of infection	Most arthropods will transmit disease in the natural environment by biting; although this will be a risk in the laboratory, the risk of skin-penetrating injuries from other sources needs to be considered e.g. use of sharps such as needles, scalpels, scissors and glass, as well as of routes other than skin-penetrating injuries such as aerosol, existing wounds etc.
Status of vector	Are there any phenotypical properties that mean the arthropod vector is more or less able to survive outside of the laboratory environment? These may be naturally occurring properties or else the vector may have been intentionally modified e.g. to be resistant to insecticides.
Risk of survival of escaping vector	If a vector escaped could it survive and reproduce in the outside environment? Although some vectors may be "exotic" i.e. not currently present in the outside environment, their ability to tolerate outside local conditions, particularly during the summer months, needs to examined. If a vector escapes and survives, could it persist, reproduce and establish in the outside environment or would this be limited by local conditions? Would the escaped vector be able to transmit other infections i.e. it may be a primary vector for one disease but is able to transmit others.
Likelihood of pathogen being present in outside environment	Are infectious agents that the vector can transmit (or has been shown to be able to transmit) circulating in the local fauna? They may be present on a permanent basis or else temporarily e.g. migratory species.
Presence of another competent vector in the outside environment	If an escaped infected vector was to transmit the infection, could this then be passed on/transmission maintained by another competent vector?
Level of exposure typically needed to result in hazard	Some agents require regular or substantial exposure to establish infection e.g. *Brugia*.

20. Where work takes place with wild-caught arthropods e.g. in field stations, the assessment should ask the same questions. The key consideration will be an assessment of the likelihood of an infecting agent being present since escape is not likely to be a concern. However, if a wild-caught arthropod is deliberately infected with a different pathogen to that which is normally present in the outside environment or else the arthropod is subsequently modified, then the appropriate containment and control measures in this guidance should be used.

21. Assessment of work with genetically modified arthropods, whether infected or not, should follow the guidance set out Part 5 of the SACGM Compendium of Guidance [and, where, when?] risks to human health and the environment will be addressed. For modified arthropods that are incapable of surviving the environment in the UK, have limited ability to transfer genetic material to UK animal species or where the genetic modification does not increase the level of risk to human health or the environment above that of the unmodified organism, it is anticipated that minimal additional containment measures will be necessary. Those modified arthropods that could either

(i) Become established outside of the containment facility;

(ii) Have a genetic modification that increases the level of risk to human health or the environment above that of the unmodified organism; or

(iii) Cause harm to humans or the environment if they escaped from the containment facility and have the ability to transfer novel genetic material to UK animal species,

may require more rigorous containment measures to be applied.

Impact of local climate on survivability

22. Although there is still uncertainty about the extent to which environmental change will affect the ability of non-native vector species to survive and establish populations that are capable of transmitting disease within the UK, the assessment of whether a species could survive will need to take into account the current epidemiological and environmental data available. However, the impact of the local environment on vector survivability is complex and will also be affected by other factors such as changes in land use or increased urbanisation as well as changes to the presence and population of animals that are hosts for some vectors and/or pathogens.

23. It is not within the scope of this guidance to consider all the various factors but rather to reinforce that any risk assessment for work with vector species will need careful consideration of the consequences of escape.

General principles of design and operation

24. A facility is likely to comprise a number of different areas dependant on the scale and type of work undertaken including areas for housing/rearing as well as procedure space. Work may take place within a defined containment boundary and comprise separate areas designated for work with infected or uninfected arthropods; or else the work with uninfected arthropods takes place elsewhere e.g. in building or site.

25. In either situation, consideration needs to be given to the best means of transfer of materials including arthropods to the containment area as well as movement within the containment boundary. For example, the installation of transfer hatches between adjacent key areas will avoid the need for movement of materials within clean areas of the facility.

26. All facilities should be designed to prevent escape of arthropods. It may not be considered necessary to prevent escape of an uninfected species that cannot survive outside of the containment boundary (not necessarily the outside environment). However, if infection work with the same species also takes place within the same facility or in neighbouring facilities, then any escapee could cause concern, as it will not be possible to determine the infection status of the escapee by sight alone.

27. Although not the primary consideration, there may also be an impact on human health from a bite from an uninfected arthropod (see Infobox2: Health considerations).

28. Walls, ceilings and floors should be light in colour to allow easy detection of escapees, with lighting levels sufficient to facilitate detection. The use of mould resistant products e.g. paints and other finishes is recommended for surfaces, particularly in areas of high humidity, especially if this is maintained at a room level.

29. Having lower ceiling heights in rearing rooms helps enables easier detection and recapture/ kill of any escapees

30. Furniture and other equipment should be minimised to limit areas where escapees can hide. Where present, storage such as cupboards, incubators and fridges/freezers should be mobile to allow for easy cleaning as well as detection of any escapees. Shelving should be minimal and the use of adjustable shelving with uprights that contain holes avoided.

Infobox2: Health considerations

Immunisation: If unscreened human blood is used for arthropod feeding, those handling the blood should be offered immunisation against hepatitis B and arrangements put in place to manage exposure to blood e.g. via a skin penetrating injury or splash onto mucous membranes (eyes/mouth/nose). The risk of other blood-borne viruses being present should also be assessed and appropriate immunisation offered if available.

Allergy/hypersensitivity: Those working in facilities known to react badly to bites may require a local re-assessment of the controls in place to ensure that they are sufficient; such individuals are advised to inform their managers/Occupational Health particularly if prompt treatment following a bite is required. Although such individuals may wear repellent in normal circumstances, this may not be possible within the research setting as it may impact on the arthropod being studied. The need for health surveillance of those working with arthropods will need to be assessed, as there are a number of known arthropod-related allergens (e.g. scales, frass, feed) to which individuals may be exposed depending on the species.

Quarantine: Many facilities will have staff working overseas in endemic or epidemic areas and returning to work in the arthropod facility[1]. An appropriate procedure should be in place to ensure that the risk (albeit low) of an escapee biting an infected individual and transmitting the infection to individuals in the facility (or to susceptible populations outside) is managed. The procedure should include:

- Defined exclusion period – this should be based on the known incubation period of the disease(s) potentially exposed to
- Identification of "risk" countries (or areas within countries) – this could follow the approach used when assessing need for travel immunisation
- Consideration of the need for diagnostic tests on return – given that this is an invasive procedure, the need for ongoing tests following an initial test on return should be based on:
    - Time spent in the endemic area and the nature of work carried out
    - Whether the individual was known to have received bites while in endemic areas
    - Whether any pre-exposure treatment, such as an antimalarial, was used and the course completed
    - Whether any relevant immunisation was given
    - Whether the disease is asymptomatic
- The need for self-monitoring of symptoms of disease (and prompt reporting of any symptoms) for a defined time-period.

[1] *Staff may also holiday in endemic areas in which case, a similar approach should be taken.*

31. Ceiling mounted lighting should be flush with the ceiling and ideally accessible from above to avoid breaching containment.

32. Any points of penetration into the facility such as air inlets/extracts, drainage from sinks and electrical service conduits should be screened and/or sealed to prevent escape of arthropods. These measures will also serve to prevent entry of animals as may also be required. If mesh is used for screening, it should be appropriate for all the different sizes of the life cycle of the arthropod being handled. Screening should be made of a material that is able to withstand both the conditions of the

local environment e.g. high humidity, as well as repeated cleaning/disinfection. It must fit well and if glued in place, the adhesive resistant to environmental conditions within the room.

33. If sinks are present, water traps should be dosed with a suitable chemical that prevents survival of all stages of the life cycle of the arthropod or else an appropriately sized mesh screen fitted.

Use of personal protective equipment when working with uninfected arthropods

34. Those working within the facility should wear appropriate personal protective equipment (PPE). The use of PPE laboratory coats may appear unnecessary especially when working with uninfected arthropods but its use will have benefits in terms of providing some protection against inadvertent bites. It will also allow easier detection of escapees that may attach to clothing. It should also be remembered that the PPE may be necessary for other purposes, for example using cleaning chemicals or handling other biological materials such as human blood for feeding.

35. The use of PPE and types of PPE must be based on risk assessment, which needs to consider the following:

- Arthropod involved and its mechanism of movement (crawling, flying, jumping etc), its physical size and the allergens it may generate
- The physical barriers already in place to prevent escape of the arthropod and exposure to operators or visitors
- The density of the arthropods being used (culture rooms may require more robust PPE than experimental areas)
- The environmental factors that may affect choices of PPE, for example working in high temperatures, low temperatures or high humidity
- Potential for those who may come into contact with the arthropod to have a strong reaction to bites or other forms of contact
- Colour – some species are attracted to particular colours and should not be used e.g. blue when working with large biting flies

Working with infected arthropods

36. The following guidance takes as a starting point the minimum requirements of the relevant legislation (COSHH, GU (CU) and SAPO) and provides information on approaches to containment and control when working with infected (or transgenic) arthropods at different containment levels. The general guidance on CL1-4 laboratories is not repeated here but should be read alongside this more specific information.

37. Where possible, similar requirements are blended; as the wording may be slightly different in the original regulations, the exact requirements are reproduced in Appendix 2. Any differences between similar requirements are shown in italics.

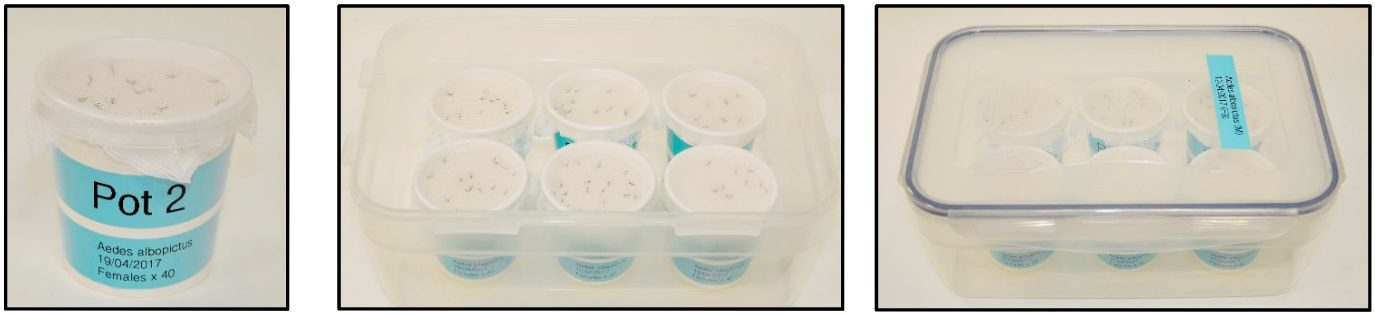
38. There are no legal minimum containment requirements under COSHH or SAPO for Containment Level 1 laboratories however, the practices, safety equipment and facilities are likely to be similar to those required at CL2. Although the Contained Use regulations do set out minimum requirements for CL1 laboratories, required measures are limited to the following:

- Bench surfaces must be easily cleaned, impervious to water and resistant to acids, alkalis, solvents, disinfectants and other decontamination agents that may be in use
- An autoclave is required on site
- Suitable protective clothing must be worn
- Specified disinfection procedures must be in place, where the risk assessment shows that they are required

- All waste material containing viable GMMs is required to be inactivated by validated means prior to disposal where the risk assessment shows that this is required
- An observation window or alternative is required where the risk assessment indicates that it is necessary
- Safe storage for GMMs is required if the risk assessment indicates that it is needed

39. **Primary containment measures** e.g. microbiological safety cabinets or equivalent and other engineered measures control the risk of exposure to the infectious agent. To control exposure at source is a key principle in protection of human health and safety. **Secondary measures** operate at the laboratory/facility level and control risks to the outside environment i.e. control the risk of escape of pathogen and/or infected arthropod.

40. **Primary arthropod containment** refers to the first layer of containment used for housing; this is not for safety reasons *per se* but a means of confining/controlling motile animals. This may be supplemented by further/extra robust layers for safety reasons, for example when moving between rooms and when arthropods have been infected.



Layers of containment

41. Work with infected arthropods will use a combination of measures but there will be more emphasis on the use of primary containment when working with human pathogens. However, use of primary containment when working with animal pathogens or modified agents/arthropods that present a risk to the environment will reinforce the secondary containment provided by the facility design alone.

Facilities

COSHH/SAPO/GM(CU)	2	3	4
Separation of the laboratory or laboratory suite from other activities in the same building.	No	Yes	Yes

42. Lobbies or anterooms act as a physical barrier to potential escapees as well as providing staff with an opportunity to check for any escapees before final exit from the laboratory.

43. Purpose built lobbies and anterooms with appropriately screened interlocked doors should be used at CL3. Doors do not have to be of a solid construction and could be mesh. Lobbies need to be suitably sized to allow for door opening and also movement of equipment in and out.

44. At CL2, although a lobby may not be required, if space allows, a simple mesh lobby could be constructed and sited at the entrance to the laboratory.

45. If live animals are used to feed arthropods, these should be housed separately (see paragraph 46) and in accordance with Home Office requirements. As pest control will be needed in such housing, careful consideration will be needed as to the impact of any insecticides used on the arthropods housed within the facility.

46. SAPO requires physical separation of the rearing room from infected animals or other infected arthropods at CL3, although it can be located within the same containment suite. Rearing rooms must be separate from any activities with SAPO4 specified animal pathogens at CL4.

COSHH/SAPO/GM(CU)	2	3	4
The workplace is to be sealable to permit disinfection (fumigation).	No	Yes	Yes

47. Although not required at CL2, the need for room decontamination and the means of decontamination should be assessed. For example, what action would be taken in the event of a release of multiple arthropods from primary containment? Although rooms could be treated with an appropriate gaseous insecticide, the impact on the room and potentially elsewhere in the facility may mean areas being taken out of use for significant periods of time.

48. At CL3 and above, the room must be sealable for fumigation. There is some evidence that formaldehyde fumigation can kill escapees but not necessarily any infecting agent so dead escapees should be located, collected and disposed of as infectious waste.

49. All penetrations to the laboratory where infectious material and infected arthropods are handled should be minimised and where possible co-located, i.e. cables routed through a purposed designed and appropriately sealed[3] cable transit system.

Infobox3: Use of attractants/traps

If used, there should be a documented procedure that sets out:

- Rationale for number, location (both within and outside of the facility) and types of traps used
- Frequency of monitoring and frequency of replacement/renewal
- Action levels set and actions to be taken in the event of numbers reaching agreed levels

The most appropriate trap/attractant for the species needs to be selected, for example different species have a preference for different colours – commercially available sticky traps come in blue and yellow, with yellow being more attractive to pollinators.

Equipment

COSHH/SAPO/GM(CU)	2	3	4
Surfaces impervious to water, easy to clean and resistant to acids, alkalis, solvents, disinfectants.	Yes, for bench	Yes, for bench and floor	Yes, for bench, floor, walls and ceiling

50. Surfaces should be light in colour to facilitate detection of escapees. This includes items such as the support framework for benching.

51. All areas should be kept clean and free from environments that could attract and/or harbour escapees. This means prompt removal of any spilt water or food materials as well as secure storage of food materials

52. Procedures should be in place to prevent the routine build-up of residues and other matter to control the risk that arthropods or pathogens may survive outside their normal culture conditions.

[3] At CL3 and above, the system needs to prevent ingress/egress of the arthropod **and** the gaseous disinfectant that may be used for fumigation of the laboratory.

Additionally, surfaces should be smooth and seamless with no cracks or crevices, as these provide hiding places for escaped arthropods.

Infobox4: Controlling the risk of escape

Physical methods include:

- Use of temperature:
    - Use of a chilled[1] air curtain or shower at the entrance/exit to areas used to house infected arthropods taking into account the size of the vector being used; larger species are less affected by such airflows and more able to cling onto clothing.
    - Temperature controlled corridors permanently set at a temperature at which the vector in use cannot survive or corridors that can be quickly cooled to such temperatures periodically, especially in the event of a known/suspected escape. A rapid change in temperature is recommended as some species can survive a gradual drop and may become acclimatised to lower temperatures. The use of cold temperatures as a means of control may have impact on the structural integrity of the building or fixtures and fittings. For example, heated door seals will require and the use of doors that do not warp when exposed to heat on one side and cold on the other so as not to cause a gap between the door and the frame. If used, cold corridors should be checked regularly for ice formation and a buddy system or similar put in place because of the risk of slips/falls.
- Use of reduced lighting or use of red light in lobbies/anterooms or corridors removes the risk of any escapees being attracted to the bright lights that may otherwise be used in such areas.
- Use of attractants/traps (see Infobox3).

Procedural methods include:

- When handling arthropods e.g. prior to deliberate infection, they should be anaesthetised. This allows for easier counting and so detection of any losses as well as facilitating delicate procedures such as intra-thoracic inoculation. Some species can be pre-chilled to immobilise before handling and work carried out over a cold mat, whereas others can be anaesthetised using CO_2. If CO_2 is used within the confines of a Class III cabinet or glove box, periodic venting through a scrubbing unit (as well as a HEPA filter) may be needed to avoid build up which could kill the arthropods. Numbers of infected arthropods handled within primary containment should be minimised so that that numbers can be counted out and back into the cages with confidence.
- Numbers of infected arthropods handled at any time should mimimised to ensure that an accurate count can be kept at all times. They should be counted out of their pots and counted back in before transport back to their housing. Records should be kept of each handling stage (with numbers) until final disposal of the infected arthropods.

[1]Does not need to be chilled – physical movement of air alone will be effective

SAPO/GM(CU)	2	3	4
Entry to laboratory via airlock	Not required	Required where and to extent the risk assessment shows it is required	Required

53. Doors on both sides of the airlock/lobby should be tight-fitting on all sides and open inwards to push any escapees back towards the containment area; brushes can be fitted at the bottom of the door. If lobby doors are not physically interlocked, management procedures should be in place to ensure only one door is open at a time.

54. An additional measure that can used in anterooms or lobbies are recapture devices. Although not specified in COSHH or GM (CU), the use of such devices is a specific measure for work with animal pathogens and whilst they are not required at SAPO CL2, they are required at CL3 (as determined by the risk assessment) and CL4.

COSHH/SAPO/GM(CU)2	2	3	4
The workplace is to be maintained at an air pressure negative to atmosphere in the immediate environment.	No	Yes except for activities where transmission does not occur by the airborne route	Yes

55. Although the use of attractants and traps is widespread in insectaries, it should be noted that they are not a control measure in themselves. They only indicate how well (or not) physical and procedural controls are working – see Infobox3 for further information on numbers, types and locations.

56. Although there is a requirement to control the release of airborne pathogens at CL3 and 4, this measure could also help control the risk of passive transfer of escaped arthropods from the containment laboratory to the outside environment. This will be dependent on how air is delivered and extracted in such areas but the use of appropriately positioned air transfer grilles may provide an air curtain which may be effective when working with smaller arthropod species (see Infobox4 for other options to control release).

COSHH/SAPO/GM(CU)	2	3	4
Input air and extract air to the workplace are to be filtered using HEPA or equivalent.	No	Yes, on extract air	Yes, on input and double on extract air

57. Given that air inlets/extracts represent a major penetration in the containment area, these need to be screened with an appropriately sized mesh but be robust enough to withstand the high airflows particularly at higher containment levels. Fans serving these air extracts will need sufficient capacity to pull the required amount of air through a HEPA filter(s) and fine mesh screen (this may also be the case on the supply side where incoming air may require HEPA filtration).

GM(CU)	2	3	4
Microbiological safety cabinet/enclosure	Required where and to extent the risk assessment	Required, and all procedures with infective materials	Required, and all procedures with infective materials

GM(CU)	2	3	4
	shows it is required	required to be contained within a cabinet/ enclosure	required to be contained within a cabinet/ enclosure
COSHH/SAPO	**2**	**3**	**4**
Infected material, including any animal, is to be handled in a safety cabinet or isolator or other suitable containment.	Yes, where aerosol produced	Yes, where aerosol produced *SAPO - required*	Yes
SAPO/GM(CU)	**2**	**3**	**4**
Specific measures to control aerosol dissemination.	Required so as to minimise	Required so as to prevent	Required so as to prevent

58. Primary containment such as a microbiological safety cabinet, glove box or isolator must be used when directly handling flying life stages of arthropods infected with human pathogens.

59. The airflows within a cabinet may affect some procedures such as feeding; certain species may struggle to feed. However, feeding can still be undertaken within the confines of a fully enclosed cabinet (e.g. a Class III), with airflows switched off during the feed and switched on following the feed. If there is a risk of escape, Class III (or I/III in Class III mode) cabinets or glove boxes/isolators can be used as the physical barrier prevents escape of the arthropods.

60. The use of a safety cabinet with a HEPA filtered extract will control the release of any airborne pathogen if being handled directly e.g. during inoculation, or likely to be disseminated during a procedure such as grinding of infected tissues. The exact containment approach used e.g. safety cabinet vs simple glove box should be selected on the basis of the risk assessment for the work.

61. Working within the confines of a Class III cabinet or glove box can be challenging in terms of dexterity but they can be constructed to include microscopes to allow delicate procedures to be carried out. These can be used directly but connection to a display screen outside the confines of the primary containment provides sufficient detail to carry out procedures although lighting within the cabinet may need to be supplemented.

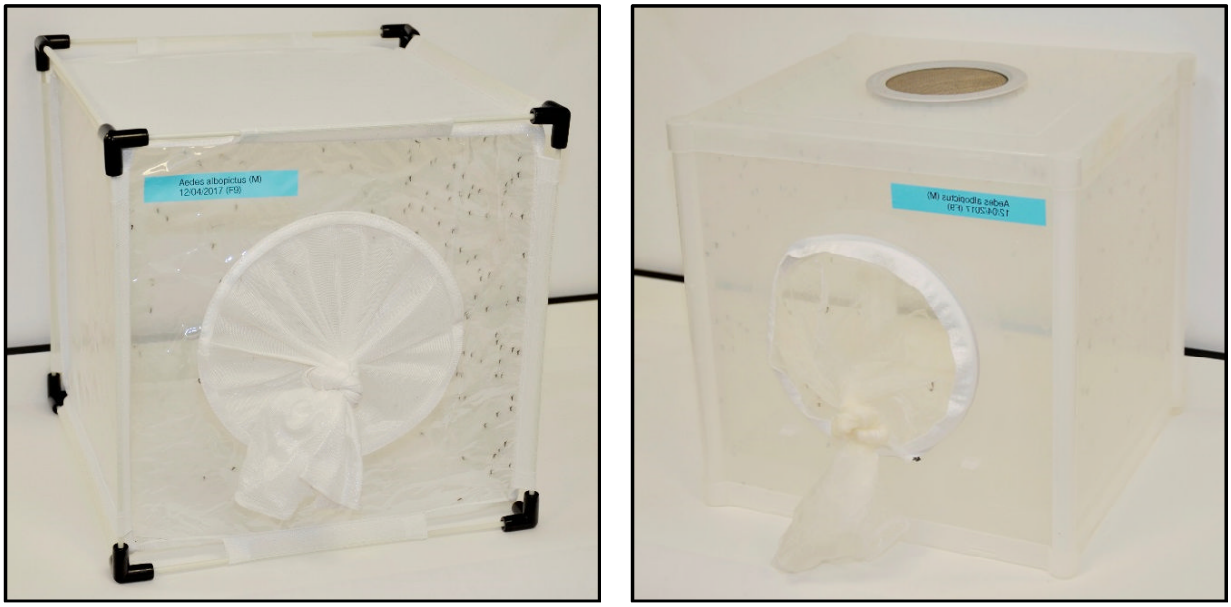
62. Work with infected arthropods outside of the containment laboratory should only take place in exceptional circumstances and be justified in the risk assessment for the activity. For example, in vivo imaging of virus replication within mosquitoes may require the use of specialist equipment located outside of the containment facility. Such work would require the use of additional controls, for example, the removal of legs and wings of infected mosquitoes to prevent escape if lids of primary containers need to be removed for imaging.

63. Containers of infected arthropods should be placed within secondary containers, such as robust, clip-top boxes (with an internal seal) when moving from incubators/rearing rooms and only opened within primary containment such as a microbiological safety cabinet.

Pots within robust secondary containment

64. At CL2 and CL3, it is preferable to maintain infected arthropods within incubators or ventilated cage racks rather than benches in a temperature/humidity controlled room. Incubators should have a glass or mesh door to allow for visual inspection without needing to open doors. Whether on the bench or in an incubator, the infected arthropods should be housed in double arthropod containments e.g. sealed pots within netted enclosures or lock and lock plastic boxes. In some circumstances, pots may be double-netted. However, mesh size and number of layers may affect local humidity, air-flows and light levels which may impact on the health of the arthropods contained within.

65. Containers and cages should be robust and easy to clean and disinfectant and be able to withstand repeated disinfection cycles.



Examples of cages used – netted and solid

SAPO/GM(CU)	2	3	4
Autoclave	Required in the building	Required in the laboratory suite	Double ended autoclave required in the laboratory suite

66. At CL3 and above, housing used for infected arthropods should be autoclaved before washing/cleaning and re-use.

System of work

COSHH/SAPO/GM(CU)	2	3	4
Access is to be restricted to authorised persons only.	Yes	Yes *SAPO/GM only: where and to the extent the risk assessment shows it is necessary*	Yes, via airlock

67. Access to the containment areas must be controlled and limited to appropriately trained staff only. Dependant on the design and nature of the facility, additional access controls may be needed on a room-by-room basis, for example where activities require specific training before work is undertaken (see requirement for written training records) and these could be linked to permissions on a proximity card access system.

SAPO/GM(CU)	2	3	4
Shower - *SAPO only*: *Shower before leaving laboratory*	Not required	Required where and to extent the risk assessment shows it is required	Required

68. Although there is no requirement in COSHH for a shower for work at CL2-4, there is a general requirement in COSHH for any work with biological agents to provide appropriate and adequate washing facilities – this usually takes for the form of a hand-wash basin located near the exit/entrance to the room.

69. At CL2 (and CL3 subject to assessment), drainage from sinks can be discharged to the main drainage system. However, work with SAPO and GMMs may require this water to be treated prior to disposal.

70. If used, sink traps should be either be treated chemically to limit survival of any escapees or fitted with an appropriate mesh screen if waste-water is not treated before discharge.

SAPO/GM(CU)	2	3	4
Protective clothing	Suitable protective clothing required	Suitable protective clothing required; footwear required where and to extent the risk assessment shows it is required	Complete change of clothing and footwear required before entry and exit

SAPO/GM(CU)	2	3	4
Gloves	Required where and to extent the risk assessment shows they are required	Required	Required

71. Howie-style laboratory coats or back-fastening gowns should be worn in the containment area. Areas of exposed skin should be minimised to control the risk of attracting and being bitten by an escapee (see also guidance on choice of colour of PPE to avoid attracting an escapee)

72. Clothing should be checked systematically before removal when exiting the facility; reusable clothing could also be removed and frozen before re-use. Mirrors should be located in anterooms/lobbies to allow for a full check or individuals could exit in pairs to check each other. A means of killing or trapping any escapees should be located in these areas (See infobox3).

COSHH/SAPO/GM(CU)	2	3	4
Efficient vector control, e.g. rodents and insects	Yes *COSHH for animal containment only*	Yes *COSHH for animal containment only*	Yes

73. Measures taken to prevent escape of infected arthropods should be sufficient to prevent ingress of pest species. The need for different types of traps to detect any incoming pest species should be considered.

74. There should be documented procedures for handling of escapees within the containment area. While every effort should be made to locate escapees, it may not always be possible. Although most species are unlikely to survive for any length of time outside of their normal housing environment, all escapees, live or dead, should located if possible. Procedures should include:

- Likely survival times outside of environmentally controlled environment
- Whether escapees are killed or trapped when located and equipment to be used e.g. pooters to collect or electric swatters to kill
- Trigger for room decontamination and means of decontamination – although rooms could be treated with an appropriate gaseous insecticide, the impact on the room and potentially elsewhere in the facility may mean areas being taken out of use for significant periods of time

Waste

COSHH	2	3	4
Specified disinfection procedure	Yes	Yes	Yes
SAPO/GM(GU)			
Inactivation of GMMs/specified animal pathogens in effluent from hand-washing sinks, showers and similar effluents	Not required	Required where and to extent the risk assessment shows it is required	Required

Inactivation of specified animal pathogens in contaminated material and waste	Required by a validated means	Required by a validated means with waste inactivated within laboratory suite	Required by a validated means with waste inactivated in the laboratory

75. All stages of the life cycle of the arthropod should be killed using a validated methodology before disposal e.g. by autoclaving or by freezing. Freezing temperatures and times should be validated; it is routine to freeze adults overnight[4] before disposal but 100% kill may be achieved in less time, dependant on the number of containment layers used.

COSHH/SAPO/GM(CU)	2	3	4
Safe storage of agents	Yes	Yes	Yes, secure storage

76. A means of safe storage is required at all containment levels. Agents must be stored in appropriate containers and appropriately labelled. Labelling should indicate whether the agents are human or animal pathogens or modified (or a combination of these). They must be stored at the highest appropriate level to their classification, for example, rabies is a SAPO4 agent but an HG3 biological agent so must be stored (and handled) at SAPO4.

COSHH/SAPO/GM(CU)	2	3	4
An observation window, or alternative, is to be present, so that occupants can be seen	No	Yes	Yes

77. If windows/vision panels in doors need to be covered to control light levels within the room, for example for experimental purposes, then an alternative means of check on the safety of the occupants will be required.

COSHH/SAPO/GM(CU)	2	3	4
A laboratory is to contain its own equipment	No	Yes, so far as is reasonably practicable	Yes

78. If the use of specialised equipment is required which is located outside of CL3, appropriate procedures need to be in place to ensure containment of the infected arthropod during transport to and from the equipment as well as during the procedure.

Other measures

GM(CU)	2	3	4
Biohazard sign on door	Required	Required	Required

79. There is a general requirement in COSHH to display suitable and sufficient warning signs, including the biohazard sign for any work with biological agents.

80. Other signage on doors could indicate the containment level of the area, permissions required to enter and other hazard warning signs such as the presence of flammable gases. Signage should be kept to a minimum with only essential information displayed.

[4] APHA require 72 hours for plant pests (96 hours for Colorado beetle)

GM(CU)	2	3	4
Written procedures and records of staff training	Required where and to extent the risk assessment shows they are required	Required	Required

81. Training records that have been signed off by an authorised trainer can be linked to entry to specific work areas as a means of controlling access. Although only a requirement for work with GM agents, it is good practice to apply this to work with human and animal pathogens too. Work with plant pathogens requires regular, documented refresher training.

Appendix 1

Summary of requirements for work with plant pathogens and vectors

Work with prohibited animals and plants is carried out by means of a licence issued by the Animal and Plant Health Agency (APHA), with the licence giving the specific conditions under which such material can be imported, moved or kept. These can include the following:

- Plants, including parts of plants and seeds
- Invertebrate plant pests (arthropods, molluscs and nematodes)
- Plant pathogens (fungi, bacteria, viruses, virus-like agents and phytoplasmas)
- Soil and other organic material
- Potatoes

In applying for a licence, the following information will be required:

- Site security – a description of:
    - All areas containing quarantine material are kept locked
    - The names of anyone who has access to quarantine areas or a set of keys
    - How quarantine areas are labelled
    - That all authorised personnel will read and sign a standard operating procedure before starting work with licensed material
- Record-keeping and labelling – a description of:
    - How dated records will be kept when new material arrives and how existing licensed material will be moved or disposed of
    - How licensed material will be labelled or distinguished at all stages of experimentation
- The lay-out of the facilities:
    - A description of the type of containment facility used e.g. glasshouses, polytunnels, laboratories
    - A description of the location of containment facilities on the premises – with room numbers or a geographic location relative to a named or numbered area and a plan of the facility if possible
    - Give details of how often authorised staff enter the containment facilities
    - A description of how much material will be kept in containment facilities at a given time
    - A description of how licensed material will be kept within three layers of secure containment to stop plant pests escaping
    - State the other material that will arrive with the licensed material (e.g. soil) and how it will be handled or disposed of
    - Whether licensed and non-licensed material will be kept in the same containment facilities
    - A description of the type of work carried out in each area of the site
    - A description of the containers used to grow plants in, if the licensed material includes plants for planting

    - A description of the traps used to detect the escape of licensed organisms
    - A detailed contingency plan to follow if organisms escape or if there is a suspected escape
- The procedures to be followed during experiments:
    - How experiments will be carried out - a step by step description of every experiment
    - Whether workers will wear dedicated protective clothing that is only used when working on licensed material and how it will be cleaned after use
    - The precautions to be taken when transporting material between containment facilities during experiments
    - The disinfectants to be used to clean containment facilities, their concentration and how they will be used
    - How licensed material will be destroyed after work has finished and before final disposal
    - A list of the scientific and technical qualifications of all personnel who will do work under the licence being applied for

Appendix 2

Minimum containment requirements – minimum legislative requirements

COSHH

Containment measures		***Containment levels***		
		2	**3**	**4**
1	The workplace is to be separated from any other activities in the same building	No	Yes	Yes
2	Input air and extract air to the workplace are to be filtered using HEPA or equivalent	No	Yes, on extract air[1]	Yes, on input and double on extract air
3	Access is to be restricted to authorised persons only	Yes	Yes	Yes, via airlock key procedure
4	The workplace is to be sealable to permit disinfection	No	Yes	Yes
5	Specified disinfection procedure	Yes	Yes	Yes
6	The workplace is to be maintained at an air pressure negative to atmosphere	No	Yes[1]	Yes
7	Efficient vector control, e.g. rodents and insects	Yes, for animal containment	Yes, for animal containment	Yes
8	Surfaces impervious to water and easy to clean	Yes, for bench	Yes, for bench and floor (and walls for animal containment)	Yes, for bench, floor, walls and ceiling
9	Surfaces resistant to acids, alkalis, solvents, disinfectants	Yes, for bench	Yes, for bench and floor (and walls for animal containment)	Yes, for bench, floor, walls and ceiling
10	Safe storage of biological agents	Yes	Yes	Yes, secure storage
11	An observation window, or alternative, is to be present, so that occupants can be seen	No	Yes	Yes
12	A laboratory is to contain its own equipment	No	Yes, so far as is reasonably practicable	Yes
13	Infected material, including any animal, is to be handled in a safety cabinet or isolator or other suitable containment	Yes, where aerosol produced	Yes, where aerosol produced	Yes
14	Incinerator for disposal of animal carcases	Accessible	Accessible	Yes, on site

NOTE

[1] In the table, COSHH Schedule 3 - Part II, the requirement for several containment measures at CL3 is risk based. For example, the need for HEPA filtration of extract air and the provision of an inward airflow is dependent on the ability of the biological agent to be transmitted via the airborne route. The *Approved list of biological agent*s helpfully identifies, with an asterisk, which biological agents are not normally transmitted via an airborne route. This information should be used in the first instance to inform the risk assessment. The actual specifics of the contained use then need to be considered to make a final decision on the extent to which it is necessary to protect workers from exposure to airborne biological agents. For example, propagation of blood-borne viruses such as the Hepatitis B virus is unlikely to require room air to be extracted through a HEPA filter or an inward airflow into the room, but would require the use of a microbiological safety cabinet. However, other containment requirements will still necessitate the laboratory being designated as CL3.

Genetically Modified Organisms (Contained Use)

Containment Measures		*Containment Levels*			
		1	**2**	**3**	**4**
Facilities					
1	Laboratory suite: isolation[1]	Not required	Not required	Required	Required
2	Laboratory: sealable for fumigation	Not required	Not required	Required	Required
Equipment					
3	Surfaces impervious to water, resistant to acids, alkalis, solvents, disinfectants and decontamination agents and easy to clean	Required for any bench	Required for any bench	Required for any bench and floor	Required for any bench, floor ceiling and walls
4	Entry to laboratory via airlock[2]	Not required	Not required	Required where and to extent the risk assessment shows it is required	Required
5	Negative pressure relative to the pressure of the immediate surroundings	Not required	Not required	Required except for activities where transmission does not occur by the airborne route	Required
6	Extract and input air from the laboratory must be HEPA filtered	Not required	Not required	HEPA filters required for extract air except for activities where transmission does not occur by the airborne route	HEPA filters required for input and extract air[3]
7	Microbiological safety cabinet/ enclosure	Not required	Required where and to extent the risk assessment shows it is required	Required, and all procedures with infective materials required to be contained within a cabinet/ enclosure	Required, and all procedures with infective materials required to be contained within a cabinet/ enclosure
8	Autoclave	Required on site	Required in the building	Required in the laboratory suite[4]	Double ended autoclave required in laboratory
System of work					
9	Access restricted to authorised personnel only	Not required	Required	Required	Required (via airlock key procedure)
10	Biohazard sign on door	Not required	Required	Required	Required

Containment Measures		*Containment Levels*			
		1	**2**	**3**	**4**
11	Specific measures to control aerosol dissemination	Not required	Required so as to minimise	Required so as to prevent	Required so as to prevent
12	Shower	Not required	Not required	Required where and to extent the risk assessment shows it is required	Required
13	Protective clothing	Suitable protective clothing required	Suitable protective clothing required	Suitable protective clothing required; footwear required where and to extent the risk assessment shows it is required	Complete change of clothing and footwear required before entry and exit
14	Gloves	Not required	Required where and to extent the risk assessment shows they are required	Required	Required
15	Efficient control of disease vectors (e.g. rodents and insects) which could disseminate GMMs	Required where and to extent the risk assessment	Required	Required	Required
Waste					
16	Inactivation of GMMs in effluent from hand- washing sinks and showers and similar effluents	Not required	Not required	Required where and to extent the risk assessment shows it is required	Required
17	Inactivation of GMMs in contaminated material and waste	Required by validated means where and to extent	Required by validated means	Required by validated means, with waste inactivated within the laboratory suite	Required by validated means, with waste inactivated within
Other measures					
18	Laboratory to contain its own equipment	Not required	Not required	Required, so far as is reasonably practicable	Required
19	An observation window or alternative is to be present so that occupants can be seen	Required where and to extent the risk assessment shows it is required	Required where and to extent the risk assessment shows it is required	Required where and to extent the risk assessment shows it is required	Required

Containment Measures		*Containment Levels*			
		1	**2**	**3**	**4**
20	Safe storage of GMMs	Required where and to extent the risk assessment shows it is required	Required	Required	Secure storage required
21	Written records of staff training	Not required	Required where and to extent the risk assessment shows it is required	Required	Required

[1] "Isolation" means, in relation to a laboratory, separation of the laboratory from other areas in the same building, or being in a separate building.

[2] Entry must be through an airlock which is a chamber isolated from the laboratory. The clean side of the airlock must be separated from the restricted side by changing or showering facilities and preferably by interlocking doors.

[3]Where viruses are not retained by the HEPA filters, extra requirements will be necessary for extract air.

[4] Where the autoclave is outside the laboratory in which the contained use is being undertaken, but within the laboratory suite, there must be validated procedures for the safe transfer of material into that autoclave, which provides a level of protection equivalent to that which would be achieved by having an autoclave in that laboratory.

SAPO

Containment measure		*Containment Levels*		
		2	**3**	**4**
1	The laboratory suite is to be separated from other areas in the same building or is in a separate building (Note 1)	Not required	Required	Required
2	Laboratory sealable to permit fumigation	Not required	Required	Required
3	Surfaces impervious to water, easy to clean and resistant to acids, alkalis, solvents, disinfectants and decontamination agents used for decontamination	Required for bench	Required for bench and floor	Required for bench, floor, walls and ceiling
4	Entry to laboratory via airlock	Not required	Required where and to extent the risk assessment shows it is required	Required
5	The laboratory to be maintained at an air pressure that is negative relative to the immediate surroundings	Not required	Required except for activities where transmission does not occur by the airborne route	Required
6	Input and extract air from the laboratory to be filtered using HEPA or equivalent (Note 2)	Not required	Required on extract air except for activities where transmission does not occur by the airborne route	Required on input air, double on extract air
7	Infected material to be handled in a safety cabinet or isolator or other suitable physical containment	Required where and to extent the risk assessment shows it is required	Required	Required
8	Autoclave	Required in the building	Required in the laboratory suite	Double ended autoclave required in the laboratory suite
9	Access to be restricted to authorised persons only (Note 3)	Required	Required and via airlock where and to extent the risk assessment shows it is required	Required via airlock
10	Specific measures to control aerosol dissemination	Required so as to minimise	Required so as to prevent	Required so as to prevent

Containment measure		*Containment Levels*		
		2	**3**	**4**
11	Shower before leaving the laboratory	Not required	Required where and to extent the risk assessment shows it is required	Required
12	Protective clothing to prevent the dissemination of specified animal pathogens	Suitable protective clothing	Suitable protective clothing required, footwear where and to extent the risk assessment shows it is required	Complete change of clothing and footwear required before entry and exit to the containment area
13	Gloves	Required where and to extent the risk assessment shows it is required	Required	Required
14	Efficient control of disease vectors which could disseminate animal pathogens	Required	Required	Required
15	Inactivation of specified animal pathogens in effluent from hand-washing sinks, showers and similar effluents	Not required	Required where and to extent the risk assessment shows it is required	Required
16	Inactivation of specified animal pathogens in contaminated material and waste	Required by a validated means	Required by a validated means with waste inactivated within laboratory suite	Required by a validated means with waste inactivated in the laboratory
17	A laboratory is to contain its own equipment	Not required	Required so far as is reasonably practicable	Required
18	An observation window or alternative is to be present so that occupants can be seen	Required where and to extent the risk assessment shows it is required	Required	Required
19	Safe storage of specified animal pathogens	Required	Required	Safe and secure storage required

Note 1 - Laboratory suite means one or more laboratories, together with the supporting infrastructure, equipment and services, including ancillary rooms such as airlocks, changing rooms, storage rooms and rooms for the inactivation or disposal of specified animal pathogens.

Note 2 - Where viruses are not retained by HEPA filters, extra requirements should be used for extract air.

Note 3 - Airlock – entry must be through an airlock which is a chamber isolated from the laboratory. The clean side of the airlock must be separated from the restricted side by changing or showering facilities and preferably by interlocking doors.

Appendix 3

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[5] Lead author