Insectaries and infection experiments: design and standardization at European level and the Infravec2 project

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The Infravec2 project

- EU-funded infrastructure project
- Infrastructure- physical but also products and reagents
- Different from research projects, but with a research component
- Research component relates to product development
- Meant as a service to the research field
- Provides services and products/reagents for free to the community to advance this type of research activity

Infravec2 provides no-cost vector resources:

Services:

- NGS sequencing (DNA, RNA) +/- bioinformatics analysis
- High-throughput drug & molecule screen in live insects
- Insecticide resistance testing
- Cuticle thickness as a marker of insecticide resistance
- Mosquito mass rearing (1k-100k)

Infravec2 is strengthening vector research

- Development of new vector resources and tools
  - New characterized vector colonies
    - Mosquito vectors of CHIKV, ZIKV, DENV, malaria, WNV, RVFV
    - *Hyalomma marginatum*, hard tick vector of Crimean-Congo HFV
  - New *Plasmodium falciparum* strains for transmission
    - Cloned gametocyte-producing lines from different geographic origins
  - Improved mosquito genetic editing tools
    - *Ae. aegypti*, *Anopheles*: New docking lines for gene insertion, improved CRISPR/Cas9 tools

Infravec2 is strengthening vector research

- Collect wild mosquitoes & adapt
- Microbiome changes during colonization (F0, 2, 6, 12)
- Genetic diversity during colonization

New Infravec2 mosquito colonies for public distribution (2018-2019)

- *An. darlingi* - French Guiana
- *An. coluzzii* – Mali
- *An. funestus* – Burkina Faso
- *An. atroparvus* – Spain AVAILABLE NOW!
- *Ae. aegypti formosus* – Senegal
- *Ae. aegypti aegypti* – New Caledonia
- *Ae. albopictus* – France
- *Cx. pipiens* - England

Infravec2 provides no-cost vector resources:

Physical access to sophisticated facilities:

- 1-week no-cost access to CL1, 2 and 3 facilities to work on your own project:
  - Experimental vector infection
  - Vector behavior and physiology experiments (EAG…)
  - Mosquito behavior in large field cages
  - Insecticides
  - …

Infravec2 :
Funding source from EU for disease vector research

*NO-COST vector products & services

- Available to researchers worldwide
- You can ask novel questions using rare and unique research tools
- Supplier (Infravec2 partner) reimbursed by EU at actual cost
  - pre-existing equipment, personnel

*Strengthening vector research

- New vector resources
- Standardized insectary procedures for cross-facility reproducibility
- Harmonized European definitions for insect containment levels (P3/P2)

Check it out!

Project contact:
infravec2@pasteur.fr
Web: www.infravec2.eu

The Infravec2 project and insectary design

- Part of a dedicated workpackage on standardization; task dedicated to design of secure insectaries

- Key aims:

1) Advice on best practice and design

2) Support development of new and existing insectaries

3) Set standards and improve experimental practices/reproducibility

Framework of the presentation

- General and legal considerations
- Setting specific considerations and interactions: make your life easier
- Design and protective measures at CL2 and CL3 levels

Before you do anything…!

- Educate, speak to the relevant people (biosafety, administration, colleagues)
- Elaborate on what you want to do and explain

What do we want to achieve in a secure insectary environment?

Disclaimer: here we are mainly talking about mosquito species and infection experiments and containment levels 2 and 3 in closed facilities!

- Manage risks to experimentators working with arthropods/pathogens
- Manage risks of infected arthropods/vectors to the environment
- Manage risks of escape of arthropods/vectors and colony establishment

Some, non-exhaustive legal context…!

Hazard groups (HGs; or risk groups) refer to the classifications (generally defined by national bodies such as: Advisory Committee for Dangerous Pathogens in the UK; ZKBS in Germany;) of micro-organisms with regards to:

1) Their pathogenicity to humans and animals

2) Risk to laboratory workers

3) Transmissibility to the environment, and whether prophylaxis/treatments are available.

HGs range from HG1 to HG4 and determine, in general terms, at which containment level micro-organisms should be manipulated. Here, a layer of complexity is added to safety procedures through the use of infected arthropods.

Some, non-exhaustive legal context…continued!

The EU defines legislation under: Council Directive 90/679/EEC of 26 November 1990 on the protection of workers from risks related to exposure to biological agents at work, OJ No. L 374, p. 1. Risks associated with **Genetic Modification** (GM) are regulated by the EU under: Directive 2009/41/EC of the European Parliament and of the Council of 6 May 2009 on the contained use of genetically modified micro-organisms.

National bodies implement these directives in different ways; your local biosafety structures and officers are key contact points.

Some, non-exhaustive legal context…continued!

Containment levels (CLs) in the context of pathogens:

1) CL1-4 (lowest to highest), or Biosafety levels (BSL-1 to BSL-4) or pathogen/protection level (P1 to P4) depending on country.

2) These link to with HG classifications.

3) Work needs to take into account national Genetic Modification regulations (of pathogens and arthropods) and associated safety levels.

Pathogens directly relevant for insectary design as presented here fall under (biased towards viruses…):

1) HG2: Semliki Forest virus, Sindbis virus

2) HG3: Dengue, West Nile viruses etc.

HG4 arboviruses are rare: tick-borne flaviviruses such as Omsk hemorrhagic fever. To my knowledge one BSL4 insectary is in existence (UTMB Galveston).

Some, non-exhaustive legal context…continued!

There are key differences between countries, and how risk is assessed.

These can be defined by setting and geography as much as legislation.

Example: Rift Valley fever virus strain MP12, BSL2 Germany but ACDP3/SAPO3 and bioterrorism agent in UK.

This is critical when moving material between countries as is often the case in vector biology/arbovirus/parasitology research.

It’s not just the pathogen….!

Besides pathogens, risk associated with arthropod vectors needs to be assessed:

1) Ecology and behaviour (introduction and survival of new species) of wild type species.

2) Genetic modification of arthropods (reporter genes, gene drives for properties such as pathogen resistance, or less resistance) and potential impact on ecology and behaviour.

It’s not just the pathogen….! GM considerations in arthropods

Example

Gene drive technology for arthropods such as mosquitoes:

Relies on nucleases such as CRISPR/Cas9 technology

** Useful in sexually reproducing species*

** Can be used for modifications of genes, “crash” populations etc.*

** Clear risk to natural populations*

Combining pathogen and virus in the insectary

Considerations when GM vectors and viruses are used simultaneously in infection experiments:

Example (from Samuel et al., PNAS, 113:13863-13868, 2016)

1) Dcr2- deletant *Ae. aegypti* mosquitoes
2) Higher yellow fever virus load

Does this pose an increased risk, even when hazard group 2 pathogens are used?

Or less risk because mosquitoes have lost a key Immune component?

Such questions need careful consideration in risk assessments.

Factors to consider in risk assessments- summary

1) Risk through mosquito escapes to the environment (GM, establishment of new species not endemic locally, etc. - climate/environmental factors, ability to complete life cycles etc).

2) Risk posed by the mosquito to the laboratory workers and other arthropod colonies (colony contamination, introduction of pathogens).

3) Risk posed by the pathogen to the laboratory workers and the environment.

4) Risk posed by the pathogen to arthropod colonies (for example contamination of non-infected cultures, or affecting fitness of the arthropod colonies).

5) Risk of pathogen transmission between individuals, also specifically from a facility worker to community.

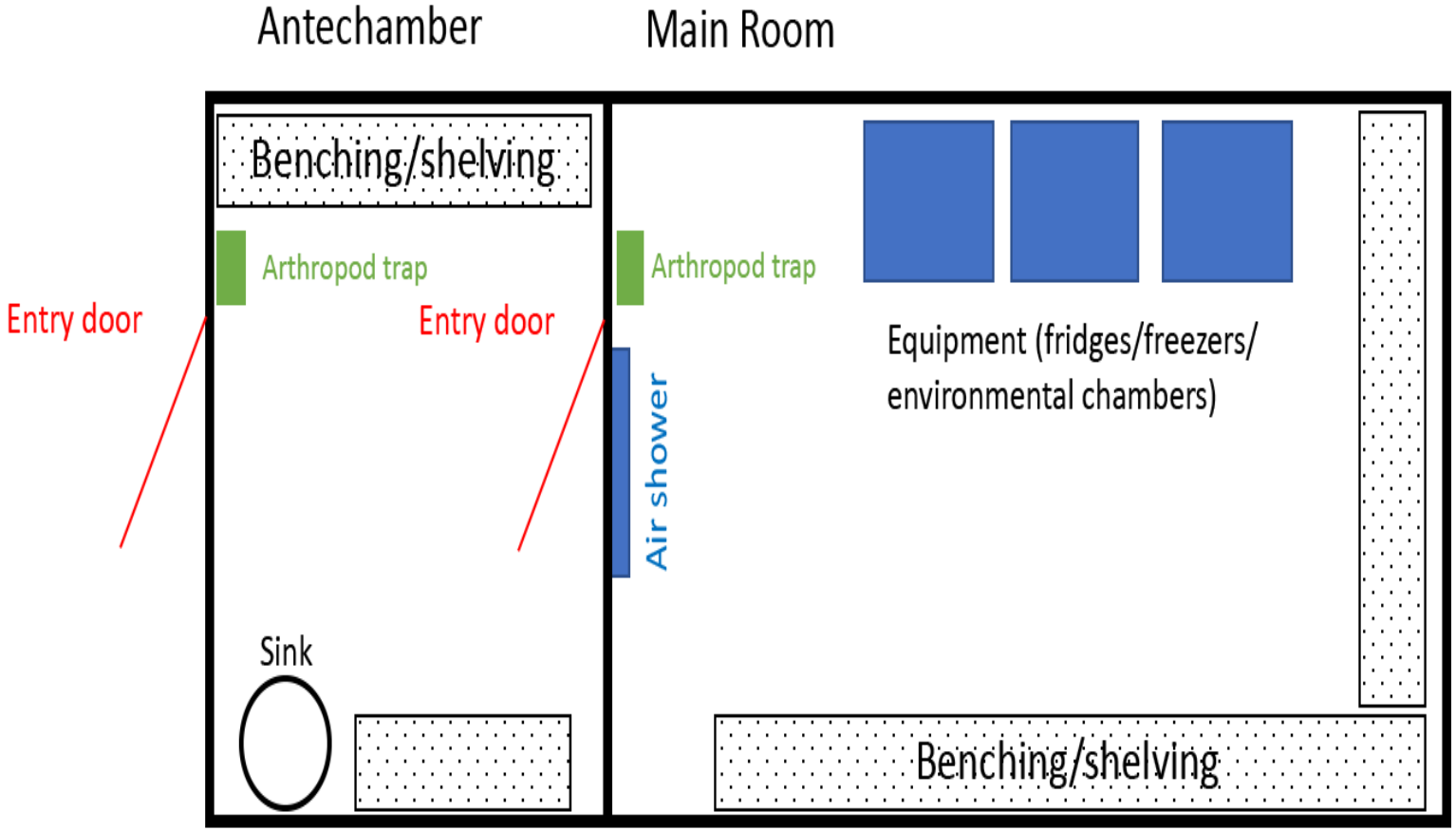
6) Availability of treatments and preventive measures eg. vaccines.

7) When manipulating mosquitoes and pathogens that are genetically modified, the **level of containment** may be upgraded and will depend on the type of experiments planned and on the nature of the transgenic modifications

Design of the basic CL2 insectary

Basic layout

A key consideration is whether main room should be controlled room or environmental chamber; sink is potential escape route.



Design of the basic CL2 insectary

Environmental chamber

Flexible, easy to clean, easy to regulate, escapes easier to control

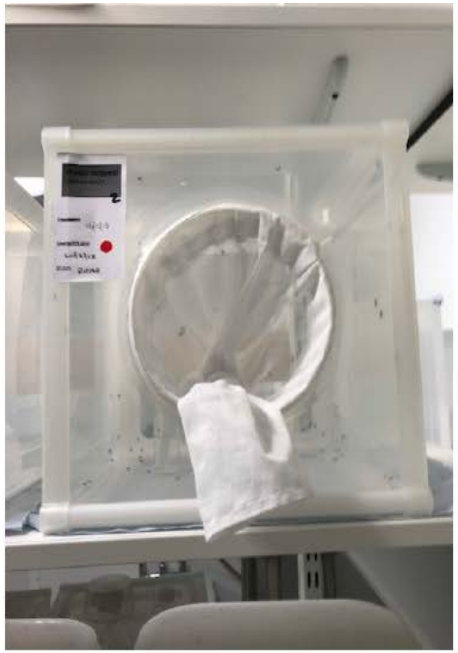
Controlled Insectary room

Space used to maximum, but more difficult to clean and control

Design of the basic CL2 insectary

Space is a key issue

Breeding pans



Cage design

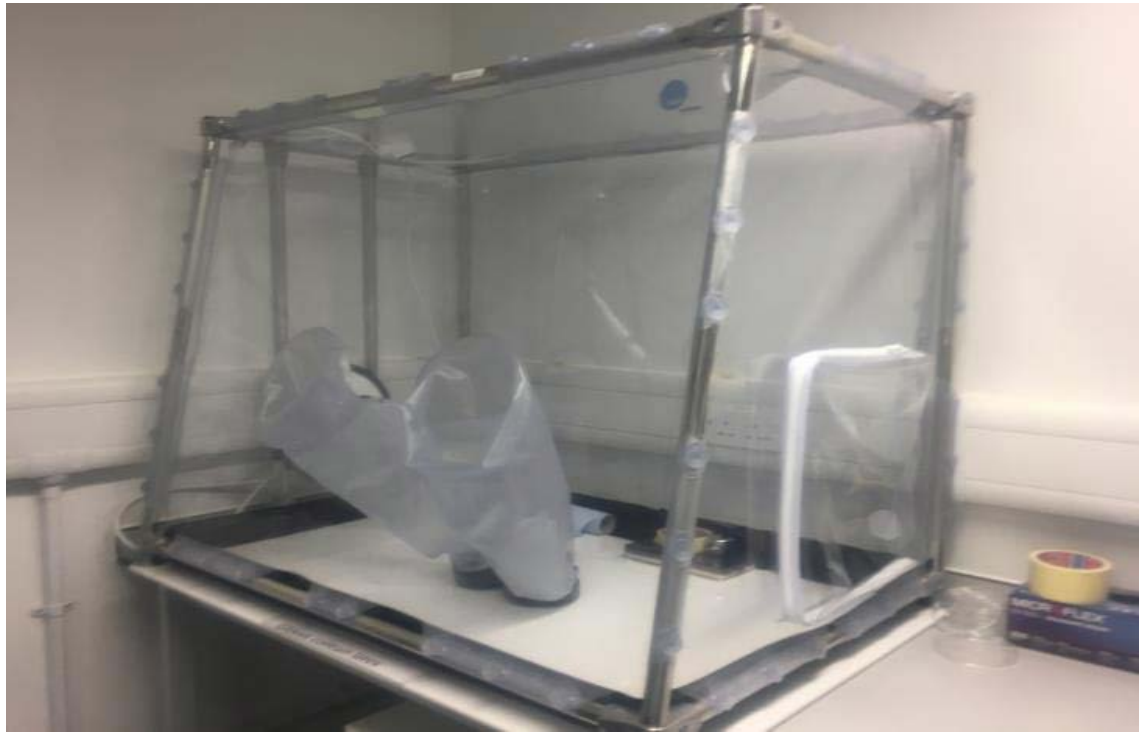
Meshed pot

Plan to use a maximum- avoid overcrowding!

It is easier to deal with escapes in a well organised insectary what ever the safety level.

Design of the basic CL2 insectary

Glovebox versus a microbiological safety cabinet



We use gloveboxes- mainly as they do not have airflow and contain any escapees.

Downside- difficult to work inside. Takes training and good organisation.

Design of the basic CL2 insectary

Manipulation room can be useful

Manipulation room with microscopes; freezers (not shown) to kill mosquitoes.

Kill/waste disposal procedures need checked locally.

Dissection room

Running of the basic CL2 insectary

1) Adequate information, instructions, training and supervision of all workers and visitors.

2) User access restrictions required (pin or key).

3) Access to autoclave in building; and if required (for example animal carcasses), access to incinerator, or tissue digester ; validated inactivation and waste disposal procedures must be in place.

4) Protocols for disinfection in place; waste streams according to national regulations.

5) Vector control measures such as traps to prevent intrusion/escape of arthropods are recommended throughout insectary rooms (light traps, attractor tape, fly catcher [electric or mechanic]).

Running of the basic CL2 insectary

6) Display (in the laboratory) of key standard operating procedures [SOPs] and emergency procedures.

7) Safe storage of biological material must be ensured.

8) If genetically modified organisms are to be used, other specific regulations such as Biohazard signs on access doors may be required.

9) Record keeping for risk assessments, standard operating procedures and other relevant records.

10) Monitoring of activities to ensure implementation and effectivity of risk assessments, controls and standard operating procedures.

Running of the basic CL2 insectary

Safety considerations: personal safety, standard personal protection for this safety level is sufficient

Depending on experiments you may want to consider a head net!

Running of the basic CL2 insectary

Waste considerations:

1) Disposal is generally regulated by national and/or local biosafety regulations.

2) It is recommended that all solid waste from mosquitoes should be discarded into autoclave bags, left for one night in a freezer (to kill all mosquitoes and avoid potential escapes before autoclaving), autoclaved and discarded.

3) Water resulting from larval rearing should be filtered [for example with Nitex cloth (50 µm)] mounted on a net (50 µm pore diameter to avoid any embryos/larvae/adults escapees) before being discarded in a sink. Boiling water can also be added to the larvae container to kill any eggs/larvae if using a sieve with a pore diameter larger than eggs/larvae, or overnight freezing at -20°C.

For infectious solids and liquids waste, inactivation by copious spraying or immersion might be required before freezing/autoclaving and disposal (for example with inactivating agents such as Virkon) though generally double bagging of waste followed by autoclaving is sufficient.

Running of the basic CL2 insectary

Waste considerations:

***Aedes ssp.* eggs are small!**

Running of the basic CL2 insectary

Dealing with escapes:

1) Individual escapes or low numbers can be dealt with easily by direct killing (handheld zappers, or fly catchers are useful).

2) Larger numbers may necessitate the use of insecticide (or even formaldehyde-based fumigation or Vapour Hydrogen Peroxide (VHP) decontamination by fumigation) but these negatively affect operations for long periods.

3) As mosquitoes require daily sugar feeding for hydratation, larger escapes may be more effectively dealt with by sealing the affected room and wait for mosquitoes to die (typically within 1-2 weeks post escape), depending on species/conditions!

Do trial runs before work begins!

Spills with pathogen-containing material need to be considered and dealt with according to local/national safety practices, for example decontamination with inactivating agents.

Running of the basic CL2 insectary

Other considerations:

1) Separation of infected and non-infected mosquitoes

2) Screening and quarantine: import of mosquitoes

3) Spatial restrictions versus design with planned work in mind?

4) Work flows around you insectary eg what does the rest of the institute do..?

The CL3 insectary

Many of the previous criteria apply- early planning, information, discussions…

1) Technical design is that of a classical CL3 laboratory with consideration for mosquito infections

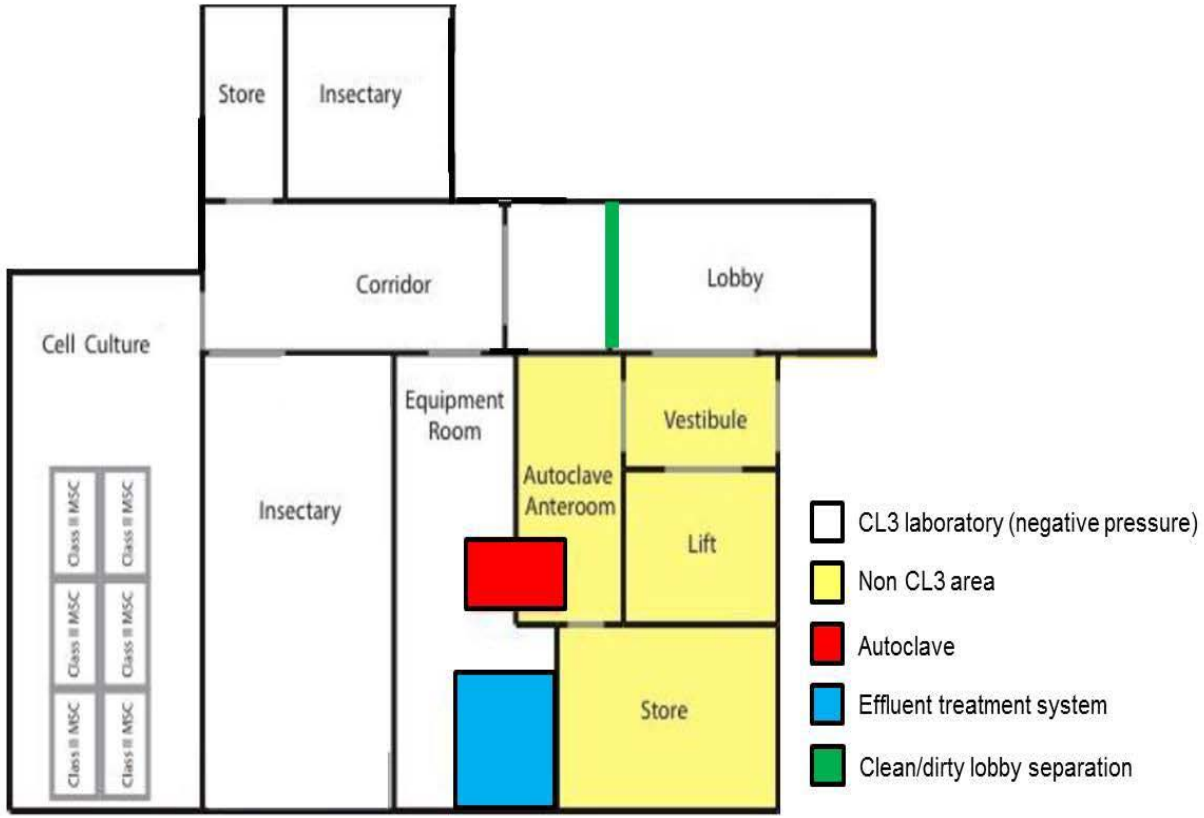
2) Some experiments involving HG2 pathogens and may be carried out at higher safety level depending on risk assessment (2+2=3?)

Design of the basic CL3 insectary

Basic layout (with cell culture)

For safety reasons we opt for environmental chambers to house arthropods. This may be influenced by individual requirements!

Attached cell culture may be useful for preparation of pathogens!



Running of the basic CL3 insectary

1) Adequate information, instructions, training and supervision.

2) User access restrictions required (pin and/or swipe card and/or fingerprint electronic readers among others) and dedicated protective clothing (PPE).

3) The laboratory must be protected with an intruder alarm in some cases according to the pathogens used and the national regulations.

4) Sealed laboratory (secondary containment or barrier) to prevent any accidental release of pathogen and HEPA filtration of air outflow.

5) Negative pressure environment with a gradient (check survival of arthropods!).

6) Access to autoclave within the CL3 suite or double-ended; and if required (for example animal carcasses), access to incinerator or tissue digester; validated inactivation and waste disposal procedures must be in place.

Running of the basic CL3 insectary

7) Protocols for disinfection in place; waste streams according to national regulations.

8) Vector control measures such as traps to prevent intrusion/escape of arthropods are recommended throughout insectary rooms.

9) Display or access to (in the laboratory) key standard operating procedures [SOPs] and emergency contacts and procedures.

10) Safe storage of biological material must be ensured. Pathogens falling under HG3 regulations can only be used within such a facility, and must be securely stored (by key or password) in dedicated spaces.

11) Biohazard signs on access doors may be required.

Running of the basic CL2 insectary…continued

12) Record keeping for training, risk assessments, standard operating procedures and other relevant records.

13) Monitoring of activities to ensure implementation and effectivity of risk assessments, controls and standard operating procedures. Personnel should ensure enough provisions in their laboratory (disinfectant, PPE, etc.) and all streams for bringing in and taking out material should be described and risk assessed.

14) Vision panels and/or web cams/CCTV to allow supervision of workers within the CL3 laboratory.

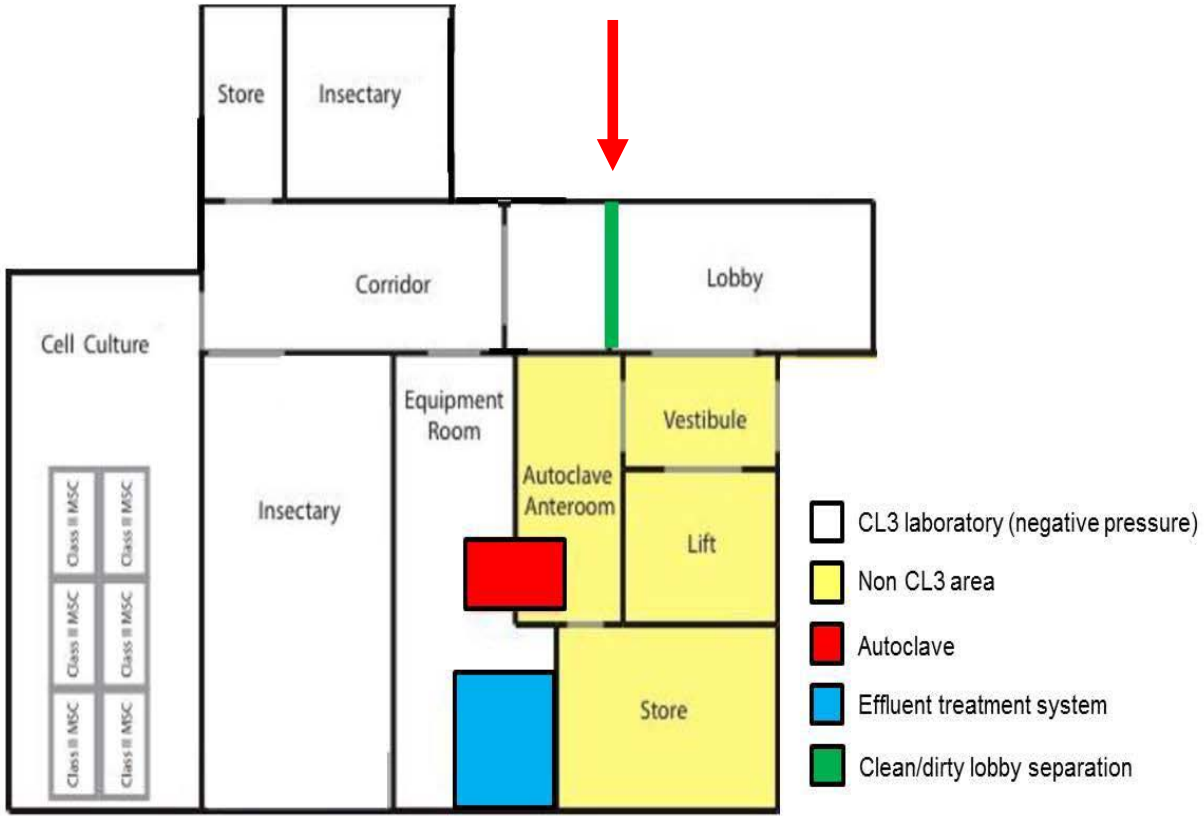
15) Transport measures (containers etc.) for movement of samples between rooms and in/out of the facility. The exit of inactivated samples from CL3 to CL2 should be done by pass-through or hatches with double door and integrated and validated disinfection/inactivation system.

Design of the basic CL3 insectary: containment

Entering the CL3 laboratory:

Controlled access with log book
Change to PPE (suit/goggles)
"Buddy" or other safety system

Entry/Exit point



Design of the basic CL2 insectary

Environmental chamber: housing mosquitoes

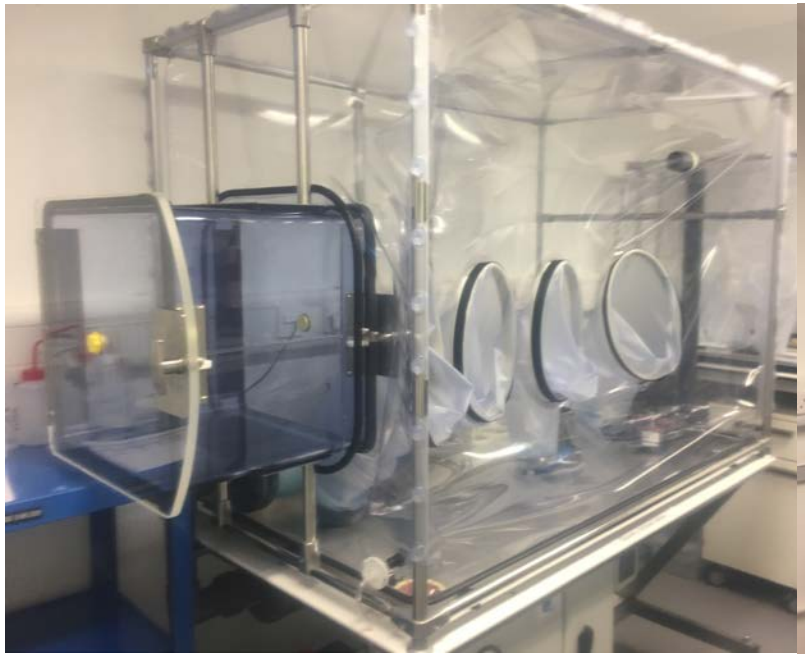
Flexible, easy to clean, easy to regulate, escapes easier to control which key in this higher safety environment.

Provide a further layer of containment.

Mosquitoes in containers should be labelled and numbers managed reasonably.

Design of the basic CL2 insectary: containment

Glovebox versus a microbiological safety cabinet



We use gloveboxes- hepafiltered airflow and contain any escapees.

Downside- difficult to work inside. Takes training and good organisation.

Design of the basic CL2 insectary: containment

Glovebox versus a microbiological safety cabinet

MSC can be useful but airflows may disturb arthropods!

Easier to work in.

Design of the basic CL2 insectary: protection of personnel

Is bench work possible? Exposure may just be a theoretical, or low risk. Some CL3 facilities operate with mask systems.

Use of respirators/filtering face pieces?

Masks may need fitting.

Even simple designs are used but depends on virus.

Easier to work with, allows good dexterity.

Design of the basic CL2 insectary: containment

Glovebox versus a microbiological safety cabinet- is either needed? An example- forced mosquito salivation.

This experiments require a degree of dexterity and space.

Easier to work on bench.

Exposure to pathogen is mainly a theoretical risk as virus amounts are minimal.

Running of the CL3 insectary

Waste considerations:

1) Disposal is generally regulated by national and/or local biosafety regulations.

2) All solid waste to be autoclaved out of the CL3 laboratory; for equipment- wiping with disinfectants may be necessary.

3) Liquid waste to be chemically inactivated or autoclaved.

Effluent treatment systems can be useful- but expensive to install and run!
But if shower facilities are planned, it may be a good addition!

Running of the CL3 insectary

Dealing with escapes:

1) Individual escapes or low numbers can be dealt with easily by direct killing (handheld zappers, or fly catchers are useful) but if infectious decontamination is necessary- potential exposure if outside containment!

2) Larger numbers may necessitate the use of insecticide (or even formaldehyde-based fumigation or Vapour Hydrogen Peroxide (VHP) decontamination by fumigation) but these negatively affect operations for long periods.

3) As mosquitoes require daily sugar feeding for hydratation, larger escapes may be more effectively dealt with by sealing the affected room and wait for mosquitoes to die (typically within 1-2 weeks post escape), depending on species/conditions!

Do trial runs before work begins! Establish emergency plans.

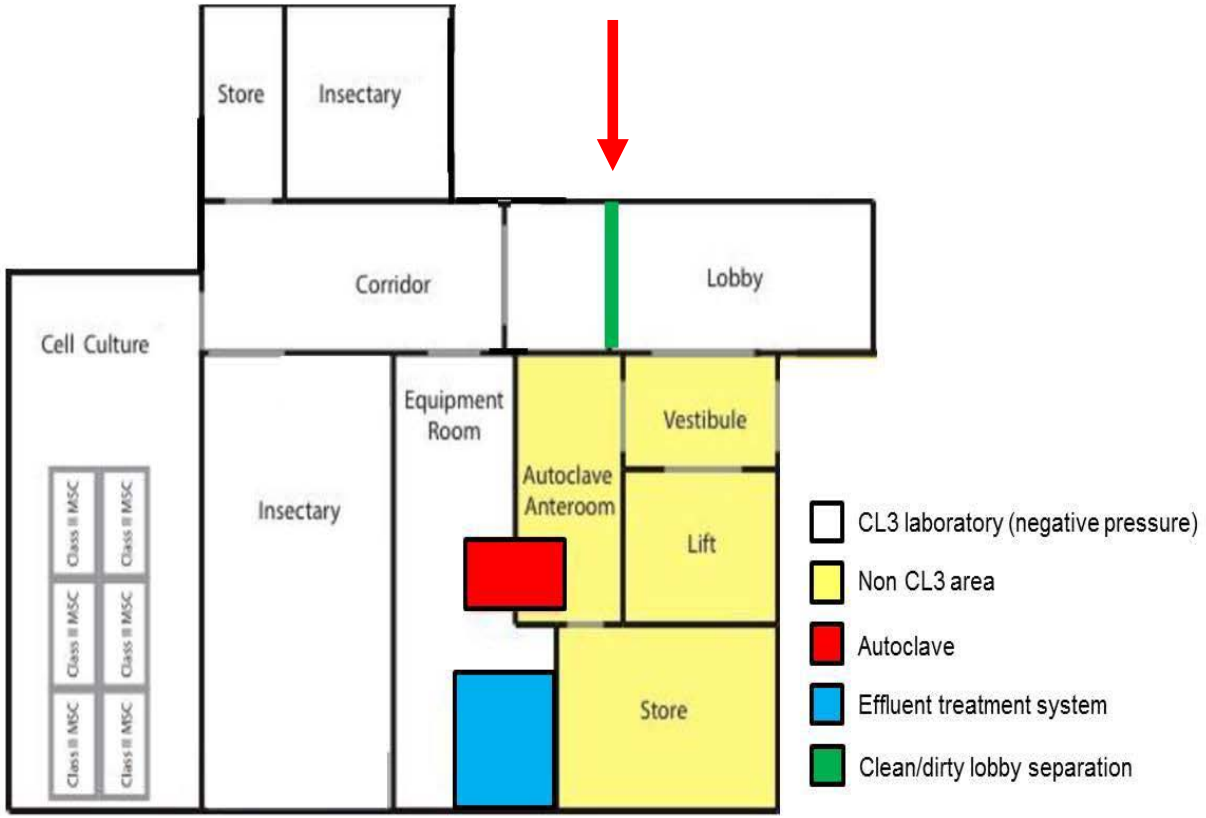
Spills with pathogen-containing material need to be considered and dealt with according to local/national safety practices, for example decontamination with inactivating agents.

Design of the basic CL3 insectary

Exiting the CL3 laboratory:

Air shower/curtain,
water shower….
or none of the above

Exit point



Design of the basic CL3 insectary

Exiting the CL3 laboratory:

Shower facilities:

Safe…..but:

Require structural adaptation for users

Water treatment may add to infrastructure costs.

Design of the basic CL3 insectary

Exiting the CL3 laboratory:

Air shower/curtain:

Efficient at removing mosquitoes from clothing

More cost effective as design can be simple
(we are producing equipment a dust free-environment)

Simple may be efficient.

Design of the basic CL3 insectary

Exiting the CL3 laboratory:

Decision on exit safety comes down to:

Regulation (some pathogens may require shower-out facilities)

Cost (water treatment and space for example)

Efficiency (in some settings, water supply may be an issue)

Effective need (at CVR, we use none of the above)

Some general considerations: training

Following theoretical training and lectures on specific aspects of biosafety related to the activities to be carried out (risk assessments, SOPs etc.).

CL2: Demonstration of procedures and then at least once under supervision or until competent with procedures.

CL3: Experience at CL2 level should be a requirement. Training should be provided in specific techniques.

Refresher training should be offered for staffs who have not been working in the facility following initial training.

Trial runs are useful for new procedures!

Some general considerations: final overview of workpractices, based on virology laboratories at CL3 level

Institut Pasteur: masks, manipulation on bench

Wageningen University: masks, manipulation on bench

IRTA: respirators, masks (depending on virus) (shower out system)

CVR: gloveboxes (hepa filtration) for all manipulation

(Gloveboxes are becoming more common though)

Beyond mosquitoes…

Midges: numbers, sizes, mesh sizes….

Ticks: can’t fly, lower numbers, but can hide…

Risk assessments need to be adaptable!