“Basic Principles in Biosafety”

Suzanne LORET (PhD)
Biosafety Officer , UNamur (SIPPT)

Rue de Bruxelles 61 – 5000 Namur
Suzanne.loret@unamur.be

UNIVERSITÉ DE NAMUR

Content

1. Context – Definitions
2. Risk Assessment
3. Risk Management

1. Context – Definitions

UNIVERSITÉ DE NAMUR

Basic principle of Laboratory safety:

A PROPER TRAINING IS MANDATORY BEFORE STARTING ANY EXPERIMENTAL WORK

Bio Safety

Biological Agent (BA)

dangerous

BA Risk Group

Use-related risk

CONTAIN the risk (facility)

CONTAIN the activity (equipment)

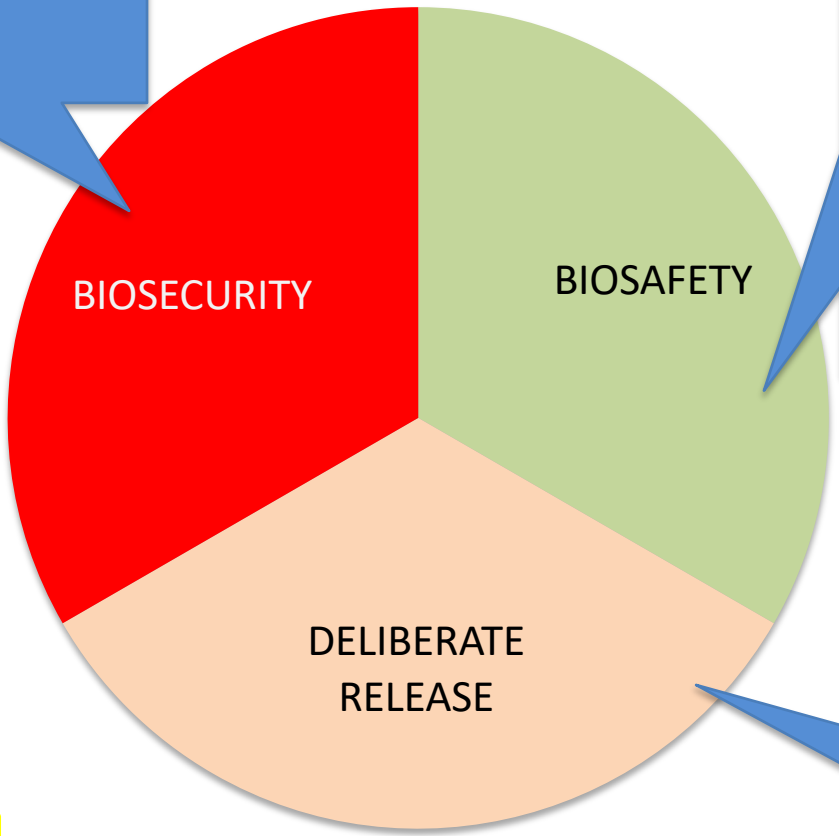
PREVENT accident/incident (GBP)

DISINFECT, STERILIZE, eliminate WASTE safely

RISK ASSESSMENT | **RISK MANAGEMENT**

4 situations creating biological hazards

1) when pathogen are used as biological weapons (**Bioterrorism**)



2) When pathogens are purposely grown (cell multiplication)
- to confirm a serious infection
- for research and development
- to infect animals experimentally

3) When organisms are purposely genetically modified

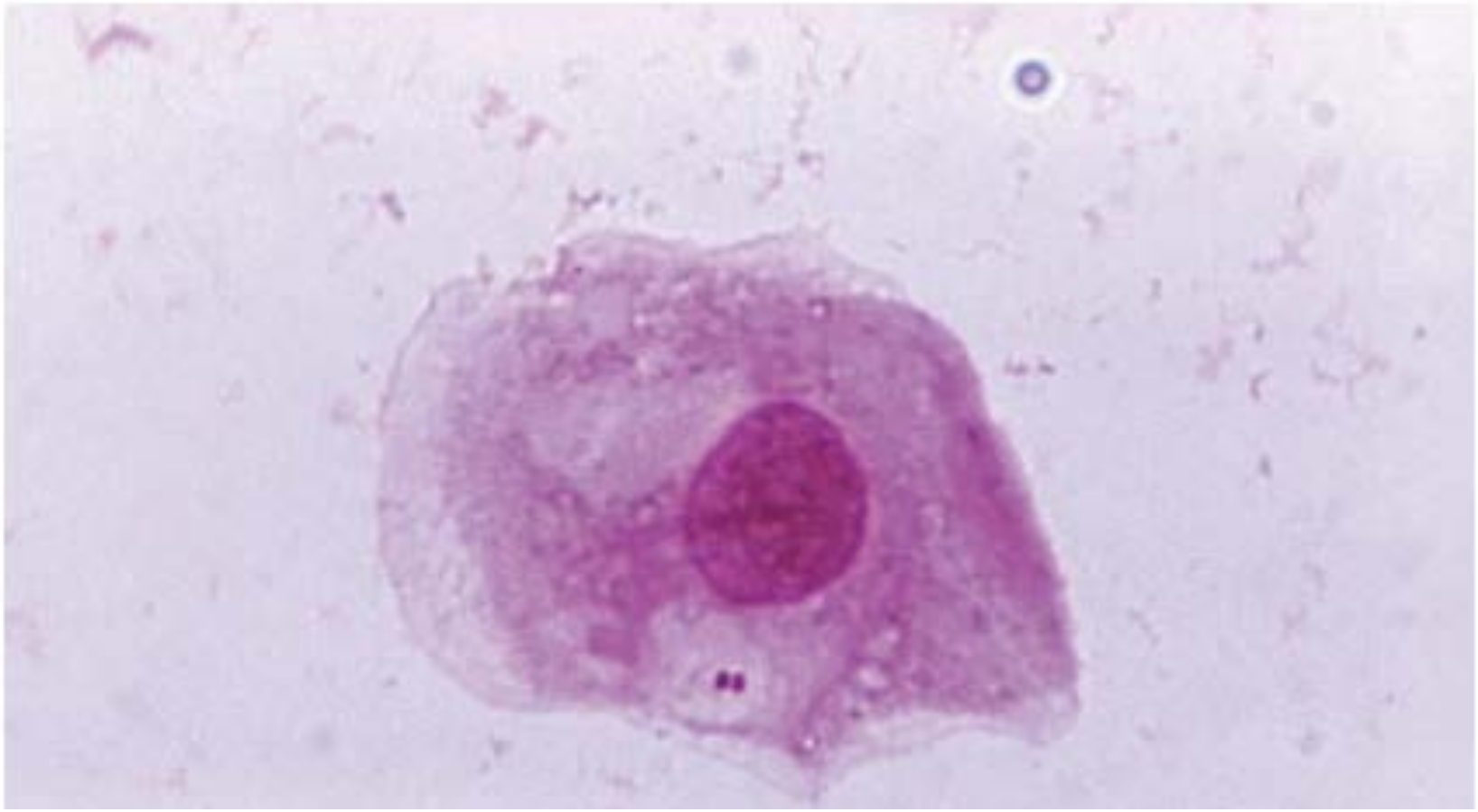
4) When OGM are exported in environment

Topics of this training

Biosafety, What for?

1) **to protect laboratory staff against LAIs**

"Researcher, 25, dies from rare bacteria in San Francisco"



Laboratory Acquired Infections (LAI)

Infection	Total no. (%) of cases reported for:			
	U.S.[a]	U.S. and world[b]	Great Britain[c,d]	NADC[e]
Brucellosis	274 (9.4)	423 (10.8)	2 (2.1)	18 (52.9)
Q fever	184 (6.3)	278 (7.1)	0	
Typhoid fever	292 (10.0)	256 (6.5)	3 (3.2)	
Hepatitis	126 (4.3)	234 (6.0)	19 (20.0)	
Tularemia	129 (4.4)	225 (5.7)	0	
Tuberculosis	174 (6.0)	176 (4.5)	24 (25.3)	4 (11.8)
Dermatomycosis	84 (2.9)	161 (4.1)	0	2 (5.9)
Venezuelan equine encephalitis	118 (4.1)	141 (3.6)	0	
Typhus	82 (2.8)	124 (3.2)	0	
Psittacosis	70 (2.4)	116 (3.0)	0	4 (11.8)
Coccidioidomycosis	108 (3.7)	93 (2.4)	0	
Streptococcal infections	67 (2.3)	78 (2.0)	3 (3.2)	
Histoplasmosis	81 (2.8)	71 (1.8)	0	
Leptospirosis	43 (1.5)	87 (2.2)	0	3 (8.8)
Salmonellosis	54 (1.9)	48 (1.2)	11 (11.6)	1 (2.9)
Shigellosis	54 (1.9)	58 (1.5)	26 (27.4)	
All reported infections	2,912	3,921	95	34

[a] 1969 data adapted from reference 151.
[b] 1976 data adapted from reference 110.
[c] 1980 to 1989 data adapted from references 51 through 55.
[d] Includes possibly attributable and attributable cases.
[e] NADC, National Animal Disease Center; 1975 to 1985 data adapted from reference 93.

SEWELL, DL (1995). CLINICAL MICROBIOLOGY REVIEWS, July 1995, p. 389–405

Biosafety, What for?

2) to protect the community (human/animal) and the environment

Pirbright fined for foot-and-mouth trial failings

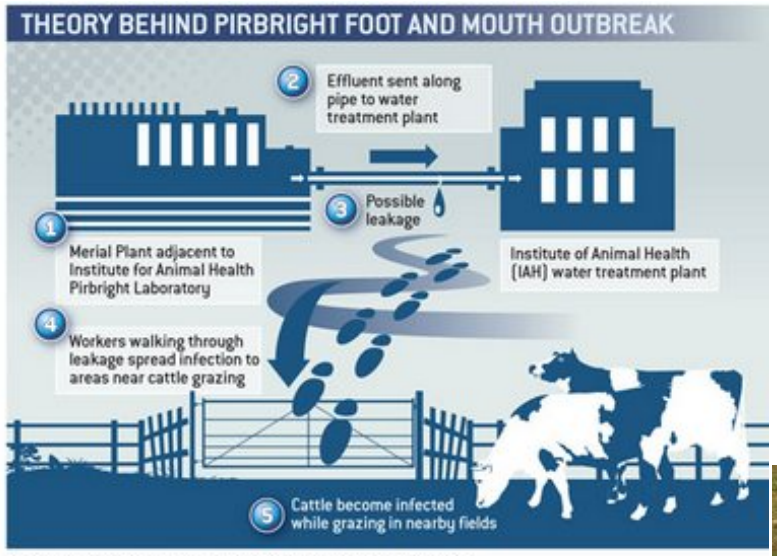
Thursday 1 May 2014 12:56 Jonathan Riley (http://www.fwi.co.uk/author/jonathan-riley)

The government's disease research centre at Pirbright has been ordered to pay fines and costs totalling £77,000 for safety failings during foot-and-mouth experiments (F&M).

The Pirbright Institute in Surrey was at the centre of the 2007 F&M outbreak after leaking drains at the research site were identified as the most likely source of the disease.

See also: Foot-and-mouth outbreak 2007 (http://www.fwi.co.uk/articles/12/09/2007/105708/foot-and-mouth-disease-fmd-2007-surrey-outbreak-farmers-weekly-interactives-special.htm)

The site is regulated by the Health and Safety Executive (HSE) which said the latest two incidents, in November 2012 and January 2013, involved the airflow into and out of parts of a facility housing infected animals.



Based on: HSE www.hse.gov.uk/news/archive/07aug/pirbright.htm

The use of biological agents could lead to damages

Certain biological agents can present Biohazards (Biorisk)

Laboratory biohazard (biorisk) : what does it mean?

Biological hazard (« biorisk ») ≈ **Consequence (disease, death, …)** ∪ **Likelihood (exposure)**

PATHOGEN ORGANISMS (PO) (GM or not)

GENETICALLY MODIFIED ORGANISMS (GMO) (P or not)

of the worker
+
his colleagues

his family
the population
+
the environment (BIODIVERSITY)

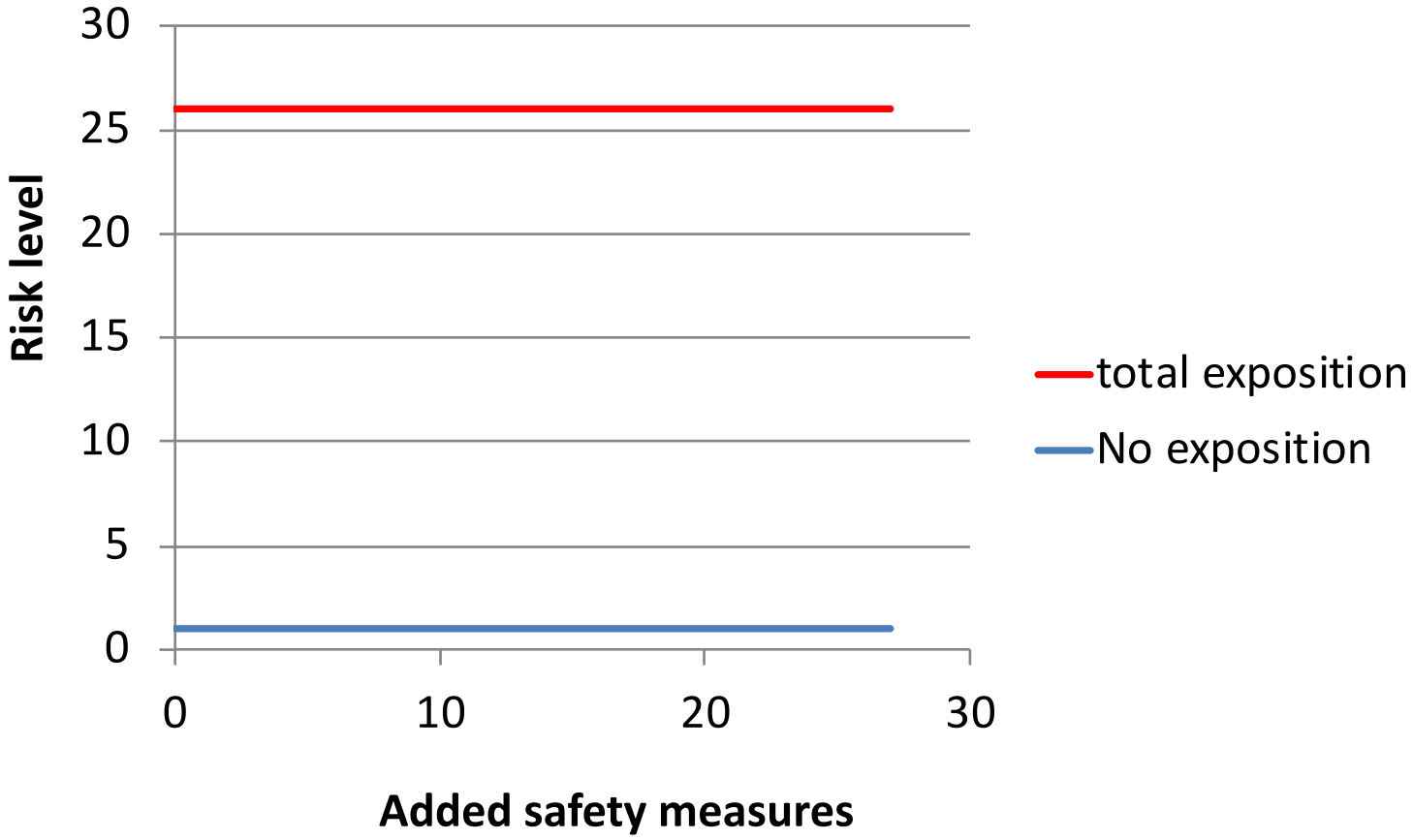
Biosafety:
is the set of measures taken to:

- protect laboratory professionals (including health workers) against Laboratory Acquired Infection (LAI)
- avoid accidental release of pathogens/GMO in the environment and consequently, to protect
  - the health of the community (i.e. humans, animals and plants)
  - the biodiversity of wild endemic species

when using - purposely - pathogenic and/or genetically modified organisms (PO and/or GMO).

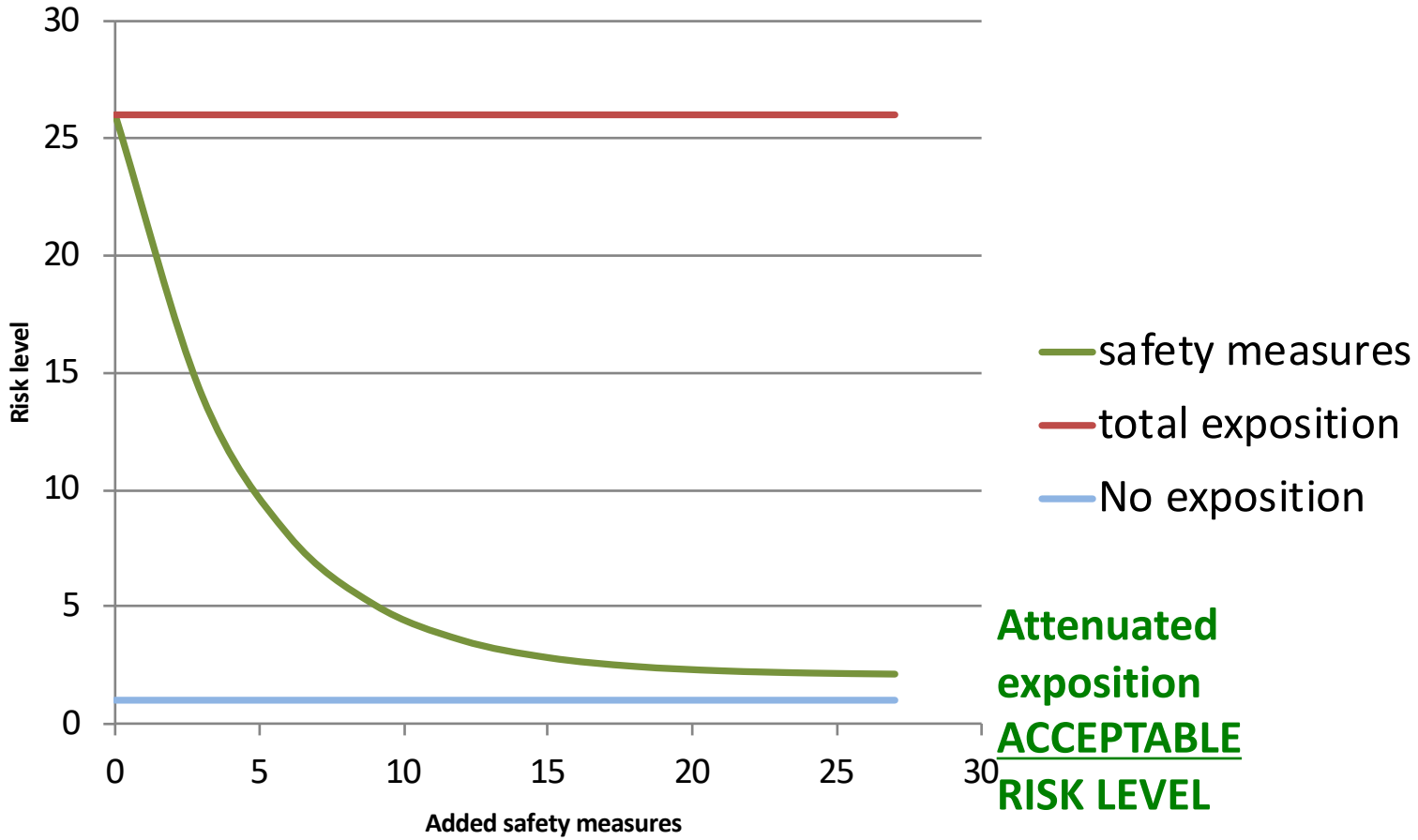
Biosafety: Why?

To reduce the risk level

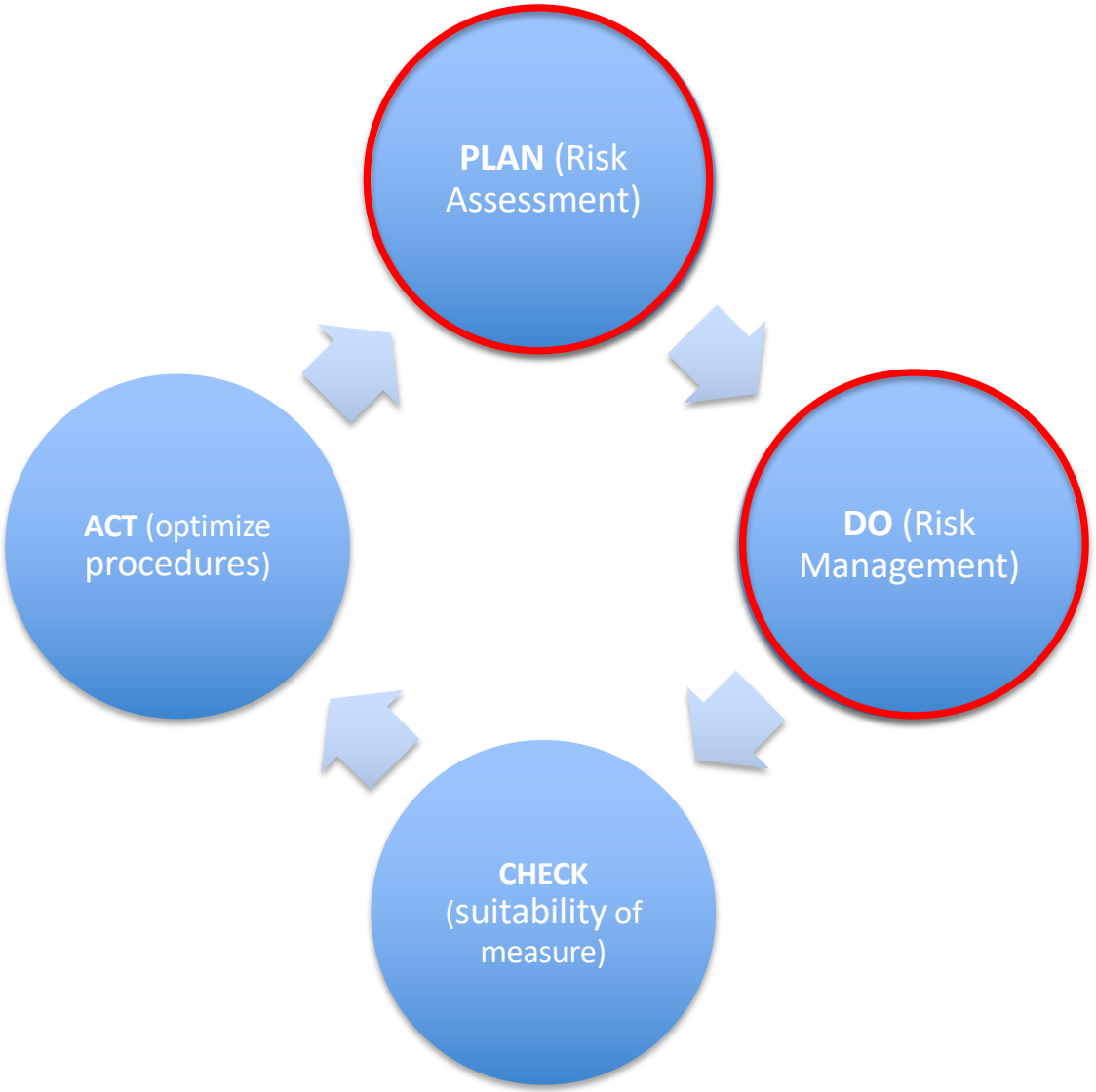


Biosafety,Why?

To reduce the risk level



Dynamic management of Biosafety: PDCA cycles (=continuous improvement)



Topics of this training

2. Risk Assessment

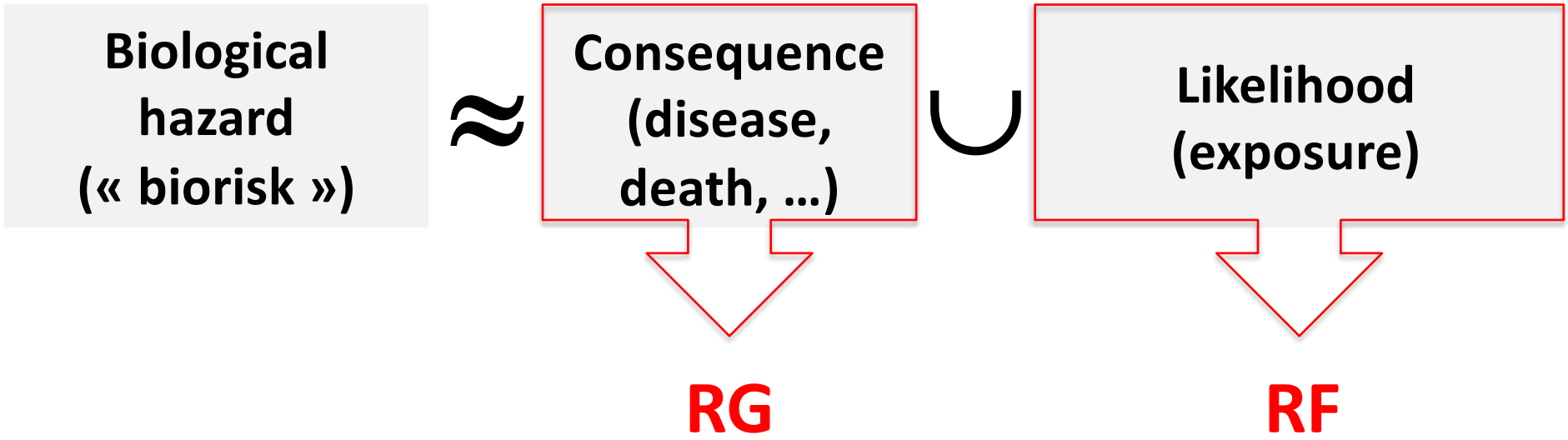
UNIVERSITÉ DE NAMUR

Biosafety measures are based on the Risk Assessment, which consists in:

- the identification of biological hazards
- the analysis of risks linked to their use (RA)
- the identification of measures to be applied to minimize the risk, including :
  - Personal protection equipment (PPE)
  - Laboratory secondary barriers (“containment”)
  - Validated methods to inactivate PO/GMO and the waste produced during their use.

First steps

1) Identify the risk of the biological organism = define the RISK GROUP (RG)
2) Identify RISK FACTORS in the activity (RF)



Organisms presenting Biological Hazards (pathogenic organisms PO):

- Bacteria
- Fungi
- Viruses
- Protozoa
- Parasites (worm-like parasites, ...)
- *Unconventional transmissible agents (UTA, ex. prions)*
- *Toxins and other noxious substances*
- *Allergens*

Identification of biological hazards: Risk Groups (RGs) of PO (4 groups = RG1, RG2, RG3, RG4)

Defined according to :

- their pathogenicity and/or harmfulness (toxicity)
- their transmissibility rate (contagion way)
- the availability of a prophylaxis and/or a treatment

In this classification, the target of the OP is the healthy worker

Risk Groups of Pathogenic Organisms (PO)

- RG 1: **no risk for human health**,

  but a possible risk for animals/plants and/or biodiversity

- RG 2: slight risk

  ➔ Causes human disease, but

  – low dispersal probability

  – effective prophylaxis/treatment is available

Risk Groups of PO

- RG 3: moderate risk
  - ➔SEVERE disease (to humans),
  - – high dispersal probability *(but not in the laboratory, under "safe condition")*
  - – "usually", effective prophylaxis or treatment

- RG4: high risk
  - ➔ SEVERE and generally FATAL disease (to humans)
  - ➔ Highly contagious disease
  - ➔ there is usually no effective prophylaxis or treatment available

Summary about PO RGs

Risk groups are defined in accordance to Immuno-competent **Humans** (/animals/plants)

Risk Group	Risk Criteria		
	Disease Severity (Human)	Infection probability in the lab (contagion way)	Treatment/Prophylaxis Availability
RG 1*	**No**	**/**	**/**
RG 2	**+**	**-**	**+**
RG 3	**++**	**+**	**+**
RG 4	**+++**	**+++**	**-**

RG1*

= riskless for workers

= (like all other RG2-4 organisms) could be a thread to other species in environment

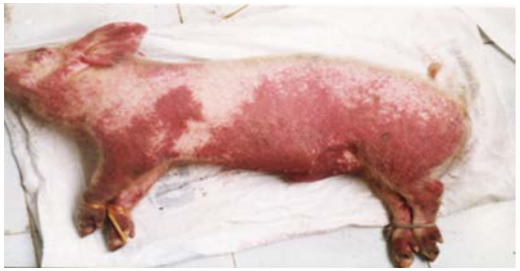
→ should not be released outside the lab

Examples of RG 1 POs for human

While non-pathogenic for humans, are highly pathogenic for healthy plants and animals

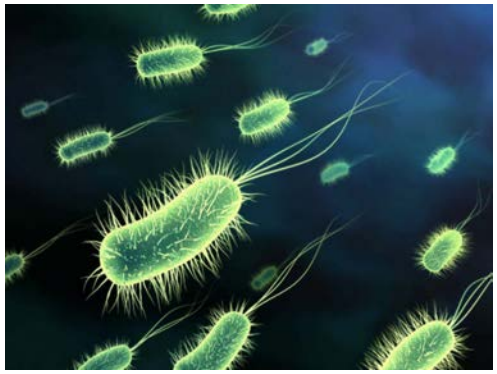
"Blue Tongue Virus"
(cattle)

"Swine pestis virus"
("classical swine fever virus")
(pork/wild boar)

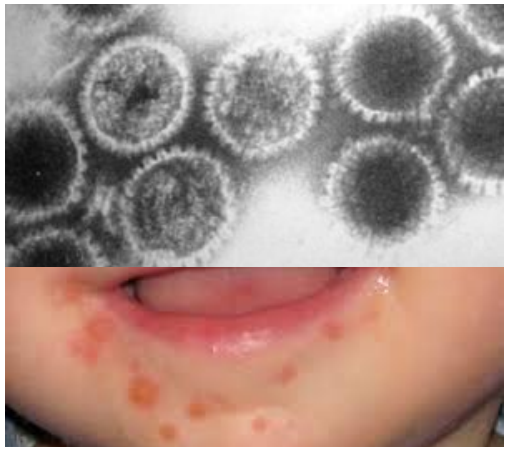


"Agrobacterium tumefaciens"
(crown gall disease)

RG 2:

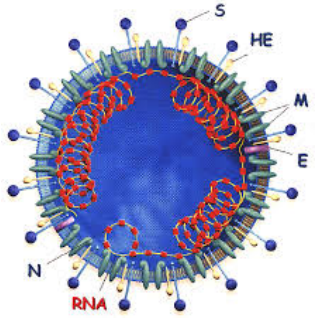


Salmonella enterica
(zoonotic PO)

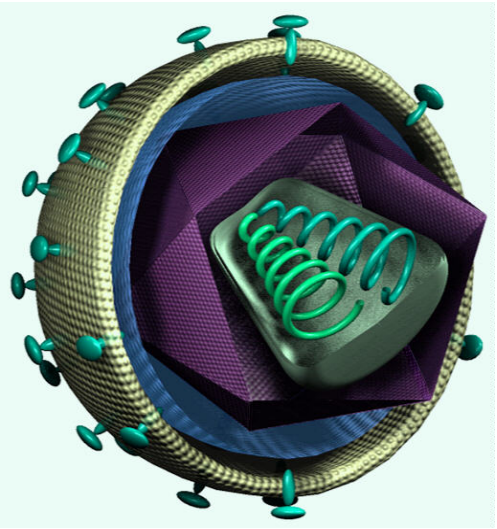


Herpes simplex virus
(human PO)

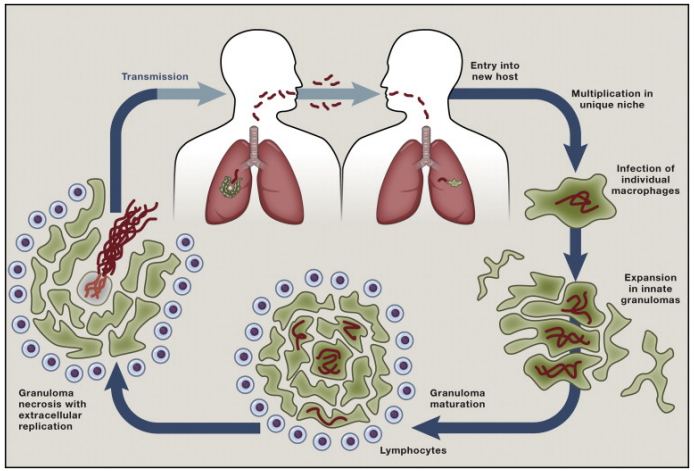
RG3:



SARS corona virus
(zoonotic PO -
2003 outbreak)



HIV (zoonotic)



Mycobacterium tuberculosis
(human PO)

Pathogenic Prion
Protein (P PrP)
(zoonotic UTA)

New Corona virus (RG3)

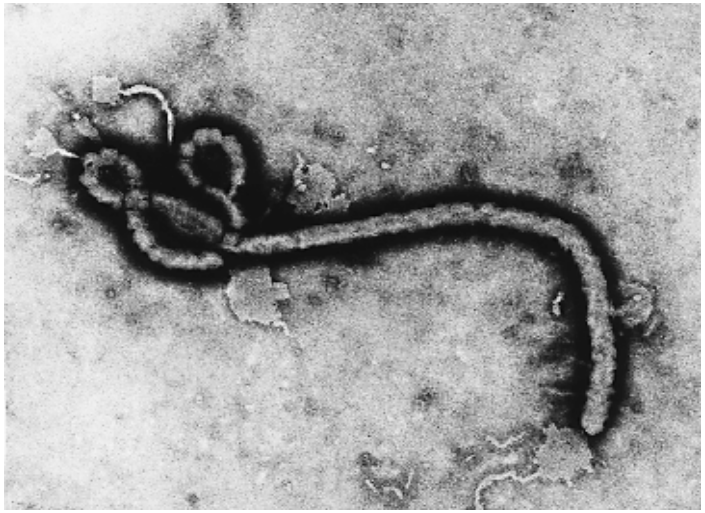
Specific Guidelines

Virus isolation in cell culture and initial characterization of viral agents recovered in cultures of SARS-CoV-2 specimens are NOT recommended at this time, except in a BSL3 laboratory using BSL3 work practices.

The following activities may be performed in BSL-2 facilities using standard BSL-2 work practices:

- Pathologic examination and processing of formalin-fixed or otherwise inactivated tissues
- Molecular analysis of extracted nucleic acid preparations
- Electron microscopic studies with glutaraldehyde-fixed grids
- Routine examination of bacterial and mycotic cultures
- Routine staining and microscopic analysis of fixed smears
- Final packaging of specimens for transport to diagnostic laboratories for additional testing. Specimens should already be in a sealed, decontaminated primary container.
- Inactivated specimens (e.g., specimens in nucleic acid extraction buffer)

RG 4 example



Ebola virus

Risk Group lists on Biosafety.be

- **Bacteria:** https://www.biosafety.be/sites/default/files/h_a_bacteries.pdf
- **Fungi:** https://www.biosafety.be/sites/default/files/h_a_fungi.pdf
- **Parasites:** https://www.biosafety.be/sites/default/files/h_a_parasites.pdf
- **Viruses and UTA:** https://www.biosafety.be/sites/default/files/h_a_virus.pdf

Contagious risk = f (infection route)

Inhalation

(air borne pathogens)

∨

Ingestion

(contaminated samples/biopsies)

∨

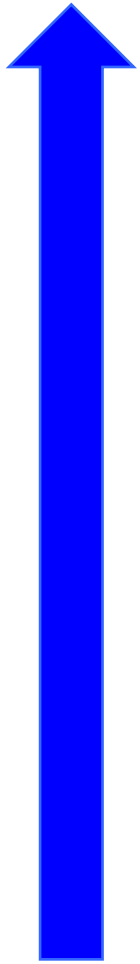
Skin contact (or eyes mucous contact)

(contaminated samples/biopsies)

∨

blood contact

(blood borne pathogens; contaminated samples/biopsies)



Safety

containment & measures

Infection routes of pathogens: a few examples

Infection route	Example(s)
Inhalation	*Brucella* bacteria, Influenza virus, Measles virus
Through Skin/ mucous membranes	Parasite fungi, EBOLA virus
Ingestion	Typhoid bacteria, poliovirus, prion protein
Blood contact	HIV, Hepatitis B virus, prion protein, EBOLA virus

Infections routes when working with animals:

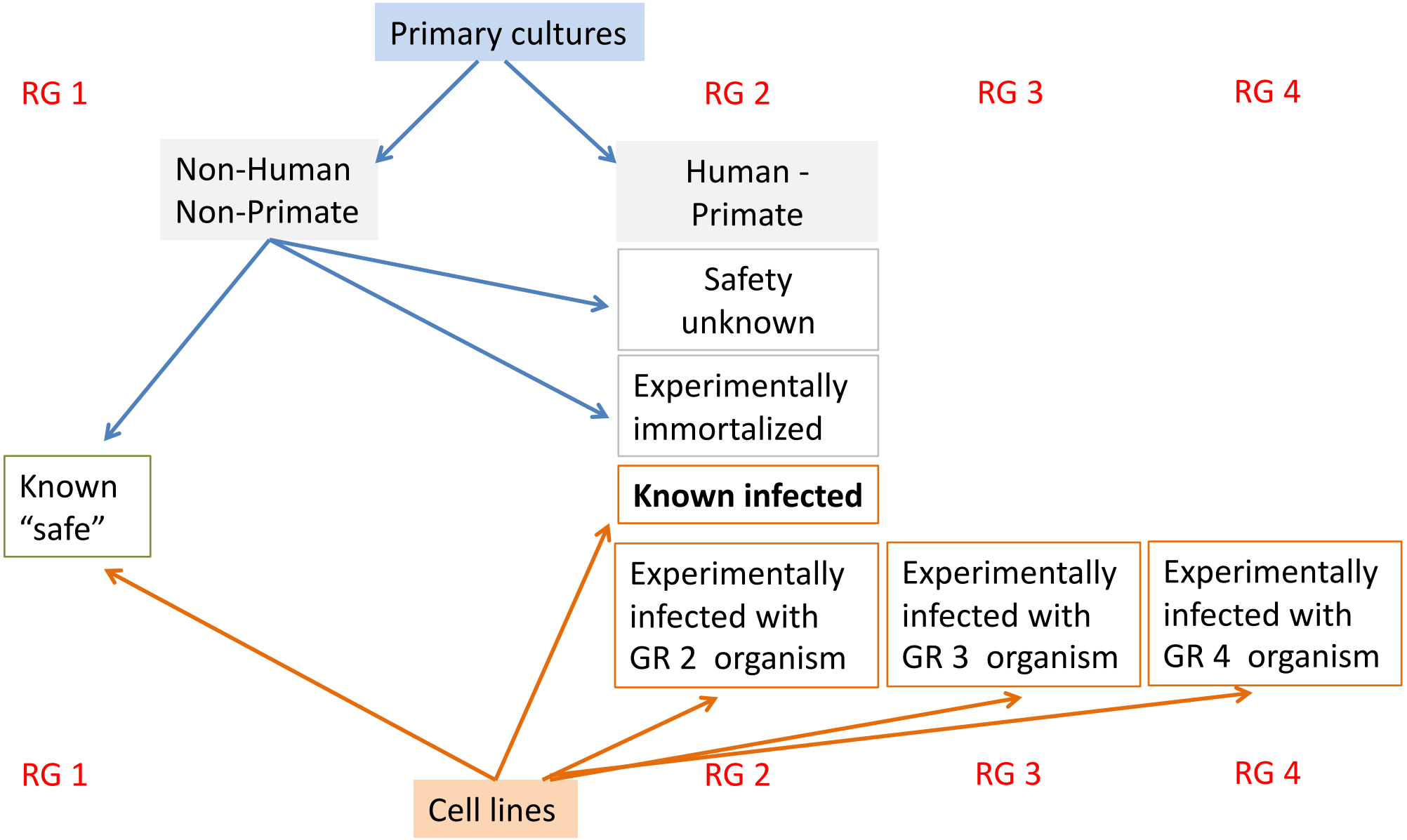
Infection routes:

- Skin wounds caused by animal biting and scratching
- Splashing in eye mucous membrane of contaminated body fluids during autopsy/chirurgic acts
- Mucous contamination (eye) by aerosols from coughing animals

Examples of infections acquired from mice and rats:

- Anthrax (skin, blood)
- Brucellosis (aerosols of blood and body fluids)

Risk groups of eucaryotic cells cultures



Risk groups of GM cells

3 risk criteria have to be taken into account:

- **Host cell RG (H RG)**

- **Plasmid RG (P RG)**
  - Most plasmids present no risk (RG 1, commercial origin)
  - RG 2 plasmids:
    - "Home made plasmids
    - Defective lentiviral vectors (oncogenic plasmids)

- **RG of the gene product of the cloned gene (GP RG)**
  - **RG 2**, if the products = toxins, allergens and other metabolically actives substances (hormones; anabolic, β-adrenergic, anti-infectious, anti-inflammatory and antiparasitic agents)
  - **RG 3**, if the product is a UTA such as the P PrP (prion)

Risk groups of (micro-)OGM

Exercise 1:

In a pharmaceutical company, a vaccine against the causative agent of haemorrhagic fever, is produced by a GMO in large culture fermenters (2000 L capacity).

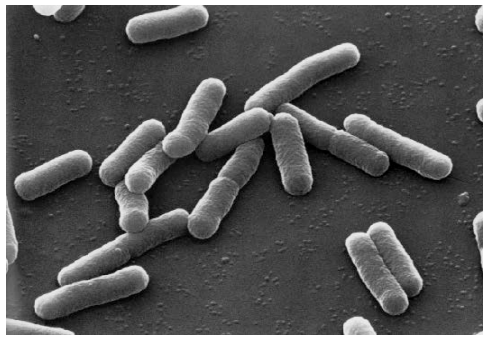
The GMO is non-pathogenic bacteria *(E. coli K-12),* transformed with a plasmid containing the gene coding for a surface antigen of the Ebola Virus in the culture medium.

The genetic transformation of the bacteria is done using a commercial plasmid (pUNO form InvivoGen).

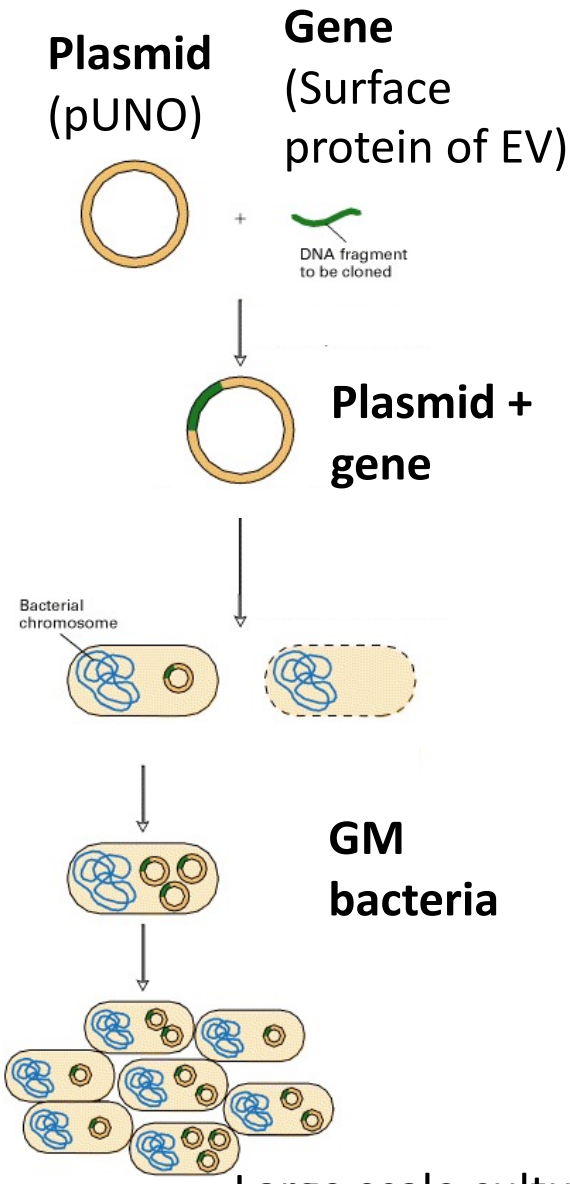
What is the RG of the transformed *E. coli*?

E. coli K-12 expressing EV antigen principle:

The different steps of **bacteria transformation** with a plasmid expressing the desired antigen



Host cell
E. Coli K-12



Large scale culture of GM bacteria

Risk groups of (micro-)OGM

Exercise 2:

In a pharmaceutical company, Insulin is produced by a GMO in large culture fermenters (2000 L capacity).

The GMO is non-pathogenic bacteria *(E. coli K-12),* transformed with expression plasmids coding for the the hormone two sub-units.

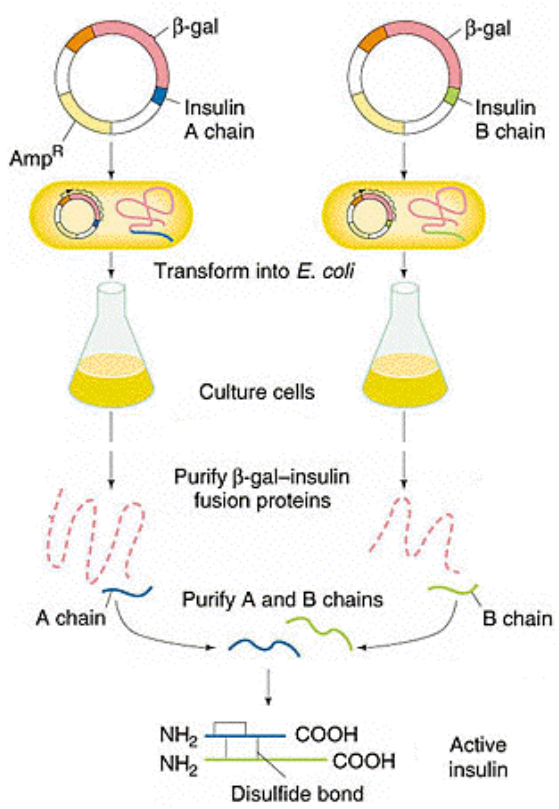
The genetic transformation of the bacteria is done using a commercial plasmid (pUNO form InvivoGen).

What is the RG of the transformed *E. coli*?

E. coli K-12 expressing Insulin sub-units principle:

Necessity of two cloning steps:

- 1 for the A chain
- 1 for the B chain



RG ?

1. **The host cell of the gene (H)**

   The RG of non-pathogenic *E. coli?* **1**

2. **The gene vector (P) : a safe commercial plasmid**

   pUNO for gene expression in *E. coli?* **1**

3. **The risk of the synthesised protein (GP)**

   Is the synthesised protein a hormone, an allergen, a toxin or a UTA? No (Half-hormone=**inactive**) **1**

The **GMO Risk group is** the highest RG level reached by one of those three components RG (H or P or GP) → **RG1**

The complex of A-B chains is a RG 2 (noxious) substance !
→ the last step (outside coli) is a level 2 Risk

The same exercise could be done using

- the yeast *S. Cerevisiae*
- the bacteria *P. pastoris*

as “insulin factories”

The conclusion will be the same: the MGM is RG 1 organism

Risk groups of (micro-)OGM

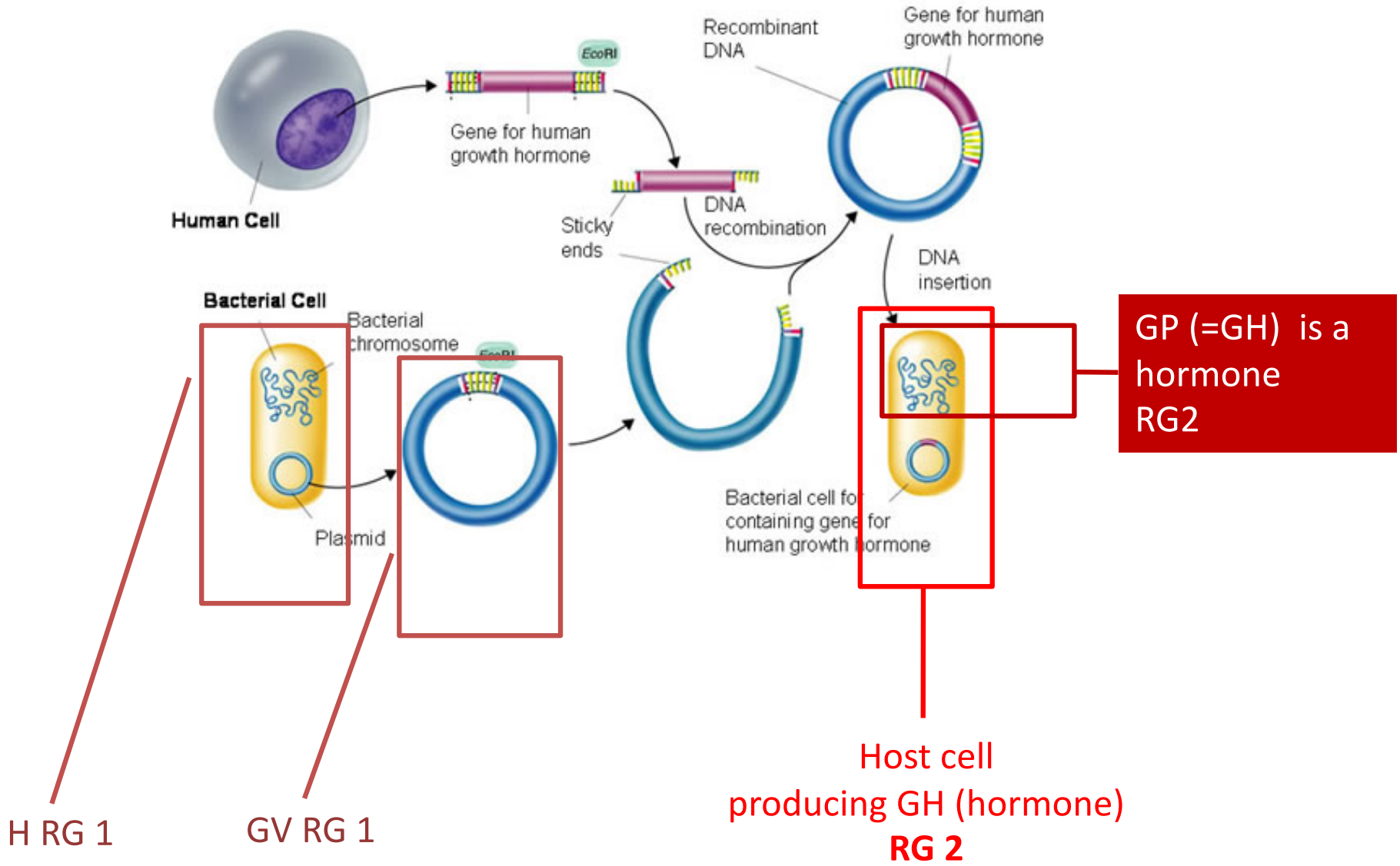
Exercise 3:

In a pharmaceutical company, **growth hormone** is produced by a GMO in large culture fermenters (2000 L capacity).

The GMO is non-pathogenic bacteria *(E. coli K-12),* engineered to secrete the hormone.

The genetic transformation of the bacteria is done using a commercial plasmid (pUNO form InvivoGen).

What is the RG of the transformed *E. coli?*



The **GMO Risk group is** the highest RG level reached by one of those three components RG (H or P or GP) → **RG2**

RG ? Take into account the risk level of 3 parameters:

1. **The host cell of the gene (H)**
   The RG of non-pathogenic *E. coli?* **1**

2. **The gene vector (P) : a safe commercial plasmid**
   pUNO for gene expression in *E. coli?* **1**

3. **The risk of the synthesised protein (GP)**
   Is the synthesised protein a hormone, an allergen, a toxin or a UTA? No **1**

The **GMO Risk group is** the highest RG level reached by one of those three components (H or P or GP) → **RG1**

BASIC RULE IN RISK ASSESSMENT OF GMO:

Never add or multiply the components' Risk levels !!!

A few examples:

H RG	P RG	GP RG	+	X	Correct RG
1	1	1	3	1	1[1]
1	1	2	4	2	2[2]
2	2	1	5	4	2

[1]: Exercises 1 and 2

[2]: Exercise 3

In exercises 1 to 3, large capacity fermenters were used.

The production of recombinant proteins in large capacity fermenters requires specific conditions called “LARGE SCALE CONTAINMENTS”

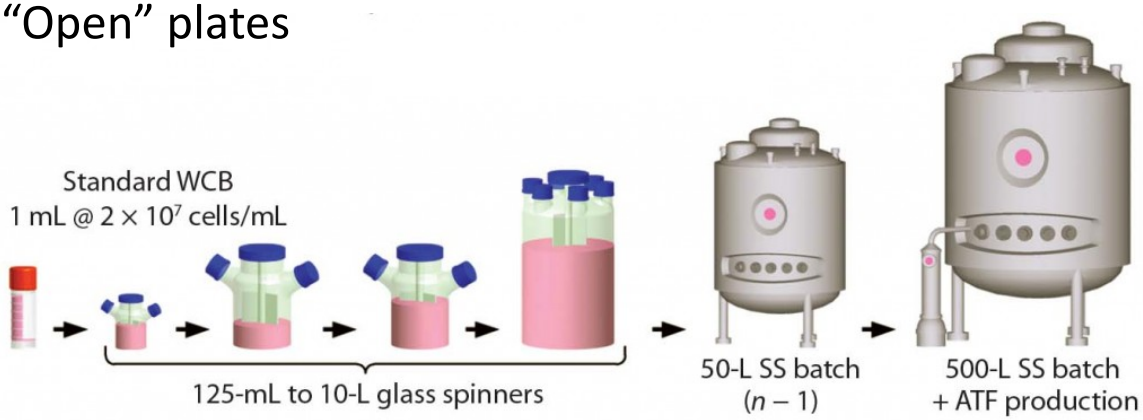
Use-related risk: an example with cell culture

Dry

Liquid

Tight flask

“Open” plates



Small scale

Large Scale

RISK of accidental release (splashing/leaking)

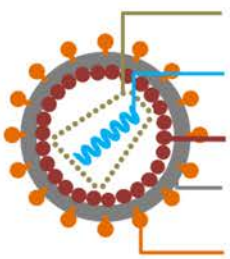
Example of a GM cell used to produce lentiviral vectors (packaging cells)

- Applications:
  - Cell transduction
  - Gene therapy

- Biosafety: the culture of these packaging cells present an oncogenic risk for the user

Packaging of Defective viral vectors by packaging cells: Principle

A ***Wild type virus***



Capsid (C)

Genetic material (DNA or RNA)

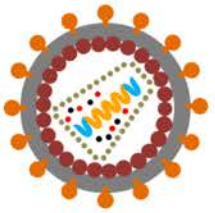
Matrix (M)

Lipid envelop (E, in enveloped viruses, only)

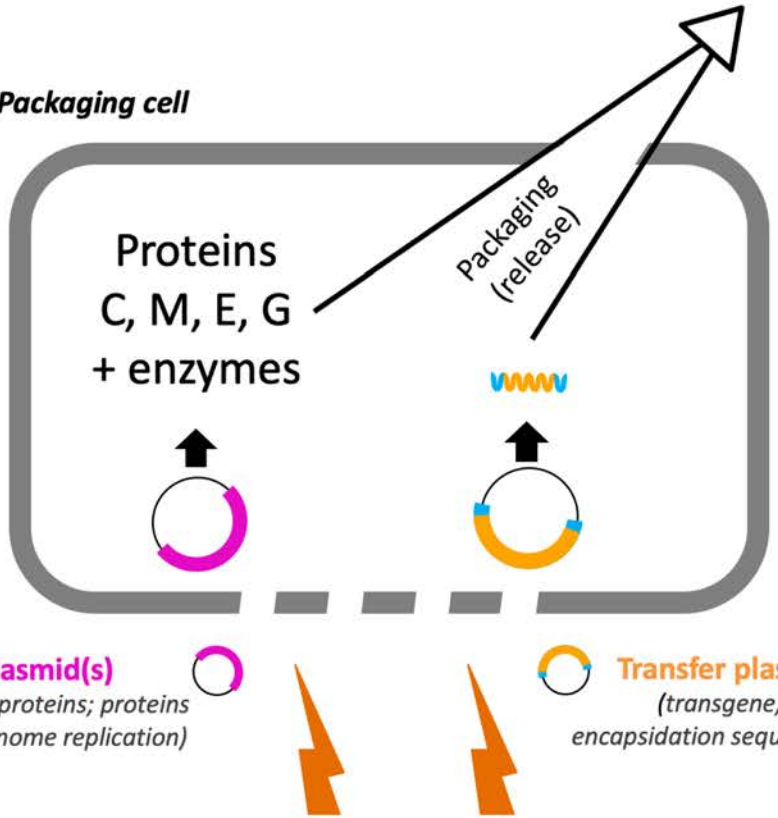
Glycoprotein (G, ligand for cell target receptor)

C ***Replication defective virus (RDV)***

- The major part of **genetic material** is replaced by the **transgene** to be transferred into the target cell
- the capsid contains **enzymes** needed ( ) for the **transgene expression** in the host cell



B ***Packaging cell***



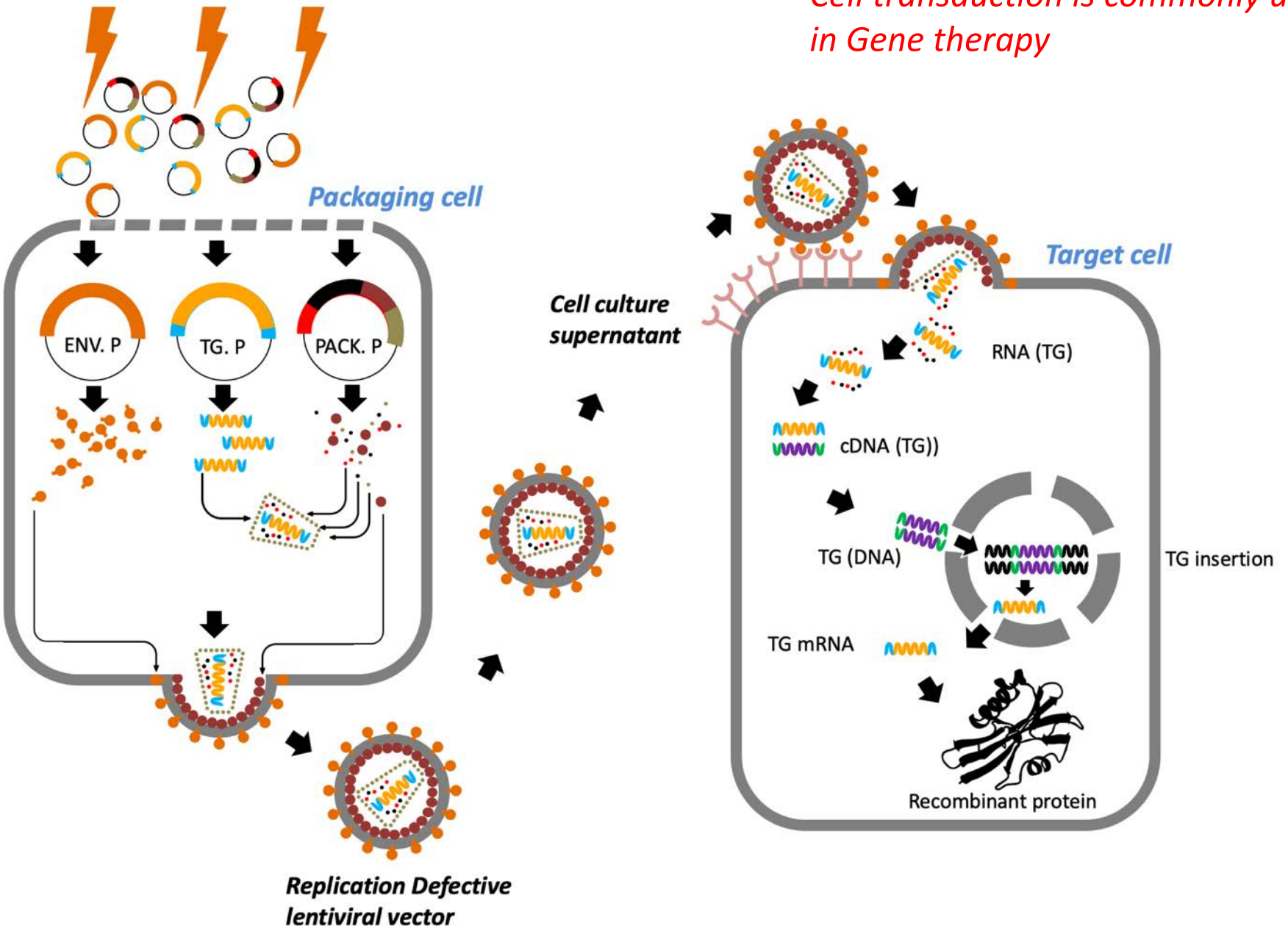
Helper plasmid(s)
(structural proteins; proteins for viral genome replication)

Transfer plasmid
(transgene; TR & encapsidation sequence)

Transfection with 2 plasmid types

Transduction of the target cell using retroviral vector produced in PC

Cell transduction is commonly used in Gene therapy



Major risks of lentiviral vectors:

1. Insertional oncogenic mutation

In the past, children with SCID were isolated in a germ-free sterile clear plastic bubbles, thus the name "bubble baby disease". [Credit: Baylor College of Medicine Archives]

(A) protection of children affected by the SCID in a sterile bubble (before developing a gene therapy; (

(B) baby cured be gene therapy (integration of the ADA gene in their blood progenitor cells) using an approach avoiding mutational insertions (and therefore decreasing the risk of cancer to the lowest possible level)

Between (A) and (B) situations: 4 children treated with bone marrow transduced by retroviral vectors developed severe leukaemia

Major risks of lentiviral vectors:

2. Recombination of defective vector into the wt virus

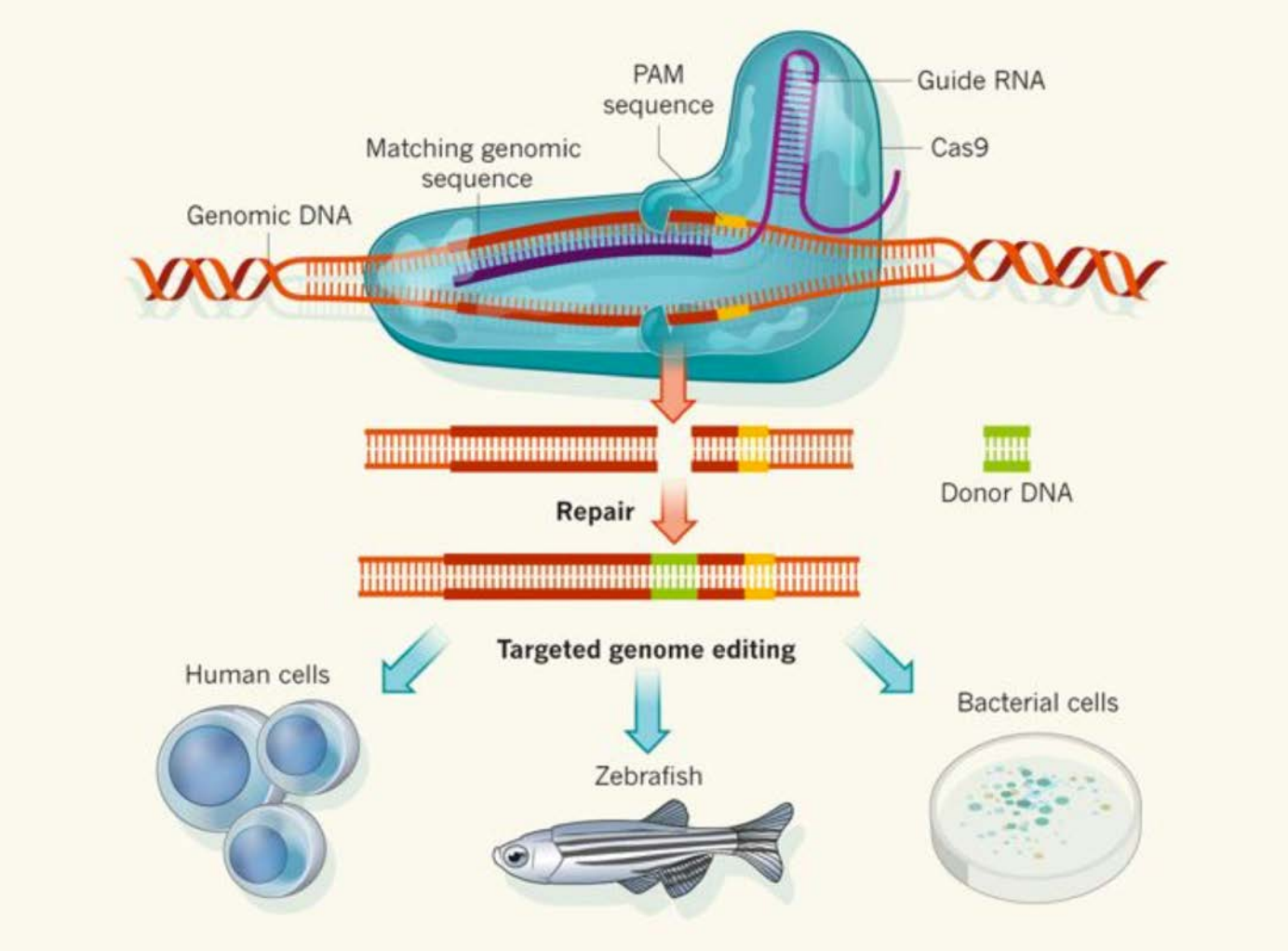
Two possibilities:

A) Recombination of the three plasmids in the packaging cell

B) Recombination with viral sequences (from past infections) in the treated organism

- Chong, H. and Vile, R. G. (1996) Replication-competent retrovirus produced by a' split-function' third generation amphotropic packaging cell line. Gene Ther. 3, 624–629. 4.
- Chong, H., Starkey, W., and Vile, R. G. (1998) A replication-competent retrovirus arising from a split-function packaging cell line was generated by recombination events between the vector, one of the packaging constructs, and endogenous retroviral sequences. J. Virol. 72, 2663–2670.
- Garrett, E., Miller, A. R., Goldman, J. M., Apperley, J. F., and Melo, J. V. (2000) Characterization of recombination events leading to the production of an ecotropic replication-competent retrovirus in a GP+envAM12-derived producer cell line. Virology 266, 170–179. 6.
- Otto, E., Jones-Trower, A., Vanin, E. F., et al. (1994) Characterization of a replication-competent retrovirus resulting from recombination of packaging and vector sequences. Hum. Gene Ther. 5, 567–575.

Safer alternative to lentiviral vectors for gene insertion:



Major advantages:

1. Very low probability of insertional mutation
2. No viral vector needed : the Cas-RNA system can be introduced using physical methods

3. Risk Management

UNIVERSITÉ DE NAMUR

Risk Management (create safe working conditions), using:

1) Biosafety equipment
2) Personal protective equipment (PPE)
3) Good laboratory practices
4) Containment Laboratory

1) Biosafety equipment

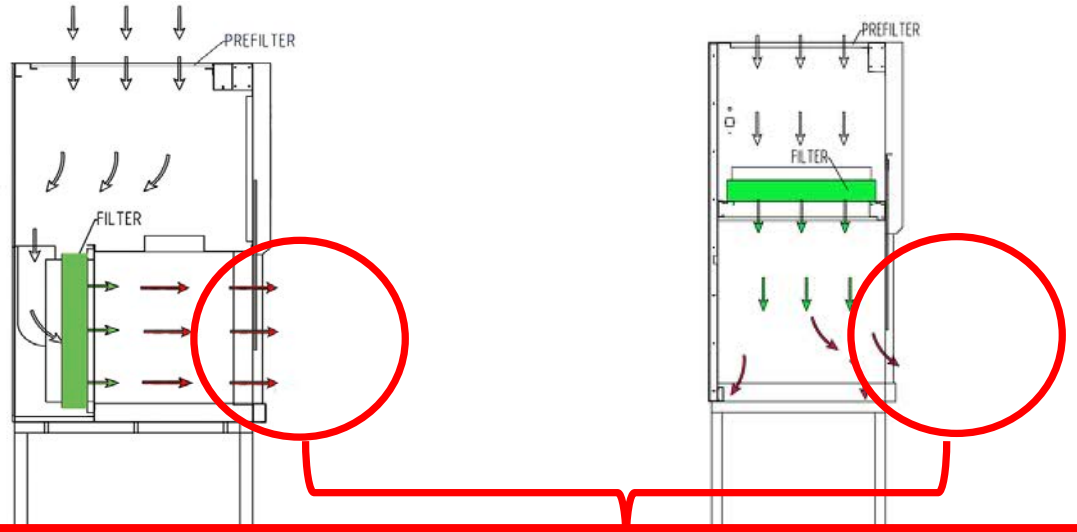
- Biosafety cabinets (BSC) (and HEPA filters)
- Autoclaves

Biosafety Cabinets (BSC)

Do not confuse
Laminar Flow
(LF, also called Clean Benchs)
with a BSC !!

How to recognize a clean bench?

The « clean bench » blows air towards the worker



Is appropriate for the handling of « clean samples » to be kept sterile (air sterelized on HEPA)

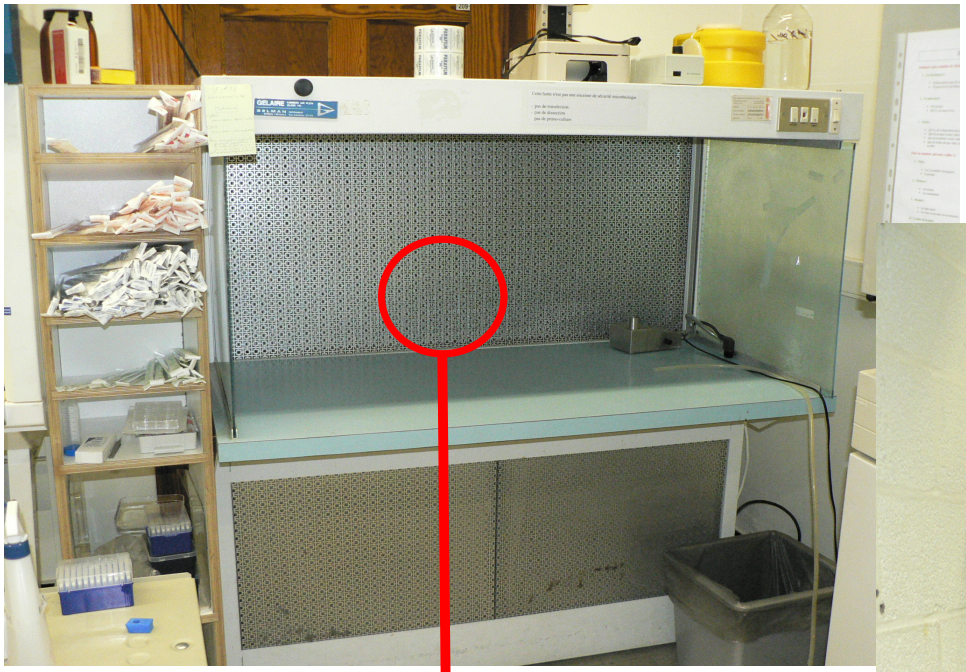
PERCEPTION OF THE AIR OUTFLOW

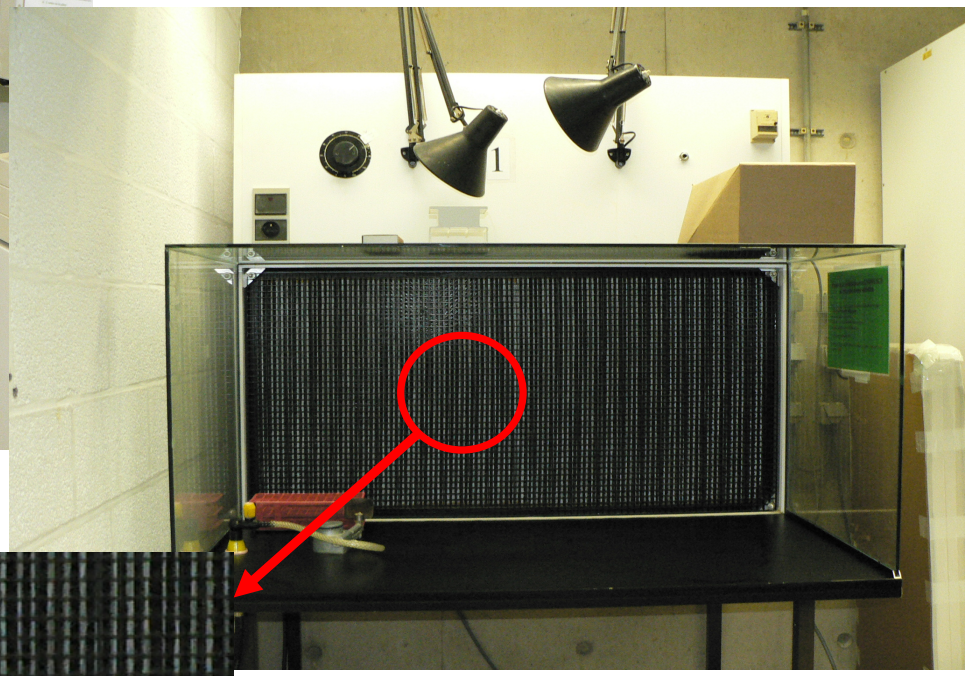
If the samples release

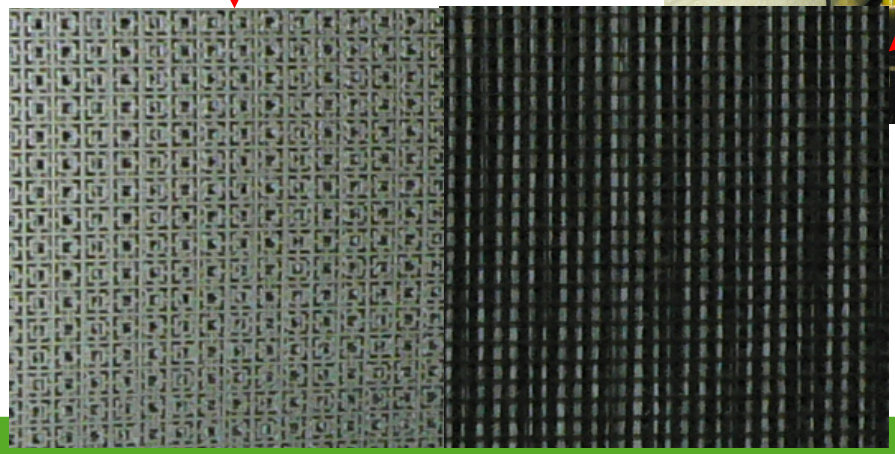
- harmful Substances (Toxic, nocive, irritant, allergenic, …)
- pathogenic microorganisms

THIS AIR OUTFLOW IS DANGEROUS

Examples of « clean bench » (horizontal laminar flows)







Chemical fume hoods (CFH)

= Safety cabinets (but not biosafety protection of Env.

The work (sample) is not kept sterile

→ the room air is aspirated on the sample.

DO NOT CONFUSE :

- The chemical fume hood

  with

- The type I BSC (type I BSC, *also called Class I BSC)*

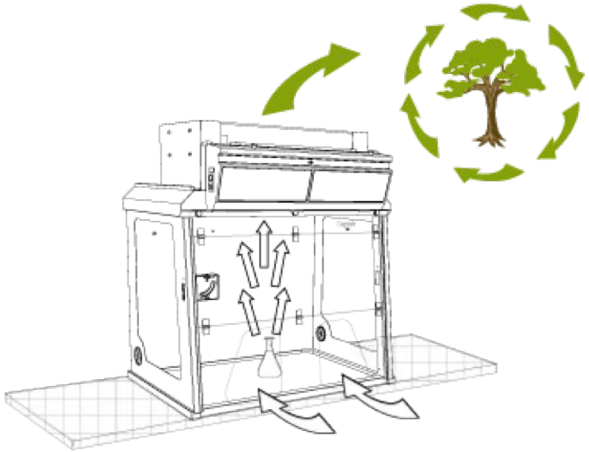
Same « look »

But there is no HEPA Filter on the CFH exhaust conduct to prevent the release of biological agents In the environment

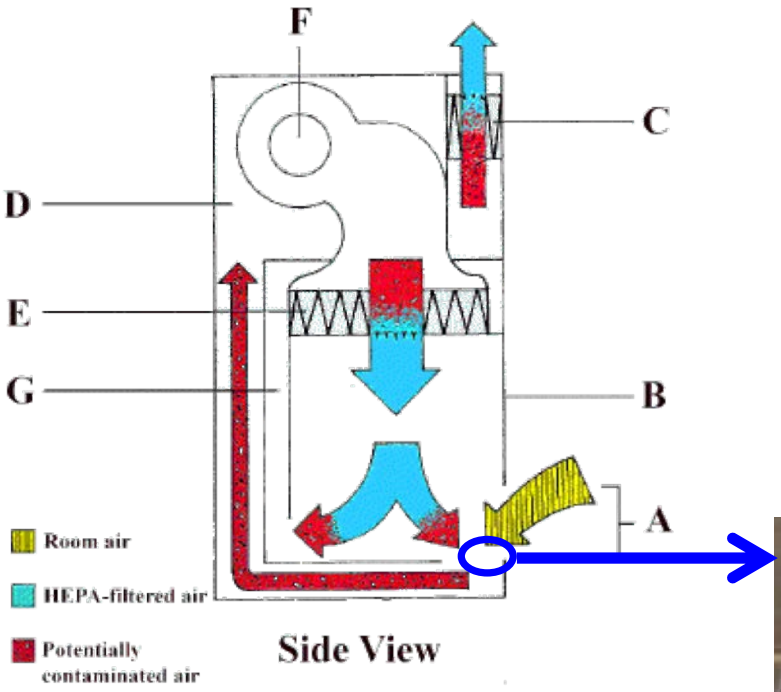
Chemical Fume Hood (CFH)
(in all laboratories: « hottes chimiques »)

≠

type I BSC:
WORKER protected, but
WORK unprotected
with HEPA filter
ENVIRONMENT protected



Type II BSC *(also called Class II BSC)*
« aspirating safety hood »
keeping the work (sample) sterile



features :

A: front aperture → **air aspirated under the working surface**

B: front window

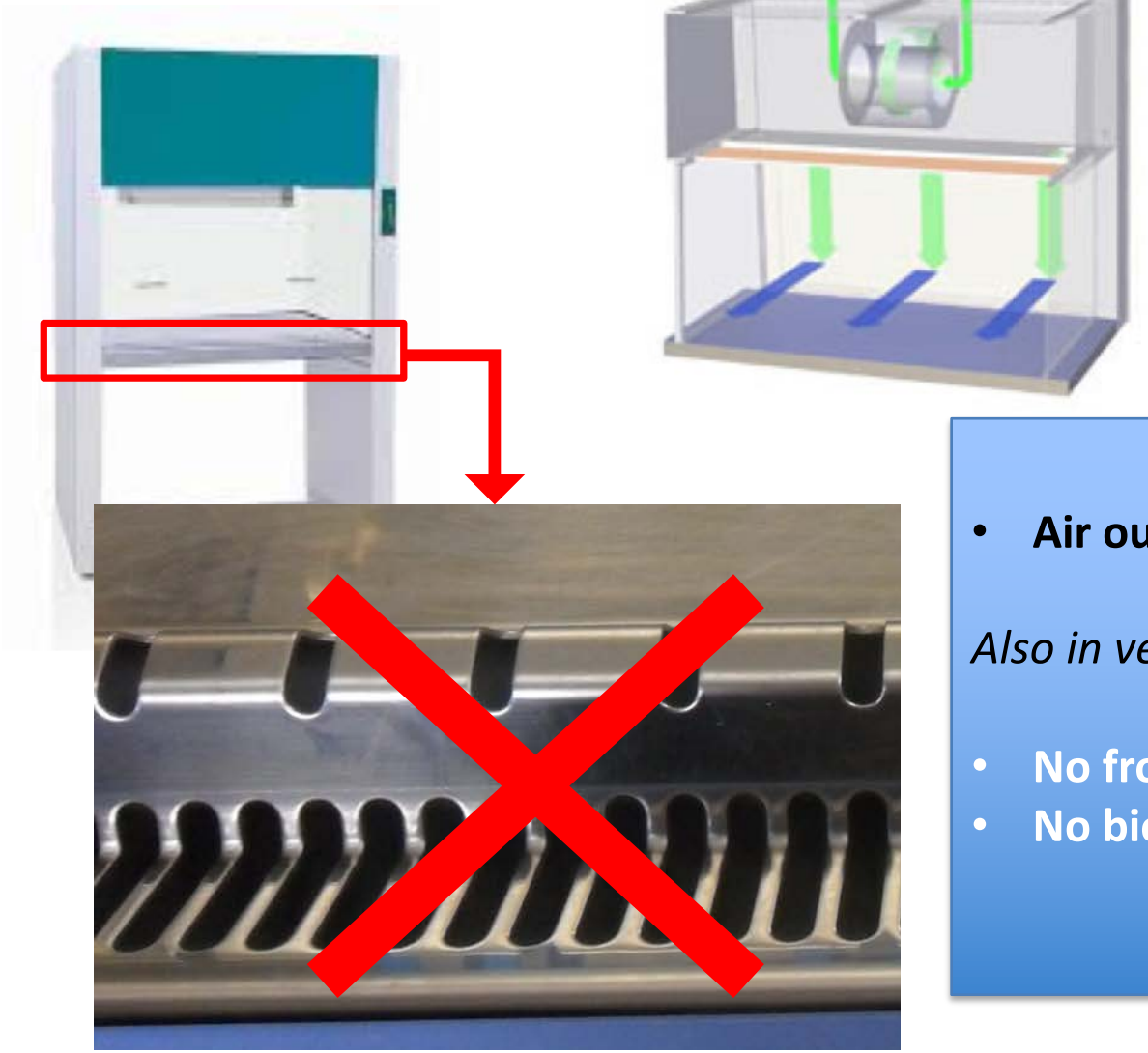
C & E: HEPA filter

D: contaminated air (negative pressure, no outleaking)

Type II BSC
« aspirating safety hood »
keeping the work (sample) sterile

Are both of them type II BSC?

How to avoid the confusion between this “vertical clean bench” and a type II BSC?



- **Air outflow**: excludes the BSC

Also in vertical clean benches:

- **No front grille**
- **No biohazard label**

Important to note :
declassified type II BSC

"Old BSC" without front window

↓

Unreliable for the

- worker complete protection
- sterility of the work

↓

consult experienced staff before starting your work

Type (class) III BSC (« glove box ») total protection

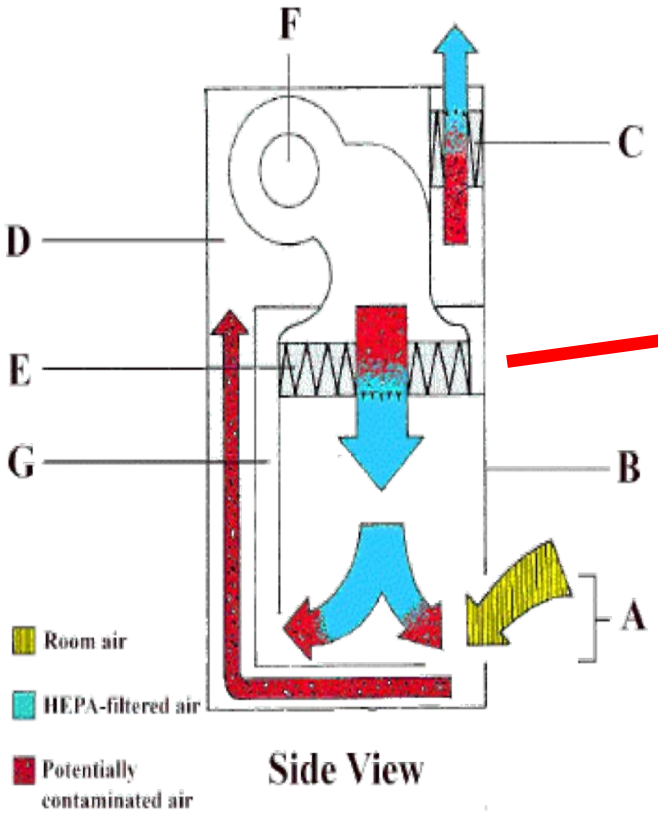
Lateral unit
For material storage and incubations

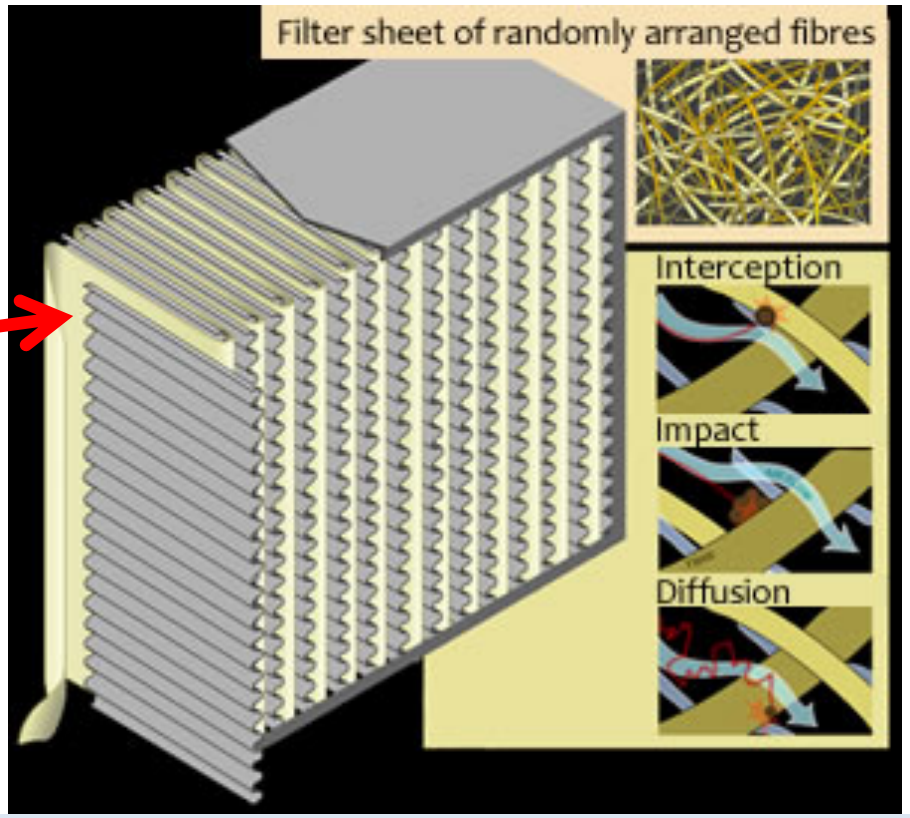
Summary of hood identification criteria and protection range

HOOD TYPE	Identification criteria			Protection range		
	Air outflow perceptible	Working bench **with a front GRILLE**	Biohazard Label 	Worker	Work (kept sterile)	Environment
Chemical fume hood	-	-	-	+	-	-
Class I BSC	-	-	+	+₊	-	+
Class II BSC	-	+	+	+	+	+
Class III BSC	-	IR*	+	++	+	+
Horizontal CB	++	-	-	-	+	-
Vertical CB	+	-	-	-	+	-

**irrelevant in the case of an hermetically closed cabinet*

HEPA Filter (BSC et L3) : (« High Efficiency Particulate Air »)





HEPA : retains 99,97 % of particles with a diameter ≥ 0,3 µm

Limits of the Type II BSC:

- Inappropriate for the use of volatile toxic chemicals (would be rejected in the room, if no ducting of of the exhausted air)
- Inappropriate for the use of uncoupled radioactive Iodine (^{125}I, as it is volatile would be rejected in the room)

  → when charcoal filter is added, the use of free iodine is allowed (example: UNamur – URPHYM)

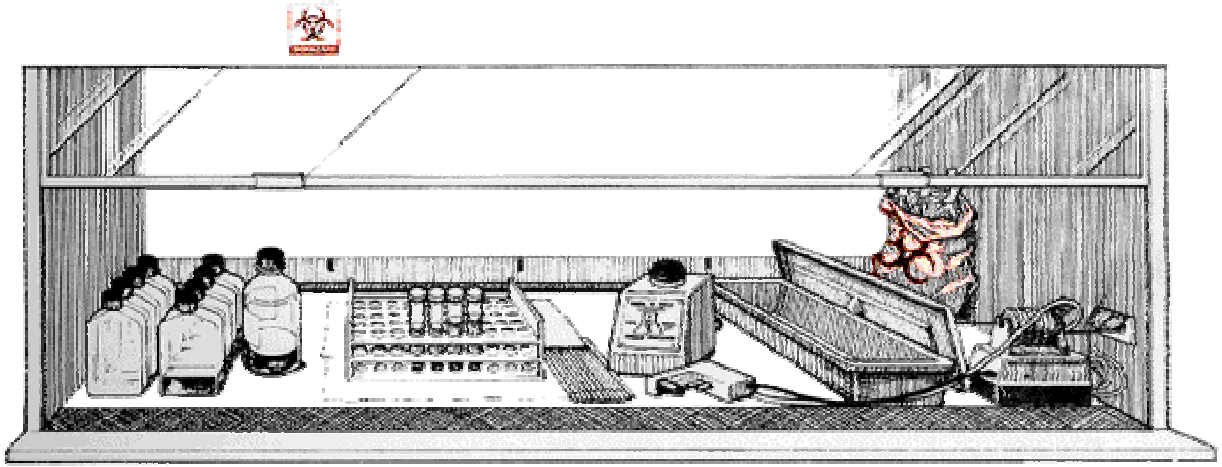
Work practice and procedure with a Type II BSC

- Place necessary materials in the BSC before beginning, to minimize the number and extent of air curtain disruptions by moving arms in and out
- Avoid other personnel activities near the BSC (keep the room doors closed, do not allow walking traffic near the BSC) to keep the cabinet air barrier
- The front grille must not be blocked with towelling, research notes, discarded plastic wrappers, pipetting devices, …

Work practice and procedure with a Type II BSC

- All materials should be placed as far back in the cabinet as possible (avoid “walls of culture flasks)
- **Avoid the open flame** in BSC, as it creates turbulence that disrupt the laminar flow
- The front window position should be as low as possible during work

Work practice and procedure with a Type II BSC



2) Personal Protective Equipment (PPE)

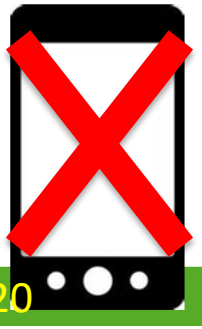
- Lab coat: **MANDATORY**, regardless of the risk level and the activity
- Gloves recommended in BSL2 (see next slides)
- Gloves, overshoes & Overalls: MANDATORY in BSL3
- Goggles eyes, mask (+ HEPA Filter) and cap: recommended in L3



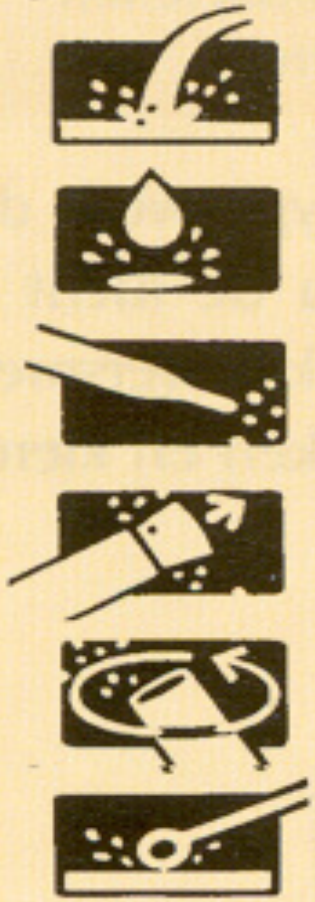


3) Good practices in Biosafety

1. Wear Lab coat (and other PPE if need)
2. Forbidden: eating and drinking, smoking, applying cosmetics, handling contact lenses
3. ALSO FORBIDDEN: using ones cell phone
4. Wash hands after work
5. No mouth pipetting
6. Clean work surfaces
7. Inactivate waste



8. Avoid aerosol formation



Pouring liquids

Droplets deposition

Pipette emptying

Tube uncapping

Tube vortexing

Colony picking with hot loops

9. Select the adapted waste container

Sorting of cultures flasks, pipettes, petri dishes, …
ACCORDING TO LOCAL RULES

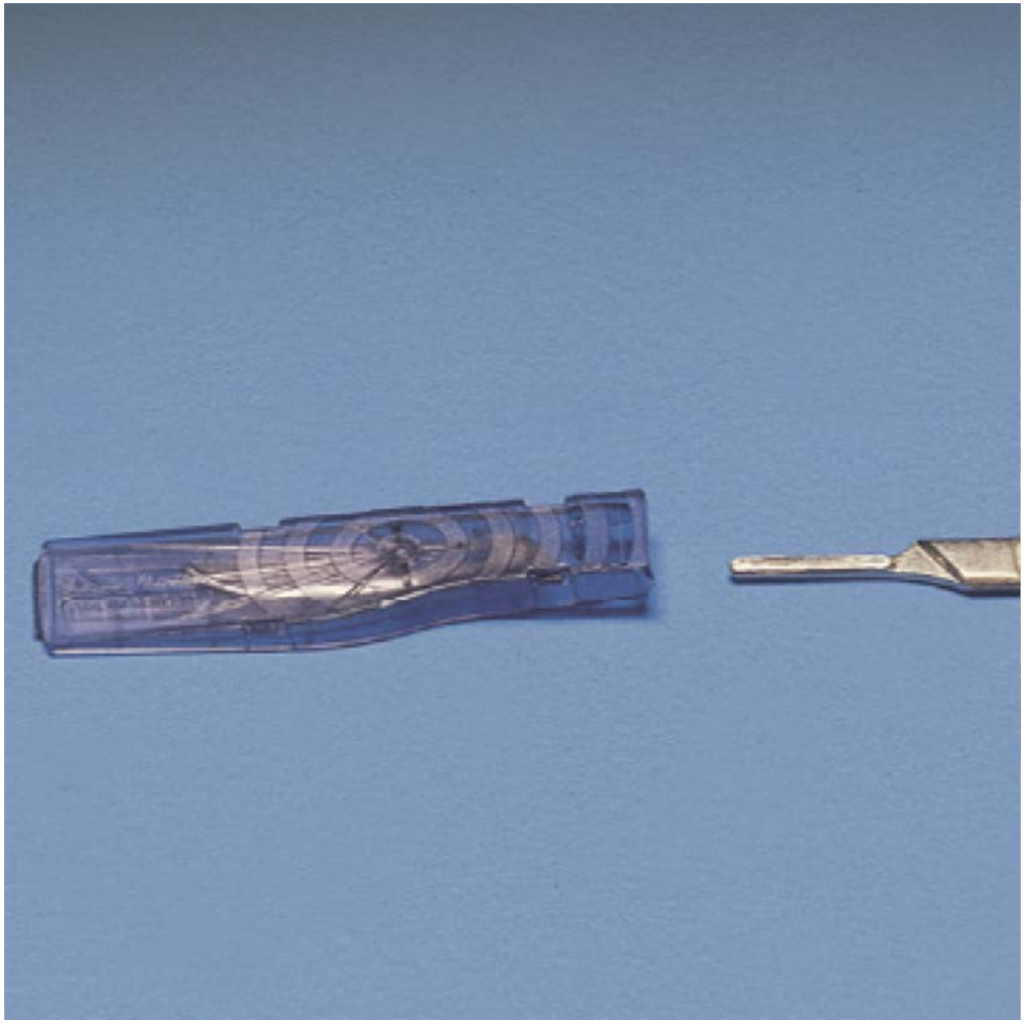
For “sharp material”

10. Dispose sharp wastes safely

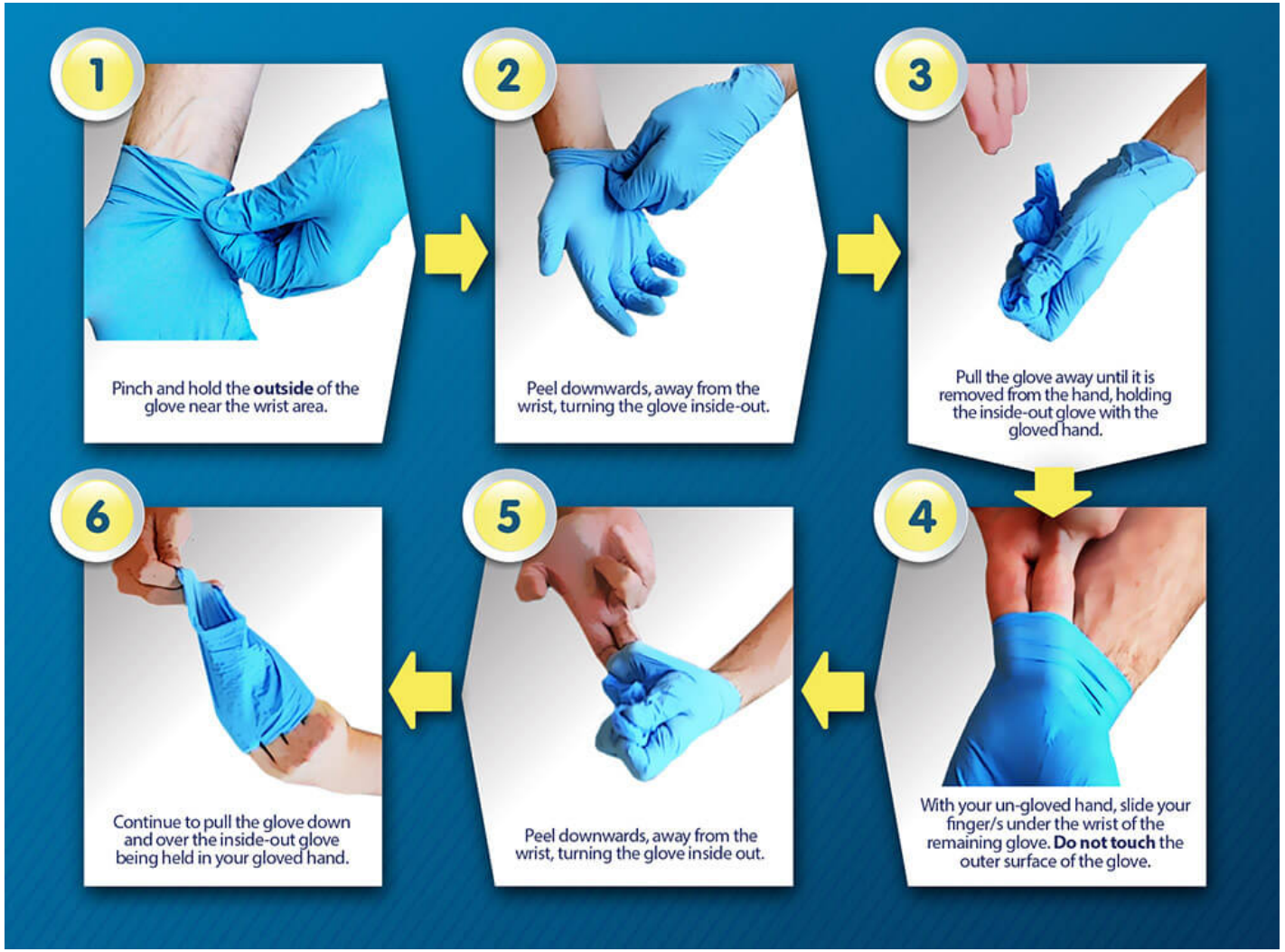
NEVER RECAP NEEDLES!!

Scalpel blades

How to remove them safely (use a remover):



Gloves Removal technique
(to avoid accidental contamination)




Proper Glove
Removal

In case of « Mixted Wastes »

The **priority cascade** to be considered in **waste management:**

Radioactive risk

↓

Chemical/Toxicological risk

↓

Biological risk

Advise: chemical inactivation of Biological agents before disposal in radioactive or chemical wastes

11. Use appropriate chemical disinfectants (to neutralize living micro-organisms)

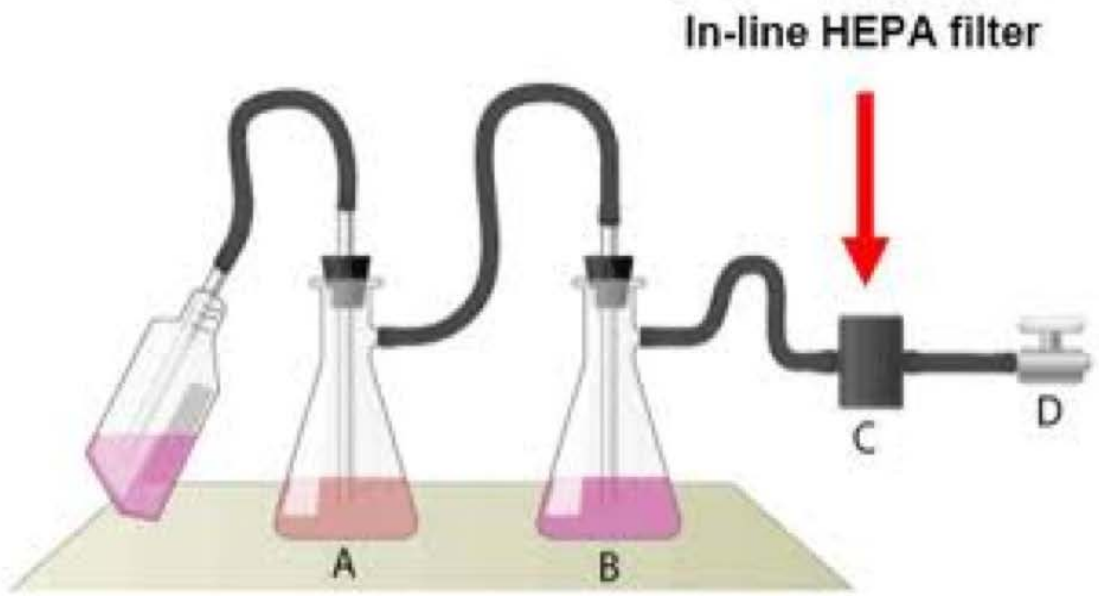
Disinfectant	Efficacy on				
	Fungi	Bacteria	Mycobacteria	Spores	Virus
Ethanol 70%	-	++	++	-	+/-
Hypochlorite (10%)	+	++	+	+	+
Formaldehyde (carcinogenic!)	++	++	++	++	+
Hydrogène Peroxide 6%	+	++	+	+	+
Quaternary ammonium compounds (QAC) *	++	++	++	++	++

1N NaOH for 'disinfection' of pathogenic prion protein

Disinfection of material soiled with pathogenic prion proteins (RG3)

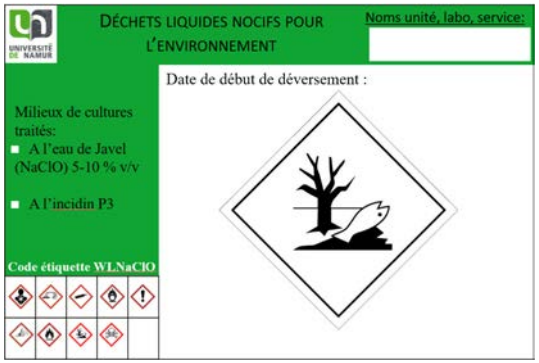
- Immerse in sodium hydroxide 1 N NaOH + heat in an autoclave at 121° C for 30 min.
- Autoclave at 134° C for 18 minutes
- Paraformaldehyde vaporization procedures do not diminish prion titres and prions are resistant to ultraviolet irradiation
- Formalin-fixed tissues should be regarded as still infectious, even after prolonged exposure to formalin.
- Histological samples containing prions are substantially inactivated after exposure to 96% formic acid for 1 h

How to neutralize potentially contaminated culture media



Vial A: with sodium hypochlorite solution (10% v/v), to neutralize aspirated culture medium
Vial B: “retention tank” in case of A overfilling

Once inactivation has occurred, liquid materials can be disposed in “NaCLO” containers



Do not let culture media “spoil” without the liquid bleach !

12. Inactivate Solid wastes in Autoclave (121° C, 20 min)

NEEDS TO BE REGULARLY CHECKED and VALIDATED

Use steam autoclave indicator tape

Avoid Chlorine Bleach In wastes !!! (causes damages to the Autoclave)

13. Management of accidents

A) Spills of contaminated solutions:

- If it occurs in BSL3, leave the place and wait 10 minutes for the filtration and replacement of the air
- Wear new gloves (and other PPE with RG3 organisms)
- Cover the spill with absorbent material
- Add disinfectant and let incubate for 10-15 minutes
- Remove the decontaminated material using dustpan
- Dispose in the biohazard waste container
- Clean de surface (and the dustpan and other tools)
- Inform the laboratory head
- Declare the event in the accidents notebook
- Ask for a medical examination (if the spill occurs in BSL3)


Biological Spill Cleanup

B) Glass breakage

- Never touch the pieces of broken glass: use forceps, dustpan and/or cardboard to collect them
- Decontaminate the spill as explained above
- Declare the event

Avoid glassware with RG3-4 organisms!

C) cuts

- Wash with running water
- Never suck !!
- apply a dressing (with help second man to L3)
- In case of significant injury, contact the rescue service
- Inform the laboratory head
- Declare the event in the accidents notebook
- Ask for a medical examination (if the spill occurs in BSL3)

KNOW YOUR EMERGENCY TELEPHONE NUMBERS

112, then 1

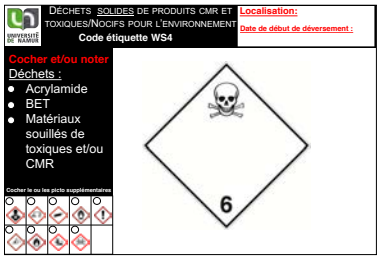
If no answer: 5000

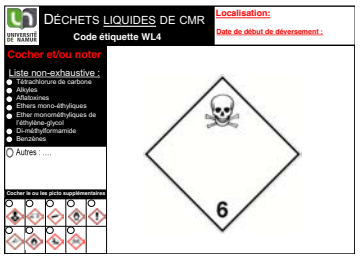
(Name of the building; Number of the room)

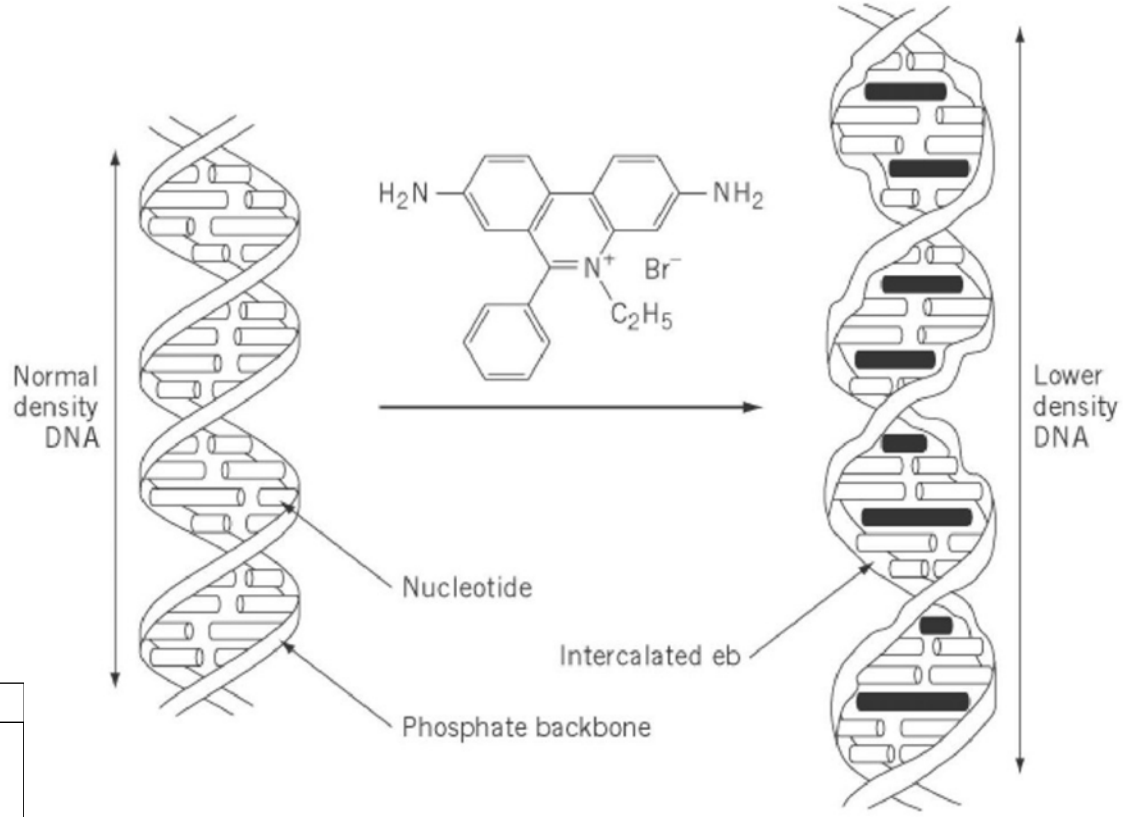
Ethidium Bromide (EB) : a carcinogenic substance often found in biology labs

Labeling of DNA and RNA, using intercalating agents: mutagenic effect

- Wear gloves
- Avoid inhalation of “boiling” solutions containing (EB) → handle them in fume hood
- Use appropriate waste containers for buffers and gels

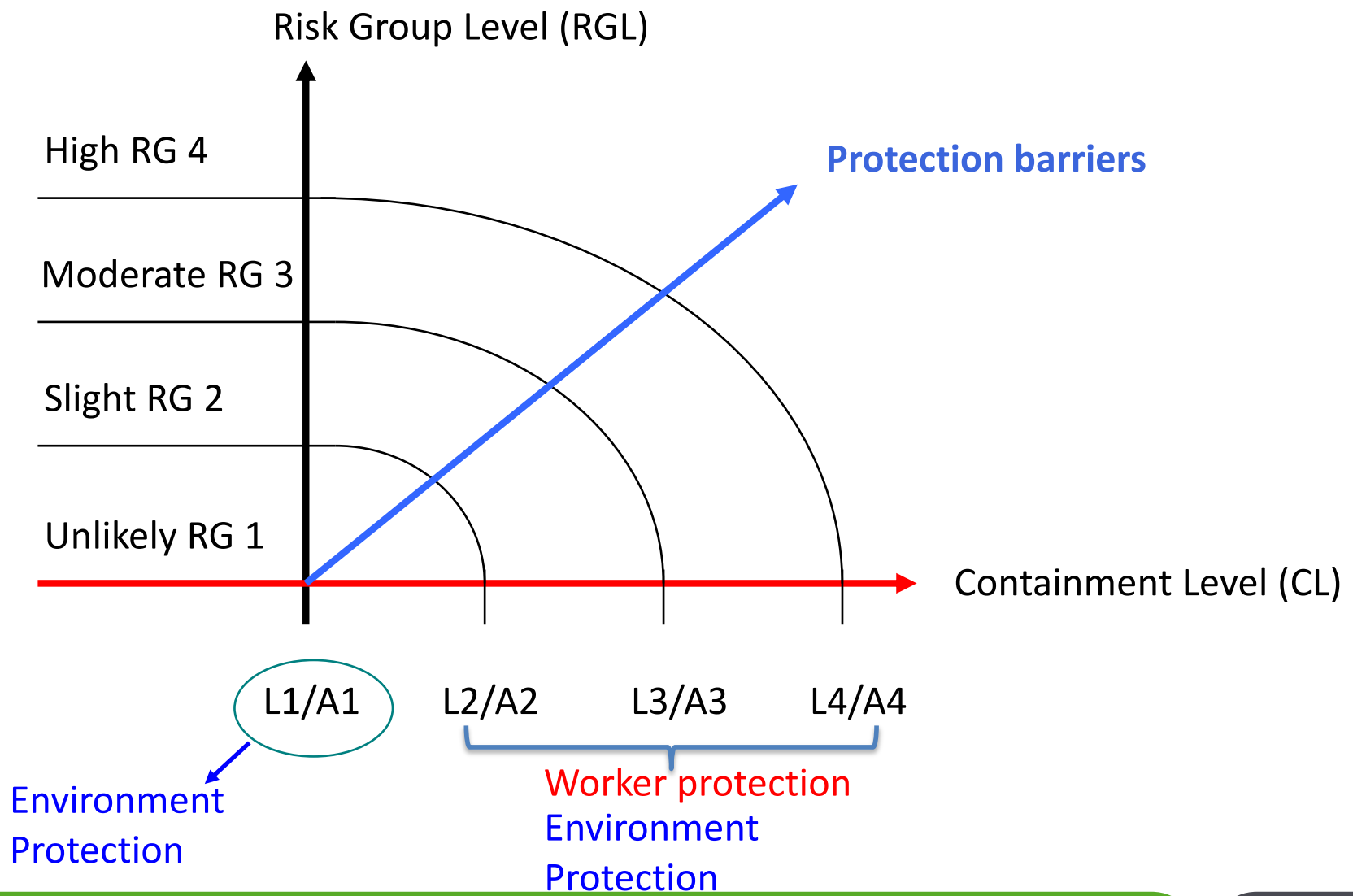






4) Containment Laboratories

L= laboratory, A= animal facility



Biosafety laboratories (BSL)
(4 levels: BSL 1 ➔ BSL 4)

Link between RG and BSL:

Risk Group level	Risk Criteria			Biosafety Laboratory Level (BSL)		
	Disease Severity (Human)	Infection Chance in the lab (contagion way)	Treatment/Prophylaxis Availability			
RG 1	**No**	**/**	**/**	BSL 1		
RG 2	**+**	**-**	**+**	BSL 2	BSL 2-Q	Erad. BSL 3
RG 3	**++**	**-**	**+**	Diag. BSL 2	BSL 3	Erad. BSL 4
RG 4	**+++**	**+++**	**-**	BSL 4		

The link between the RG and BSL is not systematic

Exceptions to the « rule RGL-BSL »

- activity type:

  BSL diagnostic < BSL culture

  (BSL 2) ←→ (BSL 3) (HIV, prions, *Brucella*, …)

- pathogen targeted by an eradication programme

  ex. Poliovirus BSL upgraded :BSL2 → BSL3

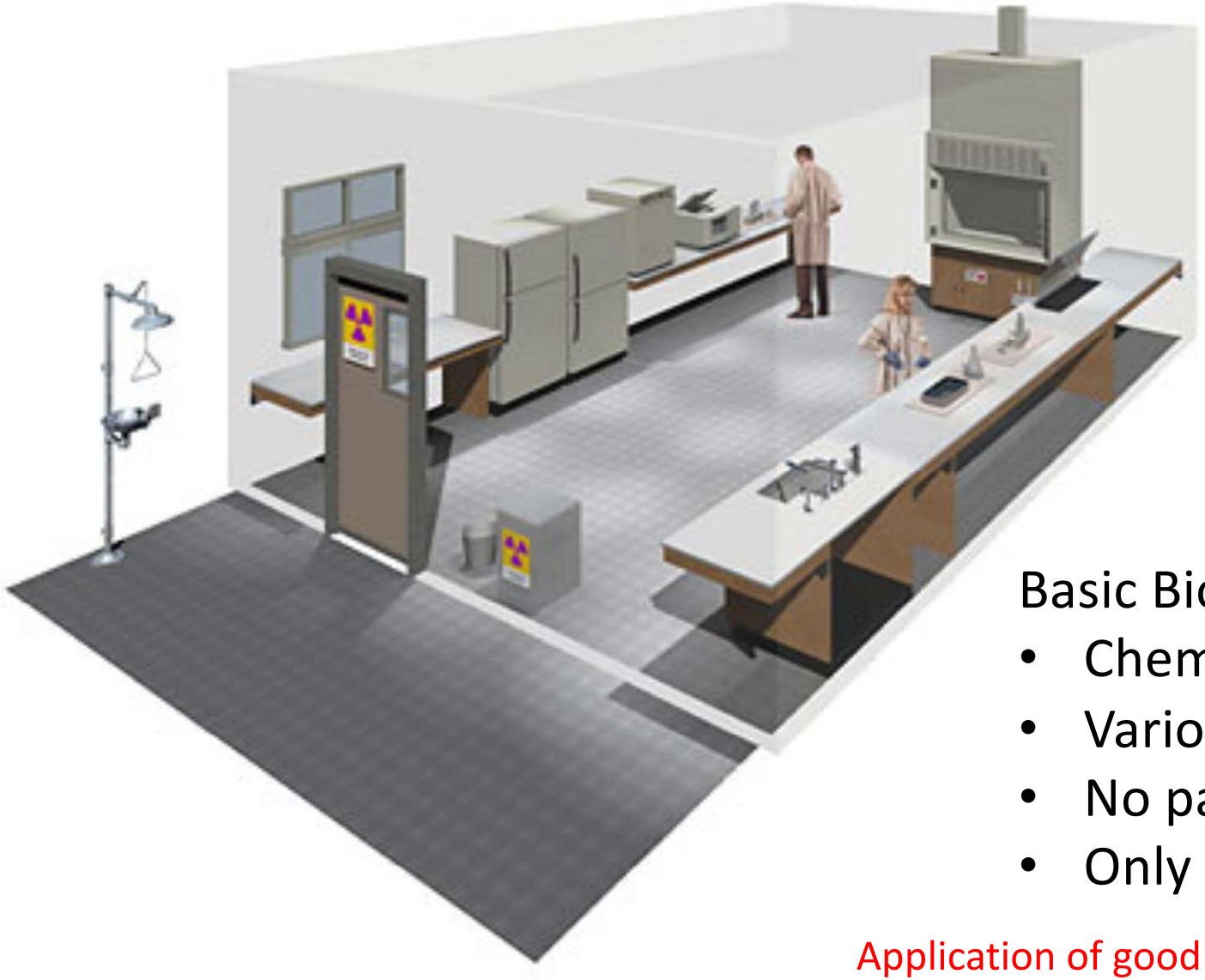
  (to avoid accidental release)

- Pathogenic organism of commercial plants: "quarantine organisms"

  QL2 (> L2)

  ex. *X. oryzae,* a rice parasite, used in a QL2

BSL 1



Basic Biochemistry lab

- Chemical hood
- Various activities
- No pathogens
- Only RG1 OGM

Application of good biosafety practices

BSL 2

Biohazard sign on the door

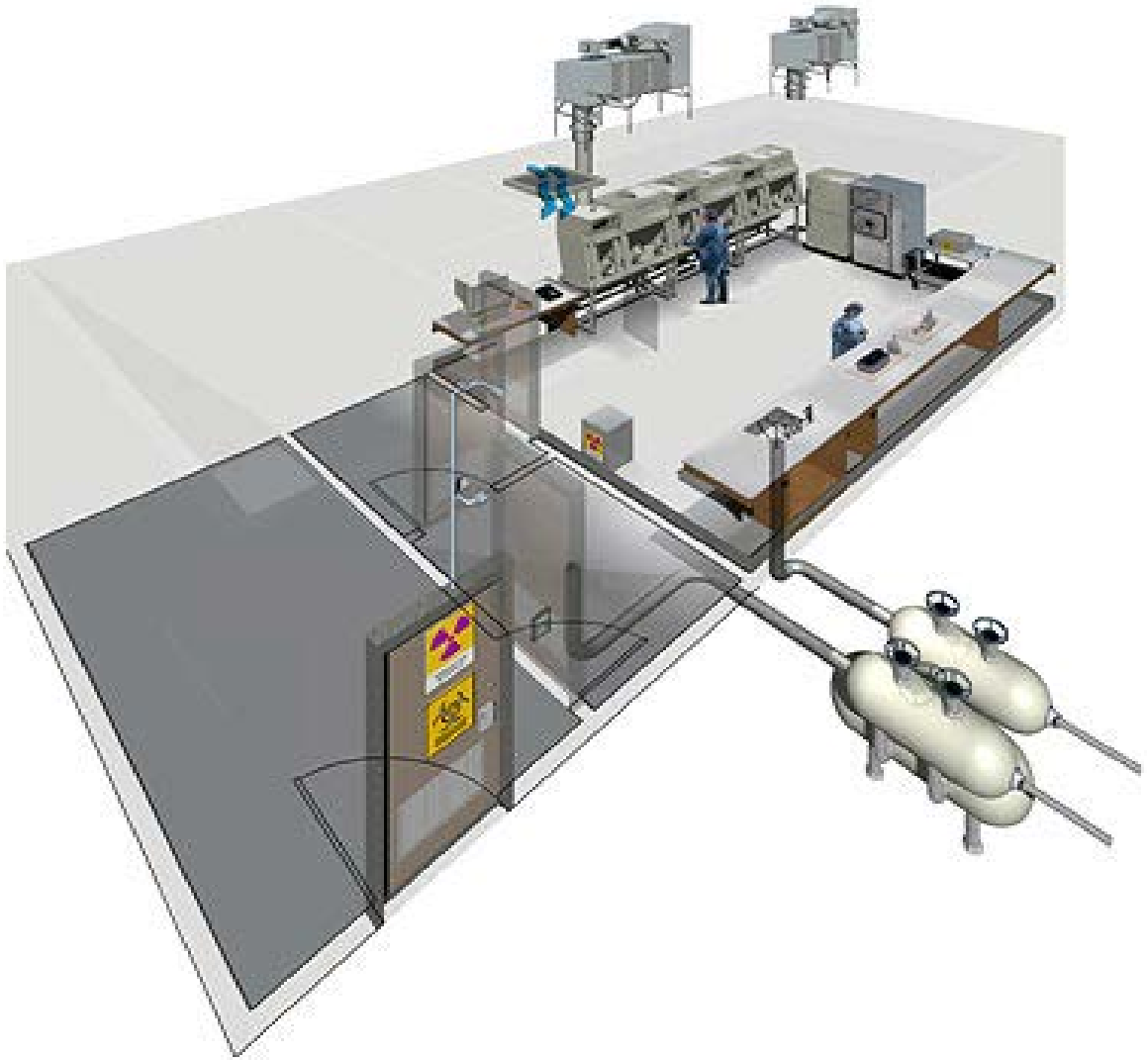
Containment of centrifugation-produced aerosols
(use of closed tubes / rotors, opened in BSC)

Operational procedures in BSL 2

- BSL 1 Good Practices, **+** :
  - Controlled Access
  - Biohazard Sign on the door
  - Protective clothing: to be removed before leaving
  - Hand wash sink
  - Suitable lab furniture and Cleanable lab
  - Clean work surfaces
  - **Avoid aerosols** (dispersion of a contaminated liquid medium as colloidal particles in air)
  - Class II BSC is recommended, but not mandatory

BSL-3

designed and provided for work with Risk Group 3 microorganisms and with large volumes or high concentrations of Risk Group 2 microorganisms that pose an increased risk of aerosol spread



BSL 3 specificities

Technical features of the facility :

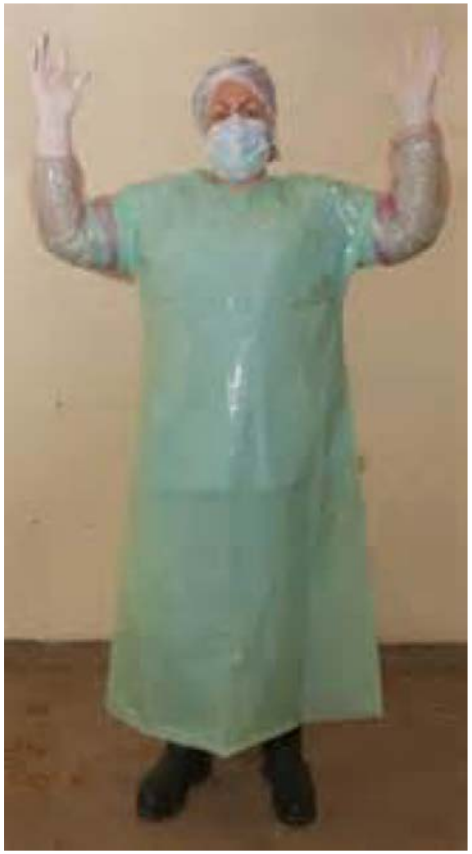
- Power autonomy
- Negative pressure (inward directional air flow, to avoid leaking out)
- Air Filtration (High-Efficiency Particulate Air HEPA)
- Anterooms, should have facilities for separating clean and dirty clothing (+ shower available, but not mandatory)
- Anteroom doors : may be self-closing and interlocking so that only one door is open at a time (to maintain the negative pressure)
- Surfaces of walls, floors and ceilings should be water-resistant and easy to clean.
- Windows must be closed, sealed and break-resistant.
- Decontamination of all effluents (including sink water)
- No exit for the material and/or equipment unless prior disinfection (waste sterilisation in double-door pass-through autoclave)
- Airtightness : to allow decontamination with gaseous disinfecting substance (formaldehyde, H_2O_2, ..)

BSL – 3 specificities

Workers :

- Access authorized after specific training
- The two-person rule should apply (“never alone in a high security laboratory”)
- Medical monitoring
- PPE: solid-front or wrap-around gowns, scrub suits, coveralls, head covering and shoe covers or dedicated shoes, two pairs of gloves
- Laboratory protective clothing must be decontaminated before it is laundered.
- *The removal of street clothing and change into dedicated laboratory clothing may be warranted when working with certain agents (e.g. agricultural or zoonotic agents)*

PPE in L3



Mandatory: gloves and lab coat

Recommended: overall + overshoes + coat, 2 pairs of gloves + mask + protective eye goggles

BSL 3 - specificities

- all potentially infectious material must be conducted within a Class II biological safety cabinets
- centrifuges safety buckets or containment rotors

Wrist skin protected

BSL3 at the university of Namur

Entrance:
2 anterooms

ENTEROOM 1

ENTEROOM 2
PPE

Access Procedues and “safe lock”

Main laboratory (collective equipment)

Individual laboratory (ABSL3) with type II BSC

“BSL2+”

Is a BSL2 dedicated to

- the use culture of cells packaging retroviral vectors (reproduction defective)
- the transduction of cell cultures by retroviral vectors (reproduction defective)

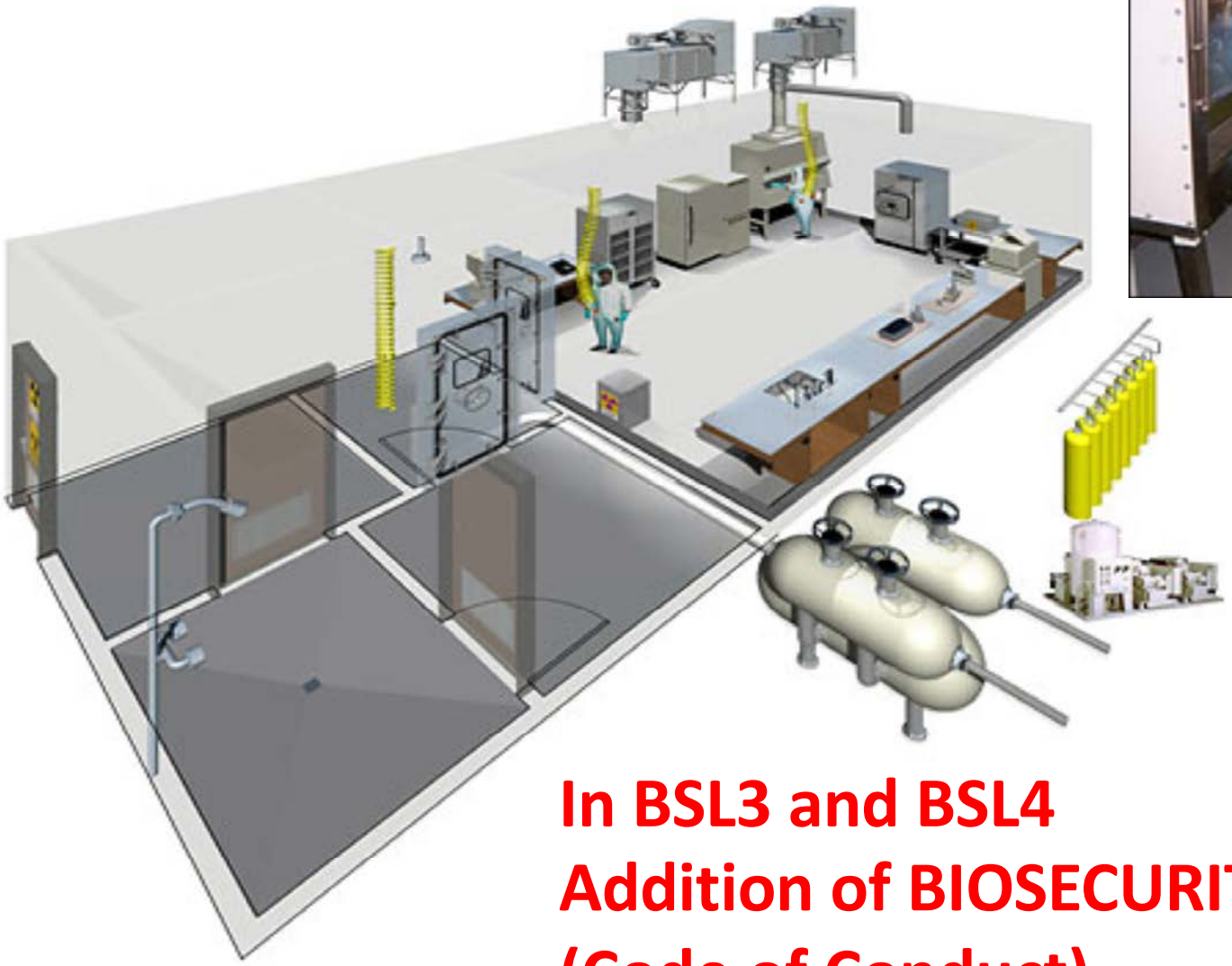
BSL2+ is a BSL2 lab where BSL3 habits are imposed:
The use class II BSC and wearing gloves are mandatory

BSL 4

designed for work with Risk Group 4 microorganisms

- BSL 4 must be located in a separate building (or in a clearly delineated zone within a secure building).
- Entry and exit of personnel and supplies must be through an airlock or pass-through system. On entering, personnel must put on a complete change of clothing; before leaving, they should shower before putting on their street clothing.
- protective supplied-air suit positively pressurized with self-contained breathing (HEPA-filtered),
- Class III biological safety cabinets ***(recommended)***
- Personnel must be trained in emergency extraction procedures in the event of personnel injury or illness.
- A method of communication for routine and emergency contacts must be established between personnel working within the containment and support personnel outside the laboratory.
- Good practices described for BSL1-3 are applied

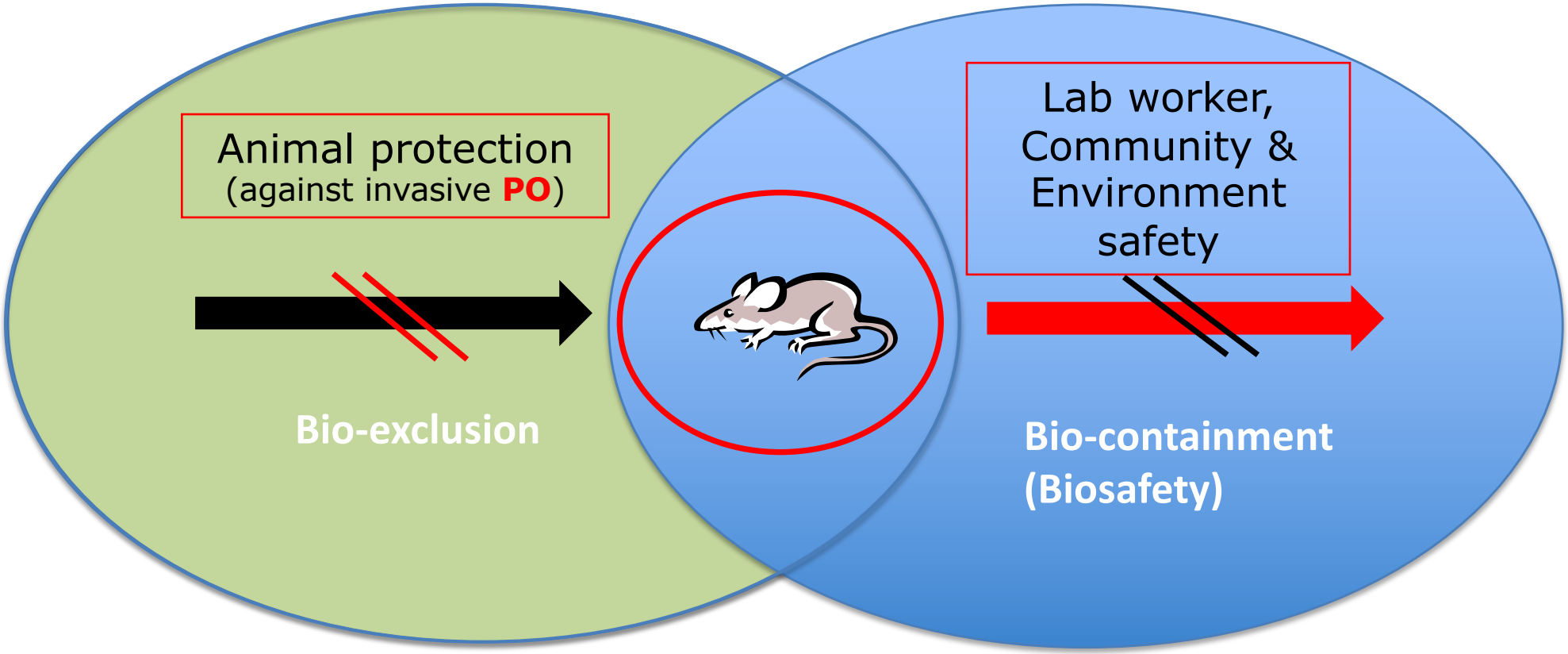
BSL 4



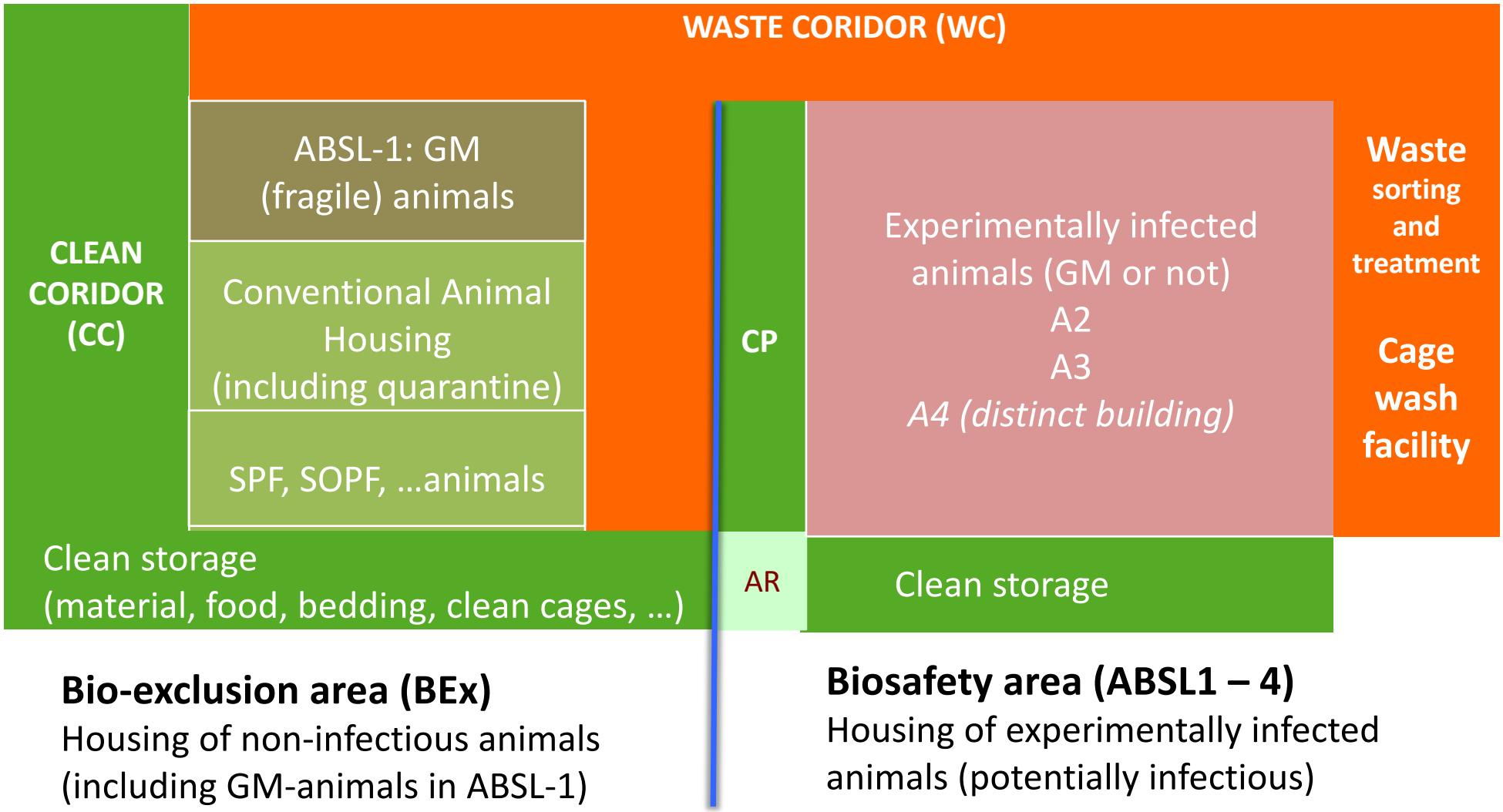
In BSL3 and BSL4
Addition of BIOSECURITY MEASURES
(Code of Conduct)

Animal Biosafety laboratories (ABSL) (4 levels: ABSL 1 ➔ ABSL 4)

Specificity of Facility: Bio-exclusion and Biosafety should go hand in hand



The ABSL is a hybrid facility, with 2 distinct areas:



Bio-exclusion area (BEx)
Housing of non-infectious animals (including GM-animals in ABSL-1)

Biosafety area (ABSL1 – 4)
Housing of experimentally infected animals (potentially infectious)

Physical separation between BEx & ABSL facilities

An anteroom (AR), is located at the entrance of the biosafety area

Bio-exclusion should be maintained in both areas,

since the control of experimental infection involves the exclusion of any other accidental infection.

Bio-exclusion in the BEx zone is obtained thanks to:

1) The strict application of **unidirectional flow** for lab workers, animals (and animal by-products) and material (i.e. never go back to cleaner areas when exiting a room of the facility);
2) The purification of the **incoming air** through a **HEPA filter**; when needed, laboratories are similar to clean rooms (positive pressure in chirurgy rooms);
3) The obligation for lab workers to take a **shower at the entrance** of the facility
4) The **disinfection of cages** (and other material that will be used in animal experimentation), bedding and food **before contact with animals**
5) In certain cases (such as **SPF condition**), the housing of animals in isolators with air in positive pressure (to avoid leaking in in case of tightness failure)

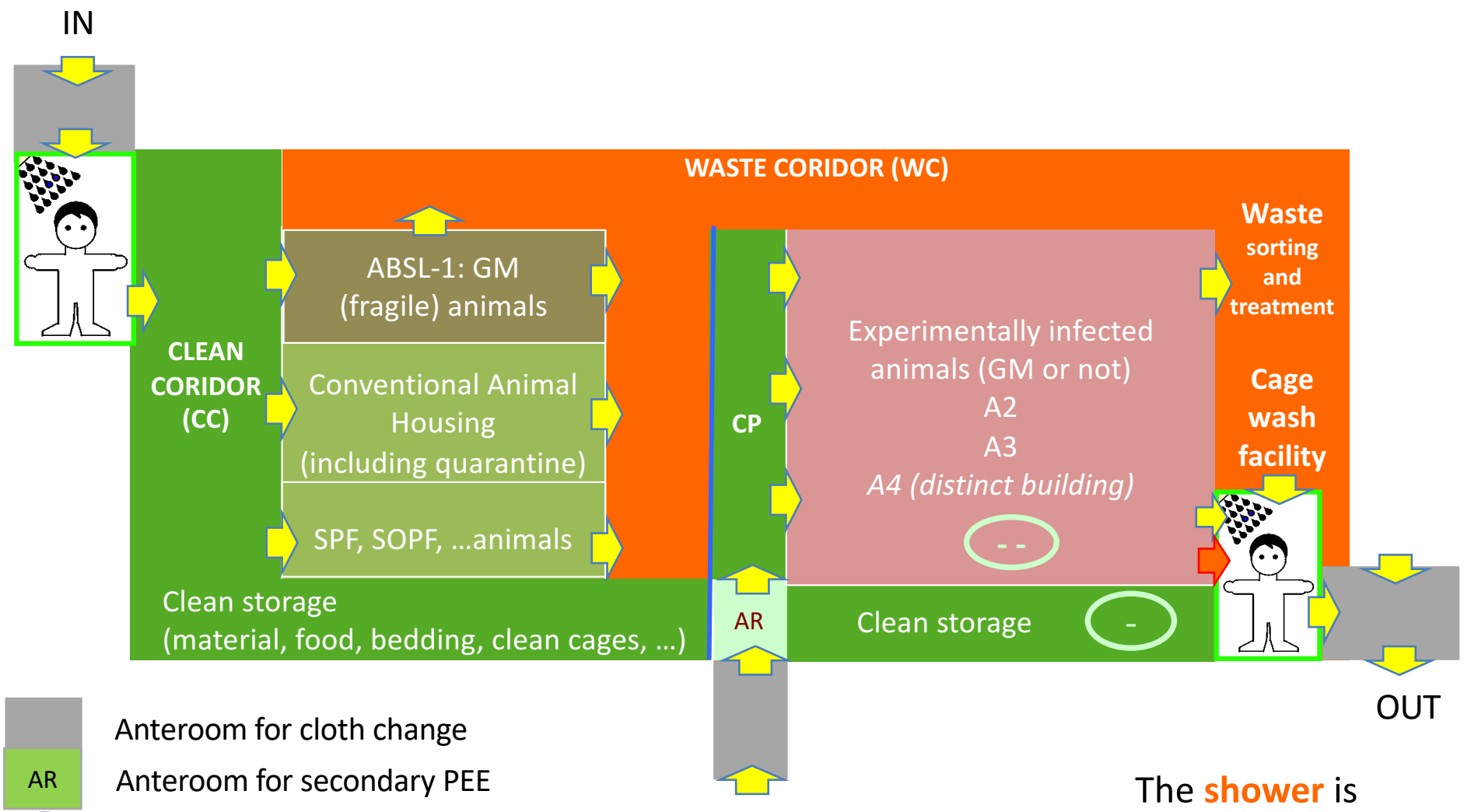
Biosafety approaches (RA and RM) are the same than those explained for BSLs.

Biosafety conditions are guaranteed thanks to:

1) The **containment characteristic**s (ABSL-1 to 4, like in the BSL series), the use of safety equipment (such as BSC, autoclave, …) and the application of BGP (including an adequate waste treatment);
2) The purification of the **outgoing air through a HE**PA filter; in ABSL-3 and -4, the air pressure is negative (to avoid leaking out);
3) The obligation for lab workers to take a **shower before exiting** the facility
4) The **disinfection** of cages (and other material that will be used in animal experimentation), bedding and food before **after contact with anim**als
5) **In certain cases** (such as ABSL-3 and -4), the housing of animals in **isolator**s with air in negative pressure (to avoid leaking out in case of tightness failure)

In the ASBL part of the animal facility the protection of animals (bio-exclusion) is obtained by the respect of the unidirectional flow (from clean to dirty corridors)

Illustration of the principle of unidirectional flow of lab workers in the facility:



The **shower** is **mandatory** when leaving ABSL-4

Biosafety measures are applied when:

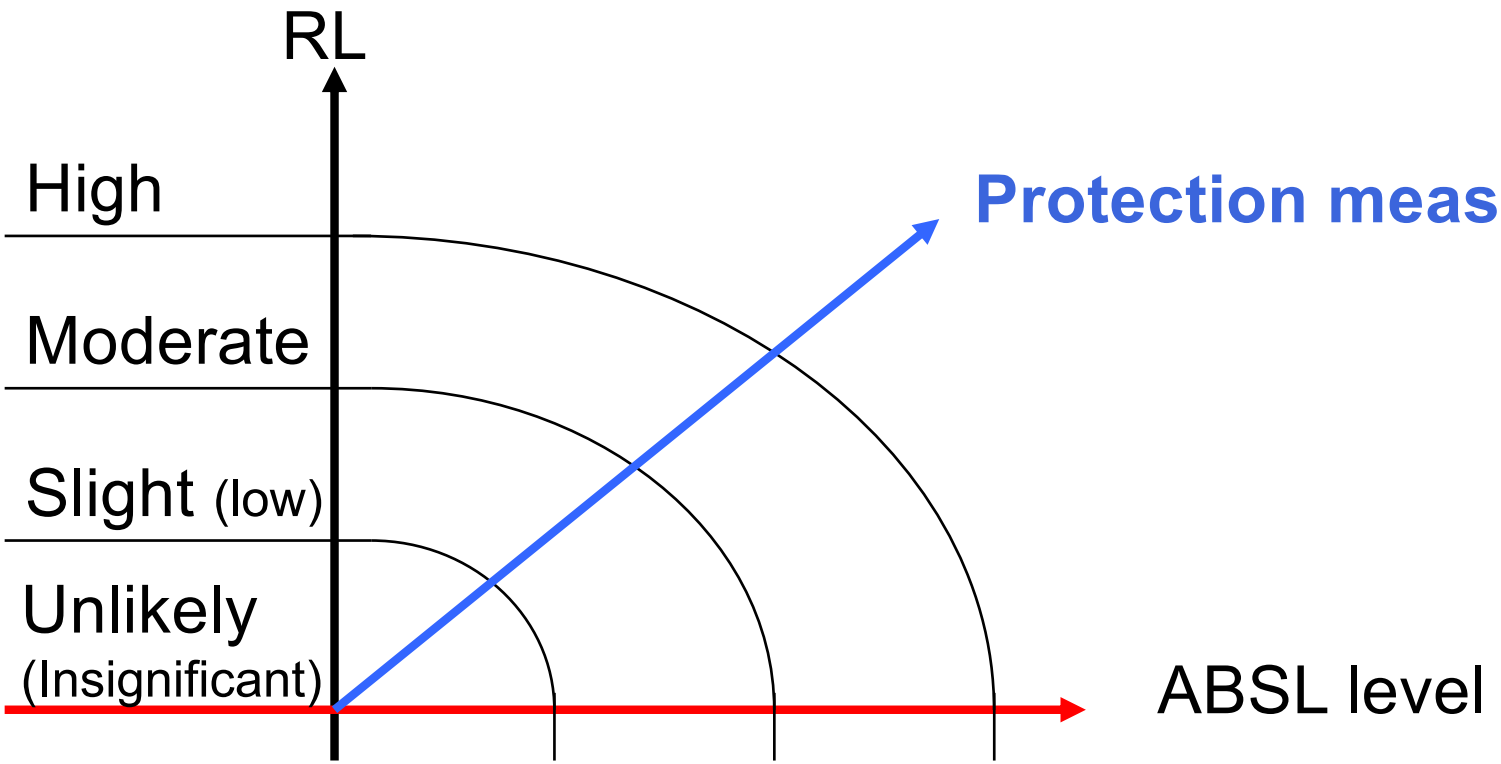
1) Animals are genetically modified

GM animals (essentially used as model animals for the study of human and animal diseases) are neither pathogen nor toxic (no known case) → RG1

2) Animals are experimentally infected or treated with plasmids (preliminary studies of gene therapy)

- The RG of experimentally infected animals corresponds to that of the used **PO** (or viral vector)
- ABSL categories correspond to **PO** (viral vector) RG

With exception of material used for animal rearing, safety equipment and practices of an ABSL (-2, -3 or -4) are those described for the corresponding BSL (-2, -3 or -4)

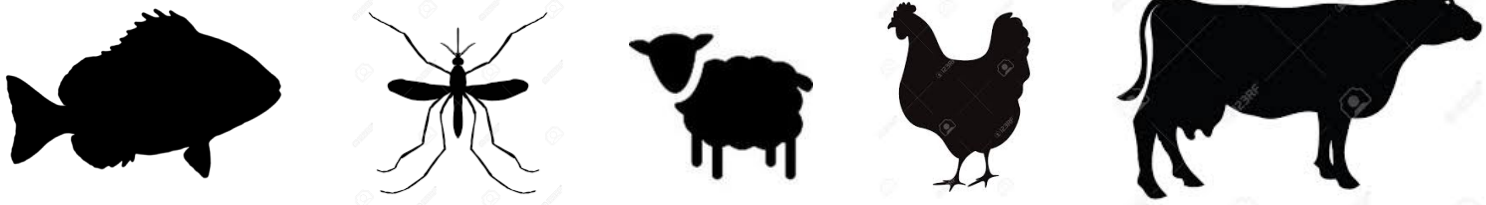


Protection of environment

DOOR

Protection of the worker, the community and the environment

Animaleries de types variés !



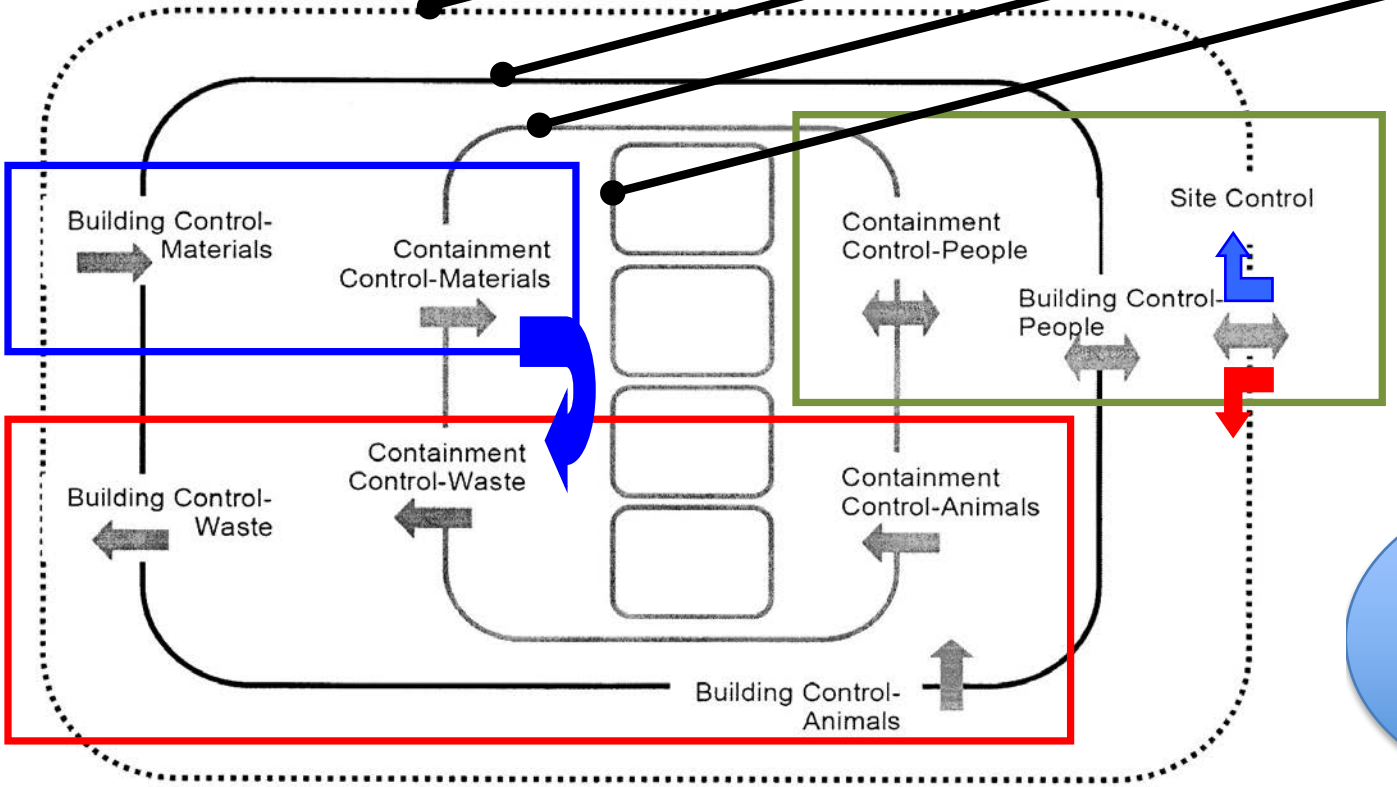
3 types of flow
In an ABSL-4

Animals

Material

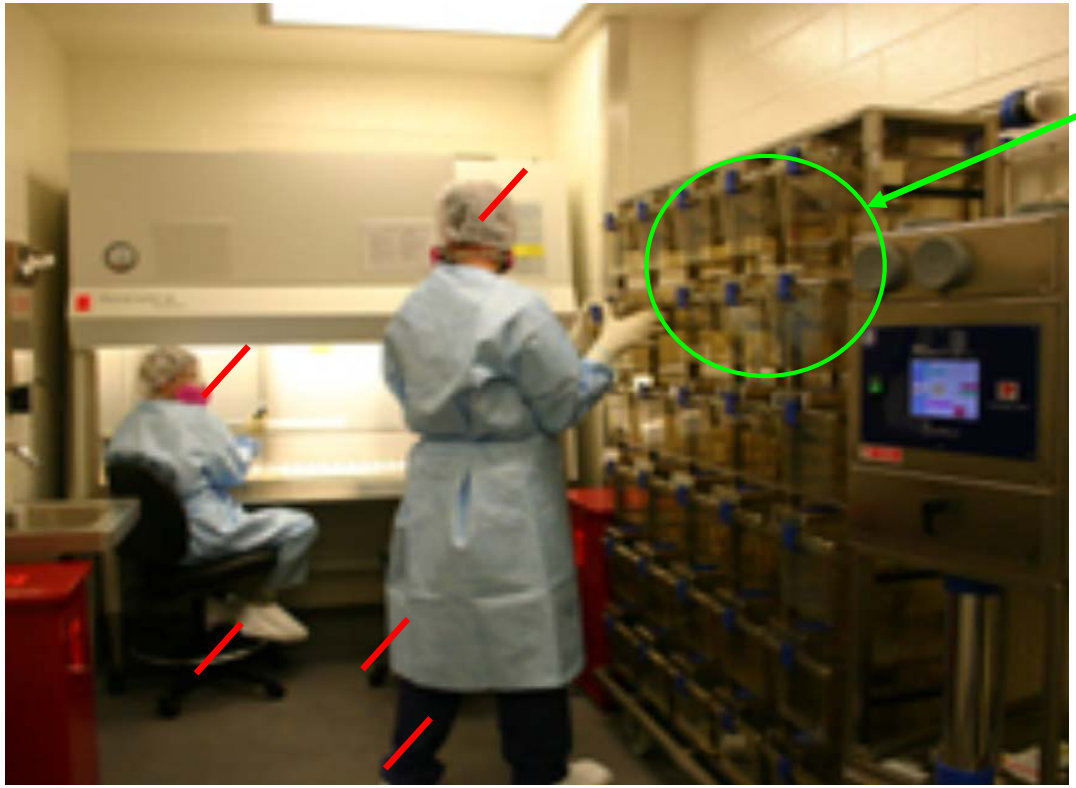
worker

4 barriers:
- Site
- Building
- ABSL
- Housing unit



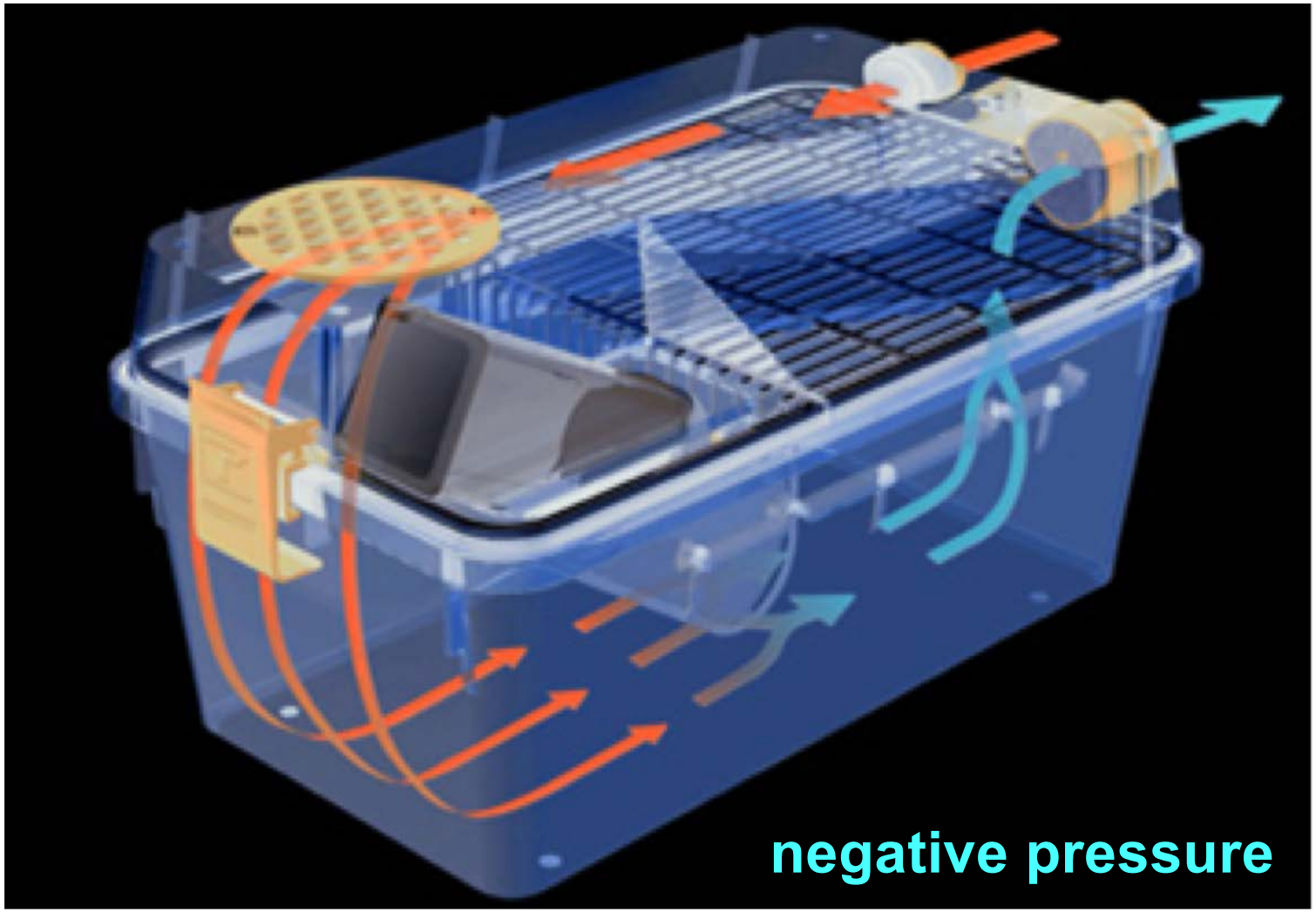
BIOSECURITY MEASURES

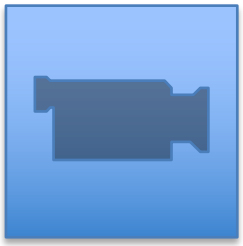
Example of material and practices specific to rodent ABSL-3



PPE including rigid shoes (to avoid accidental needle pricks) and thick gloves (to protect worker from biting and scratching)

Individually Ventilated Isolator (IVC) used in ABSL-3 and -4





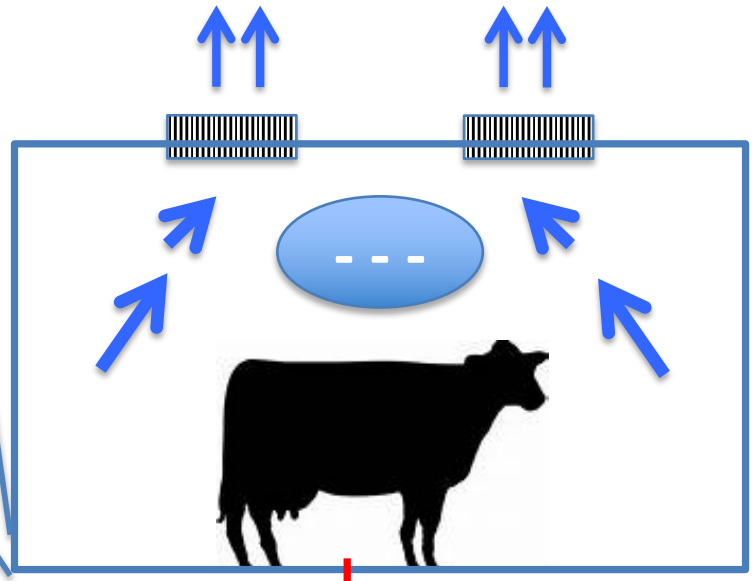
IVC, ventilation pattern

Isolators for Animals are frequently used in ABSL-4

Animal handling is always done in a Type 2 BSC (could be done in a type I BSC if the sterility is not required)

Lab worker wears a « tight » mask, as rodents fur and urine is highly allergenic

Example of bovine ABSL-3



- Exhausted air filtered on HEPA
- Negative pressure

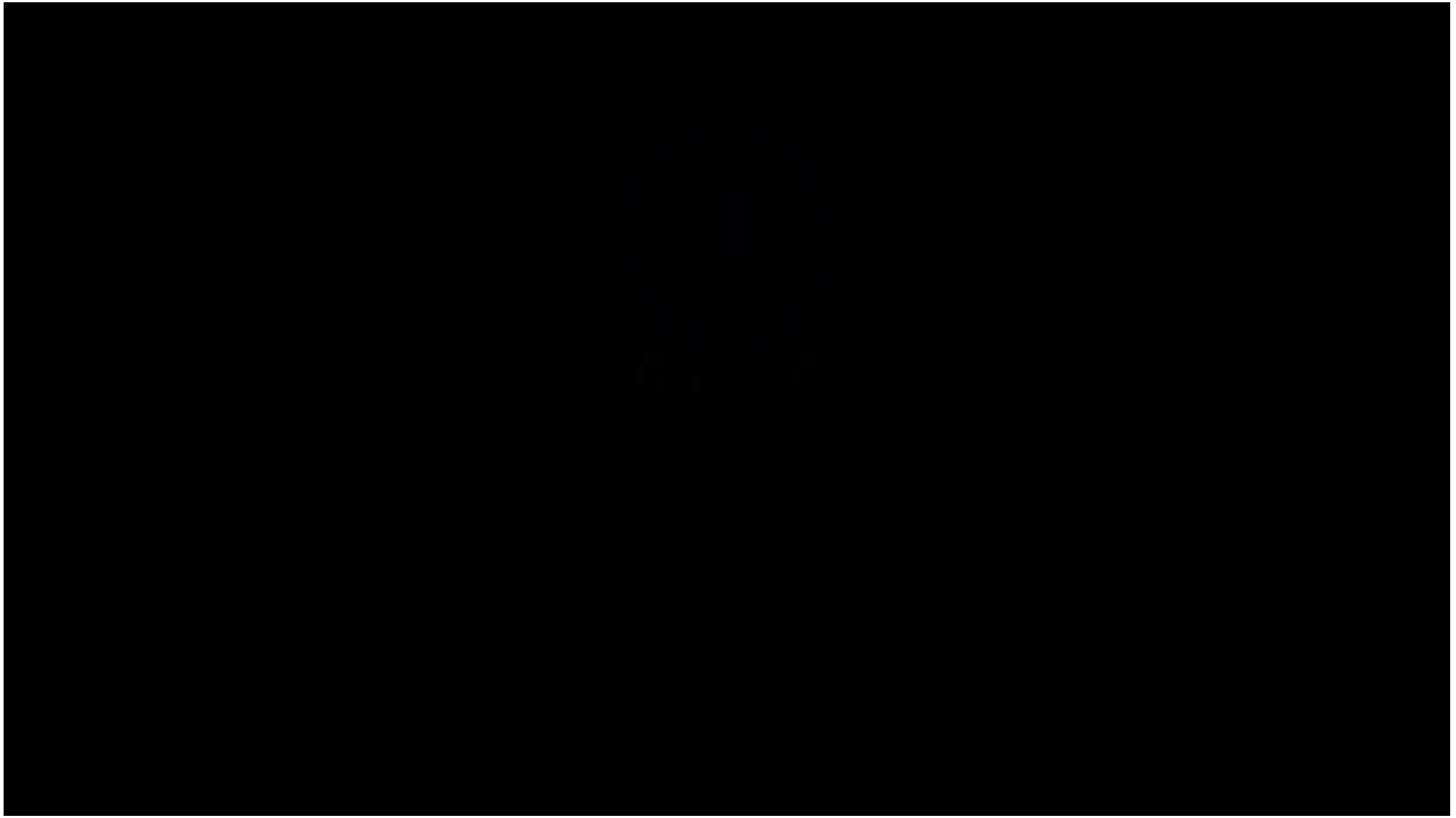
Disinfection of urine and faeces in a tank placed in the basement

Exemple of ABSL-4
Plum **I**sland
Animal **D**isease **C**enter

PIADC (NY) has served as the nation's premier defence against accidental or intentional introduction of transboundary animal diseases

Is the only laboratory in USA that can work on live Foot and Mouth Disease virus (FMDV)

PIADC Activities focus on vaccine and treatment of animal diseases



Useful links:
Biosafety experts in Belgium

Service de Biosécurité et Biotechnologie (SBB)
Rue Juliette Wytsman 14
B-1050 Bruxelles
Belgique

http://www.biosafety.be