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Extract of slides
Course of

Risk assessment of GMMs in the Laboratory, biological and regulatory aspects

by Aware Lab srl Training Center

Special edition reserved to

UNIVERSITÀ DEGLI STUDI DI MILANO BICOCCA

March 2024

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Training Course

HANDLING THE GMMs ASSOCIATED RISK IN THE LAB

General aspects, legislation aspects and fulfillment requirements

Carlotta Cancelliere, *Aware Lab Srl*

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What we are going to talk about

- Legislation
- Biohazard
- Risk assessment: general and specific aspects
- Barriers
- Vector biosafety
- GMMs and fulfillment requirements
- Waste management
- Cleaning
- Medical surveillance
- Conclusions

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ROUND THE TABLE

1 Minute for each of you to tell

- **Your name**
- **What are you expecting from this course**
- **What are the handled agents/vectors**

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Biological Aspects

Biohazard assessment

- Biological agent classification and related containment levels
- Biohazard assessment
- Aspects related to the use of cell cultures

The GMMs contained use assessment

- Methods for inducing DNA modification (mention)
- Basic concepts for GMMs evaluation: host organism, donor, vector, genetic insert
- Fields of application of GMMs regulations
- Classification and evaluation of the manipulation in a biosafety environment

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Regulatory Aspects

Risk assessment, preventive, procedural, technical, protective measures.

The authorizing notification to the Ministry of Health

Facility Notification:
- Inspection
- Outcome
- Upcoming actions

Use notification:
- Inspection and data collection
- Current situation
- Plan
- Strategy for future uses

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Legislative Decree 81 of April 9, 2008.

TITLE X - EXPOSURE TO BIOLOGICAL AGENTS

TITLE X concerns the protection of workers against risks to their health and safety, including the prevention of such risks arising or likely to arise from exposure to biological agents during work

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"Invisible" risk, not perceivable at the sensory level

Risk of contaminating treated sterile material

Absence of exposure threshold limits

Deliberate exposure

Potentian exposure

Accidental exposure

7

For each identified task define the exposure

Deliberate use or employment of biological agents

(Pursuant to Article 267 of Legislative Decree 81/2008)
when they are purposely fed into work cycles and are processed, handled or transformed as "raw material," "substrate," catalyst, "reagent" or "product".

Potential **exposure**

occasional presence of biological agents if there is: handling of biological liquids and materials; analysis of food samples; analysis of soil samples; chemical-clinical diagnostics; agricultural activity; livestock activity; veterinary activity; animal housing.

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BIOLOGICAL RISK

Aerosol inhalation

Aerosol is a widespread risk factor in laboratories.

Droplets with a diameter of less than 10 microns evaporate very quickly, so that the residues of the material dissolved in them (or the micro-organism) remain in suspension in the air as particles, known as 'droplet nuclei.' They, being infinitesimal in size, persist in suspension in the air for hours/days, constituting the so-called **PRIMARY Aerogenic Particles**.

The largest droplets with a diameter between 100 and 400 microns, precipitate quickly near their place of origin, and before the liquid can evaporate (local contamination only). Once dry, they may in turn release particles back into suspension in the air, called **SECONDARY Aerogenic Particles.**

PARTICLE TYPE.	DIMENSION [microns]	SPEED OF SEDIMENTATION [m/sec]
Droplet	100 - 400	0,30 - 2,5
Dust	10 - 100	0,003 - 0,3
Droplet cores	1 - 10 0,0 - 0,1	3.5 and^{-5} - 0.003 8.1 and^{-7} - 0.000508

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Legislative Decree No. 81 of April 9, 2008.

TITLE X - EXPOSURE TO BIOLOGICAL AGENTS

Biological agent

Any microorganism **even if genetically modified,** cell culture and human endoparasite that could cause infection, allergy or intoxication.

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Legislative Decree No. 81 of April 9, 2008.

TITLE X - EXPOSURE TO BIOLOGICAL AGENTS

Microorganism

Any microbiological entity, cellular or otherwise, capable of reproducing or transferring genetic material.

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Legislative Decree No. 81 of April 9, 2008.

TITLE X - EXPOSURE TO BIOLOGICAL AGENTS

Cell culture

The result of in vitro growth of cells derived from multicellular organisms.

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The hazardousness of biological agents is determined on the basis of:

INFECTIVITY: ability of a microorganism to penetrate and multiply in the host.

PATHOGENICITY: ability to produce disease following infection.

TRANSMISSIBILITY: ability of a microorganism to be transmitted from an infected person to a susceptible person.

NEUTRALIZABILITY: availability of effective prophylactic measures to prevent the disease, or therapeutic measures to treat it.

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NEUTRALIZABILITY: availability of effective prophylactic measures to prevent the disease, or therapeutic measures to treat it.

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Biohazard groups

Biological agents are divided into four groups according to the risk of infection

Group 1	Biological agent that is **unlikely to cause disease** in human subjects.
Group 2	Biological agent that **can cause disease** in human subjects and pose a risk to workers; unlikely to spread in the community; effective prophylactic or therapeutic measures are available.
Group 3	Biological agent that **can cause serious illness** in human subjects and poses a serious risk to workers; can spread in the community, but **effective prophylactic or therapeutic measures are** usually **available**.
Group 4	Biological agent that **causes serious illness** in human subjects and poses a serious risk to workers; may present a high risk of spread in the community; **no effective prophylactic or therapeutic measures are available**.

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Legislative Decree No. 81 of April 9, 2008.

TITLE X - EXPOSURE TO BIOLOGICAL AGENTS

Group 1 biological agent

An agent that is unlikely to cause disease in human subjects.

No bacteria, viruses, parasites or fungi are classified by the decree in this class

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Legislative Decree No. 81 of April 9, 2008.

TITLE X - EXPOSURE TO BIOLOGICAL AGENTS

Group 2 biological agent

- An agent that can cause disease in human subjects and pose a risk to workers
- It is unlikely to propagate in the community
- Effective prophylactic or therapeutic measures are usually available.

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Legislative Decree No. 81 of April 9, 2008.

TITLE X - EXPOSURE TO BIOLOGICAL AGENTS

Group 2 biological agents

BACTERIA: Actinomyces, Bordetella pertussis, Borrelia, Chlamydia, Clostridium botulinum and tetanus, Enterococci, Escherichia coli, Helicobacter pylori, Legionella pneumophila, Neisseria meningitidis, Staphylococcus aureus, Streptococci, Treponema pallidum and Treponemi, Vibrio cholerae

VIRUSES: Epstein-Barr virus, Herpes simplex, Herpesvirus varicella-zooster, Measles virus, Mumps virus, Poliovirus

PARASITES: Ancylostoma duodenale, Schistosoma, Taenia saginata

FUNGI: Aspergillus, Candida

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Legislative Decree No. 81 of April 9, 2008.

TITLE X - EXPOSURE TO BIOLOGICAL AGENTS

Group 3 biological agent

- An agent that can cause serious illness in human subjects and poses a serious risk to workers;
- The biological agent can spread in the community, but effective prophylactic or therapeutic measures are usually available.

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Legislative Decree No. 81 of April 9, 2008.

TITLE X - EXPOSURE TO BIOLOGICAL AGENTS

Group 3 biological agents

BACTERIA: Bacillus anthracis, Mycobacterium tuberculosis, Salmonella typhi, Yersinia pestis

VIRUSES: Hepatitis B and D viruses, AIDS virus, rabies virus

PARASITES: Echinococci, Leishmania donovani, Trypanosoma

FUNGI: Blastomyces dermatitidis

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Legislative Decree No. 81 of April 9, 2008.

TITLE X - EXPOSURE TO BIOLOGICAL AGENTS

Group 4 biological agent

- A biological agent that can cause serious disease in human subjects, poses a serious risk to workers, and may present a high risk of propagation in the community
- No effective prophylactic or therapeutic measures are usually available.

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Legislative Decree No. 81 of April 9, 2008.

TITLE X - EXPOSURE TO BIOLOGICAL AGENTS

Group 4 biological agents

BACTERIA: NONE

VIRUSES: Lassa virus, Crimean-Congo hemorrhagic fever virus, Ebola virus, Marburg virus, Variola (major & minor) virus

PARASITES: NONE

FUNGI: NONE

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Legislative Decree No. 81 of April 9, 2008.

TITLE X - EXPOSURE TO BIOLOGICAL AGENTS

BACTERIA and similar organisms

Biological agent Classification
Actinobacillus actinomycetemcomitans 2
Actinomadura madurae 2
Actinomadura pelletieri 2
Actinomyces gereneseriae 2
Actinomyces israelii 2
Actinomyces pyogenes 2
Actinomyces spp. 2
Arcanobacterium haemolyticum 2
Bacillus anthracis 3
Bacteroides fragilis 2
.....
.....

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Legislative Decree No. 81 of April 9, 2008.

TITLE X - EXPOSURE TO BIOLOGICAL AGENTS

VIRUS

Biological agent Classification
Adenoviridae 2
Arenaviridae: LCM-Lassa Virus complex (Old World Arenavirus):
Virus Lassa 4
Lymphocytic choriomeningitis virus (neurotropic strains) 3
Lymphocytic choriomeningitis virus (other strains) 2
Mopeia Virus 2
.....
.....

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Legislative Decree No. 81 of April 9, 2008.

ANNEX XLVI: LIST OF CLASSIFIED BIOLOGICAL AGENTS.

CELL CULTURES

This directive does not make any classification for cell cultures But It is necessary to know very well the biological agent that you are using
It is necessary to know very well the cell culture that you are using
For this reason it is necessary to perform a risk assessment

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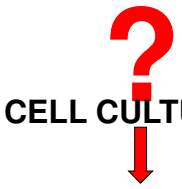

CELL CULTURES

RISK ASSESSMENT

The main purpose of risk assessment is to prevent damage to people and property.
Risk assessment must be carried out before starting any activity.
The evaluation consists of two elements:

1. **Identification and risk assessment**
2. **Defining ways to minimize or avoid risks**

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1. Identification and assessment of risks

For cell cultures of animal origin, the level of risk depends on the cell line to be used and whether it is capable of causing harm to humans.

Classification:

LOW RISK
Non-human or primate-derived continuous cell lines; Well-characterized human-derived lines

MEDIUM RISK
Poorly characterized eukaryotic cell lines

HIGH RISK
Human or primate
- Derived or blood-derived cell lines
- Cell lines with endogenous pathogens (precise classification depends on the pathogen)
- Cell lines used following experimental infection (classification depends on the infecting agent)

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CELL CULTURES

RISK ASSESSMENT

The main purpose of risk assessment is to prevent damage to people and property.
Risk assessment must be carried out before starting any activity.
The evaluation consists of two elements:

1. Identification and risk assessment
2. Defining ways to minimize or avoid risks

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2. Defining ways to minimize or avoid risks

- Containment levels
- Good laboratory practice
- Preparation and training of operators
- Restriction of staff and material movement
- Availability of SOPs (safety operating procedures)

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2. Defining ways to minimize or avoid risks

↓

Containment

↓

A cell bank such as ECACC (The European Collection of Cell Cultures) with the support of the Health Protection Agency, recommends a minimum containment level for a given cell line based on a specific risk assessment

↓

For most cell lines
an appropriate level of containment is Level 2

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ECACC European Collection of Cell Cultures
A Health Protection Agency Culture Collection

Health Protection Agency

MATERIAL SAFETY DATA SHEET

Growing Culture

Advisory Committee on Dangerous Pathogens (ACDP) Levels 1 or 2.

This MSDS has been written in accordance with the European Union Council Directive 98/24/EC of 7th April on the protection of the health and safety of workers from the risks related to chemical agents at work (fourteenth individual directive within the meaning of Article 16(1) of the Directive 89/391/EEC). Commission Directive 2001/58/EC of 27th July 2001 amending for the second time Directive 91/155/EEC defining and laying down the detailed arrangements for the system of information relating to dangerous preparations in implementation of Article 14 of the European Parliament Directive 1999/45/EC and relating to dangerous substances in Implementation of Article 27 of Council Directive 67/548/EEC (safety data sheets). (Text with EEA relevance).
Appropriate risk phrases are cited in this MSDS

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ECACC European Collection of Cell Cultures
A Health Protection Agency Culture Collection

Health Protection Agency

3. Hazards identification

Chemical Hazards: The above components are deemed NON- hazardous.

Biological hazards
Although the ECACC-supplied animal cell lines are not known to contain any agents capable of harm to healthy adult humans the possibility of a contaminant, adventitious virus cannot be excluded. Therefore it is recommended that all animal cell lines are handled as a ACDP Hazard Group 2 (Bio-safety Level 2) organism. The relevant Data Sheet includes any specific instructions that may pertain to the biohazard potential of this cell line and that should be considered by the user when performing a risk assessment. Any such information will not be inconsistent with ACDP (Bio-safety) Group 2. The user is referred to the relevant references in the attached Cell Line Data Sheet. These cell lines have not been screened for adventitious agents.

Health Effects:
Eyes: Not known; Skin: Not known; Ingestion: Not known; Inhalation: Not known

Physical Hazards
It is recommended that persons handling growing animal cell cultures should wear a laboratory overall, protective glasses and latex/plastic gloves.

This sheet does not constitute an assessment as required by the Control of Substances Hazardous to Health Regulations 1994.

The information contained in this publication is given in good faith and is accurate to the best of our knowledge.

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BALB/3T3 clone A31 (ATCC® CCL-163™)

Organism: ***Mus musculus*, mouse** / Tissue: **embryo** / Cell Type: **fibroblast**

ATCC

GENERAL INFORMATION | CHARACTERISTICS | CULTURE METHOD | HISTORY | DOCUMENTATION

Organism	*Mus musculus*, mouse
Tissue	embryo
Cell Type	fibroblast
Product Format	frozen
Morphology	fibroblast
Culture Properties	adherent
Biosafety Level	1
Disease	sarcoma
Age	embryo; 14 to 17 days gestation
Strain	BALB/c
Applications	This cell line is a suitable transfection host.
Storage Conditions	liquid nitrogen vapor phase

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HeLa 229 (ATCC® CCL-2.1™)

Organism: ***Homo sapiens*, human** / Tissue: **cervix** /

ATCC

GENERAL INFORMATION | CHARACTERISTICS | CULTURE METHOD | SPECIFICATIONS | HISTORY | DOCUMENTATION

Organism	*Homo sapiens*, human
Tissue	cervix
Product Format	frozen
Morphology	epithelial
Culture Properties	adherent (The line can be readily adapted to growth in suspension)
Biosafety Level	2 [Cells contain human papilloma virus (HPV-18)]
Disease	adenocarcinoma
Age	31 years
Gender	female
Ethnicity	Black
Storage Conditions	liquid nitrogen vapor phase

ECACC European Collection of Cell Cultures — A Health Protection Agency Culture Collection

Health Protection Agency

General Cell Collection: HeLa229

Catalogue No.:	86090201
Cell Line Name:	HeLa229
Keywords:	Human cervix carcinoma
Cell Line Description:	Derived from the parent HeLa line but differs chiefly in its relative insusceptibility to polioviruses. The presence of human papilloma virus 18 (HPV-18) sequences in HeLa cells has been reported.
Species:	Human
Tissue:	cervix
Morphology:	Epithelial
Growth Mode:	Adherent
DNA Profile:	STR-PCR Data: Amelogenin: X CSF1PO: 9,10 D13S317: 14 D16S539: 9,10 D5S818: 11,12 D7S820: 8,12 THO1: 7 TPOX: 8,12 vWA: 16,18
Subculture Routine:	Split sub-confluent cultures (70-80%) 1:3 to 1:6 i.e. seeding at 2-4x10,000 cells/cm² using 0.25% trypsin or trypsin/EDTA; 5% CO2; 37°C.
Culture Medium:	EMEM (HBSS) + 2mM Glutamine + 1% Non Essential Amino Acids (NEAA) + 10% Foetal Bovine Serum (FBS).
Karyotype:	2n = 46
Depositor:	Obtained from ATCC
Originator:	No
Country:	USA
References:	Am J Pathol 1985;119:361

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RISK ASSESSMENT

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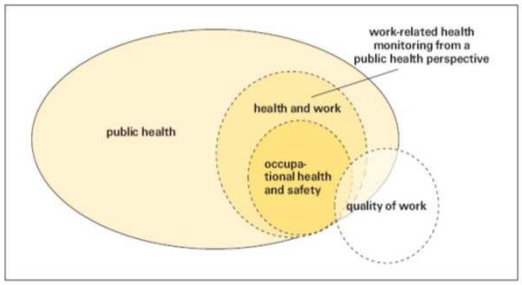
The difficulty of **assessing biological risk** in the workplace, highlights the need to develop reliable risk assessment tools.
Standards and occupational exposure limits related to biological agents need to be defined.
Because of the many links between occupational safety and other areas such as public health, environmental safety, veterinary, an interdisciplinary approach is important. (EU-OSHA)



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Bio-Ritmo

un algoritmo per valutare il rischio biologico

Un esempio applicativo del metodo realizzato da INAIL e ARPA Liguria chiarisce le modalità per adattare l'algoritmo a tutte le attività lavorative

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INAIL

Il rischio biologico negli ambulatori "Prime Cure" INAIL
Proposta di valutazione attraverso una metodologia integrata
Edizione 2013

ARPAL
Agenzia Regionale per la Protezione dell'Ambiente Ligure

Bio-ritmo method 2010

Step A
Identification of biological risk source (danger)
Identification of the exposed people

Step B
Evaluation of the risk for probability and damage entity
Identification of priority actions

Step C
Identification of actions suitable to eliminate or control the risk

Step D
Action implementation

Step E
Monitoring activity on got results

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Identification of exposed workers

Examples of potentially exposed individuals/tasks performed	Sensitive categories (Reporting by the Physician in Charge)	Type and Frequency of Exposure
Researchers/laboratory technicians/analysts Plant and instrumentation maintenance/repair workers Cleaning/disinfection workers Pest control / deratization workers For cleaning, pest control, rodent control or maintenance companies, an assessment of interference risk (DUVRI) is essential	Pregnant women Minors Individual conditions of hypersusceptibility • Injuries - skin - mucosal pathologies • Phlogosis in place • Immunological deficits • Absence of immunoprophylaxis • Immunosuppressive treatments e.g., NSAIDs, corticosteroids, radiation therapy, agents, alkylating agents, antimetabolites • Immunosuppressive diseases e.g., diabetes, chronic nephropathies, chronic hepatopathies, hemopathies, asplenia (lack of spleen), transplants, neoplasms, malabsorption, autoimmune diseases	occasional, constant, periodic

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Identification of biological agents present

BIOLOGICAL AGENTS (AB)	GROUP	EXPOSURE ROUTES	POTENTIAL HEALTH EFFECTS
Report the type of AB detected by environmental monitoring or inferred from literature for the specific risk profile	For detected or presumed biological agents report classification into groups 2,3,4 according to Legislative Decree 81/08	Describe the main modalities: direct contact, parenteral route, inhalation route, etc.	Infections, allergies, intoxication, inflammation, etc.

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Identification of biological agents present

BIOLOGICAL AGENTS (AB)	GROUP	EXPOSURE ROUTES	POTENTIAL HEALTH EFFECTS
Viruses potentially or deliberately present			
HBV virus, HCV virus	Group 3 (**), D for HBV: V	Parenteral route (needle sticks and infected sharps); mucosal contact with biological fluids (especially blood)	Hepatitis B, hepatitis C; liver cirrhosis, liver cancer
HIV virus	Group 3 (**), D	Parenteral route (needle sticks and infected sharps); mucosal contact with biological fluids (especially blood)	AIDS

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Identification of biological agents present

BIOLOGICAL AGENTS (AB)	GROUP	EXPOSURE ROUTES	POTENTIAL HEALTH EFFECTS
Bacteria potentially or deliberately present:			
Various bacteria with deliberate exposure (including genetically modified) used in research and diagnostic testing, or potential exposure	Groups 2,3 if natural; if MOGM evaluate use class according to D.Lgs 206/2001	Contact, parenteral route, inhalation route, oro-fecal route	Different pathologies according to species

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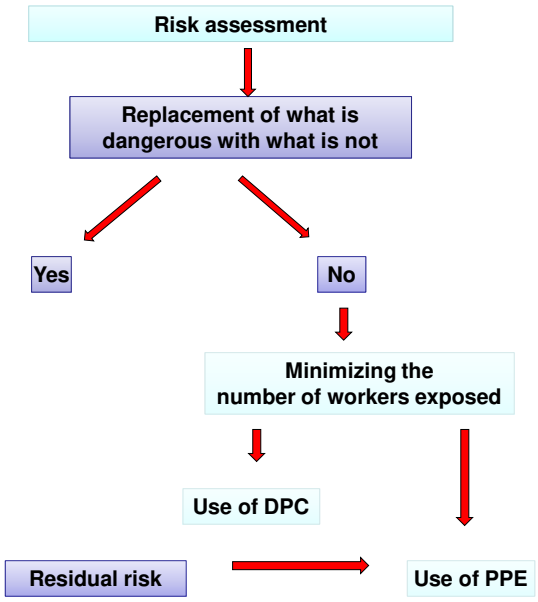

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Risk assessment

The algorithm used to calculate the risk level refers to the "matrix" method, which is widely used in Industrial Hygiene for semi-quantitative risk assessment.

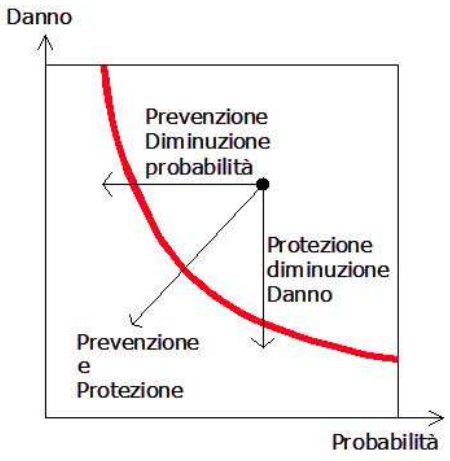
P = **probability** of occurrence of a harmful event

D = consequential **damage to** the event, should it occur

The relationship P x D gives rise to an R (Risk) value, which expresses the level of risk present in the activity under consideration,

R = P x D

The matrix used is the 4x4 matrix, that is, with 4 possible probability graduations and 4 damage graduations.



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Determination of the value related to "Probability"

Probability refers to the possibility that an individual exposed to biological agents will be contaminated and may develop an infectious disease.

Several elements contribute to determining the probability of infection, which must be analyzed individually and entered into the algorithm.

$$P = [C] \times [(F1 + F2 + F3 + F4 + F5 + F6) + 1] / 7$$

Where:

C is the PRESUMPTIVE CONTAMINATION GRADE of the sources;

F is the coefficient expressing the degree of influence of WORK FACTORS on risk exposure (F1: quantity; F2 frequency of sample handling; F3 environmental characteristics; F4 procedures adopted; F5 using of PPE, F6 training received, etc.).

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Determination of the value related to "Damage"

Once the potential biological hazards have been identified, the quantification of harm refers directly to the group to which the biological agents belong, according to the infectious risk classification in Annex XLVI of Legislative Decree 81/08;

The damage is quantified as equal to the highest possible group identified.

GROUP XLVI	DESCRIPTION OF THE EXTENT OF DAMAGE	DAMAGE VALUE D
4	Biological agent that can cause serious disease in human subjects and poses a serious risk to workers and may present a high risk of propagation in the community; no effective prophylactic or therapeutic measures are usually available	4 GREAT
3	Agent that can cause serious illness in human subjects and poses a serious risk to workers; biological agent can spread in the community, but effective prophylactic or therapeutic measures are usually available	3 GRAVE
2	Agent that can cause disease in human subjects and pose a risk to workers; unlikely to spread in the community; effective prophylactic or therapeutic measures are usually available	2 MODEST
1	Agent that is unlikely to cause disease in human subjects	1 LIEVE

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Risk assessment

- Esempio di Matrice di Valutazione del Rischio: R = P x D

P - Probabilità	D - Danno 1	2	3	4
4	4	8	12	16
3	3	6	9	12
2	2	4	6	8
1	1	2	3	4

R > 8	Azioni correttive indilazionabili	Priorità P1
4 ≤ R ≤ 8	Azioni correttive necessarie da programmare con urgenza	Priorità P2
2 ≤ R ≤ 3	Azioni correttive e/o migliorative da programmare nel breve medio termine	Priorità P3
R = 1	Azioni migliorative da programmare non richiedenti un intervento immediato	Priorità P4

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Risk estimation and prevention measures

Risk estimation

From the combination of the previous two factors (PROBABILITY and DAMAGE), the ***Magnitude of RISK*** for each task as potentially exposed to biohazard is derived as shown in the Assessment Matrix above
With regard to the definition of prevention and protection measures to be implemented, the information used to calculate the F coefficients makes it possible to infer the critical issues, the improvement actions to be taken and the relative priority scale.

0.5 < DxP ≤ 1	1 < DxP ≤ 2	2< DxP ≤ 8	8 < DxP ≤ 10	10 < DxP ≤ 16
ACCEPTABLE	LOW	MID	HIGH	UNACCEPTABLE
1	2	2	3	4

Identification of preventive measures

Numerical value	Risk level	Prevention and protection measures to be taken
0.5 < DxP ≤ 1	ACCEPTABLE	General hygiene standards
1 < DxP ≤ 2	LOW	General hygiene standards
2< DxP ≤ 8	MID	General hygiene rules + Specific prevention and protection measures
8 < DxP ≤ 10	HIGH	Specific urgent prevention and protection measures
10 < DxP ≤ 16	UNACCEPTABLE	General hygiene rules + Specific urgent prevention and protection measures

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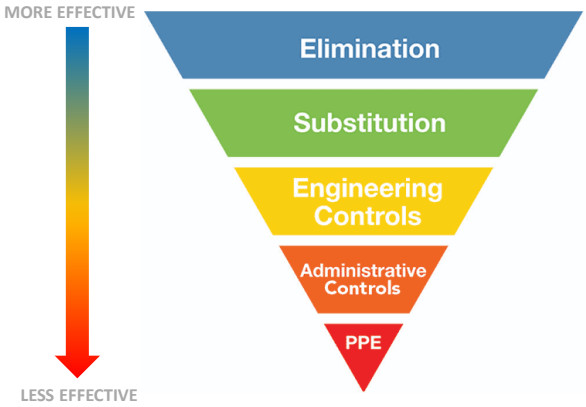
HIERARCHY OF ACTIONS

Hierarchy of control

Let us look together at the meaning of the various hierarchical levels, starting with the most effective one :

- **Elimination** aimed at physically removing the hazard (elimination of methods using radioactive material)
- **Substitution** of the hazard (replacing a highly toxic reagent with one of lower toxicity)
- **Protection technologies** to separate the worker from the hazard (installation of DPCs such as a chemical fume hood)
- **SOPs** to change the way of working (training interventions)
- **PPE** to protect the worker (protective gloves).



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Definitions and aggravating factors of biohazard in the laboratory

the confusion, the chaotic flows of staff in cramped spaces among poorly arranged furniture

risky operations performed outside the primary barriers

he incorrect use of collective and personal protective equipment

The contamination of commonly used surfaces

The failure or incorrect periodic inspection of equipment

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Definitions and aggravating factors of biohazard in the laboratory

Not using the specific PPE prescribed for the activity performed.

The improper use of waste containers.

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Primary and secondary protection barriers

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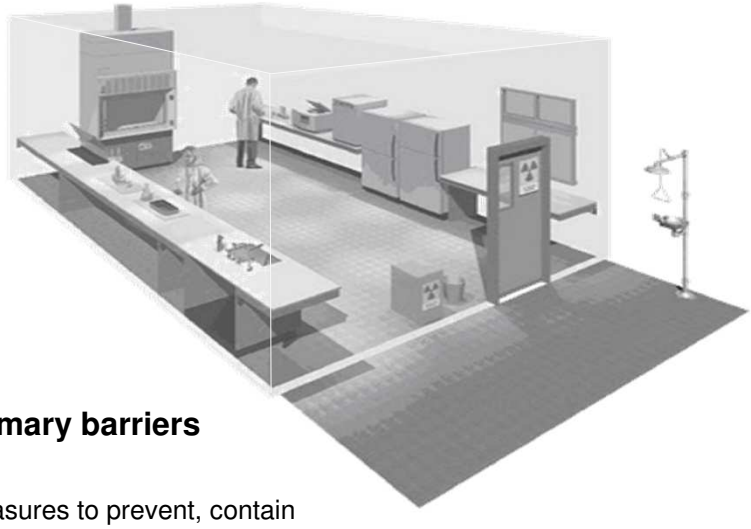
PRIMARY BARRIERS OF PROTECTION



DPC
Chemical Hood
Biohazard Cabinet

PPE
Body
Hands
Eyes and face
Airways

Hygiene standards
Vaccinoprophylaxis
Sterilization

Primary barriers

measures to prevent, contain
or eliminate the risk of exposure
workers and contamination of the workplace.

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Banchi sterili e Cappe BioHAZARD

BIOHAZARD

BIOLOGY HOODS

STERILE BENCHES
(CLEAN BENCHES)

ISOLATORS

MICROBIOLOGICAL SAFETY CABINETS
(BioHAZARD HOODS)

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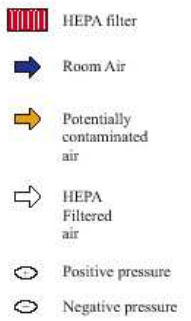
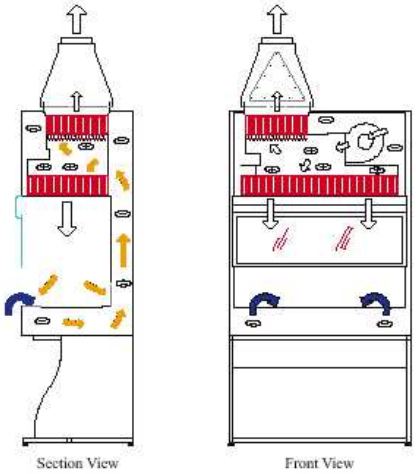
Biohazard Class II Type A2 Hoods





- Good operator protection
- Good environmental protection
- Good sample protection
- Frontal barrier of 0.4 m/s
- Airflow: 30% exhaust into the room and 70% recirculation on the product after HEPA filtration

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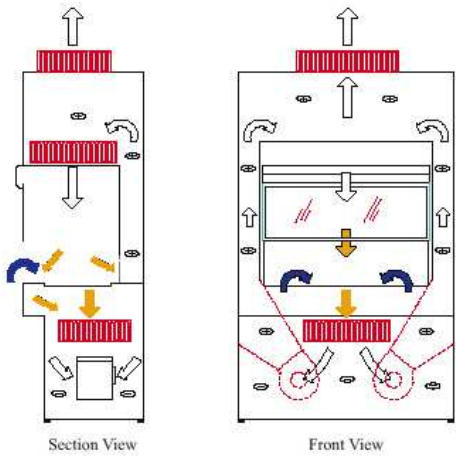
Biohazard Class II Type B1 Hoods



- Good operator protection
- Good environmental protection
- Good sample protection
- Frontal barrier of 0.52 m/s
- Airflow: 70% expulsion into the room and 30% recirculation on the product after HEPA filtration

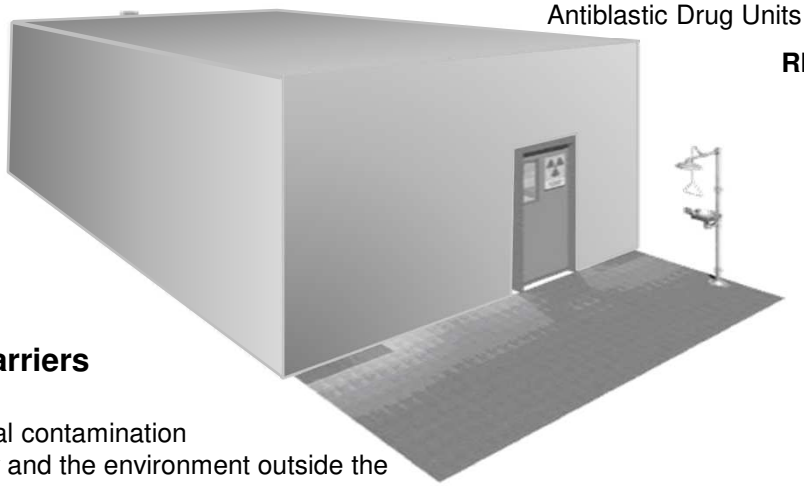
56

SECONDARY PROTECTION BARRIERS

PCL /**BSL** physical containment laboratories

Antiblastic Drug Units **(UFA/UMaCA)**

RIA Laboratories

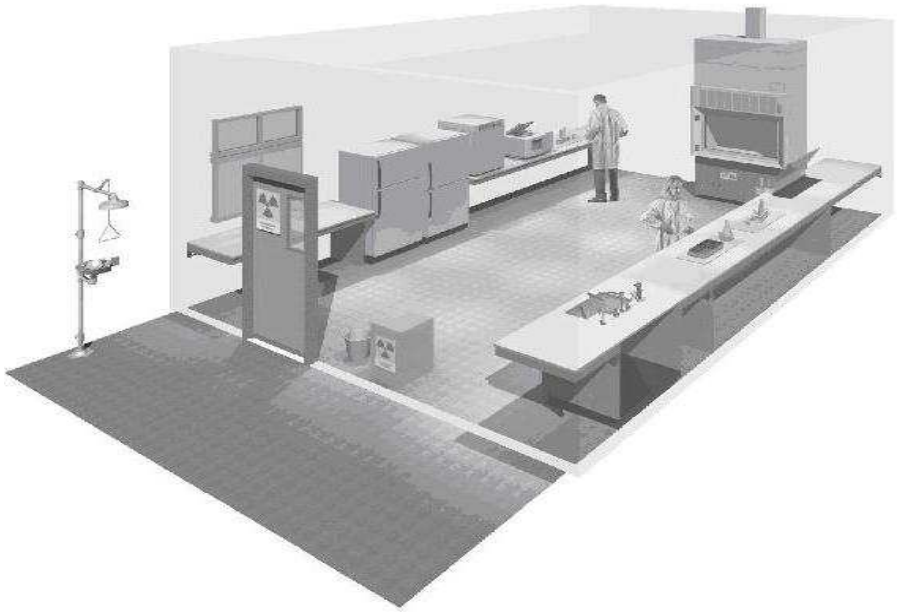


Secondary barriers

prevent accidental contamination of the community and the environment outside the laboratory (casing)

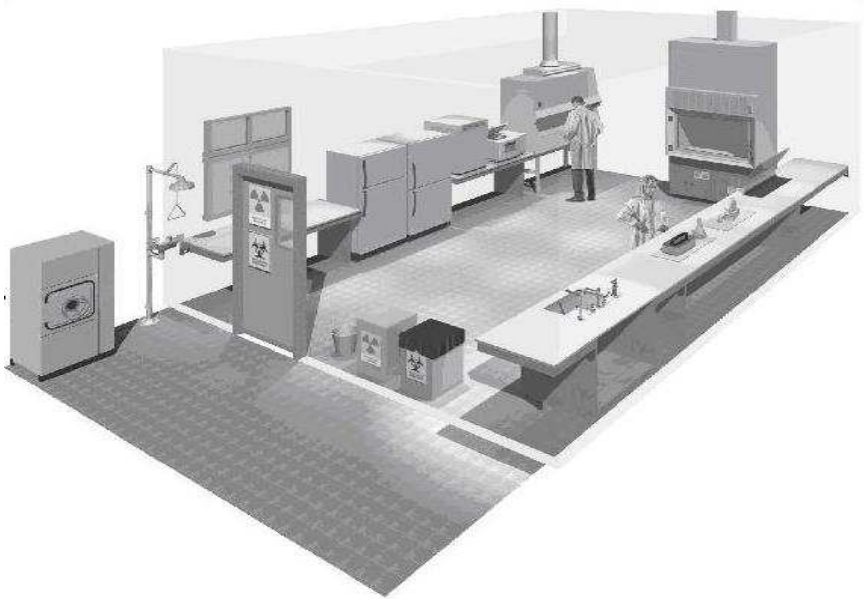
57

Biosafety Laboratory Level 1



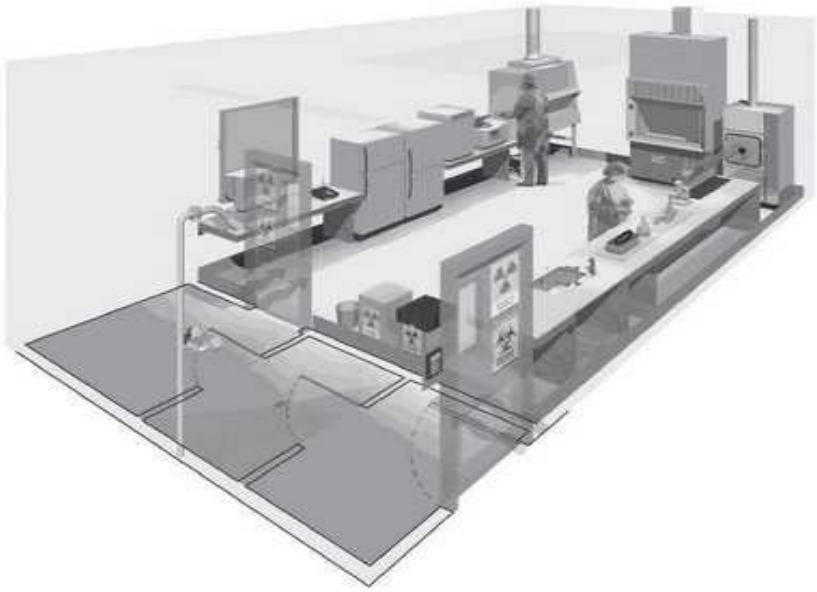
58

Biosafety Laboratory Level 2



59

Biosafety Laboratory Level 3



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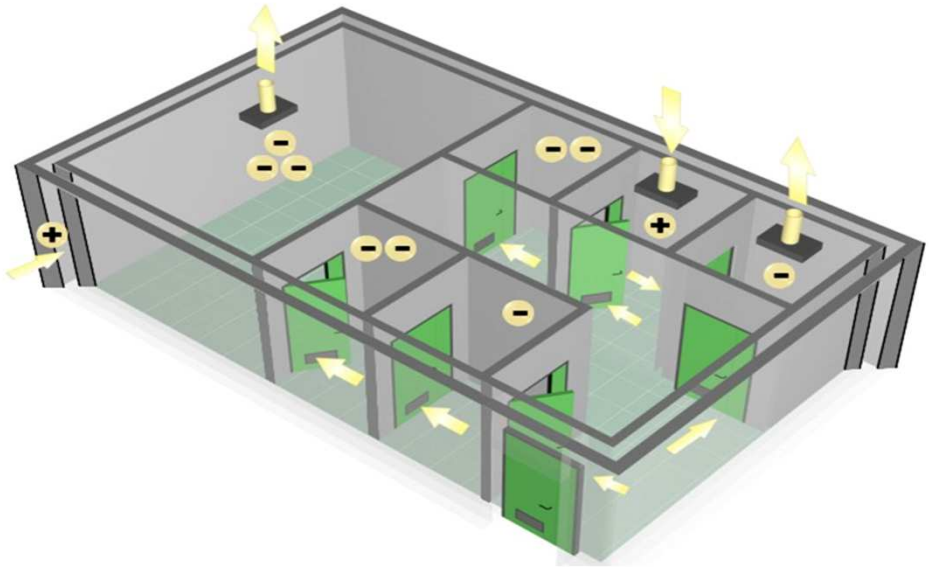
Biosafety Laboratory Level 4



61

Legislative Decree No. 81 of April 9, 2008 ANNEX XLVII : BIOLOGICAL AGENTS - SPECIFICATIONS ON CONTAINMENT MEASURES AND LEVELS.

A. Containment measures	B. Containment levels		
	2	3	4
1. The work area must be separated from any other activity in the same building	No	Recommended	Yes
The air fed into the work area and the air extracted must be filtered through an ultrafilter (HEPA) or similar filter	No	YES, on air extracted	YES, on air in and air out
3. Access must be limited to authorized persons	Recommended	Yes	Yes through an airlock
4. The work area must be able to be closed tightly to allow disinfection	No	Recommended	Yes
5. Specific disinfection procedures	Yes	Yes	Yes
6. The work zone must be maintained at a negative pressure relative to atmospheric pressure	No	Recommended	Yes
7. Effective vector control, e.g., rodents and insects	Recommended	Yes	Yes
8. Water-repellent and easy-to-clean surfaces.	Yes, for the workbench	Yes, for the workbench and the floor	Yes, for the workbench, furniture, walls, floor and ceiling

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Legislative Decree No. 81 of April 9, 2008 ANNEX XLVII : BIOLOGICAL AGENTS - SPECIFICATIONS ON CONTAINMENT MEASURES AND LEVELS.

A. Containment measures	B. Containment levels		
	2	3	4
9. Surfaces resistant to acids, alkalis, solvents, disinfectants	Recommended	Yes	Yes
10. Safe storage for biological agents	Yes	Yes	Yes, safe deposit
11. Inspection window or other device that allows its occupants to be seen	Recommended	Recommended	Yes
12. Laboratories must contain the equipment necessary for them	No	Recommended	Yes
13. Infected materials, including animals, must be handled in safety booths, isolators or other appropriate containers	Where appropriate	Yes, when the infection is airborne	Yes
14. Incinerators for the disposal of animal carcasses.	Recommended	Yes (available)	Yes, on site
15. Means and procedures for waste treatment	Yes	Yes	Yes, with sterilization
16. Wastewater treatment	No	Optional	Optional

63

Legislative Decree No. 81 of April 9, 2008 ANNEX XLVII : BIOLOGICAL AGENTS - SPECIFICATIONS ON CONTAINMENT MEASURES AND LEVELS.

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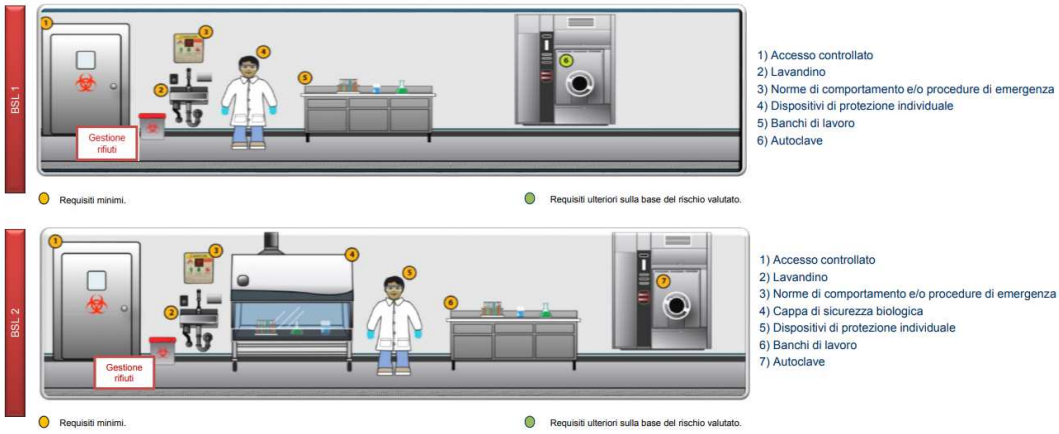
67

Legislative Decree No. 81 of April 9, 2008 ANNEX XLVII : BIOLOGICAL AGENTS - SPECIFICATIONS ON CONTAINMENT MEASURES AND LEVELS.



68

Legislative Decree No. 81 of April 9, 2008 ANNEX XLVII : BIOLOGICAL AGENTS - SPECIFICATIONS ON CONTAINMENT MEASURES AND LEVELS.

1) Due porte interbloccate (zona filtro)
2) Accesso controllato
3) Doccia personale
4) Norme di comportamento e/o procedure di emergenza
5) Lavandino
6) Scarico a tenuta stagna
7) Cappa di sicurezza biologica
8) Dispositivi di protezione individuale
9) Banchi di lavoro
10) Autoclave

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7) Dispositivi di protezione individuale
8) Banchi di lavoro
9) Autoclave
10) Doccia chimica
11) Doccia personale

Immagine modificata da *The infographic "4 biosafety lab levels"* reperibile on line http://www.cdc.gov/phpr/documents/bsl_infographic_final.pdf

69

Personal Protective Equipment PPE

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Personal Protective Equipment

First Category	Rischi di danni fisici di lieve entità di cui la persona che usa i DPI abbia la possibilità di percepire il progressivo verificarsi degli effetti lesivi	Azioni lesive di lieve entità prodotte da strumenti metallici Azioni lesive di lieve entità causate da prodotti detergenti Contatto o urti con oggetti caldi che non espongano ad una temperatura superiore ai 50 °C Ordinari fenomeni atmosferici nel corso di attività professionali Urti lievi e vibrazioni inidonei a raggiungere gli organi vitali ed a provocare lesioni di carattere permanente Azione lesiva dei raggi solari	Simbolo CE (Dichiarazione di conformità del fabbricante o mandatario)
Second Category	Tutti i rischi non coperti dalle altre categorie		Simbolo CE (Attestato di certificazione rilasciato da organismo notificato previa verifica del prototipo)
Third Category	Rischi di morte o di lesioni gravi e di carattere permanente di cui la persona che usa i DPI non abbia la possibilità di percepire tempestivamente la verificazione istantanea degli effetti lesivi	Inquinamento dell'atmosfera respirabile o deficienza di ossigeno nella stessa Aggressioni chimiche e radiazioni ionizzanti Temperatura d'aria non inferiore a 100°C o non superiore a –50 °C Cadute dall'alto Tensioni elettriche pericolose	CE + n° di riconoscimento dell'organismo notificato che ha rilasciato la certificazione o ha effettuato le verifica annuale del sistema di qualità del fabbricante (Attestato di certificazione)

Mandatory training and training in use for third category PPE.

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Personal Protective Equipment

Legislative Decrees 81/08 and 106/09 and PPE.

PPE aimed at protection
Of the exposed **body parts**

ANNEX VIII
PERSONAL PROTECTIVE EQUIPMENT

General directions regarding to special protections

head	(helmets, hoods, headphones)
eyes and face	(goggles, face shields, masks, goggles for protection against X-rays, laser beams, ultraviolet, infrared, visible radiation)
hearing	(earplugs, headphones)
respiratory tract	(filtering facepieces, respirators)
hands and arms	(gloves against physical, chemical and biological agents, mittens, cut-resistant gloves, barrier creams)
feet and legs	(shoes, clogs, boots)
trunk and abdomen	(vests, aprons)
whole body	(fall arrest systems and devices)

81

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Personal Protective Equipment

IPR Information Note

a) instructions for storage, use, cleaning, maintenance, overhaul and **disinfection**

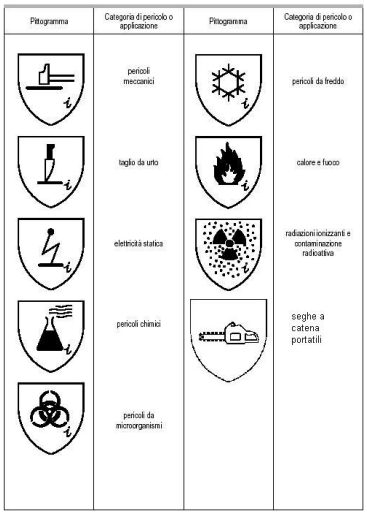
(b) the performance obtained during the various tests **conducted** to verify the levels or classes of protection of PPE

(c) the **accessories that can be used** with the PPE and the characteristics of the appropriate spare parts;

(d) the **classes of protection** appropriate to different levels of risk and the corresponding limits of use

(e) the date or **expiration** date of the PPE or some of its components

f) the appropriate type of **packaging** for transporting the PPE

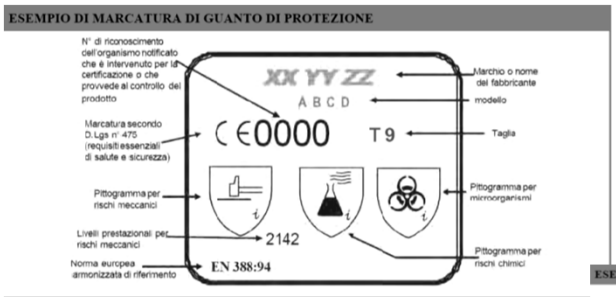


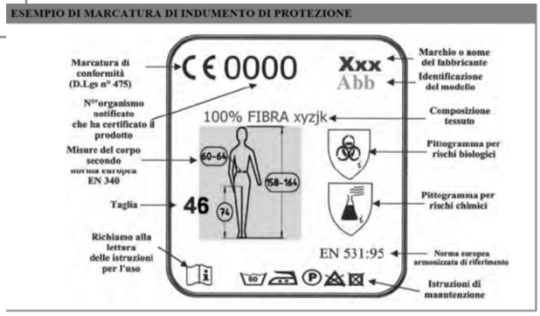
Cleaning, maintenance or disinfection products recommended by the manufacturer must not have within their mode of use any harmful effect on the PPE or the user.

73

Personal Protective Equipment

PPE marking





74

PPE: Signage

75

Personal Protective Equipment

Protective Clothing for Biological Hazard

These protective garments must have a CE marking for protection from chemical biological agents and compliance with the technical standard UNI EN 14126:2004
And be classified as a **third category**.

with elastic cuffs

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Personal Protective Equipment

- The hands are the most contaminated part of the body and are the first to become contaminated
- Personal hygiene is prerequisite for working safely
- Hand washing before and after wearing gloves
- In each laboratory protective gloves in diff. sizes and of different materials.

HAND PROTECTION

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Personal Protective Equipment

EN 374-1/2/3 GUANTI DI PROTEZIONE CONTRO I PRODOTTI CHIMICI E I MICROORGANISMI

La prestazione alla resistenza chimica è determinata da 3 fattori:
Penetrazione: consiste nel movimento di una sostanza chimica o di un microrganismo attraverso materiale poroso, cuciture buchi o altre imperfezioni del guanto ad un livello non molecolare.
Degradazione: cambiamento irreversibile e deleterio di una o più proprietà meccaniche del materiale del guanto dovuto al contatto con una sostanza chimica.
Permeazione: processo secondo il quale la sostanza chimica si muove attraverso il materiale del guanto di protezione a un livello molecolare (coinvolge le fasi di assorbimento, diffusione ed espulsione). Il relativo indice di permeazione misura il passaggio della sostanza chimica nel corso del tempo attraverso il materiale del guanto.

Indice di protezione EN	0	1	2	3	4	5	6
Tempo di permeazione	< 10	10	30	60	120	240	> 480

Nota: 480 minuti equivalgono a 8 ore di lavoro in immersione simulate in laboratorio (condizioni di prova standard), il riutilizzo di un guanto deve essere soggetto a tutte le valutazioni e cautele del caso.

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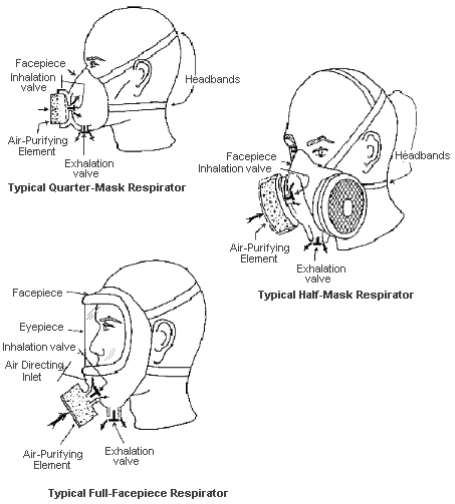
Personal Protective Equipment

Protection of airways

Technical standard UNI EN 529:2016

selection, use, and maintenance of respiratory protection equipment

APVR, **respiratory protection equipment**



APVR filtering
(Filtering Facials,
Half-masks, Whole masks)

79

Personal Protective Equipment

Face and eye protection

Reference standard goggles and visors
UNI EN 166
For general laboratory uses, the following is indicated.
***Type 3**, for protection against drops and splashes of liquids,*
***Optical class 1**, to reduce visual stress*

optical radiation reference standards:

- UNI EN 170 (UV)
- UNI EN 171 (IR)
- UNI EN 172 (sunlight)
- UNI EN 208 (laser)

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Personal Protective Equipment

A useful reminder:

1. PPE should be worn **before** performing a risky operation and maintained until the hazard finishes.
2. They must be adapted to the worker's morphology *(shape, size, adjustment, closing)*
3. Must not pose an additional danger *(touch, mobility, tic)*
4. They must be compatible with each other *(e.g., filtering face mask and goggles, glove and gown)*
5. They must be hygienic and not cause problems for the worker (allergies, frequent replacement)

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Personal Protective Equipment

6. If reusable, they must be sanitized regularly and between workers (by DDL)
7. Adoption by the DDL is mandatory.
8. It is mandatory for the worker to wear them and care for them
9. It is the obligation of the RADRL and the supervisor to see that they are used (and set a good example!).
10. Using injuries and near misses to raise awareness among employees
11. Train operators in the use of PPE *(training)*

PPE should be worn for one's own safety and not only because it is an obligation

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D.lgs. 12th april 2001, n. 206 implementation of European Council Directive 98/81/CEE which modifies Directive 90/219/CEE regarding the «contained use» of genetically modified microrganisms (GMMs)

It establishes the measures for the contained use of GMMs in order to safeguard human health and the environment

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DEFINITIONS

84

D.lgs. 12th april 2001, n. 206

DEFINITION

Micro-organism: any microbiological entity, cellular or non-cellular, capable of replication or of transferring genetic material, including viruses, viroids, animal and plant cells in culture.

Genetically modified micro-organism (GMMs): a micro-organism in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination

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DEFINITIONS

(D.Lgs : 206/2001 in attuazione della Direttiva 98/81/CE che modifica la Direttiva 90/219/CE)

For **Contained Use** we shall mean "any activity in which micro-organisms are genetically modified or in which such GMMs are cultured, stored, transported, destroyed, disposed of or used in any other way, and for which specific containment measures are used to limit their contact with the general population and the environment"

For **User (Utilizzatore)** we shall mean "any natural or legal person responsible for the contained use of GMMs"

For **Facility Director (Titolare dell'impianto)** we shall mean "*the employee as defined in the art.2 of D. Lgs 81/2008*"

For **Notification** we shall mean "the presentation of the required information to the competent authorities of Italy (Ministry of Health)"

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Techniques/methods to obtain a GMMs

(D.Lgs : 206/2001 implemetation of European Council Directive 98/81/CE which modifies the Directive 90/219/CE)

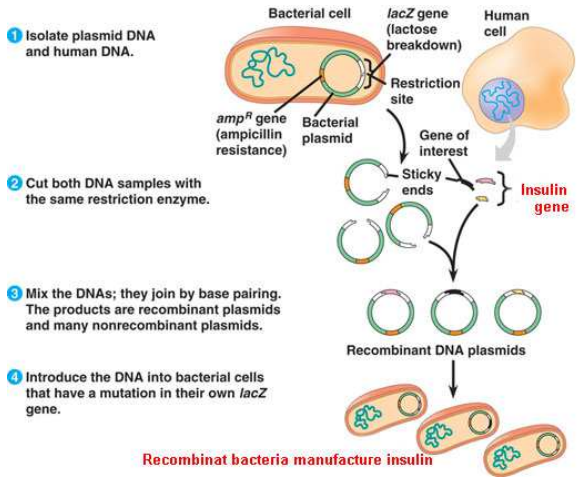
Techniques/methods for genetic modification:

In the **Annex I Part A** of the Directive are specified which techniques or methods are suitable in order to obtain a GMMs:

Recombinant nucleic acid techniques involving the formation of new combinations of genetic material by the insertion of nucleic acid molecules produced by whatever means outside an organism, into any virus, bacterial plasmid or other vector system and their incorporation into a host organism in which they do not naturally occur but in which they are capable of continued propagation

Techniques involving the direct introduction into a micro-organism of heritable material prepared outside the micro-organism including **micro-injection, macro-injection and micro-encapsulation**

Cell fusion or hybridisation techniques where live cells with new combinations of heritable genetic material are formed through the fusion of two or more cells by means of methods that do not occur naturally



87

D.lgs. 12th april 2001, n. 206

Techniques and methods of genetic modifican: esclusions and exceptions

Out of the scope of application as they are not considered genetic modification techniques:

- in vitro fertilization
- natural processes such as: conjugation, transduction, transformation
- induction of polyploidy. Provided that they do not involve the use of recombinant nucleic acid molecules or genetically modified microorganisms produced with techniques or methodologies other than those excluded from Annex II Part A

Genetic modifications obtained using the techniques or methodologies reported in Annex II Part A of Legislative Decree 206/2001 that do not fall within the scope of application:

- Mutagenesis
- Cellular fusion of prokaryotic species that exchange genetic material through known physiological processes
- Cellular fusion of cells of any eukaryotic species, including hybridoma production and plant cell fusion
- Microorganisms obtained through self-cloning as long as they are not pathogenic for humans, animals or plants

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BIOVECTORS SAFETY

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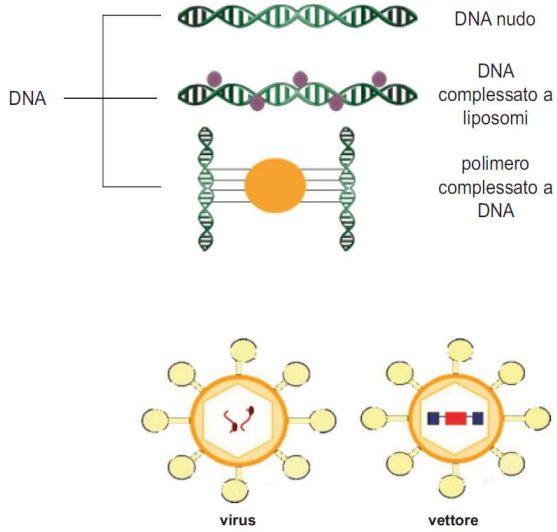
BIOVECTORS



Non-viral vectors

Viral vectors

- Retrovirals
- Lentivirals (HIV)
- HSV (herpes simplex)
- Adenoviral
- Adenoassociates (AAV)

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PHYSIOLOGICAL BARRIERS

CELL MEMBRANE
Viral vectors maintain the specialization of the original virus in transferring genetic information into the cell

Viruses are stripped of all components that can induce pathogenicity downstream of infection

91

BIOVECTORS

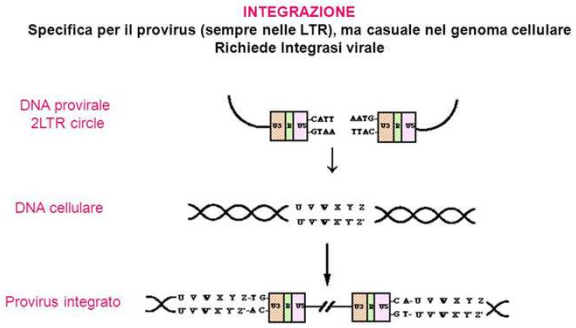
INTEGRATING viral vectors
- Retrovirals and lentivirals
- Adenoassociates (AAV)

NON-INTEGRATING Viral Vectors
- HSV (herpes simplex)
- Adenoviral

92

INTEGRATION

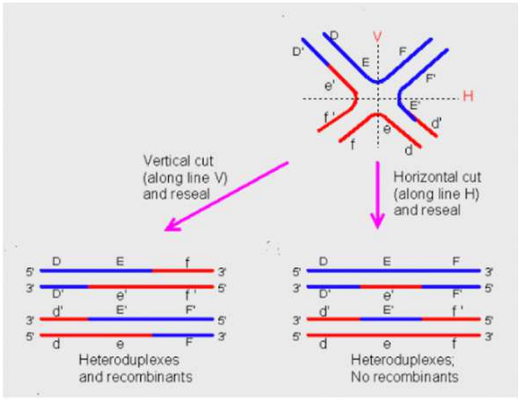
INTEGRATION is the ability of the carrier to integrate genetic information into the host cell's DNA within the nucleus



93

RECOMBINATION

RECOMBINATION is the ability of the viral vector to recombine in vivo and can generate replication competent virus (RCV) particles



94

PSEUDOTYPING

PSEUDOTYPIZATION is the modification of the protein expression profile on the viral pericapside in order to achieve a more suitable tropism for the host spectrum of different cell types to be infected

Modification of envelope proteins can help bind different protein components of host cells

95

REPLICATION CAPACITY

The ability of the viral vector to replicate which is inhibited by replacing the genetic information necessary for the vector to function and by "splitting" it into multiple plasmids. The associated recombination capacity is thus reduced

The use of **SIN vectors,** in which only the 30 nucleotides that ensure integration into the target cell genome are retained, further lowers the possibility of subsequent vector replication

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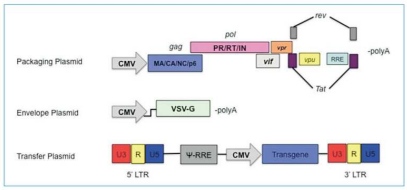
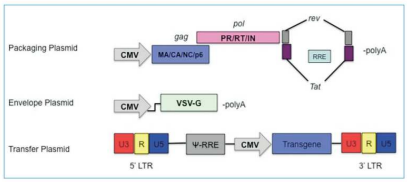
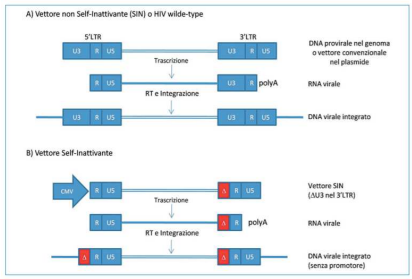
BIOSECURITY OF LENTIVIRAL VECTORS



First generation

All viral proteins except Env are contained in a single packaging plasmid

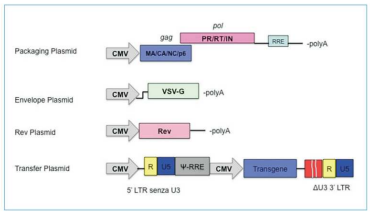




SIN vs. NON-SIN Carriers

Second generation

All accessory proteins are excluded from the packaging vector - Glycoprotein and transgene are provided by different plasmids

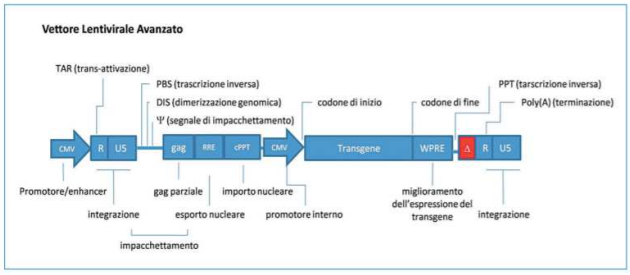


Third generation

Rev protein is encoded by a different plasmid

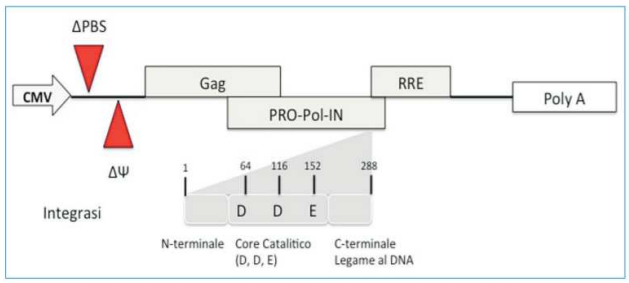
97

BIOSECURITY OF LENTIVIRAL VECTORS



Advanced Lentiviral Vector

WPRE was inserted to increase transgene expression in cells, while cPPT, implicated in PIC transport, increases the transduction efficiency of SIN vectors.



Packaging Plasmid vector IDLV

All sequences coding for unnecessary proteins are removed. The integrase protein (IN) is modified to avoid integration of the vector into the cell genome.

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FACTORS AFFECTING BIOSAFETY

- Carrier selection
- Carrier design
- Physical containment in the laboratory
- Good laboratory practices
- SOPs
- Staff training

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RISK FACTORS IN LABORATORY ACTIVITY

- Carrier preparation
- Handling of VL suspension (high titer)
- In vivo inoculation in laboratory animals
- Cellular Transduction
- Handling of transduced cell cultures

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CONFINED EMPLOYMENT: BARRIERS AND FLOWS

Equipaggiamento di sicurezza (**barriera primaria**) →
- Cappe biologiche
- Glove box
- Altre modalità per minimizzare aerosols

Equipaggiamento di progettazione (**barriera secondaria**)
- Il grado di protezione dipende dalla natura dell'agente infettivo e dai rischi associati alla produzione (aerosols)
- All'aumentare del rischio di trasmissione via aerosol maggiori devono essere i livelli di contenimento primario e secondario

→
- Impianto progettato per trattamento asettico
  - ✓ Superfici lisce, senza soluzione di piastrelle, ecc.
  - ✓ Facile pulizia con rifiniture non articolate
  - ✓ Ridotta capacità di diffusione
- Aria filtrata tramite filtri HEPA attraverso unità di trattamento separato
- Separazione della produzione da altre zone della struttura
- Ingresso separato / zona pulita controllata
- Equipaggiamento utilizzato per la produzione non dovrebbe essere usato in attività non legate alla produzione
- Laboratori con contenimento dell'aria
- Nessun movimento di personale tra area di produzione del vettore e area di trasduzione
- Stanze per aree di supporto
  - ✓ preparazione buffer, terreni, lavaggio vetreria
  - ✓ aree di stoccaggio per materie prime, banche cellulari adiacenti alla area di produzione, se possibile, per ridurre al minimo il traffico in entrata e in uscita dalla struttura
- Aree di produzione dovrebbero essere separate da quelle per altre attività (ricerca, testing)

Definire dei **flussi di percorso** per il materiale e il personale per ottimizzare l'efficienza e minimizzare potenziali cross-contaminazioni tra fasi dello stesso processo e tra processi diversi →
- Stabilire flussi unidirezionali, ove possibile
- Controllati anche attraverso le modalità operative e la temporalità

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CONFINED EMPLOYMENT: SOPs AND TOOLS

Stretta aderenza a protocolli standard operativi
- lavorare su una linea cellulare alla volta
- usare adeguate procedure di flusso operativo
- adeguata separazione di attività concomitanti
- attuare procedure per evitare la contaminazione crociata

→ potenziali vie di contaminazione crociata

Centrifughe
- processare un campione alla volta
- pulire/sanificare la centrifuga tra campioni diversi

Pipettatori
- procedure di pulizia
- filtri

Incubatori

102

CONFINED EMPLOYMENT: EMERGENCY MANAGEMENT

Piano di emergenza	Disposizioni per i casi di emergenza derivanti dal: • trasporto al centro di trattamento • trasporto al sito di trattamento all'interno di centro • manipolazione di OGM contenenti il prodotto nel centro di trattamento • accidentale contatto con la pelle, con gli occhi e punture accidentali • metodi di prevenzione per l'infezione, il trattamento e metodi di prevenzione per le malattie / disponibilità di vaccini
SOP e piano di emergenza	Diversi gli aspetti riguardanti l'uso di un OGM può richiedere SOP o un piano di emergenza, e.g. smaltimento dei rifiuti, o incidenti da punture accidentali. La valutazione del rischio ambientale deve indicare se misure speciali, al di fuori di procedure standard cliniche, devono essere prese affinché i rischi ambientali siano tollerabili
Trattamento dei rifiuti	Può essere necessario utilizzare disinfettanti e successivo trattamento dei rifiuti. Le questioni ambientali che derivano valutazione di rischio ambientale. Dovrebbe anche essere considerata: la disinfezione dei materiali utilizzati per il trasporto, la preparazione o la somministrazione, comprese superfici, strumenti, cappe, abbigliamento, e guanti; l'efficacia dei metodi di disinfezione proposta; le misure adottate a seguito di un rilascio
Monitoraggio post-commercializzazione	Di seguito devono essere considerati anche: • effetti attesi su altri individui; su animali e / o delle piante, in materia di ambiente; • un piano di monitoraggio o motivazioni per non realizzarlo; • istituzione di metodi di monitoraggio per prevenire la diffusione al personale medico o persone per l'ambiente

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SAFETY ASPECTS OF LV VECTORS

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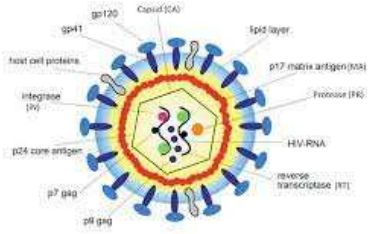
In recent years, **lentiviral vectors** have become commonly used in numerous Italian laboratories, making it crucial to thoroughly understand the origin of these significant biological tools for the delivery of genetic information and the potential biosafety implications, as outlined in Title X of Legislative Decree April 9, 2008, No. 81, and subsequent amendments, as well as Legislative Decree April 12, 2001, No. 206.

The choice of a specific vector is often guided by functional considerations, such as increased efficiency in cellular transduction and gene transfer. However, it is essential to conduct a careful **risk assessment** to ensure the safety not only of those benefiting from therapy but also of the operators handling them.

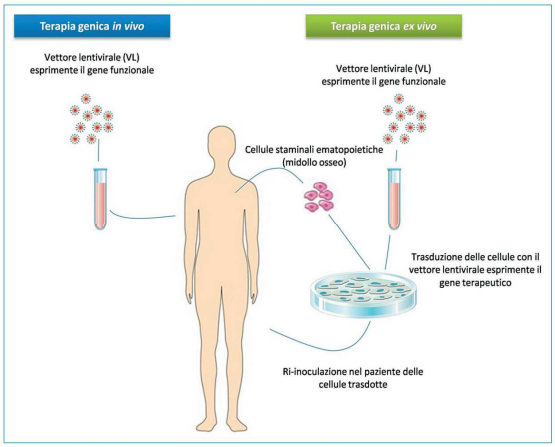
Considerable efforts have indeed been made to develop **vectors that are both more efficient and**, at the same time, **safer**.



105

Thanks to their ability to **stably integrate** into quiescent cells and ensure long-term expression of the transgene, lentiviral vectors (LVs) have become widely used in molecular biology laboratories in recent years.

They serve as gene transfer vehicles in both research and gene therapy **applications**.
In vitro applications include the transduction of eukaryotic cells and the production of recombinant proteins.
In vivo applications involve pre-clinical and clinical development of vectors used in gene therapy for animal models. Additionally, they play a crucial role in developing new delivery systems for the production of next-generation vaccines.



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Main Applications

Gene Therapy

- The treatment of X-linked Adrenoleukodystrophy (ALD), a severe brain disorder caused by demyelination of neuronal cells.

Recently, in Italy, Metachromatic Leukodystrophy (MLD), a hereditary disease due to the incorrect lysosomal accumulation of sulfatide, has also been successfully treated.

Vaccines

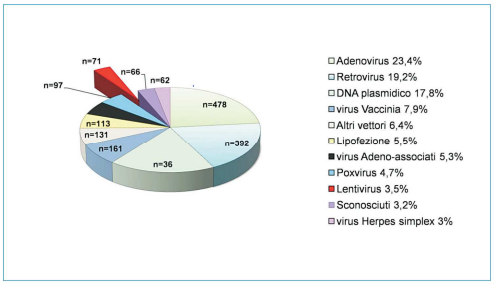
- Defective integrase lentivectors (IDLV), which have a mutation in the integrase gene that renders them unable to integrate into the host genome.

One of the most prevalent is genital herpes, generally caused by Herpes simplex-2 (HSV-2), or in infections from human immunodeficiency virus (HIV-1 and 2).

- Developing vaccines against tumors (Bobisse, 2009; Esslinger, 2003; Iglesias, 2007) and infectious diseases (Dai, 2009; Lemiale, 2010).

Immunotherapy

- Furthermore, there is significant development in the applications of adoptive immunotherapy with lymphocytes targeted against tumors through the **introduction of exogenous T-cell receptors or chimeric receptors for tumor antigens** via viral vectors.



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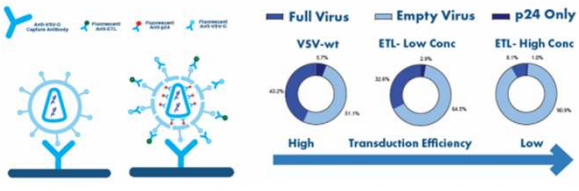
Analysis of Carcinogenic Risks Associated with the Use of Lentiviral Vectors

The use of lentiviral vectors for research and gene therapy purposes is associated with **carcinogenic risks** that require careful assessment, particularly regarding the inserted genes and their potential impact on host cells. This analysis is **crucial** to ensure that the application of lentiviral vectors is safe and free from undesirable oncogenic risks.

Most potential adverse effects attributed to lentiviral vectors are, in fact, common to all retroviral vectors, as **LVs share the same replicative cycle as retroviruses**, based on the integration of the viral genome into the host cell genome.



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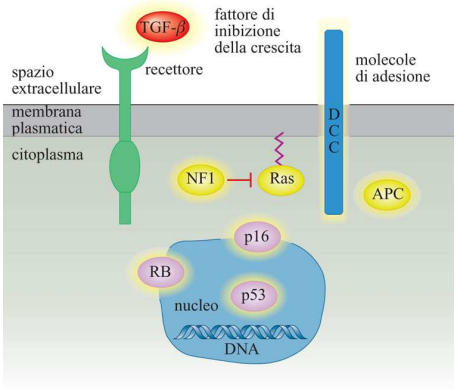
Inserted Genes and Oncogenes

In lentiviral vectors, genes are often inserted to facilitate the transport and expression of the desired genetic material. However, some of these genes may possess oncogenic characteristics or have the potential to cause malignant transformation of host cells.

Careful analysis of the inserted genes is crucial. **The presence of oncogene sequences or elements that could activate cancer-related pathways in host cells should be assessed**.



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Risk Analysis in the Use of Viral Vectors -1

1. One of the main hazards that must be considered is the potential **generation and spread of replication-competent lentiviruses (RCL) particles during vector production**.

Homologous recombination effects

2. Another event common to all retroviruses is the **integration** of viral DNA into the host genome, which is associated with the risk of **insertional mutagenesis and/or transactivation of genes adjacent to the integration site**.

Integration position

3. In several studies, adverse events, including tumor development, have occurred following the **retrovirus integration into the host cell genome**.

Scientific evidence

4. In vivo **genotoxicity studies** on murine models have shown a lower risk of oncogenesis associated with lentiviral vectors compared to first-generation retroviral vectors (Montini, 2009).

Integration position for γ-retroviruses near proto-oncogenes, cancer genes and cellular growth genes

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Risk Analysis in the Use of Viral Vectors -2

5. Lentiviral vectors, on the other hand, tend to **integrate into the body of genes**, and thanks to inactivated LTRs, they have a low tendency to activate genes near the insertion.

6. **The propensity of LVs to integrate into the bodies of transcriptionally active genes can cause genotoxicity in terms of loss of heterozygosity or inactivation of tumor suppressor genes**.

7. In vitro studies also suggest that the **presence of promoter-enhancer** elements with strong transcriptional activity within the vector can still lead to the **long-distance activation of proto-oncogenes near the integration site**, representing a potential transforming mechanism linked to LV integration.

8. Another undesirable event to consider in the use of LVs is the possible **mobilization of the vector and its spread to cells or tissues that were previously not transduced**.

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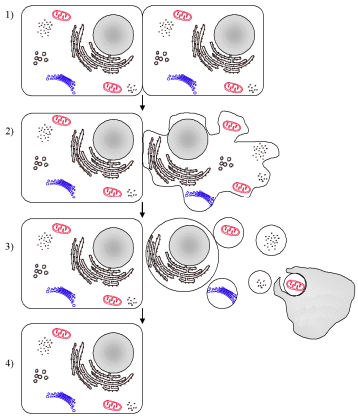
Potential Impact on Host Cells

Once the lentiviral vector has integrated its genetic material into host cells, it is crucial to assess its effect on them.

The possibility of triggering carcinogenic processes, such as uncontrolled proliferation, resistance to programmed cell death (apoptosis), or loss of cell cycle regulation, must be **considered**.

Carefully analyzing the behavior of host cells is essential to identify any signals of malignant transformation and intervene promptly.



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Risk Assessment in Laboratory Activities Involving the Use of Lentiviral Vectors

In addition to identifying intrinsic hazards associated with LVs, risk assessment must consider the handling conditions of the vectors themselves. Typical laboratory procedures include:

1. **Manipulation of cell cultures transduced by LVs**
2. **Handling of LV suspensions**
3. **In vivo experimentation involving laboratory animals inoculated with LV suspensions or LV-transduced cells**
4. **Preparation of the vector itself**

Exposure risk must be identified for each procedure, paying particular attention to the potential accidental contamination of the host during in vivo experiments or the presence of potentially existing endogenous retroviral sequences in unstabilized cell cultures.

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Long-Term Monitoring:

Studies involving lentiviral vectors should include long-term monitoring of treated cells. This monitoring can reveal any changes in the cellular phenotype that might indicate the onset of a potential carcinogenic state. The assessment should extend to multiple cell generations to identify any long-term effects that may emerge after a latency period.

Technological Improvements:

Genetic engineering and lentiviral vector technology are continually evolving. Technological advancements aimed at minimizing carcinogenic risks, such as the use of vectors with self-cleaning elements or the design of inserted genes with low oncogenic potential, are crucial for enhancing safety.

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Summary Chart for the Biosafety Assessment of Lentiviral Vectors and Corresponding Risk Levels

Valutazione della biosicurezza e livelli di rischio		
Valutazione della Biosicurezza	**Elevato**	**Basso**
Disegno del vettore	Vettore e funzioni di *packaging* separate su due vettori	Vettore e funzioni di *packaging* separate in plasmidi multipli
	Espressione di geni virali	Delezione di geni virali
Transgene	Oncogene	Non-oncogene
Produzione del vettore Animale Ospite	Elevate quantità Ospite permissivo Animali inoculati con cellule umane	Quantità di laboratorio Ospite non-permissivo
Manipolazione Animale	Somministrazione del vettore (es. uso di siringhe o materiale tagliente durante l'inoculo)	Stabulazione e allevamento (senza uso di aghi, ecc.)

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Prevention measures un using lentiviral vectors

Risk control related to the use of lentiviral vectors is achieved through the definition and adoption of appropriate preventive measures, such as:

- **Adequate containment levels for the facilities used in the processes**
- **Proper working equipment**
- **Appropriate laboratory behavior standards**
- **Suitable collective and/or individual protection measures to eliminate or reduce the risk of contamination**

Furthermore, the professionalism, training, experience, and common sense of the operator are of fundamental importance.

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Biosafety Containment Levels in the Use of Lentiviral Vectors

Both Biosafety Level 2 (BSL2) and Biosafety Level 2 with extra precautions (**BSL2+**) are considered appropriate for operations using lentiviral vectors that exhibit multiple safety features and involve segregating the vector and packaging functions on four or more plasmids (third-generation vectors and self-inactivating).

BSL2+ containment level includes specific attention to sharps (and where possible, the use of safety needles) and the use of specific protective devices to prevent worker mucosal exposure.

Specific extra precautions include **double gloving, a face shield, and a face mask**, especially for operations conducted outside the biosafety cabinet.

In most guidelines, these containment levels are considered adequate even when working with large volumes (>10 L) of lentiviral vectors from HIV.

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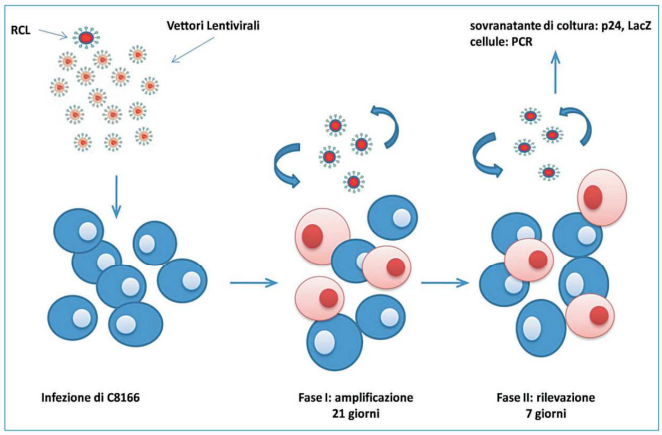
Test for the evaluation of the presence of replication-competent lentiviral vectors (RCL).



Note: La linea cellulare permissiva C8166 viene infettata con il campione di LV da testare. Dopo 21 giorni di coltura (fase I: amplificazione), il sovranatante viene raccolto ed utilizzato per infettare la linea cellulare MAGI (fase II: rilevazione). In seguito, dopo 7 giorni, il terreno di coltura viene analizzato per la presenza dell'antigene p24 e della proteina LacZ, mentre le cellule vengono analizzate per la presenza di retrovirus tramite PCR.

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Test for the evaluation of the mobilization of lentiviral vectors.

For the evaluation of possible mobilization of LVs, an in vitro test utilizing a detection marker, the Marker Rescue Assay (MRA), can be employed.

This test involves the use of the 293 cell line, stably transduced with an integrated copy of a lentiviral vector expressing the NeoR gene, providing resistance to the antibiotic Neomycin.

Cells are infected with the LV preparations to be tested for mobilization, and the supernatant is collected after 24 and 72 hours to be titrated for the transfer of the NeoR marker onto HeLa and 293 cells.

The number of positive colonies will indicate the count of vectors whose genome has been restored and mobilized.

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Considerations in Health Surveillance of Personnel in the Use of Lentiviral Vectors.

The health surveillance of personnel involved in the use of lentiviral vectors is crucial to ensure the safety and well-being of those working in the laboratory.

The potentially hazardous nature of lentiviral vectors necessitates special precautions.
Here are some crucial aspects to consider in health surveillance:

1. Risk Assessment:
Every operator should be aware of the risk assessment before starting work with lentiviral vectors and be informed about good laboratory practices.
The assessment should take into account **training, experience, pre-existing health conditions** (under the supervision of the Occupational Health Physician), as well as preventive measures and protection to be adopted with their corresponding containment levels.

2. Adequate Training
Ensuring that personnel have received comprehensive **training on laboratory safety practices** is essential.
This training should include the safe handling of lentiviral vectors, the use of personal protective equipment (PPE), and emergency procedures.
It is important to train personnel to recognize symptoms potentially related to exposure to lentiviral vectors. This may include symptoms such as fever, general malaise, or specific symptoms related to vector manipulation.

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3. Health Monitoring:
The Occupational Health Physician will assess whether it is necessary to implement regular health monitoring programs in the health surveillance protocol, which may include periodic medical examinations and blood tests.
These checks aim to identify any potential adverse health effects arising from exposure to lentiviral vectors at an early stage.

Antibody Analysis:
Periodic antibody analyses may be necessary to check for potential exposure to the lentiviral vector. The presence of antibodies may indicate a potential infection or exposure, even if asymptomatic.

In the specific context of occupational exposure to lentiviral vectors, there are currently no implemented or under-study plans for biological monitoring of workers to assess this occupational risk.
It is important to emphasize that, for preventive purposes, biological monitoring, through the detection of specific markers, would allow the definition of exposure levels before any adverse health effects manifest.

Post-Exposure Monitoring:
The Health Surveillance protocol should include a specific post-exposure monitoring protocol in the event of accidents or accidental exposure. This should involve immediate medical assessments and appropriate corrective measures.

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4. **Incident Recording and Monitoring:**
Maintain a detailed record of incidents and exposures, investigate each case, and make changes to procedures or safety practices based on root cause analysis.

5**. Ongoing Updates:**
Keep personnel informed of any new information, scientific discoveries, or safety recommendations regarding the use of lentiviral vectors.
Continuous updates are crucial for adapting to best practices and new knowledge.

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GMMs SPECIFIC RISK ASSESSMENT

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Risk Assessment for the GMMs contained use

ANNEX III D.lgs. 12 aprile 2001, n. 206 :
Principle to follow for the contained use

Decree 25 settembre 2001:
Guidelines for the risk assessment

124

User's responsability

Risk assessment

+

Containement measures necessary to control the risk

=

Assessment of the contained use

Assignment of the contained use in one of the 4 classes

Application of the assigned containement level

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User's responsability

Writing of the Risk assessment document

Sending the risk assessment document to the employee

Employee's responsability

Storing the document at the facility
Upon request the document should be available to:
Min. Sanità, Min. Ambiente, Min. Lav. e Prev.Soc., Organi Vigilanza di cui art. 17

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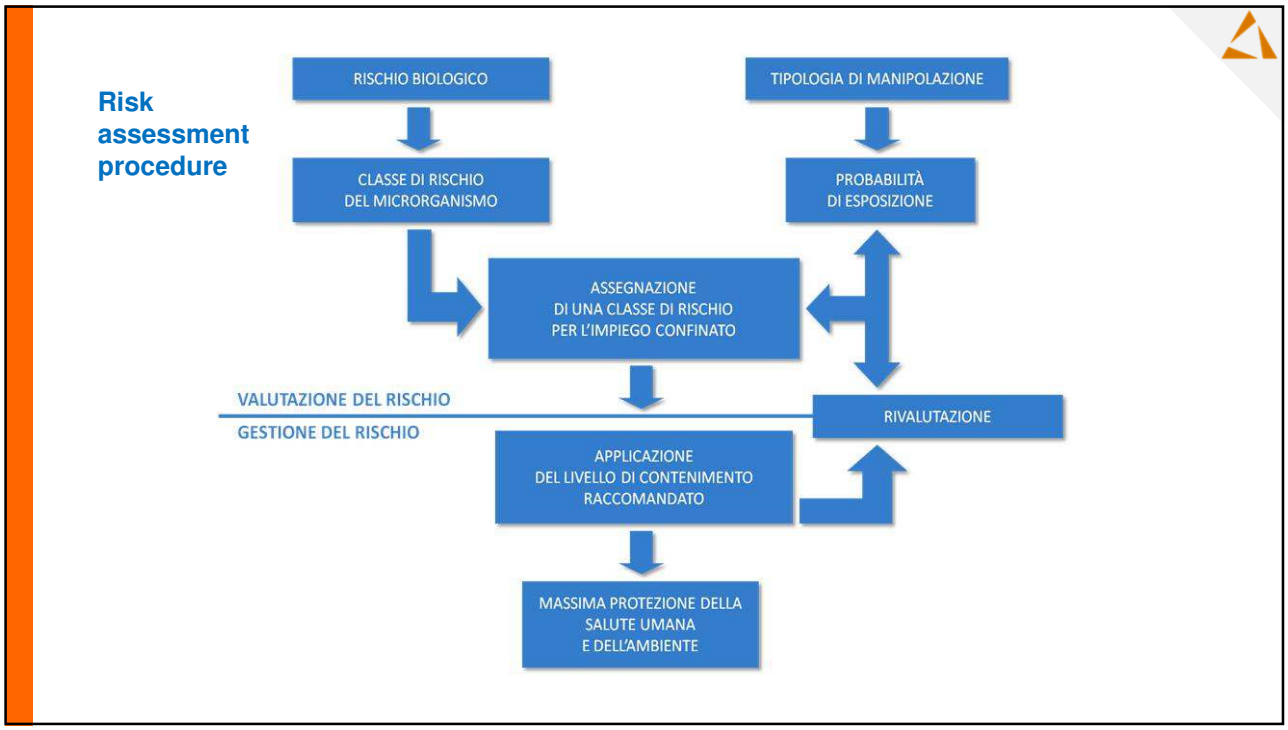
Risk assessment procedure
RISCHIO BIOLOGICO
TIPOLOGIA DI MANIPOLAZIONE
CLASSE DI RISCHIO DEL MICRORGANISMO
PROBABILITÀ DI ESPOSIZIONE
ASSEGNAZIONE DI UNA CLASSE DI RISCHIO PER L'IMPIEGO CONFINATO
VALUTAZIONE DEL RISCHIO
GESTIONE DEL RISCHIO
RIVALUTAZIONE
APPLICAZIONE DEL LIVELLO DI CONTENIMENTO RACCOMANDATO
MASSIMA PROTEZIONE DELLA SALUTE UMANA E DELL'AMBIENTE

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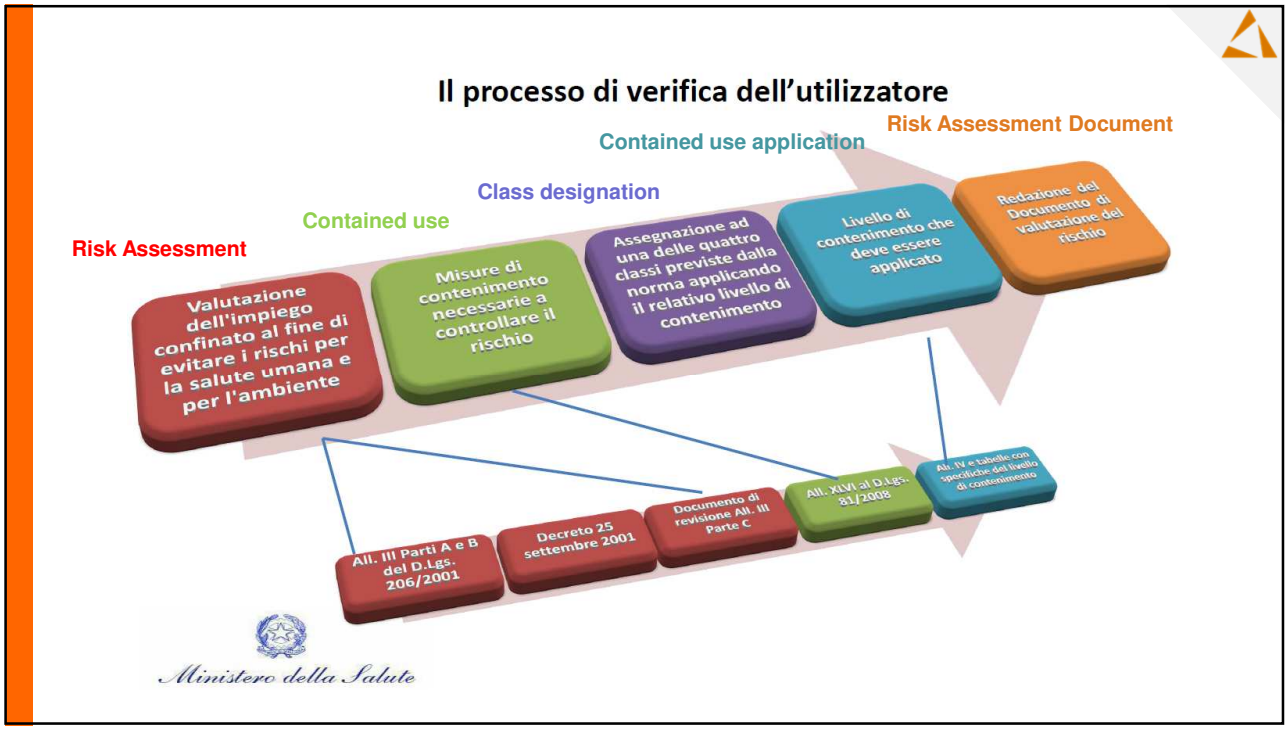
Il processo di verifica dell'utilizzatore
Risk Assessment Document
Contained use application
Class designation
Contained use
Risk Assessment
Valutazione dell'impiego confinato al fine di evitare i rischi per la salute umana e per l'ambiente
Misure di contenimento necessarie a controllare il rischio
Assegnazione ad una delle quattro classi previste dalla norma applicando il relativo livello di contenimento
Livello di contenimento che deve essere applicato
Redazione del Documento di valutazione del rischio
All. III Parti A e B del D.Lgs. 206/2001
Decreto 25 settembre 2001
Documento di revisione All. III Parte C
All. XLVI al D.Lgs. 81/2008
All. IV e tabelle con specifiche del livello di contenimento
Ministero della Salute

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GMMs Activities Classification

Class 1:	activities of no or negligible risk, that is to say activities for which level 1 containment is appropriate to protect human health as well as the environment.
Class 2:	activities of low risk, that is to say activities for which level 2 containment is appropriate to protect human health as well as the environment.
Class 3:	activities of moderate risk, that is to say activities for which level 3 containment is appropriate to protect human health as well as the environment.
Class 4:	activities of high risk, that is to say activities for which level 4 containment is appropriate to protect human health as well as the environment.

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ELEMENTS TO BE CONSIDERED FOR RISK ASSESSMENT

130

According to your knowledge, what are the elements you might consider for the risk assessment

Have you ever tried to make /been involved in the risk assessment

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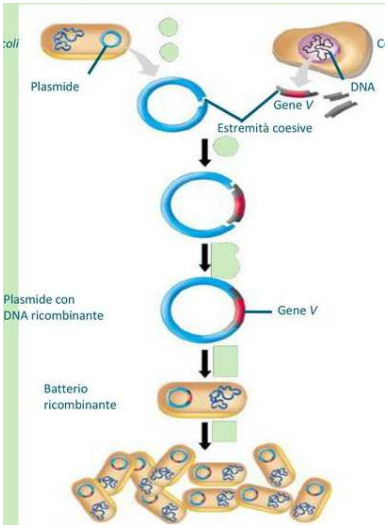
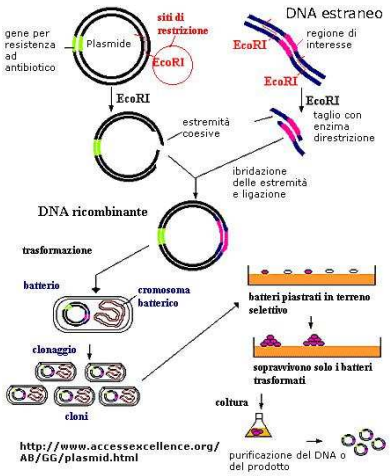
Donor – vector - host





The **risk assessment** associated to the GMMs contained use should be based on the **identification of the effects which are potentially harmful for the human health and for the environment**, associated to the receiving microorganism (host), the transfered genetic material (coming from a donor), the vector, the donor microorganism

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Risk Assessment for the GMMs contained use

ELEMENT TO BE CONSIDERED

The risk assessment should be based on these elements:

a) Identification of all potentially harmful effects of the contained use, in particular those associated with:
- The receiving microorganism
- The transfered genetic material (coming from a donor organism)
- The biovector
- The donor microorganism (in case it is part of the manipulation)
- The deriving GMMs

b) Characteristics of the contained use

c) Gravity of the potentially harmful effects

d) Probability that the potentially harmful effects take place.

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Risk Assessment for the GMMs contained use

Receiving organism (host)

- **pathogenicity** and **virulence**, **infectivity**, **allergenicity**, **toxicity** and possibility to **act as a vector** for pathogens
- the **characteristic** of the **WT vectors** where the risk of a mobilization of the transfered genetic material is present
- **frequency** of mobilization of the transfered genetic material
- **characteristics** and **stability** of the potential disabling mutation
- **previous** genetic modifications;
- **variety** of host microorganism;
- potential **phenotype** which can be altered and the stability of the resulting GMMs;
- natural environment (**habitat**) and the geographic distribution
- being **part** of the natural processes (like: nitrogen fixation or pH regulation)
- **interaction** with other organism present in the environment and their effects on them, including competitivity, pathogenicity or symbiotic processes
- ability to form **survival structures** (like spores or sclerotia)

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Risk Assessment for the GMMs contained use

Donor organism

There should be take in considerations for fusion experiments or experiments where the genetic material is not very well characterized:

- pathogenicity, virulence, infectivity, toxicity and possibility to act as a vector of pathogen agents
- characteristic of the endogen vectors, like:
  - sequence
  - mobilization frequency and specificity
  - presence of genes which confer resistance to antibiotics
- Variety of the receiving hosts.

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Risk Assessment for the GMMs contained use

Insert

- **Identity** and **specific functions** of the insert (genes)
- The **expression level** of the transfered genetic material
- The **origin** of the genetic material, the identity of the donor microorganisms and their characteristics;
- The hystory of **previous** genetic modifications
- The **place of the insert in the host genoma** (possibility of gene activation or disactivation in the host organism following the insertion)

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Risk Assessment for the GMMs contained use

Vector

- Characteristics and origin of the vectors
- Structure and quantity of the vector and/or donor nucleic acid which stays in the final construct of modified microorganism
- Mobilization frequency of the inserted vector (if present in the final GMM) and its ability to transfer the genetic material

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Risk Assessment for the GMMs contained use

Derived GMM

The aspects to be considered in relation to **human health** are the following:

- The estimated **toxic** or **allergenic effects** GMM related, including metabolites
- The **comparison** between the GMM pathogenicity and the pathogenicity of the receiving host or the pathogenicity of the **WT** organism
- The ability to **colonize** new cells
- The possibility that the GMM is **pathogen** for immunocompetent human people
- The **illness** induced by the GMM and the transmission mechanism
- The invasiveness level and the virulence
- The infective **dose**
- The possible changes in the **infection** way
- The possible changes in the **tissue** specificity
- The GMM **survival** possibility outside of a human organism
- The **biological stability**
- The **antibiotic resistance**
- **Allergenicity and toxicity**
- The presence of suitable **therapies** or suitable prophilaxys

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Risk Assessment for the GMMs contained use

Derived GMM

The aspects to be considered in relation to **the environment** are the following:

- The ecosystem **equilibrium** in case of accidentally release outside the contained facility
- The GMM expected **survival capacity**, the **replication rate** and the extension of the release in the identified environment
- The expected consequences related to the **interaction** between the GMM and the organisms or microorganism that enter in contact with the GMM in case of accidental release
- The known effects or the predictable **effects on plants and animal** like pathogenicity, toxicity, allergenicity, pathogen transmission, modification of antibiotic resistance, tropism alteration or modification of the specificity for the hosts, colonization
- The known or predictable **involvement** in biogeochemical processes

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D.lgs. 12th april 2001, n. 206

Similarly to biological agent classification (group 1>> 4) they are ipotized 4 classes of risk associated to the GMM, which corrispond to 4 possible contained uses. They are related to **the risk for human health and for the environment** associated to a particular GMM used.

"The assessment referred to in paragraph 2 shall result in the final classification of the contained uses in four classes applying the procedure set out in Annex III, which will result in the assignment of containment levels in accordance with Article 6:

Class 1: activities of no or negligible risk, that is to say activities for which level 1 containment is appropriate to protect human health as well as the environment.

Class 2: activities of low risk, that is to say activities for which level 2 containment is appropriate to protect human health as well as the environment.

Class 3: activities of moderate risk, that is to say activities for which level 3 containment is appropriate to protect human health as well as the environment.

Class 4: activities of high risk, that is to say activities for which level 4 containment is appropriate to protect human health as well as the environment.

"Where there is doubt as to which class is appropriate for the proposed contained use, the more stringent protective measures shall be applied unless sufficient evidence, in agreement with the competent authority, justifies the application of less stringent measures»

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Assessment of the contained use

In the majority of cases, the containement measures necessary to control the risk and the class of the derived GMM that need to be applied, are the same

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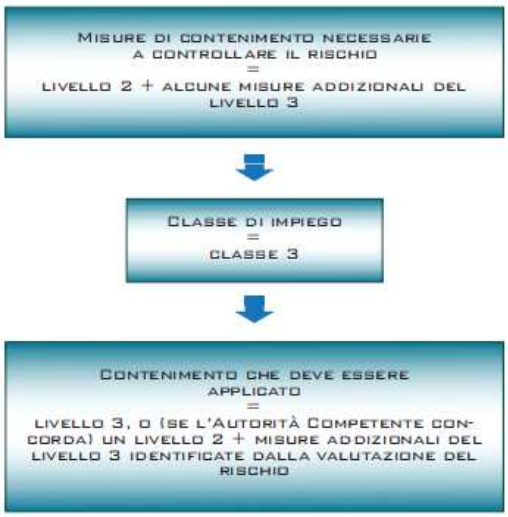
Assessment of the contained use

In some cases the containement measures which are used to control the risk, can be jointed between two different levels



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CHARACTERISTICS OF THE CONTAINED USE

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ANNEX IV : CONTAINEMENT MEASURES AND OTHER PROTECTION MEASURES

LAB ACTIVITIES - Facility

Containment and other protective measures for laboratory activities

	Specifications	Containment levels			
		1	2	3	4
1	Laboratory suite: isolation (1)	Not required	Not required	Required	Required
2	Laboratory: sealable for fumigation	Not required	Not required	Required	Required

(1) Isolation = the facility is separated from other zones of the same building or it is situated in a separate building

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ALLEGATO IV : MISURE DI CONTENIMENTO E ALTRE MISURE DI PROTEZIONE

LAB ACTIVITIES - Equipments

3	Surfaces resistant to water, acids, alkalis, solvents, disinfectants, decontamination agents and easy to clean	Required (bench)	Required (bench)	Required (bench, floor)	Required (bench, floor, ceiling, walls)
4	Entry to lab via airlock (2)	Not required	Not required	Optional	Required
5	Negative pressure relative to the pressure of the immediate environment	Not required	Not required	Required except for (3)	Required
6	Extract and input air from the laboratory should be HEPA-filtered	Not required	Not required	Required (HEPA) (4) – extract air except for (3)	Required (HEPA) (5) – input and extract air
7	Microbiological safety post	Not required	Optional	Required	Required
8	Autoclave	On site	In the building	En suite (6)	In lab = double-ended

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ALLEGATO IV : MISURE DI CONTENIMENTO E ALTRE MISURE DI PROTEZIONE

LAB ACTIVITIES – System of work

9	Restricted access	Not required	Required	Required	Required
10	Biohazard sign on the door	Not required	Required	Required	Required
11	Specific measures to control aerosol dissemination	Not required	Required minimise	Required prevent	Required prevent
13	Shower	Not required	Not required	Optional	Required
14	Protective clothing	Suitable protective clothing	Suitable protective clothing and (optional) footwear	Suitable protective clothing	Complete change of clothing and footwear before entry and exit

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ALLEGATO IV : MISURE DI CONTENIMENTO E ALTRE MISURE DI PROTEZIONE

LAB ACTIVITIES – System of work

Specifications		Containment levels			
		1	2	3	4
15	Gloves	Not required	Optional	Required	Required
18	Efficient vector control (e.g. for rodents and insects)	Optional	Required	Required	Required

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ALLEGATO IV : MISURE DI CONTENIMENTO E ALTRE MISURE DI PROTEZIONE

LAB ACTIVITIES – Waste Management

Waste					
19	Inactivation of GMMs in effluent from hand-washing sinks or drains and showers and similar effluents	Not required	Not required	Optional	Required
20	Inactivation of GMMs in contamined material and waste	Optional	Required	Required	Required

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ALLEGATO IV : MISURE DI CONTENIMENTO E ALTRE MISURE DI PROTEZIONE

LAB ACTIVITIES – Other measures

21	Laboratory to contain its own equipment	Not required	Not required	Optional	Required
23	An observation window or alternative is to be present so that occupants can be seen	Optional	Optional	Optional	Required

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SUMMARY

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JOURNAL OF VIROLOGY, July 2005, p. 8410–8421 Vol. 79, No. 13
0022-538X/05/$08.00+0 doi:10.1128/JVI.79.13.8410–8421.2005

Mobilization and Mechanism of Transcription of Integrated Self-Inactivating Lentiviral Vectors

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Current Gene Therapy, 2009, *9*, 459-474 459

State-of-the-Art Lentiviral Vectors for Research Use: Risk Assessment and Biosafety Recommendations

Katia Pauwels[1,*], Rik Gijsbers[2], Jaan Toelen[2], Axel Schambach[3], Karen Willard-Gallo[4], Céline Verheust[1], Zeger Debyser[2] and Philippe Herman[1]

Table 1. Comparison of Lentiviral Production Systems (*)

	First generation [40]	Second generation [49]	SIN third generation [50, 60] (1)	Lenti-X™ [56] (2)	Translentiviral™ [56] (3)	Super-split system [55]
Number of plasmids	3	3	4	6	6	7
Deletion in the 3'LTR ("self-inactivation")	No	No	Yes	No	Yes	Yes
Number of packaging plasmids containing HIV genes	1	1	2	3	3	6
Accessory genes *vif, vpr, vpu, nef*	All present	All absent	All absent	All absent, except for non functional *vpr* that is fused to coding sequence of Pol and is packed into the particles formed as a fusion protein with RT and IN	All absent, except for non functional *vpr that* is fused to coding sequence of Pol and is packed into the particles formed as a fusion protein with RT and IN	*Vpu* and *nef* are absent, *vpr* is fused to PR and RT/IN-Vif functions which are delivered on separate plasmids
Sequences encoding Tat and Rev protein	Tat and Rev are present on single packaging construct	Tat and Rev are present on single packaging construct	Tat is absent, Rev is expressed from a separate, non-overlapping construct	Tat and Rev are present on a single separate construct	Tat and Rev are present on a single separate construct	Tat and Rev are present on two separate constructs
Overlapping Gag and Pol polyprotein structures	On the same plasmid	On the same plasmid	On the same plasmid	Split over 2 plasmids	Split over 2 plasmids	Split over 3 plasmids
Requirement for RCL formation	2 recombinations	3 recombinations	4 recombinations, between plasmids without homology and pick up of a promoter to complement 'Sin' deletion	4 recombinations, between plasmids without homology, recombination with transfer-vector, repair of point mutations, pick up of a promoter function to allow expression of Tat, Rev, Gag and Pro	4 recombinations, between plasmids without homology, recombination with transfer-vector, repair of point mutations, pick up of a promoter function to allow expression of Tat, Rev, Gag and Pro	more than 4 recombinations

(*) lentiviral expression systems described in this table all have a separate construct expressing Vesicular Stomatitis Virus G glycoprotein (VSV-G) instead of the *env* gene encoding the HIV-1 envelope.
[40] Burns JC *et al.*, 1993, [49] Zufferey R. *et al.*, 1997, [50] Dull *et al.*, 1998, [55] Westerman KA *et al.*, 2007, [56] Kappes *et al.*, 2001,[60] Zufferey *et al.*, 1998.
(1) Commercially available : ViraPower™ from Invitrogen™
(2) Commercially available : Lenti-X™ Expression System from Clontech Laborstories (3) Commercially available : Trans-Lentiviral ™ from Open Biosystems

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Table 2. Biosafety Recommendations and Guidelines Addressing Lentiviral Vector Manipulations

Type of Manipulation	Level of Containment	Additional Measures or Conditions	Reference No.
In vitro			
Manipulation of cells transduced with SIN vectors of third generation	BSL 1	Conditions on the inoculum (see Table 4)	[115,116]
Research using systems with vector packaging functions on more than two plasmids	BSL 2		[119]
Manipulation/production of SIN-vectors devoid of regulatory proteins Vpr, Vpu, Vif and Nef, volume < 200 ml + manipulation of cells transduced with such vector	BSL 2	Gloves, PPE [a]	[114]
Manipulation of cells transduced with non-SIN-vectors or vectors not devoid of regulatory proteins Vpr, Vpu, Vif and Nef	BSL 2	p24 Elisa test is negative	[114]
Research using Lentivirus vector with vector packaging functions on 2 plasmids	BSL 2 enhanced	Attention to sharp tools (use of safety needles), PPE [a] when producing large volumes (> 10L)	[119]
Manipulation/production of non-SIN-vectors or vectors not devoid of regulatory proteins Vpr, Vpu, Vif and Nef or use of vector in volumes > 200 ml	BSL 3		[114]
In vivo			
Housing of animals inoculated with LV with vector packaging functions on more than two plasmids, 1-7 days after inoculation	BSL 1	Animal is not permissive for lentiviral infection, site of inoculation has been cleaned, bedding is changed	[119]
Housing of animals inoculated with i) SIN vector devoid of regulatory proteins Vpr, Vpu, Vif and Nef or ii) cells transduced with this vector	BSL 1	Used vectors show negative P24 Elisa test when transduced in C8166 cells	[114]
Inoculation of animals with i) non- SIN vector or vector not devoid of regulatory proteins Vpr,Vpu, Vif and Nef or ii) cells transduced with this vector	BSL 1	p24 Elisa test is negative	[113]
Inoculation of animals with systems with vector packaging functions on more than two plasmids	BSL 2		[119,see also 118]
Inoculation of animals with i) SIN vector devoid of regulatory proteins Vpr, Vpu, Vif and Nef or ii) cells transduced with this vector	BSL 2	With use of biosafety cabinet type II	[114,see also 118]
Transplantation of transduced cells in primates using SIN 'third generation' lentiviral vectors	BSL 2	Conditions on inoculum dose to minimize presence of free vector particles.	[117]
Inoculation of animals with vector with packaging functions on 2 plasmids	BSL 3	Minimize the risk of autoinoculation	[119]
Animals engrafted with human cells or animals permissive for lentiviral replication	BSL 3	Attention to sharp tools (use of blunt-end needles), PPE [a]	[119]

a : intended to reduce potential for mucosal exposure.
PPE: Personal Protective Equipment
Overview of biosafety recommendations and guidelines specifically addressing the risk assessment of lentiviral vectors. Minimal containment requirements are given for LV for which the nature of the transgene insert poses no additional risks and the production of the vectors occurs on a laboratory scale.

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FACILITY AND USE NOTIFICATION

Do you know the dynamics of the facility and use notification?

Have you ever filled a template?

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Facility and Use Notification

Ministero della Salute

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Notification

- **Facility Notification** → **Employee**
- **Use Notification** → **User** → **+ visto** → **Employee**

→ **Ministry of Health**

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Facility and Use Notifications

The facility notification: the employee shall notificate the risk class of the uses which are intended to be implemented inside the facility, giving evidence of their compliance
Responsability: facility director

The use notification has to be written for each contained use and it should include a accurate evaluation of the use class. It has a temporal deadline. Different levels of authorization are necessary. At the deadline it has to be renewed and updated
Responsability: user (researcher)

IMPORTANT
It is allowed to present to the Ministry of Health a uniq document if:

- The GMMs are referred to a same system vector/host and to a variety of insert or GMMs are referred to a same vector/insert and to a variety of hosts
- The experimental manipulations are implemented in the same facility by the same user (GMMs are produced under the same research project)

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The Facility Notification

The facility notification has to be filled in before writing the use notification, with the proper **template** that is available on the Ministry of Health Web Site

The facility notification contain the **structural information of the facility**, as specified in the part A of the Annex V of the Legislative Decree 206/2001.

It does **not** contain information relative to GMMs, which are part of the use notification and which are mandatory for classes 2, 3 and 4 GMMs. Only for class 1 uses, for which no notification is necessary, the facility notification will contain a summary of the risk assessment and the information regarding the waste management

For all uses, included those of class 1, the risk assessment complete documents must to be **stored** at the facility archive. The employee is responsable of informing the Ministry of Health of all **modification** in the facility information, part of the notification

The facility notification is filled in **once** before the first use, except for updates

Relevant **modification** in the structure of the facility will need to fill a new facility notification.

In case of class 4 uses it is expected (art. 11, commi 6 e 7) a procedure which allows interested **people** to express their opinion in relation to facility authorization

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The Facility Notification

What do you need?

The facility notification MUST be presented to the Ministry of Health attaching the **invoice** of the related fee payment

If you do not attach the **fee** payment invoice, the notification will not be taken into consideration

The fee are due once for the different section of the same facility upon condition of:

- The different section belong to a same department or institute
- The different section should be next each other (structurally and functionally connected)
- The same employee is responsable of all different sections

For the facility dedicated to gene therapy uses in class 1 there is a specific template: «*Modulo di impianto e impiego terapia genica con MOGM appartenenti alla classe di rischio 1*»; for the facility dedicated to gene therapy uses in class 2, 3 e 4 you must fill in the following template: «*Modulo di notifica di impianto destinato ad impieghi di MOGM di classe/i relative*»

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The Facility Notification

How to present the facility notification:

certified E-mail address (PEC): dgprev@postacert.sanita.it

Subject: Facility notification of class # …..

Additional instruction: You have to use a certified E-mail address (PEC) to send the notification

All the communications, included the authorisations, will be mailed by PEC to the address used for sending the facility notification

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The Facility Notification

How much time you need

Facilities dedicated to class 1 uses:
within **45 days** upon receiving the facility notification from the Ministry of Health (silenzio-assenso)

Facilities dedicated to class 2 uses:
within **60 days** upon receiving the facility notification from the Ministry of Health (authorization will be send to employee)

Facilities dedicated to class 3 and 4 uses:
within **90 days** upon receiving the facility notification from the Ministry of Health (authorization will be send to employee)

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The Facility Notification

How much it costs
Fee: 1. research and development facilities: euro 1.316,96 ; 2. industrial facilities: euro 2.917,98
Certificate release: euro 51,65
See «TARIFFE.pdf» on the website

Payment
Money transfer
Payee C/C: Tesoreria Provinciale dello Stato di Viterbo
IBAN: IT30X0760103200000058299009
Object: Notifica di impianto

Payee C/C: Tesoreria Provinciale dello Stato di Roma
IBAN: IT02U0100003245348020258204
Object: "Entrate di pertinenza del Ministero della Salute" Capo XX Capitolo 2582 art.4 per ispezione OGM

Postal
#: c/c postale n. 58299009
Payee C/C: Tesoreria Provinciale dello Stato di Viterbo
Object: Notifica di impianto

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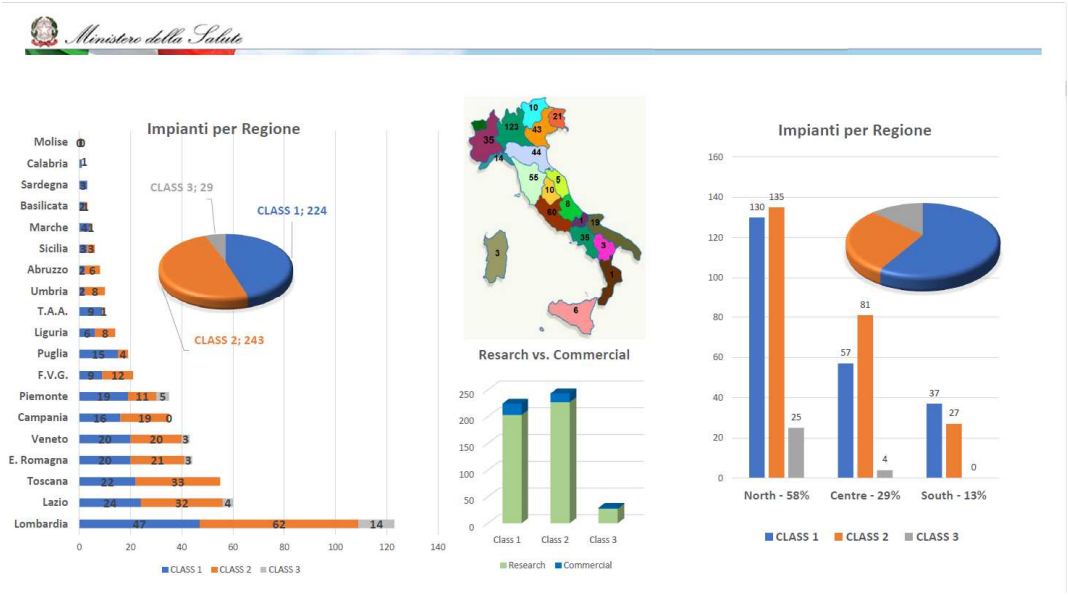
The Facility Notification: authorized facilities in Italy



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Use Notification

The use notification or a specific GMM (indicating the host, the insert and vector used) has to be filled in by the **user**, which is normally the scientific PI or group leader. He is responsable of the contained use.

It can be a researcher who coordinated the research activity with the specified GMM (or similar person for industrial field)

He is responsable of the risk assessment and of the class assignment

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Use Notification

User further duties

For all the time in which the GMM is used in the appropriate containment level, the user should **guarantee** that the containment and protection measures are strictly applied. He/she has to store and archive the notebooks or the files in which are registered the experimental activities with the GMM

From time to time, the user should review (once a year for uses of class 3 and 4; every three years for uses of class 1 and 2) the risk assessment and he/she has to write an **updated** document for the employee and for the Ministry of Health

In case of **accident**, the user is responsable, because he/she is able to evaluate the consequences of an accidental GMM release. He/she should inform in writing and immediatelly the Ministry of Health

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Use Notification

For each class use there is the corrispondent template:

- **Class 1 uses:** No notification is requested if the use is carried out in a authorised facility
- **Class 2 uses**: Template for class 2
- **Class 3 and 4 uses**: Template for class 3 or 4
- **Gene therapy class 2, 3 e 4 uses**: Templates for class 2, 3 o 4 uses

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Use Notification

How to present the use notification (PEC)

Certified E-mail address (PEC): dgprev@postacert.sanita.it

Subject: Uff. 4 DGPREV – Impiego di MOGM di classe (2, 3 o 4, in case of gene therapy: specify)

Additional instructions: All the communications, included the authorisations, will be mailed by PEC to the address used for sending the use notification

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Use Notification

How much time you need

Class 2 uses:
For facilities not notified for class 3 or 4 uses: within **60 days** upon presenting the use notification (silenzio-assenso) or in a shorter time in case of authorization

For facilities notified with a previous notification for uses of upper class and when the expected obligations are respected: class 2 use can be performed after presenting the notification, except the possibility for the user to ask the Ministry of Health for a formal authorization which must be released within 45 days upon sending the use notification

Class 3 and 4 uses:
For facilities notified with a previous notification for uses of class 3 and 4 and when all the expected prescription from the previous authorization are respected for contained uses of the same calss or upper class: within 60 days upon sendinf the use notification

in the other cases**:** within **90 days** upon sending the use notification

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Use Notification

How much it costs

Notification:

1. For research and development purposes: euro 1.316,96
2. For industrial purposes: euro 2.375,70

Certified authorization release: euro 51,65

review:

1. For research and development purposes : euro 1.316,96
2. For industrial purposes : euro 2.349,88

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Use Notification

GMM contained use authorization deadline and renewal

The user at the authorization deadline (maximum 5 years) for a contained use of a specific GMM, has to stop the experimental activities

If the user wants to carry on in the GMM use, he/she has to ask for the authorization renewal to the Ministry of Health in the 6 month before the deadline, giving the following information:

A) If it is the first renewal, in relation to the previous authorization
B) If it is a following renewal:

In the case in which the proposed modifications and/or the use conditions and/or the safety applied measures are no more suitable to allow the contained use, it should be ask for a new authorization

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From time to time (every year for class 3 and 4 uses and every three years for class 1 and 2 uses) the user should review the risk assessment, the containment measures and the protection measures

- To give to the Employee (for class 1 and 2 uses)
- To give to Employee and sent to the Ministry of Health (for class 3 and 4 uses)

The review is expected also in case of:

- Technical and scientific updates
- In case of accident
- Upon justified request of Ministry of Health

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Use Notification

Structure:

I) General Information
II) Information about the host
III) Information about the genetic modification
IV) Information about the insert donor
V) Information about the insert
VI) Information about the GMM
VII) Experimental operation description
VIII) Description of containment measures and protection measures to apply
IX) Summary or the risk assessment in regards of human health
X) Summary Table of the used GMMs

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III) Informazioni sulla modificazione genetica

1) tipo di modificazione genetica:
a) Inserzione di materiale genetico X
b) Delezione di materiale genetico X
c) Sostituzione di basi
d) Fusione cellulare
e) Altro, specificare.....

2) Scopo della modificazione genetica..... Es: Assemblaggio delle particelle lentivirali ricombinanti difettive per replicazione tramite cotrasfezione dei plasmidi X, Y, Z ....della linea cellulare 293T.....

3) Metodo utilizzato per introdurre l'inserto nella cellula ricevente/parentale:

a) Trasformazione X
b) Elettroporazione
c) Microiniezione
d) Microincapsulazione
e) Infezione
f) Altro, specificare .....

4) Se è stato impiegato un vettore nel processo di modificazione:

a) specificare il tipo di vettore (plasmide, virus, cosmide, fasmide ecc.): Vettore plasmidico
b) Identità del vettore....X, Y, Z....
c) Dimensione del vettore (in coppie di basi).....
d) Mappa di restrizione del vettore completa di indicazioni su funzione e posizione di:

i) geni strutturali.....
ii) geni marcatori di resistenza ad antibiotici.....
iii) geni marcatori di resistenza a metalli pesanti..... Allegare mappa di restrizione
iv) geni marcatori d'immunità ai fagi.....
v) altri geni, specificare.....
vi) elementi regolatori.....
vii) siti utilizzati per l'inserzione.....
viii) origine di replicazione.....
ix) origine, funzione e sequenza di altri elementi presenti nel vettore.....

e) Gamma di ospiti del vettore
.....Es: E. Coli – ceppo…..

f) Caratteristiche di mobilità del vettore:.....
i) E' difettivo per fattori di mobilizzazione e trasferimento?.....
ii) Se il vettore è un batteriofago, un cosmide o un fasmide è reso non lisogenico?.....
iii) Il vettore può trasferire marcatori di resistenza ad altri microorganismi?.....
.....Es: Può trasferire resistenza ad antibiotico in batteri.....

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IV) Informazioni riguardanti l'organismo donatore:

1) Nome scientifico Es: Mus musculus
Tassonomia .....
Altri nomi.....Topo domestico

2) L'organismo è patogeno o comunque nocivo in qualche modo (compresi i suoi prodotti extracellulari), vivo o ucciso?

SI NO NON SO

In caso affermativo, specificare:
a) Verso quale organismo:
i) uomo ii) animali iii) piante
b) in quale modo esplica la sua patogenicità?

i) infettività
ii) tossicità
iii) virulenza
iv) allergenicità
v) vettore per altri patogeni
vi) attivazione di virus latenti
vii) capacità di colonizzazione verso altri organismi
viii) altro (specificare).....

c) Le sequenze clonate sono in qualche modo coinvolte nella patogenicità e nocività dell'organismo?

SI NO NON SO

d) Le sequenze clonate sono sufficienti come determinanti di patogenicità?

SI NO NON SO

3) Il donatore ed il ricevente possono scambiare naturalmente materiale genetico?

SI NO NON SO

4) Tipo di materiale genetico utilizzato per il clonaggio:
a) DNA totale.....
b) cDNA totale.....
c) cDNA da tessuti specifici.....
d) altro..Es: regione codificante per proteina di membrana/enzima /recettore/canale.....

5) Funzione prevista per il materiale genetico utilizzato nella modificazione genetica.....
.....Breve descrizione funzione del gene.....

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V) Informazioni sull'inserto

a) Dimensioni dell'inserto, mappa di restrizione e sequenza.....
..Allegare mappe di restrizione e sequenza.....

b) Origine e funzione specifica di ciascuna parte dell'inserto.....
...Es: Promotore inducibile-gene X-IRES-EGFP.....
c) Metodo utilizzato per il clonaggio.....
Breve descrizione
d) Informazioni sui geni strutturali presenti nell'inserto.....
Breve descrizione
e) Informazioni sugli elementi regolatori presenti nell'inserto.....
Breve descrizione.....
f) L'inserto è stato completamente sequenziato? SI NO

g) L'inserto contiene sequenze non necessarie alla funzione desiderata? SI NO
In caso affermativo specificare.....

h) L'inserto contiene parti la cui funzione è sconosciuta? SI NO
In caso affermativo, specificare.....

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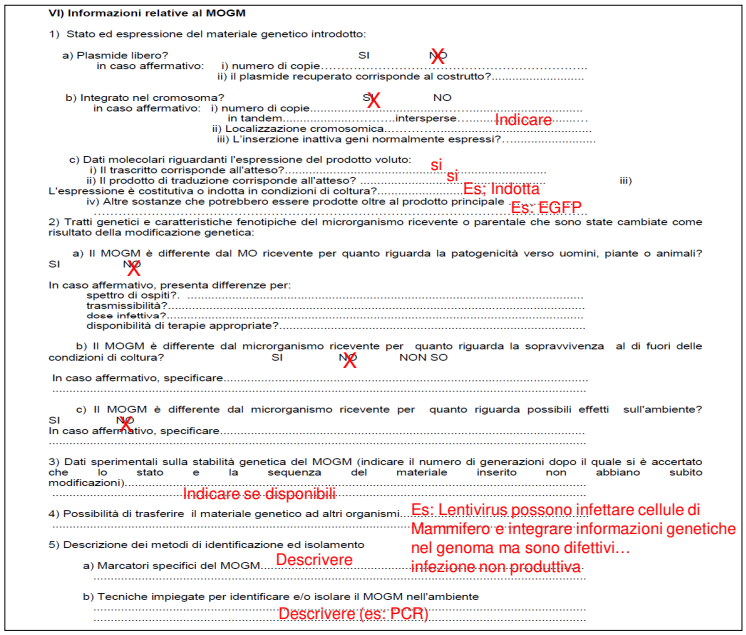

VI) Informazioni relative al MOGM

1) Stato ed espressione del materiale genetico introdotto:

a) Plasmide libero? SI NO X
in caso affermativo: i) numero di copie.....
ii) il plasmide recuperato corrisponde al costrutto?.....

b) Integrato nel cromosoma? SI X NO
in caso affermativo: i) numero di copie.....
in tandem.....intersperse..... Indicare
ii) Localizzazione cromosomica.....
iii) L'inserzione inattiva geni normalmente espressi?.....

c) Dati molecolari riguardanti l'espressione del prodotto voluto:
i) Il trascritto corrisponde all'atteso? si
ii) Il prodotto di traduzione corrisponde all'atteso? si iii)
L'espressione è costitutiva o indotta in condizioni di coltura? Es: Indotta
iv) Altre sostanze che potrebbero essere prodotte oltre al prodotto principale Es: EGFP

2) Tratti genetici e caratteristiche fenotipiche del microrganismo ricevente o parentale che sono state cambiate come risultato della modificazione genetica:

a) Il MOGM è differente dal MO ricevente per quanto riguarda la patogenicità verso uomini, piante o animali?
SI NO X
In caso affermativo, presenta differenze per:
spettro di ospiti?.....
trasmissibilità?.....
dose infettiva?.....
disponibilità di terapie appropriate?.....

b) Il MOGM è differente dal microrganismo ricevente per quanto riguarda la sopravvivenza al di fuori delle condizioni di coltura? SI NO X NON SO
In caso affermativo, specificare.....

c) Il MOGM è differente dal microrganismo ricevente per quanto riguarda possibili effetti sull'ambiente?
SI NO X
In caso affermativo, specificare.....

3) Dati sperimentali sulla stabilità genetica del MOGM (indicare il numero di generazioni dopo il quale si è accertato che lo stato e la sequenza del materiale inserito non abbiano subito modificazioni).....
Indicare se disponibili

4) Possibilità di trasferire il materiale genetico ad altri organismi..... Es: Lentivirus possono infettare cellule di Mammifero e integrare informazioni genetiche nel genoma ma sono difettivi… infezione non produttiva

5) Descrizione dei metodi di identificazione ed isolamento
a) Marcatori specifici del MOGM..... Descrivere
b) Tecniche impiegate per identificare e/o isolare il MOGM nell'ambiente
Descrivere (es: PCR)

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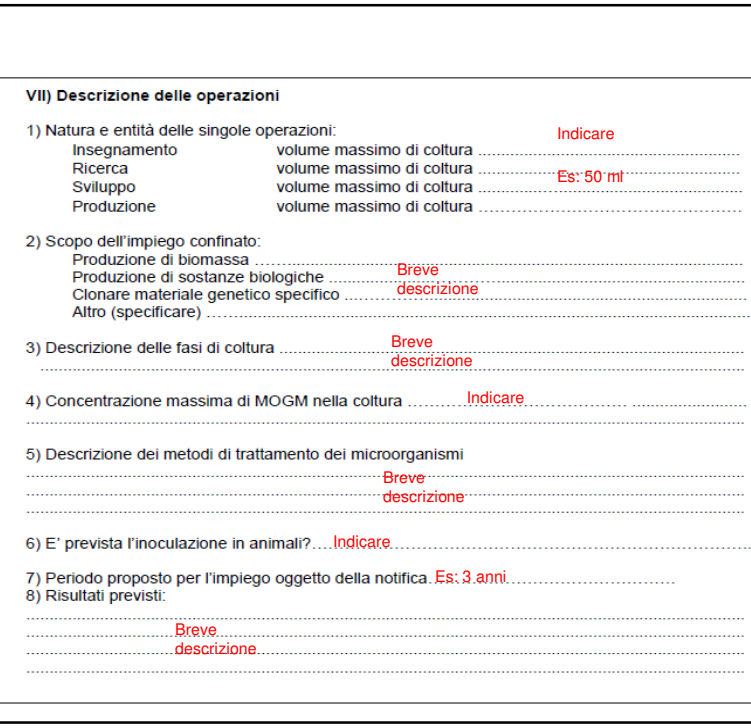

VII) Descrizione delle operazioni

1) Natura e entità delle singole operazioni: Indicare
Insegnamento volume massimo di coltura .....
Ricerca volume massimo di coltura ..... Es: 50 ml
Sviluppo volume massimo di coltura .....
Produzione volume massimo di coltura .....

2) Scopo dell'impiego confinato:
Produzione di biomassa .....
Produzione di sostanze biologiche ..... Breve descrizione
Clonare materiale genetico specifico .....
Altro (specificare) .....

3) Descrizione delle fasi di coltura ..... Breve descrizione

4) Concentrazione massima di MOGM nella coltura ..... Indicare

5) Descrizione dei metodi di trattamento dei microorganismi
..... Breve descrizione

6) E' prevista l'inoculazione in animali?.... Indicare

7) Periodo proposto per l'impiego oggetto della notifica. Es: 3 anni
8) Risultati previsti:
..... Breve descrizione

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VIII) Descrizione delle misure di contenimento e delle altre misure di protezione da adottare per tutta la durata dell'impiego confinato

Allegare la copia della descrizione data al punto D della notifica di impianto relativamente alla/e sezione/i in cui si svolge l'impiego oggetto della presente notifica, con indicazione del livello di contenimento previsto per ciascuno dei vani utilizzati (inclusi eventualmente stabulari, serre ecc.).

1) Adozione di buone pratiche di laboratorio e norme di comportamento:

Breve descrizione (Es: rispetto standard per il livello di contenimento 2)

2) Descrizione delle informazioni fornite al personale addetto:
scritte corsi di aggiornamento periodici
Es: POS Es: annuali

3) Descrizione dei metodi per minimizzare la produzione di aerosol.

Breve descrizione (Es: uso di pipettatori automatici)

4) Procedure e programmi di pulizia, disinfezione, decontaminazione dei locali e delle apparecchiature:

Breve descrizione (Es: uso di Alcool o altri disinfettanti/detergenti

5) Procedure e piani per la verifica dell'efficacia permanente delle misure di contenimento (specificare periodicità):

Es: revisione completa delle procedure ogni 6 mesi

6) Gestione dei rifiuti:

a) rifiuti solidi:
descrizione del metodo di inattivazione degli MOGM: Breve descrizione (Es: uso di ipoclorito di sodio)
destinazione finale: ditta autorizzata altro (descrivere)

b) rifiuti liquidi
descrizione del metodo di inattivazione degli MOGM: Breve descrizione (Es: uso di ipoclorito di sodio)
destinazione finale: ditta autorizzata altro (descrivere)

7) Informazioni necessarie all'autorità competente per valutare i piani di intervento qualora il mancato funzionamento delle misure di contenimento possa comportare rischi per individui al di fuori dell'impianto e/o per l'ambiente

Breve descrizione

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IX) Sintesi della valutazione dei rischi per la salute dell'uomo, degli animali, delle piante e per l'ambiente in generale, conseguenti sia alle normali attività che ad eventi accidentali ipotizzabili (cfr. art. 5 del D. L.vo 206/01).

Valutazione del Rischio per l'impiego confinato di MOGM

X) Tabella sinottica di riepilogo del/dei MOGM che si intende utilizzare:

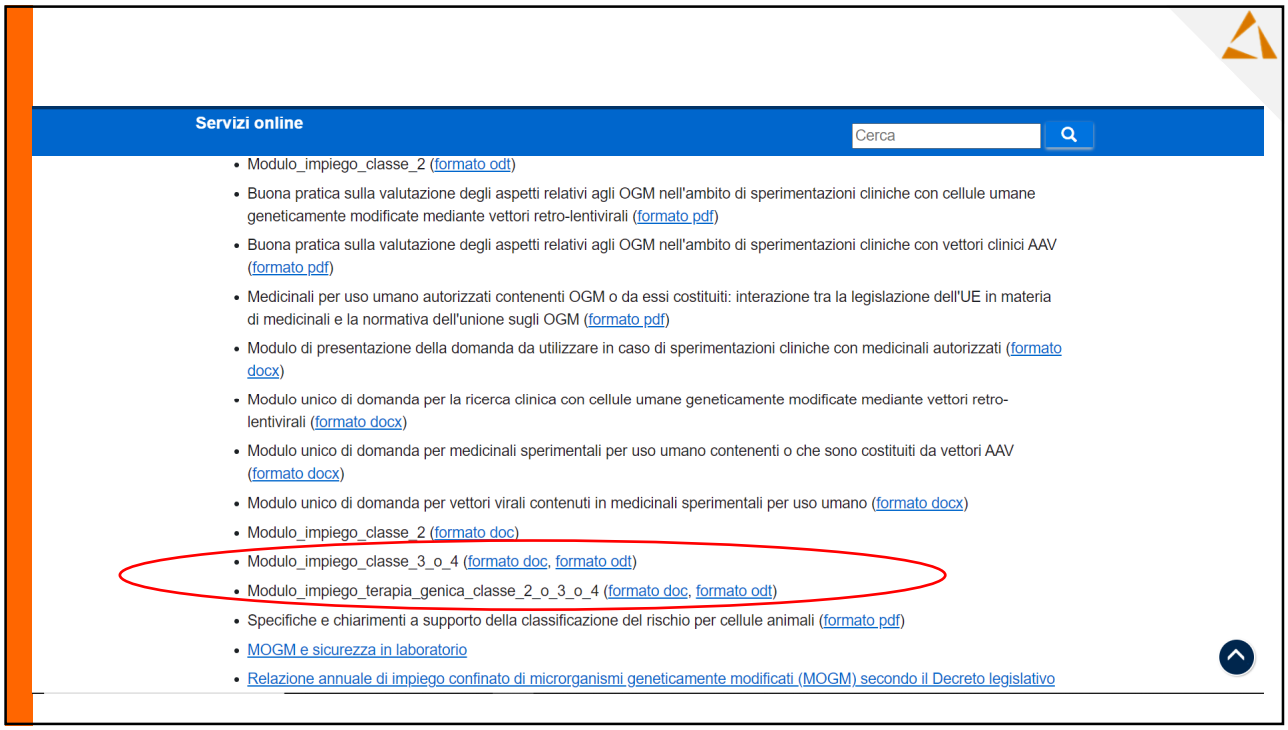
NOME DEL MOGM E PROGETTO	VETTORE	INSERTO	RICEVENTE	CLASSE DI RISCHIO
1.				
2.				
3.				
4.				

Si dichiara contestualmente la conformità alla normativa vigente, sia a livello nazionale che regionale, relativa allo smaltimento dei rifiuti.

Firma dell'utilizzatore

Per presa visione della notifica di impiego: Timbro e firma del titolare dell'impianto

180



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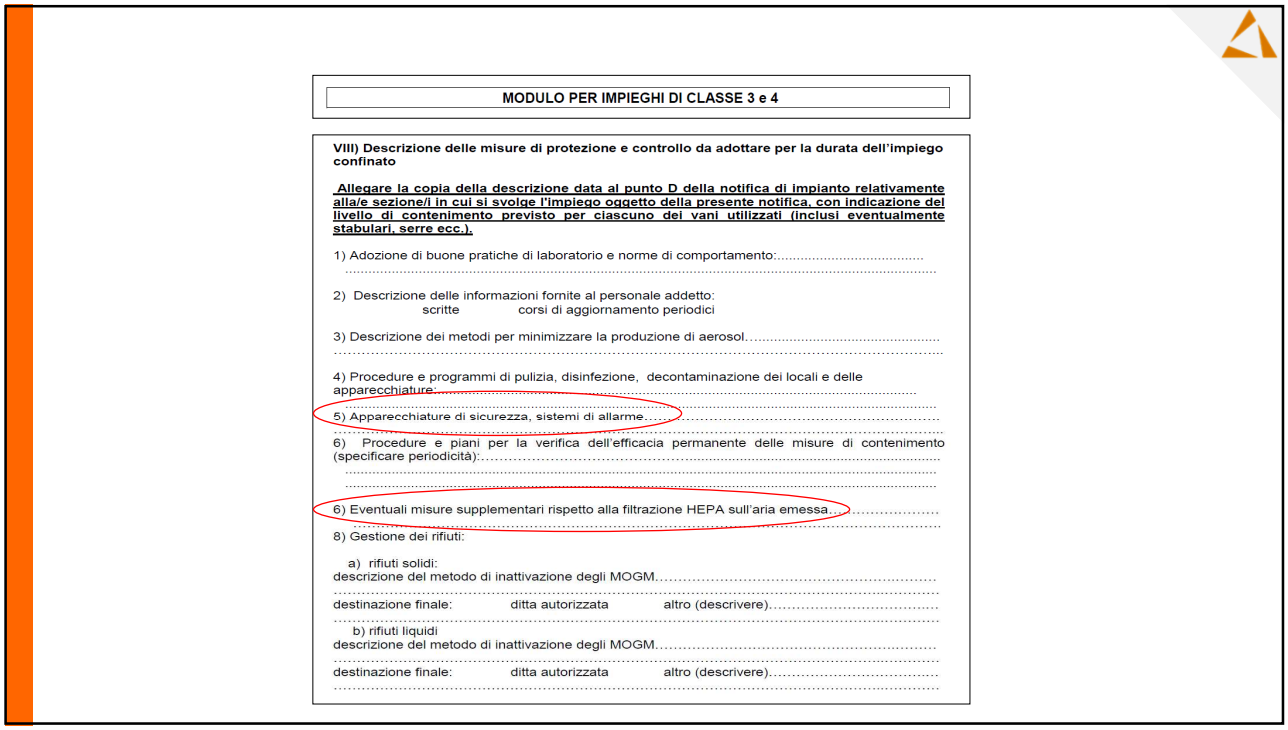

MODULO PER IMPIEGHI DI CLASSE 3 e 4

VIII) Descrizione delle misure di protezione e controllo da adottare per la durata dell'impiego confinato

Allegare la copia della descrizione data al punto D della notifica di impianto relativamente alla/e sezione/i in cui si svolge l'impiego oggetto della presente notifica, con indicazione del livello di contenimento previsto per ciascuno dei vani utilizzati (inclusi eventualmente stabulari, serre ecc.).

1) Adozione di buone pratiche di laboratorio e norme di comportamento:.....

2) Descrizione delle informazioni fornite al personale addetto:
scritte corsi di aggiornamento periodici

3) Descrizione dei metodi per minimizzare la produzione di aerosol.....

4) Procedure e programmi di pulizia, disinfezione, decontaminazione dei locali e delle apparecchiature:.....

5) Apparecchiature di sicurezza, sistemi di allarme.....

6) Procedure e piani per la verifica dell'efficacia permanente delle misure di contenimento (specificare periodicità):.....

6) Eventuali misure supplementari rispetto alla filtrazione HEPA sull'aria emessa.....

8) Gestione dei rifiuti:

a) rifiuti solidi:
descrizione del metodo di inattivazione degli MOGM.....
destinazione finale: ditta autorizzata altro (descrivere).....

b) rifiuti liquidi
descrizione del metodo di inattivazione degli MOGM.....
destinazione finale: ditta autorizzata altro (descrivere).....

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MODULO PER IMPIEGHI DI CLASSE 3 e 4

IX) Prevenzione incidenti

1) Condizioni nelle quali potrebbero verificarsi incidenti (specificare):.....
.....
.....

2) Eventuali pericoli derivanti dall'ubicazione dell'impianto.....
.....

3) Attrezzature di sicurezza presenti (specificare): .....
.....

4) Informazioni necessarie all'autorità competente per valutare i piani di intervento in caso di emergenza, qualora il mancato funzionamento delle misure di contenimento possa comportare rischi per individui ali di fuori dell'impianto e/o per l'ambiente.....
.....
.....
.....

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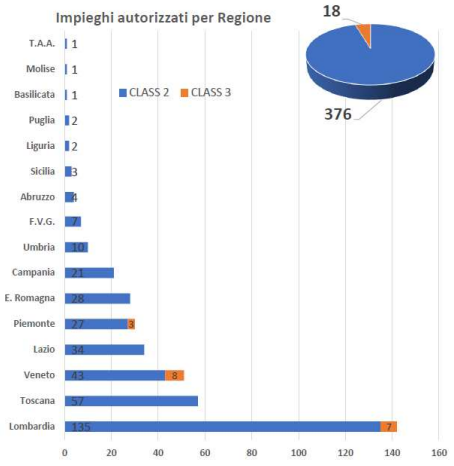
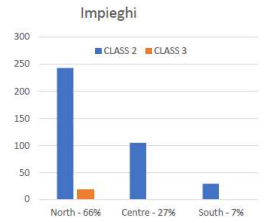
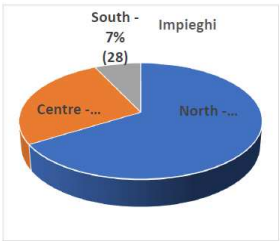
Use Notification in Italy

Ministero della Salute







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Current Legislation

- DECRETO legislativo 206/2001 Attuazione della direttiva 98/81/CE che modifica la direttiva 90/219/CE, concernente l'impiego confinato di microrganismi geneticamente modificati
- DECRETO del Ministero della Salute - 18 dicembre 2007 - Proroga dell'autorizzazione alla produzione di medicinali per terapia genica e cellulare somatica di cui al decreto 5 dicembre 2006.
- DECRETO del Ministero della Salute - 13 gennaio 2006 - Note orientative da integrazione dell'allegato II parte B del decreto legislativo 12 aprile 2001, n. 206.
- DECRETO del Ministero della Salute - 02 marzo 2004 - Istituzione di una banca dati per il monitoraggio della terapia genica e la terapia cellulare somatica. Proroga dell'autorizzazione alla produzione di medicinali per terapia genica e cellulare somatica di cui al decreto 5 dicembre 2006.
- DECRETO del Ministero della Salute - 13 gennaio 2006 - Note orientative da integrazione dell'allegato II parte B del decreto legislativo 12 aprile 2001, n. 206.
- DECRETO del Ministero della Salute - 02 marzo 2004 - Istituzione di una banca dati per il monitoraggio della terapia genica e la terapia cellulare somatica.
- DECRETO del Ministero della Salute - 13 gennaio 2006 - Note orientative da integrazione dell'allegato II parte B del decreto legislativo 12 aprile 2001, n. 206.
- DECRETO del Ministero della Salute - 02 marzo 2004 - Istituzione di una banca dati per il monitoraggio della terapia genica e la terapia cellulare somatica.

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THE WASTE MANAGEMENT IN LABORATORY

Waste management is a very important parte of the lab safety:
It has to be considered a real **PROCESS for**:

- **Risk prevention**
- **Damage protection**

- For laboratory operators
- For people in charge of waste disposal

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LEGISLATION

- **D.Lgs. 81/2008**
- **D.Lgs 206/2001**
- **DPR 254 del 15/7/2003** (if GMMs are inactivated)

- **UNI EN 12641:2000** – Biotecnology – Production processes– Guidelines on waste treatment, inactivation and control
- **UNI EN 12740:2001** – Biotecnology – Research, development and analysis laboratories - Guidelines on waste treatment, inactivation and control

- **D. Lgs 152/2006** (Testo Unico Ambientale – Parte quarta)
- **Normativa ADR** (Trasporto su strada dei rifiuti pericolosi)
- **Elenco Europeo dei Rifiuti** (EER)

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DEFINITION OF WASTE

Any substance or material which is part of categories present in

Annex A (Part IV of D.Lgs. 152/06)

and whose the owner decides or needs to discard

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THE WASTE MANAGEMENT IN THE LAB

The right waste management foresees:

- **Identification** of the waste (special waste)**
- Identification of the **physical** state of the waste (liquid or solid)
- The correct waste **separation**

**material, substance or object, produced or used in teaching activities, research activities, service activities and sanitary activities, for which the current legislation foresees particular methods for collection, storage, transportation and final treatment and disposal

THE INITIAL IDENTIFICATION AND THE LABELLING WITH THE CONSEQUENT CODE SHOULD BE DONE ON THE BASES OF THE MOST RELEVANT RISK

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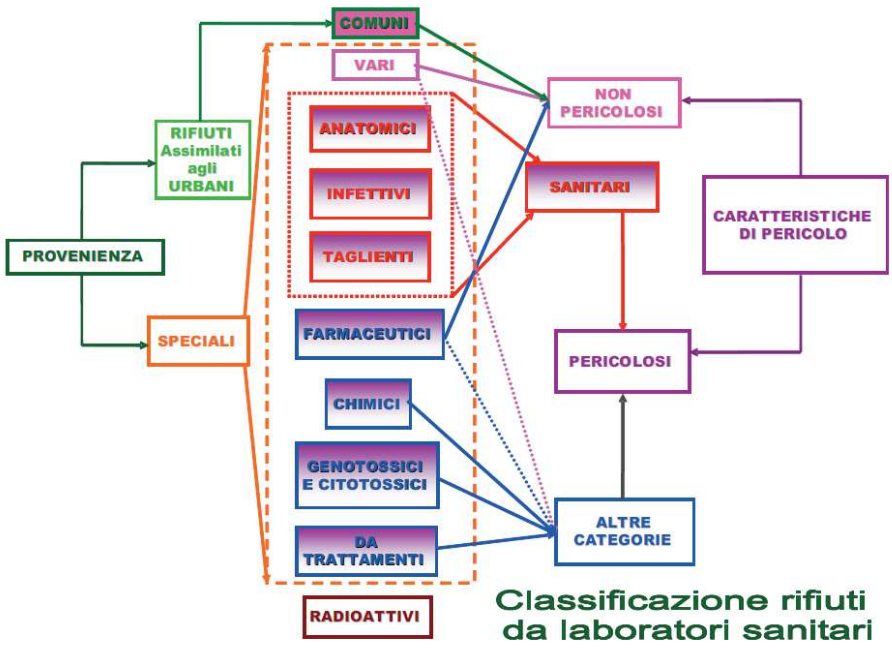
D.Lgs 152/2006
PART IV
Annex D



Classificazione rifiuti da laboratori sanitari

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EER and CER CODING LIST

The European list of wastes (EER) is a catalog which assign the CER codes for the right identification of every waste kind

Valid from 07/10/2020 EER (Vedi http://www.aerecologia.it/cer_rifiuti.htm)

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EER con codici CER settore Sanitario e Ricerca

18	**RIFIUTI PRODOTTI DAL SETTORE SANITARIO E VETERINARIO O DA ATTIVITÀ DI RICERCA COLLEGATE (tranne i rifiuti di cucina e di ristorazione non direttamente provenienti da trattamento terapeutico)**
18 01	**rifiuti dei reparti di maternità e rifiuti legati a diagnosi, trattamento e prevenzione delle malattie negli esseri umani**
180101	oggetti da taglio (eccetto 18 01 03)
180102	parti anatomiche ed organi incluse le sacche per il plasma e le riserve di sangue (tranne 18 01 03)
180103*	rifiuti che devono essere raccolti e smaltiti applicando precauzioni particolari per evitare infezioni
180104	rifiuti che non devono essere raccolti e smaltiti applicando precauzioni particolari per evitare infezioni (es. bende, ingessature, lenzuola, indumenti monouso, assorbenti igienici)
180106*	sostanze chimiche pericolose o contenenti sostanze pericolose
180107	sostanze chimiche diverse da quelle di cui alla voce 18 01 06
180108*	medicinali citotossici e citostatici
180109	medicinali diversi da quelli di cui alla voce 18 01 08
180110*	rifiuti di amalgama prodotti da interventi odontoiatrici
18 02	**rifiuti legati alle attività di ricerca e diagnosi, trattamento e prevenzione delle malattie negli animali**
180201	oggetti da taglio (eccetto 18 02 02)
180202*	rifiuti che devono essere raccolti e smaltiti applicando precauzioni particolari per evitare infezioni
180203	rifiuti che non devono essere raccolti e smaltiti applicando precauzioni particolari per evitare infezioni
180205*	sostanze chimiche pericolose o contenenti sostanze pericolose
180206	sostanze chimiche diverse da quelle di cui alla voce 18 02 05
180207*	medicinali citotossici e citostatici
180208	medicinali diversi da quelli di cui alla voce 18 02 07

torna all' indice CER SMALTIMENTO RIFIUTI

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SHARP WASTE

It is forbidden needle recapping

Needles should be collected in the proper way in order to avoid any release in the environment before incineration.

It is forbiddenn to discard glass Pasteur pipettes, needles and glass slides in the plastic bags; these materials must be discarded in the suitable rigid small container for sharps.

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THE CONTAINER

Factor to be considered in the container choise:

- **Physical** characteristic of the waste (solid-liquid-sharp)
- Methods and procedures for **handling** and **transportation** of the waste (closing-handles-cart)
- **Treatment** methods
- Container **sanification**
- Waste **identification** methods
- Containment **capacity** (adsorbent materials are higly suggested for liquids)

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THE WASTE MANAGEMENT IN CASE OF GMMs

THE WASTE CODING SHOULD BE PERFORMED IN BASE OF THE RELEVANT RISK

In presence of infectious material or GMMs contaminated material for which inactivation by sterilization is foressed, the code should be assigned on the base of relevant risck and it should be notify on the container the sterilization and inactivation need.

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THE WASTE MANAGEMENT IN CASE OF GMMs

Good Practises

Infectious waste should be

- Collected
- Handled
- Temporarly Stores
- Transported

Using a disposal bag with the following label:

- «**Dangerous GMMs waste**»
- «**Biohazard waste symbol**»

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THE WASTE MANAGEMENT IN CASE OF GMMs

Good Practises

The container should be **resistant to impact and other stresses** due to their handling and transportantion and should be easily distinguishable from other kinds of wastes by a color code.

The should be **clearly labelled by a permanent writing**. On the label should be present the **biohazard risk symbol**. The closing system should guarantee the **complete closure** of the container (complete closure, plastic or metal cable ties, thermal sealing).

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THE WASTE MANAGEMENT IN CASE OF GMMs

Good Practises

In case of infectious waste, with high content of liquid and humidity, it is higlhy suggested using the **adsorbent materials** to avoid accidental releases, inside the container, even if the weight is higher. This procedure is foreseed from ADR legislation. The adsorbent material should be present in a quantity able to adsorb the liquid volume. The container should be able to retain liquids even if it is dedicated to solid waste.

The container should be easily **sterilized**.

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THE WASTE MANAGEMENT IN CASE OF GMMs

Good Practises

The container characteristics are defined by **ADR legislation** for the following aspects: color code, materials, shape and dimensions, (Annex X). The ADR legislation give indications also for waste transportation outside the laboratory.

The filling level of the container should not exceed **3/4 of the total volume;** after removing them from the facility, the container should be temporarly stored in a DTR (temporarly waste deposit) from which the company in charge of their disposal can take them.

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THE WASTE MANAGEMENT IN CASE OF GMMs

Good Practises

- Seal and close the container at ¾ of the total volume (do not leave them open for a long time)
- Remove them from the facility and leave them in the DTR for a maximum time of 5 days

DTR requirements

- Biohazard risk symbol
- Separated from the facility
- Easily accessible
- Easily cleanable
- To authorized personnel only

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WASTE TOWARDS CONTAINEMENT LEVELS

D.Lgs 206/2001
All. IV

ATTIVITA'	SPECIFICHE		LIVELLI DI CONTENIMENTO			
			1	2	3	4
Laboratorio, Serre e camere di crescita, Stabulari (Tabelle I a, I b e I c)	8	Autoclave	Nel sito	Nell'edificio	Sul piano (4)	In laboratorio – a doppia entrata
	20	Inattivazione dei MOGM negli effluenti dei lavandini, degli scarichi o delle docce, se presenti, o in effluenti analoghi	Non necessario	Non necessario	Necessario	Necessario
	21	Inattivazione dei MOGM nei materiali e nei rifiuti contaminati	Se necessario	Necessario	Necessario	Necessario
Diverse da quelle di laboratorio (Tabella II)	22	Inattivazione dei MOGM negli effluenti dei lavandini e delle docce o in effluenti analoghi	Non necessario	Non necessario	Se necessario	Necessario
	23	Inattivazione dei MOGM nei materiali e nei rifiuti contaminati compresi gli effluenti di processo prima dello scarico finale	Se necessario	Necessario, con mezzi convalidati	Necessario, con mezzi convalidati	Necessario, con mezzi convalidati
(4) In base a procedure convalidate che consentano il trasferimento sicuro del materiale in un'autoclave al di fuori del laboratorio e che forniscano un livello di protezione equivalente						

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HOW TO INACTIVATE GMMs WASTE

TERMINE	DPR 15/7/2003	UNI EN 12461:2000	UNI EN 12470:2001	NIH guidelines (4/2002)	DHHS CDC – NIH BMBL (2/2007)
Inattivazione		processo usato per raggiungere uno stato libero da microrganismi di processo vitali	distruzione parziale o completa di una data attività fino alla distruzione del sistema microbiologico	qualsiasi processo che distrugge la capacità di uno specifico agente microbiologico o cellula eucariotica di autoreplicarsi	
Decontaminazione			rimozione o riduzione a livelli accettabili della contaminazione microbiologica		rende un'area, un dispositivo, un articolo o un materiale sicuro da manipolare, riducendo il livello di contaminazione microbica in modo da eliminare la trasmissione delle infezioni.
Disinfezione	drastica riduzione della carica microbica effettuata con l'impiego di sostanze disinfettanti	processo atto a ridurre il numero di microrganismi vitali mediante vari metodi fisici e chimici	processo di riduzione del numero di microrganismi vitali mediante vari metodi fisici e chimici	processo attraverso cui agenti microbiologici trasmissibili o cellule eucaristiche sono ridotti ad un livello tale da rendere improbabile l'induzione di malattie in uomini sani, animali o piante	processo meno letale della sterilizzazione che elimina quasi tutti i microrganismi riconosciuti come patogeni, ma non necessariamente tutte le forme microbiche (ad es. spore batteriche) presenti su oggetti inanimati

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HOW TO INACTIVATE GMMs WASTE

Disinfettante		agente chimico in grado di ridurre il numero dei microrganismi vitali			
Sterilizzazione	abbattimento della carica microbica tale da garantire un SAL (Sterility Assurance Level) (*) non inferiore (+) a 10-6 (§)	processo adottato per ottenere lo stato sterile	processo usato per ottenere lo stato sterile		processo per uccidere tutti i microrganismi, incluso un elevato numero di endospore batteriche, non così categoricamente definibile da un punto di vista operativo se non come un processo a seguito del quale la probabilità che un microrganismo sia sopravvissuto su un oggetto sottoposto al trattamento è inferiore ad 1 su 1 milione (10-6) (definizione di SAL*)
Sterile		stato di assenza di microrganismi vitali (1) e (2)	libero da microrganismi vitali (1) e (2)		esente da qualsiasi microrganismo vivente o virus. La definizione è categorica e assoluta

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HOW TO INACTIVATE GMMs WASTE

TERMINE	DPR 15/7/2003	UNI EN 12461:2000	UNI EN 12470:2001	NIH guidelines	
Sterilizzatrici	apparecchiature dedicate esclusivamente alla sterilizzazione dei rifiuti sanitari pericolosi a rischio infettivo (ç)				
Validazione / convalida		procedimento documentato per ottenere registrare ed interpretare i risultati necessari per dimostrare che un processo ottiene costantemente un prodotto conforme a una specifica predeterminate	procedura documentata per ottenere registrare ed interpretare i risultati necessari per dimostrare che un processo fornisce costantemente un prodotto conforme a specifiche predeterminate	dimostrazione dell'efficacia di una procedura sull'organismo che funge da ospite per la propagazione della molecola di DNA ricombinante	

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DISINFECTION

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DISINFECTION

General Principles

- It is different from sterilization
- Different factors influent the efficacy:

1. **Product characteristics** – Range of action and activity
2. **Microorganism characteristics** – microbial load, microbial kind and life cycle phase, resistance
3. **Use** - Concentration, action time, temperature, pH, solvent characteristics, dirty levels and inactivation properties
4. **Organic substance present**
5. **Devices kinds and material conditions to disinfect**

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DISINFECTION

Definitions

- **Germicidal:** agent direct toward pathogens
- **Disinfectant:** germicidal agent which inactivate pathogens but not all microbial types
- **Antiseptic:** chemical germicidal for skin and tissues, it cannnot be used for objects decontamination

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DISINFECTION

Kind of disinfection

First level
Elimination of the majority of bacteria, fungi and viruses (no *Mycobacterium tubercolosis* e spores)

Second level
Elimination of all bacteria (included *M. tubercolosis*), the majority of virus and fungi, not always for spores

Third level
Elimination of allmicroorganism except some spores

** N.B. prions are resistant to sterilization

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DISINFECTION

Chemical agents – Alogens

Cloro: wide antimicrobial range, rapid action, antiviral activity

- Candeggina, Amuchina, Milton

Chemical agents – NH_3

Ammonia salts:

- They inhibit spores, bacteria, mycobacter at low concentrations
- They kill bacteria, virus, fungi at medium concentrations
- Efficacy is reduced by organic material.
- They are good for floor walls and surfaces cleaning

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DISINFECTION

Chemical agents - Alcohols

Ethylic alcohol, isopropilic alcohol: medium range of activity disinfectant.

- They evaporate quicly: the time of contact with the surface and the efficacy are reduced
- Protein coagulation: they cannot be efficace in case of presence of organic material.
- Antiseptic action on skin. They can be used for non critical objects
- Disinfection of law level for the surfaces

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DISINFECTION

Tipo di disinfettante	Attivo contro *)							Interferenza negativa da			Attività, livelli concentrazione e tempo d'azione	Aspetti positivi	Aspetti negativi
	Funghi	Batteri		Microbatteri	Spore	Virus lipidici	Virus non lipidici	Proteine	Acqua dura	Detergenti			
		Gram-positivi	Gram-negativi										
Composti fenolici	XXX	XXX	XXX	XX	-	X	v	+	+	C	intermedia 0,4-3%, rapido	biodegradabili e scarsamente volatili (fenoli sintetici)	maleolenti, irritanti, tossici, inattivabili da materiale organico
Ipocloriti	X	XXX	XXX	XX	XX	X	X	+++	+	C	intermedia 0,5%, rapido	basso costo, fortemente attivi contro l'epatite virale, deodoranti	altamente instabili, corrosivi per i metalli, inattivabili da materiale organico, irritanti e lesivi
Alcoli	-	XXX	XXX	XXX	-	X	v	+	+	-	intermedia 70%, rapido		rapida evaporazione (riduzione tempi contatto), incapacità di penetrare il materiale organico residuo
Formaldeide	XXX	XXX	XXX	XXX	XXX[a]	X	X	+	+	-	alta, 6-8%, non determinato		cancerogena (sconsigliata dal Ministero della Sanità con Circolare n.57/83)
Glutaraldeide	XXX	XXX	XXX	XXX	XXX[b]	X	X	+	+	-	alta - intermedia, variabile (2%) – da 30' a 3h		tossica
Iodofori	XXX	XXX	XXX	XXX	X[c]	X	X	+++	+	A	intermedia		irritanti, si inattivano a T>43°C

XXX: buono — v: dipendente dal virus
XX: adeguato — a: >40°C
X: leggero — b: >20°C
-: nullo — c: su tempi di esposizione lunghi
*) se i dati del fabbricante sono rispettati

+++: molto — C: cationico
++: parzialmente — A: anionico
+: debolmente
-: nullo

Nota – Si richiama l'attenzione su tossicità e/o allergenicità dei disinfettanti e sul loro impatto ambientale

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DISINFECTION

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STERILIZATION

General Principles

SAL – Sterility Assurance Level

A material is defined sterile if SAL is lower than 10^{-6}; when the probability to find a microorganism are less than 1 million

Autoclaves: special device to sterilize infectious material and reagents. The efficacy and the methods are established by the standard UNI 10384/94, I part

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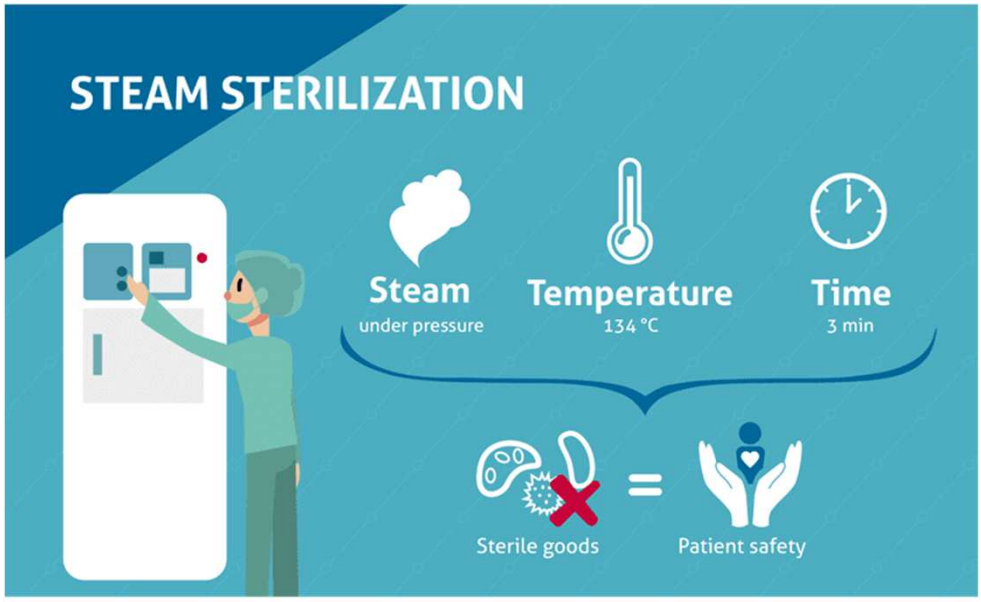
STERILIZATION



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STERILIZATION

- Sterilization cycle should be automatic
- The control system should work in continous
- There should be an alarm system for malfunctions
- The time/temperature conditions should be obtained in the critical point of the autoclave load and in the entire chamber; they should be kept for the requested time and they should be reproducible
- Effluent should be treated
- Sterilization cycle should be validate
- A report should be present and validate

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IN CASE OF EMERGENCY

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EMERGENCY PROCEDURE

In the emergency management we have to consider the possible infection ways:

HIGH RISK EXPOSURE

- Stings or injection by needles
- Ingestion
- Contact with mucous membranes (eye, mouth, nose)
- Contact with skin wounds

LOW RISK EXPOSURE

- Bite from inoculated small animals
- Percutaneous contact with animal fluids
- Aerosol exposure

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EMERGENCY PROCEDURE

FIRST AID

- Skin contact: immediately wash hands with water and soap. Do no use aggressive antiseptic agents
- Skin wound: immediately wah wound with water and soap. Dry the wound
- Contact with eyes, mouth and nose: immediately wash with flowing water for at least 10 minutes
- Clothes contamination: remove the contaminated garments and autoclave them in a appropriate plastic bag

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EMERGENCY PROCEDURE

AREA DECONTAMINATION IN CASE OF ACCIDENTAL RELEASE

In the event of an accidental spill of VL in the work environment the area must be decontaminated using disinfectants. Disinfectants can be used hospital or a 1% sodium hypochlorite solution (for contact of at least 10 minutes) or ethanol. Physical inactivation of small volumes of serum can be done at 56°C for 30 minutes.

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