Biosafety Manual

Handling hazardous biological agents, genetically modified organisms and quarantine organisms

Change log: version 8.0	
Part	Description
-	This edition has been extensively revised and restructured. All content no longer relevant for VU has been deleted. Referenced tools, like forms and checklists, have been removed as annexes and are now available on a Surfdrive site VU_GMO/Q. The document is published in English only. This revision is too extensive to record the changes in this log, but future changes will be listed here.

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Preface

This manual provides guidance on managing the biorisks associated with handling biological agents and toxins that can be hazardous to people and/or the environment, including genetically modified organisms.

The guidance applies to all departments and personnel associated with the VU, which is referred to as “VU” in this document.

This manual was established to ensure that biological agents and materials are used in compliance with the safety, environmental and security policies of the VU within the (inter)national regulatory framework on biosafety and biosecurity. It outlines the organization, regulations, safe handling requirements, and incident reporting and response that together comprise the biorisk management system of VU.

The documents referred to in this manual are listed in **Annex 1** and are available from:

> VU_GMO, a Surfdrive site providing the tools, like forms and checklists, and documents referenced in this manual; the notifications and licenses on contained use of GMO available for the VU, approved risk assessment reports and master lists of hosts, animals, vectors and donorsequences and the tools to register biological agents and animals and to carry out risk assessment for contained use of GMO.

Research group leaders, accountable staff members and biosafety experts get access to the Surfdrive by default. Anybody else who needs access can request it from the coordinating biosafety officer (C-BSO).

Procedures on the use of experimental animals are not included in this manual. Those can be found on the website of the Amsterdam Animal Research Center. Access to this website can be requested from the webmaster.

Links to referenced intranet and internet sites are listed in **Annex 2**. There are many more valuable resources on biorisk management available on the internet, but references have been limited to essential sites to reduce the workload of keeping this annex up to date.

Please contact the coordinating BSO with remarks or questions concerning this manual.

Explanation of abbreviations and terms

Abbreviation	Explanation
AARC	Amsterdam Animal Research Centre, formerly Universitair Proefdier Centrum (UPC)
ABV	Departmental Biosafety Expert or Departmental Biosafety Officer (afdelingsdeskundige bioveiligheid)
ADR	Accord Européen relative au transport international de merchandises Dangereuses par Route
AP	Equipment room (apparatenruimte)
AUMC	Amsterdam Universitair Medische Centra
Bbd	Animal biotechnology decree (Besluit biotechnologie bij dieren)
BSC	Biosafety cabinet
BSO	Biosafety Officer (biologische veiligheidsfunctionaris, BVF)
C-BSO	Coordinating biosafety officer
CCD	Central Committee on Animal Experimentation (Centrale Commissie Dierproeven)
CCMO	Central Committee on Research Involving Human Subjects (Centrale Commissie Mensgebonden Onderzoek)
Cgtb	Dutch Board for the Authorization of Plant Protection Products and Biocides (Commissie toelating gewasbeschermings-middelen en biociden)
CP	Contact person
COGEM	The Netherlands Commission on Genetic Modification (Commissie Genetische Modificatie)
D	Animal housing facility
DEC	Animal Experiment Committee (Dierexperimentencommissie)
DM	Microbiological animal housing facility
ERA	Environmental risk assessment
ESO	Environmental Safety Officer (milieuveiligheidsfunctionaris, MVF)
EU	European Union
FCO	Facility Services of VU (Facilitaire Campus Organisatie)
GMM	Genetically modified microorganism
GMO	Genetically modified organism (genetisch gemodificeerd organisme, GGO)
GMO Decree	Genetically modified organisms decree (Besluit GGO)
GMO Regulation	Genetically modified organisms regulation (Regeling GGO)
HEPA-filter	High Efficiency Particulate Air filter
HRMAM	Human Resource Management Health & Environment of VU
IATA-DGR	Dangerous Goods Regulations of the International Air Transport Association
ICAO-TI	Technical Instructions of the International Civil Aviation Organization
ILT	Inspectorate of the Human Environment and Transport (Inspectie Leefomgeving en Transport)
IVC	Individually ventilated cage
IVD	Animal Welfare Body VU-VUmc (Instantie voor Dierenwelzijn VU-VUmc)
MAT	Mutually agreed terms
MCB	Master Cell Bank
ML	Microbiological laboratory

NVWA	Dutch Food and Materials Authority (Nederlandse Voedsel en Waren Autoriteit)
ODG	Remaining part of contained use of GMO area (Overig deel GGO-gebied)
OECD	Organization for Economic Coorperation and Development
OL	Research Lead (Onderzoeksleider)
Q	Quarantine organisms, like plants, soil and plantmaterial
PG	Risk class of a biological agent (pathogene groep, PG1-4)
PIC	Prior informed consent
PPE	Personal Protective Equipment
RA	Risk assessment and evaluation
RA Report	Risk assessment report on contained use of GMO (verslag risicobeoordeling)
SOP	Standard operating procedure
SZA	Specific hospital waste
TGW	Team Gezond Werken
UN	United Nations
VHP	Vapourized hydrogen peroxide
VLG	Dangerous goods on land transport act (Wet vervoer gevaarlijke goederen over land)
VM	Responsible Investigator (verantwoordelijk medewerker)
VU	Stichting Vrije Universiteit
VUmc	Stichting Vrije Universiteit medisch centrum
WCB	Working Cell Bank
WHO	World Health Organization
Wod	Experiments on animals act (Wet op de dierproeven)

1 Organization of biosafety

1.1 Departments

Health, safety, environment and animal welfare at the VU are supported by:

- ***Faculty of Science, housing department***. Biosafety Officer, Radiation Safety Expert and Working conditions- and Environment Coordinator provide support focused on the health of VU employees.
- ***Facility Campus Organization*** (FCO). This department provides various services such as housing, maintenance and environmental management, waste handling and security.
- ***Human Resource Management Arbo & Milieu (HRMAM).*** This department advises the VU organization on several safety issues such as Occupational Hygene, Fire safety, emergency response, Chemical safety and waste handling.

1.2 Roles at site level

For contained use of GMO activities, the Dutch law implementing EU regulations on GMO usage, the *GMO Decree,* requires the appointment of a **biosafety officer** (BSO) approved by the national authorities (Bureau GGO). The BSO has the task to ensure handling of GMOs in the organization is in compliance with the regulations. VU has appointed a Coordinating BSO (C-BSO) as well as several Biosafety Experts at the departmental level (ABV). It has been arranged that the C-BSO of the VU and the C-BSO of the VU will support each other as needed.

The *Occupational Safety and Health Act* requires organizations to arrange additional support by a certified **safety officer**, an **occupational hygienist**, and a **medical doctor**. At the VU, the safety officer is based at the HRMAM, with the two other experts at the faculty of Science, department of Housing Science)

The *Experiments on Animals Act* requires that an **animal welfare officer** is appointed. VU and VUmc have arranged that this role is fulfilled by a single joint expert, who is part of the IVD.

For use of *Quarantine organisms, imported plant soil or Plants* according to the Fytorichtlijn 2000/29/EG requires the appointment of a **biosafety officer** (BSO) as well. Within the VU, this is the duty to the C-BSO.

Contact information can be found in the institutional phone directory (Vunet).

Essential contacts are listed in table 1-1.

Table 1-1. Institutional contacts on health, safety and environment

Staff	Contact	Phone	Email
Coordinating Biosafety Officer	Marjanne Markerink	06 46705552	m.markerink-van.ittersum@vu.nl
Biosafety Officer	Marleen Schiffler		m.m.w.schiffler@vu.nl
Coordinator environment and health Housing dept. Faculty of Science	Anne Marie Brand	06-15283390	a.m.brand@vu.nl
Radiation Safety Expert, Housing dept. Faculty of Science	Judith Houben	06-21538987	j.h.p.m.houben-weerts@vu.nl
Housing dept. Faculty of Science	Paul Goossens	06-35056188	p.e.goossens@vu.nl
HR, Health, Safety and Environment policy officer	Mart van der Steeg	020-5989032	m.vander.steeg@vu.nl
Environmental Coordinator HRMAM	Peter van Kesteren	020-5989029	p.van.kesteren@vu.nl
Security VU	Security Desk	020-59 85854	Beveiliging.fco@vu.nl
VU/VUmc: Animal Welfare Body Instantie voor Dierenwelzijn)	Secretariat	(020 59)89038	Ivd.amd@vu.nl
HRM Arbo & Milieu	Secretariat	(020 59)89008	arbo-en-milieu.amd@vu.nl
Coordinating Biosafety Officer VUmc	Hans van Driel	06 11263765	j.vandriel1@vumc.nl
VU: Meld- en bedieningscentrum (MBC)	MBC Desk	020-5982222 or 22222	
FCO (voor klachten en tekortkomingen aan gebouw of gebouwgebonden installaties)	Service desk FCO		Servicedesk.fco@vu.nl

1.3 Roles at departmental level

Heads of departments where contained use of GMO activities and/or activities with hazardous biological agents are carried out must assign a **Departmental Biosafety Expert** (ABV). Depending on the circumstances, also an experienced employee or the C-BSO may fulfill this role.

The GMO Decree requires that for contained use of GMO activities that do not require a license (level I or II-k) one or more **research leaders** (OL, onderzoeksleiders) must be appointed who are responsible for overseeing day-to-day operations. OL are appointed in writing by the VU Faculty Managing Directors on behalf of the Board of University. OL are not registered with the national Bureau GGO. For activities requiring a GMO license, one **accountable staff member** (VM, verantwoordelijk medewerker) per license is required who is appointed by the VU Faculty Managing Director on behalf of the Board of the University. VM are registered with Bureau GGO in order to serve as the official contact for the permit.

The aforementioned tasks at departmental level are assigned in consultation with the C-BSO who keeps an institutional register of the persons to which these roles have been assigned. The head of the department is responsible for allocating sufficient time and budget so that the ABVs, OLs and VMs can fulfill the tasks and responsibilities associated with their roles.

The Fytorichtlijn requires that for use of Quarantine organisms an **accountable staff member** (VM, verantwoordelijk medewerker) is appointed. The organization here is quite similar to the roles for contained use of GMO's i.e. the VM is appointed in writing by the VU Faculty Managing Directors on behalf of the Board of University.

1.4 Tasks and responsibilities for activities with GMO

For contained use of GMO as well as for Q the tasks and responsibilities of the OL/VM, ABV and C-BSO are given in table 1-2 on page 13. The references given between brackets refer to the corresponding articles in the GMO Decree and the GMO Regulations.

1.5 Meetings

For contained use of GMO activities, ABV meet periodically to discuss biosafety issues. This meeting is chaired by the C-BSO. When needed, the C-BSO will also initiate meetings with OL and VM.

Table 1-2. Contained use of GMO: Responsibilities and tasks of Responsible Staff Member, ABV and Coordinating BSO.

Tasks / Responsibilities	**Responsible Staff Member** (OL/VM)	**Departmental Biosafety Expert (ABV)**	**Coordinating Biosafety Officer**
Daily management of activities involving GMOs (GMO Regulation, art. 8.2)	Oversees day-to-day operations involving contained use of GMO.	N.A.	N.A.
Writing standard operating procedures and safety instructions (GMO Regulation, art. 9.2e, 9.3)	Manages working experimental protocols concerning contained use of GMO or Q	Manages departmental procedures and instructions concerning contained use of GMO to ensure compliance with regulatory requirements and institutional policy.	Manages institutional procedures and instructions concerning contained use of GMO to ensure compliance with regulatory requirements and institutional policy.
Monitoring compliance of GMO activities with internal policies based on the law (GMO Regulation, art. 9.2a)	Oversees daily activities to ensure compliance with procedures, instructions and experimental protocols.	Carries out inspections to ensure compliance with procedures and instructions.	Carries out audits to verify compliance with regulatory requirements and institutional policy. Ensures that departmental procedures and instructions are adequately implemented.
Maintaining facilities (GMO Regulation, art. 24)	N.A.	Inspects contained facilities and equipment to ensure their proper construction, lay-out and performance.	N.A.
Responding to deviations from rules/ Incidents/ Accidents (GMO Regulation, art. 9.1)	Investigates causes of deviations and implements improvement measures in cooperation with the ABV and C-BSO.	Informs management, OL/VM and C-BSO about deviations, records deviations in the incident registration system and advisors and informs the aforementioned parties on their follow-up.	Escalates incidents according to their (potential) severity, notifies authorities when required, and may impose corrective and/or preventive controls.
Ensuring worker qualification (GMO Regulation, art. 8.4, 9.2d, 10.2c)	Takes care that employee is qualified to carry out the activities. Ensures that employee is informed about the biological risks of their work, and the associated control measures. Supplies mentoring as long as needed.	Manages the departmental biosafety program, ensures timely attendance, and authorizes employees to access contained facilities.	Informs the organization about changes in regulations, institutional procedures, and compliance. Provides instructional material at institutional level, and assesses whether departmental biosafety programs cover the relevant biorisks and control measures (audit item).
Records management (GMO Regulation, art. 10.1, 10.2)	Maintains departmental records according to the task arrangements made at departmental level with respect to the role of OL/VM and ABV.	Maintains departmental records according to the task arrangements made at departmental level.	Maintains administrative data at institutional level.
Performing Risk assessment (GMO Decree, art. 2.5)	Carries out the risk assessment for contained use of GMO activities.	Supports OL/VM in carrying out risk assessment.	Verifies risk assessments, and the associated data on hosts, vectors and donor sequences.
Submitting notifications (GMO Decree, art. 2.15 a.o.)	N.A.	N.A.	Submits notifications to the authorities.

Tasks / Responsibilities	Contact Person	Biosafety Officer
Assess environmental risks (GMO Regulation, art. 27.1.g)	Performs and documents the environmental risk assessment (ERA) on deliberate release of GMO. Keeps the ERA up to date.	Authorizes the ERA and submits it to the competent authority.
Issue procedures and instructions (GMO Regulation, art. 27.1.a, 27.1.g)	Maintains the procedures and work instructions needed to implement the regulatory requirements and any additional requirements stated in the deliberate release permit.	Reviews procedures and work instructions.
Regulatory Compliance (GMO Regulation, art. 27.1.a, 27.1.g, 27.2.a)	Supervises the activity and performs inspections to ensure compliance with procedures and work instructions.	Performs audits to ensure compliance with procedures and work instructions.
Manage deviations (GMO Regulation, art. 27.1.c, 27.1.g, 27.2.b)	Decides appropriate response in case of deviations (like incidents), changes and unforeseen circumstances. Informs and consults the ESO.	Gives guidance on deviation control, evaluates deviation management and assesses whether a deviation must be reported to the authority. Reports deviations to the authority when necessary.
Manage competencies (GMO Regulation, art. 27.1.f, 27.1.g)	Determines the competency requirements necessary to carry out the activity. Ensures that only competent employees are authorized to carry out the work.	Declares employees competent to perform the activity.
Provide safety instructions (GMO Regulation, art. 27.1.d, 27.1.f))	Arranges that relevant employees are informed on the hazards and control measures associated with the activity before they start the work.	Informs relevant employees on the health and environmental hazards associated with the activity and the control measures. Supplies instruction and training addressing these aspects when necessary.
Qualify facilities (GMO Regulation, art. 27.1.g)	Selects facilities that are capable of carrying out the activity according to the procedures and work instructions.	Inspects and approves the facilities where the deliberate release activities will be carried out.
Issue Plan of Activities & Description of Activities	Assembles a Description of Planned Activities (Beschrijving van voorgenomen werkzaamheden, BVW) and a Report of Performed Activities (Verslag van verrichte werkzaamheden, VVW) for each year deliberate release activities are carried out.	Reviews the BVW and the VVW and submits these documents to the authority.
Records management (GMO Regulation, art. 28.1, 28.2.a, 28.2.b)	Maintains the required records and records, except when kept at institutional level by the ESO.	Maintains the required records and records that according to the regulations must be kept at institutional level.

2 The regulatory framework

This chapter gives an overview of the regulatory framework established for activities involving hazardous biological agents and GMO. It describes not only legislation, which in most cases is based on European regulations or directives, but also international conventions and treaties. Detailed requirements imposed by the regulatory framework are described elsewhere in the manual when relevant. Current Dutch legislation is available on the official government website, and European legislation is available on the official EU website (see Annex 2 for the url).

2.1 Handling biological agents hazardous to human health

Protection of personnel against health risks due to activities with biological agents hazardous to humans, e.g. pathogenic or expressing toxins, is regulated by European Directive 2000/54/EEC. In the Netherlands, this directive is implemented by legislation called the Working Conditions Act (Arbeidsomstandighedenwet) and the Working Conditions Decree (Arbeidsomstandighedenbesluit). The scope includes genetically unmodified as well as genetically modified hazardous biological agents (microorganisms, cell cultures, endoparasites, viruses and prions). The national Ministry of Infrastructure and Water Management monitors and enforces compliance with these regulations.

The regulations define four groups of increasing risk, PG1 to PG4 (see chapter 4 Risk assessment). Risk assessment of the activities is required. Four containment levels are described that must be adhered to depending the (highest) risk group of the agent that is handled (see chapter 6 Physical containment). There are administrative requirements such as record keeping (see chapter 5 Administrative record keeping). The regulations do not impose a licensing system, but requires initial or case-by-case notification (see par. 3.1 Use of hazardous biological agents).

2.2 Contained use of genetically modified organisms

The Decree on Genetically Modified Organisms, in short the GMO Decree (Besluit GGO), implements European Directive 2009/41/EC on contained use of genetically modified microorganisms (GMM). The GMO Decree aims to provide protection of the environment and human health by defining risk assessment principles and procedures in order to impose appropriate containment levels for activities involving GMO. The decree has an even wider scope than the EU directive, since it covers creation and use of any genetically modified organism (GMO), not only microorganisms. The GMO Decree adheres to the definitions in the EU directive regarding whether an agent is classified as genetically modified or whether a modified agent is out of the scope of the regulatory framework (see Info Box for more details). The GMO Decree also outlines whether or not a license is needed for a contained use activity; some contained use activities only require official notification of Bureau GGO.

The Regulation on Genetically Modified Organisms, in short the GMO Regulation (Regeling GGO), provides detailed guidance on how to implement the requirements of the GMO Decree. It details the organization of responsibilities (including the appointment and tasks of OL/VM and BSO), administrative requirements, and risk assessment rules (Annex 5 and 8). It also describes for various types of contained facilities (containment categories) so-called containment levels: sets of requirements on construction, lay-out, equipment and working instructions (Annex 5 and 9). The Contained Use of GMO Audit Questionnaire details the requirements imposed by the GMO legislation that are relevant for VU (see GMO_VU at Surdrive).

INFO BOX – When do you create a genetically modified microorganism?
Annexes I and II of EU directive 2009/41 specify what techniques do or do not result in a GMO by definition.

ANNEX I, PART A: GMO by definition
Part A of Annex I declares that use of specific techniques will by definition result in the creation of a GMO:

1 Recombinant nucleic acid techniques involving the formation of new combinations of genetic material by the insertion of nucleic acid molecules produced by any 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.

2 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.

3 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 use of methods that do not occur naturally.

ANNEX I, PART B: No GMO by definition
Annex I, Part B of this directive states that specific techniques, although these do meet the definition of genetic modification technique, by definition do not result in creation of a GMM, as long as no use is made of recombinant-nucleic acid molecules or GMM made by techniques other than those excluded by Annex II, Part A.
1 In vitro fertilisation;

2 Natural processes such as: conjugation, transduction, transformation;

3 Polyploidy induction.

ANNEX II, PART A: Exempted GMO
This part of the annex lists techniques or methods of genetic modification that yield micro-organisms that are excluded from the implementation of the directive on condition that they do not involve the use of recombinant-nucleic acid molecules or GMMs other than those produced by one or more of the techniques/methods listed below:
1 Mutagenesis;

2 Cell fusion (including protoplast fusion) of prokaryotic species that exchange genetic material by known physiological processes;

3 Cell fusion (including protoplast fusion) of cells of any eukaryotic species, including production of hybridomas and plant cell fusions;

4 Self-cloning consisting in the removal of nucleic acid sequences from a cell of an organism which may or may not be followed by reinsertion of all or part of that nucleic acid (or a synthetic equivalent), with or without prior enzymatic or mechanical steps, into cells of the same species or into cells of phylogenetically closely related species which can exchange genetic material by natural physiological processes where the resulting micro-organism is unlikely to cause disease to humans, animals or plants. Self-cloning may include the use of recombinant vectors with an extended history of safe use in the particular micro-organisms.

The Bureau GGO reviews risk assessments, issues GMO permits (GGO-vergunning) on behalf of the ministry and provides a help desk. The Netherlands Committee on Genetic Modification (COGEM) is an independent committee that provides advice to the Ministry of Infrastructure and Water Management, municipalities, provinces, Bureau GGO and the inspection body the Human Environment and Transport (ILT).

ILT carries out compliancy audits and its inspectors are authorized to impose a range of sanctions for violations, like issue non-compliancy warnings, shut down of activities, fines or withdrawal of permits. However, ILT does not inspect whether the building facilities comply with regulations. This is the responsibility of the Environmental Authority North Sea Canal Territory (Omgevingsdienst Noordzeekanaalgebied). This body issues permits under the Environmental Management Act authorizing type and number of work-locations containing GMO activity within an institution, and enforces rules regarding set-up for these places (see par. 2.4).

2.3 Phytosanitary Regulations

Regarding the use of soils and plant material from third countries (i.e. non EU member states) the EU policy Phyto legislation provides detailed guidance to avoid spread, escape and establishment of risk bearing material into the environment. Starting point of the EU regulations is: no import unless the risks must be well contained and therefore the regulations resemble in some points the GMO Regulations. EU directive 2008/61/EC gives Member states the options to make exemptions on the banned for import, traffic and research at materials. To be allowed to handle materials listed in EG 2000/29/EG users must have an import license, which can be obtained at NVWA.

2.4 Use of experimental animals

The use of experimental animals is regulated by the Act on Animal Health and Welfare (Gezondheids- en welzijnswet voor dieren) and the Experiments on Animal Act (Wet op de dierproeven, Wod). The first act sets the general requirements designed to guarantee the health and welfare of animals, including experimental animals. It is based on the principle that use of animals is allowed only for approved purposes. Experimental use is an acknowledged purpose, and the Wod act sets strict criteria for this. The act stipulates that animals shall not be used for experiments unless there is good reason and no reasonable alternatives.

The Wod act also requires that any organization that keeps, breeds or supplies animals for experimental purpose must have a license issued by the Netherlands Food and Consumer Product Safety Authority (Nederlandse Voedsel- en Warenautoriteit, NVWA). Furthermore, an Animal Welfare Body (Instantie voor dierenwelzijn, IVD) with an Animal Welfare Officer (IVD officer, Wod, article 14) must be appointed at each organization that has a NVWA license. The IVD officer assists in submitting new project proposals to the Animal Experiments Committee (Dierexperimentencommissie, DEC) and the national Central Animal Experiments Committee (Centrale Commissie Dierproeven or CCD). The IVD officer also oversees animal welfare, approves experimental work protocols, and assesses compliance with legal requirements. The VU and VUmc have established a shared IVD office.

For each research project that entails the use of animals, a CCD license must be obtained in a process that involves multiple organization. First, researchers must submit a proposal for evaluation to VU/VUmc IVD, which evaluates the technical and experimental aspects of the animal experiment. Upon approval, the request is submitted to the VU/VUmc DEC, which is comprised of a number of affiliated and independent experts on experimental animal well-being and ethics. A DEC must be acknowledged by the CCD and must meet various requirements, one of which is that it must be chaired by a member that is not employed by the organization for which the committee assesses experiments. The DEC determines if the value of the experiment is enough to justify the use of experimental animals, and if the experimental procedures promote using as few animals as possible in a way that minimizes discomfort. Finally, the CCD will decide to issue a license upon a favorable review by the DEC. See paragraph 3.4 for more information on the notification procedures on use of experimental animals.

Finally, experiments must be carried out by trained and accredited scientific staff (Wod, article 9), and the animals must be cared for by trained and accredited staff as well (Wod, article 13.f). Undergraduate students are not eligible for this certification.

2.5 Genetic modification of animals

Genetic modification of animals is not only within the scope of the GMO Decree. The Animals Biotechnology Decree (Besluit biotechnologie bij dieren, Bbd) of the Animal Health and Welfare Act (Gezondheids- en welzijnswet voor dieren) aims to control potential animal health and welfare aspects of (biotechnological) animal experiments. A license must be obtained from the NVWA for the following applications:

- Genetic modification of germ cells, fertilized egg cells or embryos using host-vector systems;
- Introduction of genetic material into an animal, fertilized egg cell or embryo that has been prepared outside of the host by methods including the use of micro-injection and micro-encapsulation;
- Cell fusion or cell hybridization, if this results in an animal with altered genetic traits;
- Cloning using nucleus transplantation;
- Construction of chimeric animals that are capable of transferring the new trait to progeny.

When you need a license, consult the IVD Officer on how to proceed.

2.6 Use of animal by-products

The use of materials derived from animals can pose human and animal health risks. For application of such materials as human food or animal feed, specific legislation is in force. When animal products are used for purposes other than consumption, they are called animal by-products and their derivatives. The Animal Products Decree (Besluit dierlijke producten) and the Animal Products Regulation (Regeling dierlijke producten) regulate the use of animal by-products and derivatives and implement European directives. Directive 1069/2009/EC sets the general framework, while Directive 142/2011/EC gives detailed requirements for implementation. This legislation regulates collection, transport, storage, handling, processing and disposal of animal by-products and products derived from by-products. Cell lines are not considered to be animal by-products because they are living biological agents. The regulations describe three risk categories of animal by-products:

- **Category 1** represents the potentially most hazardous material, e.g. substances that might have a risk of containing prions which can cause transmissible encephalopathy and remains of experimental animals also belong to this category. Substances of this category must be processed using a regulatory approved destruction method, e.g. incineration.
- **Category 2** materials are of intermediate risk. These include manure and content of the gastrointestinal tract, meat unsuitable for consumption, and other by-products that do not belong to category 1 or 3. Manure and bedding originating from experimental animals are examples of category 2 animal by-products.
- **Category 3** materials are considered as having low risk to human and animal health. An example is a product intended for consumption, but used instead for other purposes, e.g. research or diagnostics.

The NVWA keeps a register of organizations that carry out activities with animal by-products and enforces compliance with the laws. Specific operations need approval from the NVWA, e.g. incinerators. For usage of animal by-products for research, diagnostics and education no permit is required, but organizations must obtain approval from the NVWA. Also importing animal by-products into the EU is restricted and it requires an import permit. See chapter 3.5 for more detail on obtaining approval to use animal by-products and chapter 9.5 for additional information on import into the EU. The BVF Platform has issued a guidance document on use of animal by-products for research, diagnosis and education (see Biosafety Resource).

2.7 Control of environmental risks

Any organization where contained use of GMO activities are carried out needs an environmental license according to the Environment Licensing (General Provisions) Act (Wet algemene bepalingen omgevingsrecht). This license determines what containment levels and containment categories the organization (applicant) is allowed to have for contained use of GMO, and where and how many contained facilities are allowed on site. The VU has an environmental permit that was issued on July 13, 2015 and has permit number HZ_WABO-2012-010749. The research facilities of VU in buildings of the VUmc fall within the environmental permit of AUMC, location VUmc. Both permits have been issued by the municipality of Amsterdam. For both permits the Environmental Authority Northsea Canal Territory (Omgevingsdienst Noordzeekanaalgebied) is the inspection body. This body also inspects if the contained use facilities meet the construction and lay-out requirements of annex 9 of the GMO Regulation.

2.8 Shipping biological agents

Hazardous biological agents and GMO, including waste that contains or may contain these biological agents, are considered to be dangerous goods for transport. Transport of such goods is regulated for shipment by air, road, rail, water and mail. Since shipment by air, road and mail are mainly used at the VU to transport biological agents, this handbook addresses these types of transport only. Moreover, only the most relevant requirements are described. Details, including specific exemptions, can be found in a guide (Extern Transport van Biologische Materialen) issued by Biosafety Platform and made available in the Surfdrive folger “GMO-VU”

2.8.1 Shipment by road

In Europe shipment of dangerous goods by road is regulated by a treaty, the Accord Européen relative au transport international de merchandises Dangereuses par Route (ADR). This treaty is based on the *Recommendations on the Transport of Dangerous Goods of the United Nations, and is implemented in The Netherlands as the* Act on Transport of Dangerous Goods by Land (Wet vervoer gevaarlijke goederen over land, VLG). It defines nine hazard classes of dangerous goods and imposes requirements on packaging, labeling, documentation, as well as the construction, equipment, and use of vehicles to transport them. Biological agents are further classified in the ADR according to their infectivity. Category A "is an infectious substance which is carried in a form that, when exposure to it occurs, is capable of causing permanent disability, life-threatening or fatal disease in otherwise healthy humans or animals”. This includes biological agents of Dutch defined risk group 2, 3 and 4 (see par. 4.1) and GMO that must be handled at containment level II or higher. These are classified into ADR hazard class 6.2 and assigned a United Nations identification number:

- Class 6.2, UN 2814, “Infectious substance, affecting humans”;
- Class 6.2, UN 2900, “Infectious substance, affecting animals only”.

Hazardous biological agents that do not meet the definition of Category A are classified as Category B. These agents belong to the following classes and assigned identification number:

- Class 6.2, UN 3373, “Biological Substance, Category B”;
- Class 9, UN 3245, “Genetically modified microorganisms” or “Genetically modified organisms”.

Category B includes all cultures shipped for scientific or diagnostic purposes and diagnostic samples that do not contain biological agents of Category A. Note that containment level I GMO are assigned to ADR substance class 9.
For transportation, it is the ADR substance category (A or B) that determines how the biological agent should be shipped, not the ADR hazard risk class. Organizations that transport dangerous substances by

road need no accreditation, but the driver must have an ADR certificate. However, when packaged in compliance with the ADR rules, a driver shipping substances of UN identification number UN 3373 or UN 3245 does not need an ADR certificate, which eases shipment by VU personnel. UN 3373 substances must be packaged in accordance with instruction P650, UN 3245 substances in accordance with instruction P904.

2.8.2 Shipment by air

Shipment of dangerous goods by air is regulated by the Technical Instructions of the International Civil Aviation Organization (ICAO-TI). These also implement UN guidelines. However, in everyday practice the Dangerous Goods Regulations of the International Air Transport Association (IATA-DGR) are used, because these regulations specify the operational requirements applied by individual aviation companies to implement the ICAO-TI. The IATA-DGR are updated yearly and are available by payment only.

By default, air shipments of dangerous substances must be prepared by a party accredited by ILT. On its website ILT keeps a list of accredited providers (see Annex 2 for url). When not accredited, like VU, one must seek help from such a party. However, when shipping Category B substances UN 3373 or UN 3245, there is no need for an accredited shipper when these substances are packaged in compliance with the IATA-DGR packaging instructions PI 650 or PI 959 respectively.

2.8.3 Postal services

Postal services have their own policies regarding dangerous goods. For most dangerous substances sending by mail is prohibited. PostNL accepts certain diagnostic samples, cultures and GMO, including human blood and blood products. However, sending GMO by mail is not allowed in some countries, while transportation by air mail of dangerous substances is not allowed at all. More detail on shipping of biological agents is given in chapter 9 Transport.

2.9 Use of disinfectants

Most disinfectants are biocides and are by their very nature detrimental to living organisms. While necessary for the control of organisms that are harmful to human and animal health, they pose a risk to humans and the environment. The European Regulation 528/2012 aims to improve functioning of the biocides market while ensuring a high level of protection for humans and the environment. It stipulates that approved biocides should only be used for their proven biocidal effect while limiting environmental damage. The regulation has been implemented in Dutch legislation in the Crop Protection Substances and Biocides Act (Wet gewasbeschermingsmiddelen en biociden). In summary, it requires that a biocide must be approved by the Dutch Board for the Authorization of Plant Protection Products and Biocides (Commissie toelating gewasbeschermingsmiddelen en biociden, Ctgb) before release to the market. An approved product receives an ID (N-number), which must be indicated on the product label. Only registered biocides are allowed on the market and usage must be in accordance with their Regulatory User instructions (wettelijk gebruiksvoorschrift). This instruction states the approved purpose of use (e.g. to disinfect hard surfaces), the types of biological agents for which it is approved (e.g. bacteria excluding spores), dosage, contact time and methods of application. The instruction can also impose requirements on waste handling (e.g. prohibition of discharge into sewage systems). Biocides with labeled user instructions indicating that the product can be used in “spaces meant for people” or “hospitals and other health care organizations and laboratories” are fit to be used at VU laboratories. For use in animal housing facilities, biocide user instructions must include latter phrase or specifically indicate that the product is allowed to be used in animal housing facilities. On the website of the Ctgb, a public database is available to check all approved biocides, one can find labeled user instructions. The NVWA is the governmental agency

responsible for monitoring compliance with this act. At surfdrive a list is made available of approved biocides, used at VU.

2.10 Benefit sharing for use of genetic resources

Terms have been agreed among countries at international level for the conservation and sustainable use of plants, animals and micro-organisms, known as 'genetic resources'. The aim is to create a framework to regulate access to genetic resources and promote the fair and equitable sharing of benefits arising from their utilisation.

The rules are laid down in the Nagoya Protocol and have been incorporated by the European Union into Regulations EU 511/2014 and EU 2015/1866. The Netherlands has committed itself to these rules by its ratification of the Nagoya protocol. Accordingly, each user (institution) must ascertain whether the country of origin of the genetic resource has established rules for access. Users can therefore be required to agree terms with the provider for the fair sharing of benefits arising from the utilisation of the genetic resources or traditional knowledge associated with such genetic resources.

The Netherlands have ratified the UN Convention on Biodiversity. The 2010 Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization (Nagoya Protocol in short) is a supplementary agreement to this convention. It aims to ensure the fair and equitable sharing of benefits that may result from the use of genetic resources with the countries from which these resources originated. In other words, any use of a genetic resource obliges the user to share benefits from that use with the country of origin. The protocol has been implemented in the EU by European Regulation 511/2014. Since a European regulation, unlike a directive, needs no national regulatory implementation, there is no Dutch legislation to refer to. All genetic resources, including traditional knowledge of these genetic resources, obtained after October 12, 2014 are within the scope of the convention, as well as any benefits arising from their utilization. In addition, further genetic or biochemical modification and following application or commercialization are subjected to this regulation.

The Nagoya Protocol has three main principles: access to resources, benefit sharing and measures to maintain compliance. To implement these principles in the Netherlands, the ABS Focal Point of the Netherlands (www.absfocalpoint.nl) provides guidance, including definition of legal terms e.g. prior informed consent (PIC). Grants applications and other requests for funding frequently require proof that genetic resources will be used in compliance with the Nagoya Protocol.

2.11 Biosecurity

Hazardous biological agents, e.g. agents of risk class 3 and 4, available for research purposes can also be misused (dual use), e.g. for bioterrorism. Therefore, their presence requires biosecurity measures to prevent the misuse of agents. In 2007, the Working Party Biosecurity of the Royal Dutch Sciences Academy issued the Code of Conduct on Biosecurity (see Biosafety Resource). Many other organizations, including the World Health Organization (WHO) and the Organization of Economic Cooperation and Development (OECD) have issued guidance on biosecurity. The Bureau Biosecurity is the national information center for the Dutch government and for organizations who work with biological materials classified as high risk. On its website (see Annex 2), many publications on biosecurity are available, including the aforementioned documents. It also provides free of charge the Vulnerability Scan and the Biosecurity Self-scan Toolkit that anyone can use to assess the biosecurity status of an organization. The toolkit addresses eight biosecurity principles: awareness, employees, transport, information, material, incident management, organization and physical access. A short checklist on biosecurity is available in the Biosafety Resource.

3 Notification and licensing

3.1 Use of hazardous biological agents

The Occupational Safety and Health act requires that the ILT is notified of the use of hazardous biological agents in risk group 2 and 3, genetically modified or not. The act requires notification only, there is no permit system. However, agents that are not (yet) included in the European classification system and considered by the user as risk group 3 require individual notification for each agent. Use of each agent with risk group 4 classification also requires individual notification. In all instances, notification is only necessary in case of deliberate use of biological agents, e.g. when culturing, processing or storing them.

Notifications of the use of hazardous biological agents must be submitted online at least 30 days before start of the activity through the website of ILT. Please consult the occupational hygienist of Team Gezond Werken on how to proceed.

3.2 Contained use of genetically modified organisms

The Ministry of Infrastructure and Water Management, through the Bureau GGO, must be notified of all activities involving the contained use of GMO. For activities that require containment level I and for most activities that require containment level II, the notification will not result in a permit. The “permit-free” activities of level II are indicated as “IIk”, while the fraction at this level that requires a permit is indicated as “IIv”. All activities at containment level III and IV do require a permit.

For level I, the notification is only necessary at the start of work involving GMO (initial notification), while for level IIk, notifications must be filed for whenever changes are made (change notification).

The containment level that should be applied for an activity must be determined by risk assessment (see 4.3). Based on the risk assessment data, the BSO will assemble a notification when needed, and submit it on behalf of the Management Council to Bureau GGO.
When the initial notification of activities of containment level I has been done, changed activities within the scope of this notification are allowed to proceed directly after approval of the risk assessment by the BSO. Activities within the scope of a IIk-notification are allowed to proceed when the letter of confirmation of Bureau GGO has been received. Starting new activities that are within the scope of a permit (IIv, III and IV) must wait until the updated permit has been received.

The notification procedure is summarized in figure 3A. Detailed information on notification and permit procedures is available on the website of Bureau GGO (see Annex 2 for url).

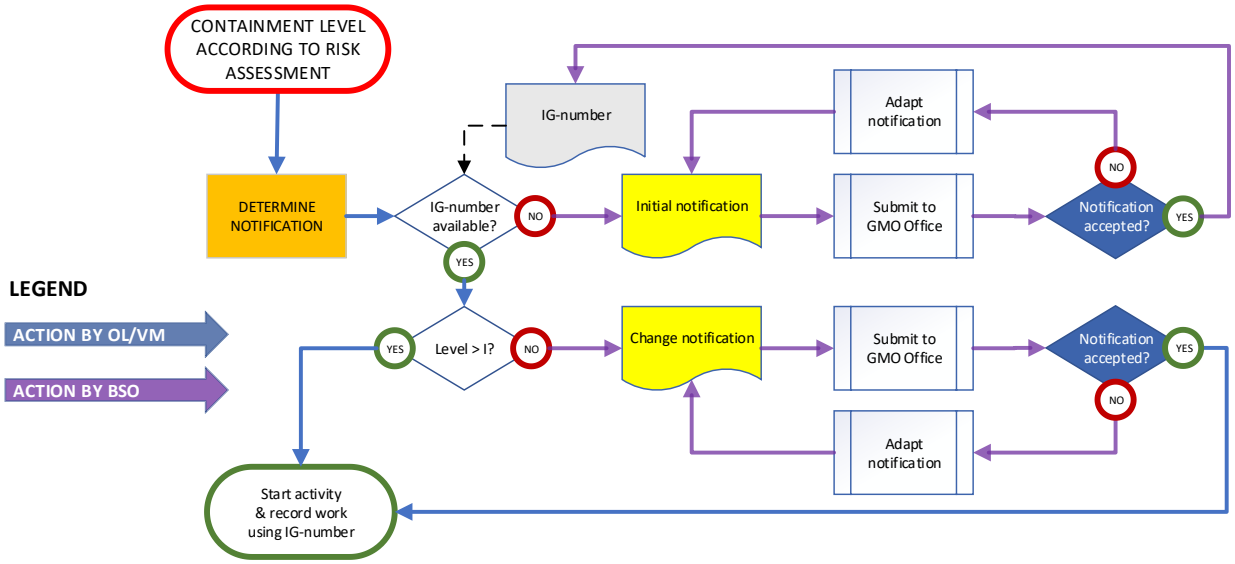


Figure 3A: Procedure at VU to notify contained use of GMO activities. See chapter 4 for the risk assessment procedure preceding this notification procedure.

3.3 Phytosanitairy Regulations

To be implemented.

3.4 Use of experimental animals

For each research project that entails the use of experimental vertebrate animals, a CCD license must be obtained, a process that involves multiple organization. First, researchers must submit a proposal for evaluation to VU/VUmc IVD, which evaluates the technical and experimental aspects of the animal experiment. Upon approval, the request is submitted to the VU/VUmc DEC, which is usually comprised of a number of affiliated and independent experts on animal experiments, well-being and ethics. A DEC must be acknowledged by the CCD and must meet various requirements, one of which is that it must be chaired by a member that is not employed by the organization for which the committee assesses experiments. The DEC determines if the project proposal meet the following criteria:

- There is no realistic option to achieve the aim of the study without the use of animals;
- The number of animals is to be minimized;
- The benefit of the study outweighs the degree of discomfort caused to the animals

When these criteria are met, the DEC advises the CCD on its approval or refusal of the project proposal. When a project is approved, researchers must submit detailed protocols for carrying out the experiments under the project license to the IVD. This body will check whether the experiment fits within the project and meets the animal welfare criteria. If approved, the notification form is signed and returned to the researcher.

Nevertheless, additional approval of the BSO is required when animal experiments create or use genetically modified animals, or use animals in association with genetically modified microorganisms or cells. In this case, the experiment must also meet the requirements for contained use of GMO. A contained use of GMO and risk assessment report must be written to support the proposed containment level and approved by the BSO. At the VU, facilities are available for containment levels D-I, DM-I or DM-II. The experimental activity must also be administrated under the correct contained use GMO license number. For this purpose, the notification procedure to the IVD includes questions about whether or not GMO are involved, which contained use license number applies and at what containment level the experiments must be carried out. These experiments are only allowed to start when the BSO has approved the protocol. Procedures, work instructions and notification forms are available on the internet portal of the Amsterdam Animal Research Centre (AARC, also known as UPC), see Annex 2 for url). Contact the experimental animal coordinator of your department to get access to this portal.

3.5 Use of animal by-products

No license is required for organizations to use animal by-products, but they must be registered at the NVWA. In addition, use of animal by-products for research, diagnostics or education requires permission of the NVWA (the so-called “ODO toestemming”). VU is registered at the NVWA and has obtained permission for usage of all three categories (see 2.6) of animal by-products for research, diagnostics, and education (number **35850**, see “GMO-VU”). The BSO is responsible for its renewal every three years.
VU is also registered as shipper of animal by-products of all three by-product categories. The registration number is **35850**. This registration allows VU personnel to ship materials themselves in addition to using a registered third party.

In case of animal by-products of category 1 and 2, the importing party must possess an import permit from the NVWA, because EU regulation 1029/2009 prohibits import of animal by-products of these categories by default. VU holds a permit to import animal by-products for purposes of research and

diagnostics (see “GMO-VU”). Renewal of this two-year permit is the responsibility of the BSO. See section 9.5 Import of animal by-products into the EU for additional information.

4 Risk assessment

4.1 Risk classification of biological agents

In The Netherlands the classification of biological agents is embedded in the Working Conditions Act, which makes employers responsible for assessing if an employee is at risk when carrying out assigned duties. This act uses the classifications of Annex III of EU directive 2000/54 to establish the risk group of biological agents. This classification takes into account virulence/pathogenicity, contagiousness, transmissibility and availability of treatment. With this approach, four risk groups with four corresponding containment levels are defined (table 4-1). If a pathogenic agent is not listed in Annex II, the user is responsible for its provisional classification.

Table 4-1. Classification of biological agents into four risk groups*.

Classification	**Ability to cause disease in humans** (virulence)	**Hazard to workers**	**Spread to the community** (transmission)	**Availability of effective treatment**
Risk Group 1	Unlikely	No hazard	Not applicable	Not applicable
Risk Group 2	Likely disease	Potential Hazard	Unlikely	Available
Risk Group 3	Likely severe disease	Serious Hazard	Likely	Available
Risk Group 4	Severe disease	Serious hazard	High risk	Not available

* Ref: RIVM Letter Report 205084002/2012, M.R. Klein, Classification of biological agents.

The European classifications of biological agents hazardous to humans or animals is in accordance with the four risk groups as defined by the WHO:

- Risk Group 1: A biological agent which is unlikely to cause illness in humans. In general, activities with agents of this risk group are carried out at the lowest containment level: ML-I, D-I or DM-I if GMO, and BSL1 in case of the use of non-genetically modified agents;
- Risk Group 2: A biological agent that can cause illness in humans and may endanger the health and safety of personnel, but is unlikely to spread in the population and for which an effective prophylaxis or treatment exists. Activities with agents of this risk group should be carried out in at least at containment level 2: ML-II, DM-II if GMO and BSL2 if case of the use of non-genetically modified agents;
- Risk Group 3: A biological agent that may cause severe illness in human and may severely endanger health and safety of personnel, that may spread in the population and for which there is not an effective prophylaxis or treatment. Activities with agents of this risk group should be carried out at least at containment level 3: ML-III, DM-III if GMO and BSL3 in case of the use of non-genetically modified agents;
- Risk Group 4: A biological agent that will cause severe illness in human and will severely endanger health and safety of personnel, that will very likely spread in the population and for which no effective prophylaxis or treatment exists. Activities with agents of this risk group must be carried out at the highest containment level 4: ML-IV, DM-IV in case of GMO and BSL4 in case of the use of non-genetically modified agents. Activities involving agents of risk group 4 are not allowed under the current environmental permit of VU.

The GMO Decree requires risk assessment for activities with GMO that take into account the risks of release into the environment. The GMO Regulation elaborates the risk assessment criteria. Multiple

criteria are taken into account, including the risk group of the hosts, vectors and donors. Depending on the outcome of the risk assessment, an approriate containment level must be assigned to control the risks. The hosts (bacteria, viruses, fungi and parasites) and vectors listed in Annex 2 of the regulation are considered low risk and are therefore allowed to be used at the lowest containment level when not applied in combination with inserts coding for hazardous gene products. In Annex 4, the GMO Regulation presents a classification of hazardous biological agents that do not qualify for containment level I by default.

Still other regulatory lists of biological agents do exist, e.g. a list of animal pathogenic organisms according to the Activities Regulation on Environmental Management (Activiteitenregeling milieubeheer), a list of biological agents that are considered to be so-called dual use goods, and a list of quarantined plant pathogens. All lists mentioned are available on the website of Bureau Biosecurity (see Annex 2 for url), including a combined list providing classification in terms of biosafety and biosecurity.

4.2 Working with hazardous biological agents

In the Netherlands, employers are responsible for assessing if personnel are at risk in carrying out assigned duties. Deliberate use of hazardous biological agents is considered an activity that requires a risk assessment (RA), regardless of whether GMO are involved. If an employee is at risk of being exposed to biological agents, a general RA and evaluation (risico-inventarisatie en –evaluatie) must be made to assess the nature, extent and duration of exposure in order to determine the hazard for the employee. If activities are considered to be at elevated levels of risk, an in-depth RA is required. At VU, the RA are carried out by the occupational hygienists of the Beta Housing Department. The RA reports are available at the individual departments. The RA reports indicate the activities that are carried out with biological agents, the potential hazards that are associated with the agents and the measures to control them. The RA must evaluate whether these control measures are sufficient and, if not, a Plan of Action for improvement must be included.

4.3 Contained use of GMO

The GMO Decree requires that the containment level that will be applied must be underpinned by a risk assessment (RA) and that risk assessment reports are reviewed once every five years.

No RA report is required for activities using this combination: hosts included in List 1 of Annex 2, vectors included in List 2 of Annex 2, and donor sequences that do not meet the criteria listed in List 3 of Annex 2 of the GMO Regulation.

The RA procedure of VU is outlined in Figure 4A. It involves two steps:

- **STEP 1: Document agents.** Check if your hosts, vectors and donorsequences are included in the Master Lists that can be consulted in the GMO-VU folder. There are Master Lists for microorganisms, cells, animals, vectors and donor sequences. If not listed yet, record them in the corresponding Recording Table that is available on this sharepoint also. Whenever possible, each agent should be assigned to a group of an equal risk profile (risk profile group). Recording the agents is the responsibility of the OL/VM. The C-BSO will move data to the corresponding Master List after positive review.
- **STEP 2**: **Determine and underpin the containment level**. The containment level for an activity must be determined using the criteria given in Annex 5 of the GMO Regulation. The criteria that are applied must be justified in a risk assessment report. Report forms in pdf-

format are available on the website of the Bureau GGO. Each containment level has a dedicated form.
Approved RA reports are available in the GMO-VU folder. To keep oversight, the C-BSO also maintains a table of approved RA reports. If no appropriate RA report is available, the OL/VM should write a new one or adapt an existing report and submit it for review to the C-BSO. The C-BSO will review new or adapted RA Reports and will include approved reports in the Master RA List.

The containment level also determines the procedure that has to be followed in order to apply for GMO activities. Therefore, when the RA has been finioshed, always check if an initial or change notifification has to be submitted (see par. 3.2).

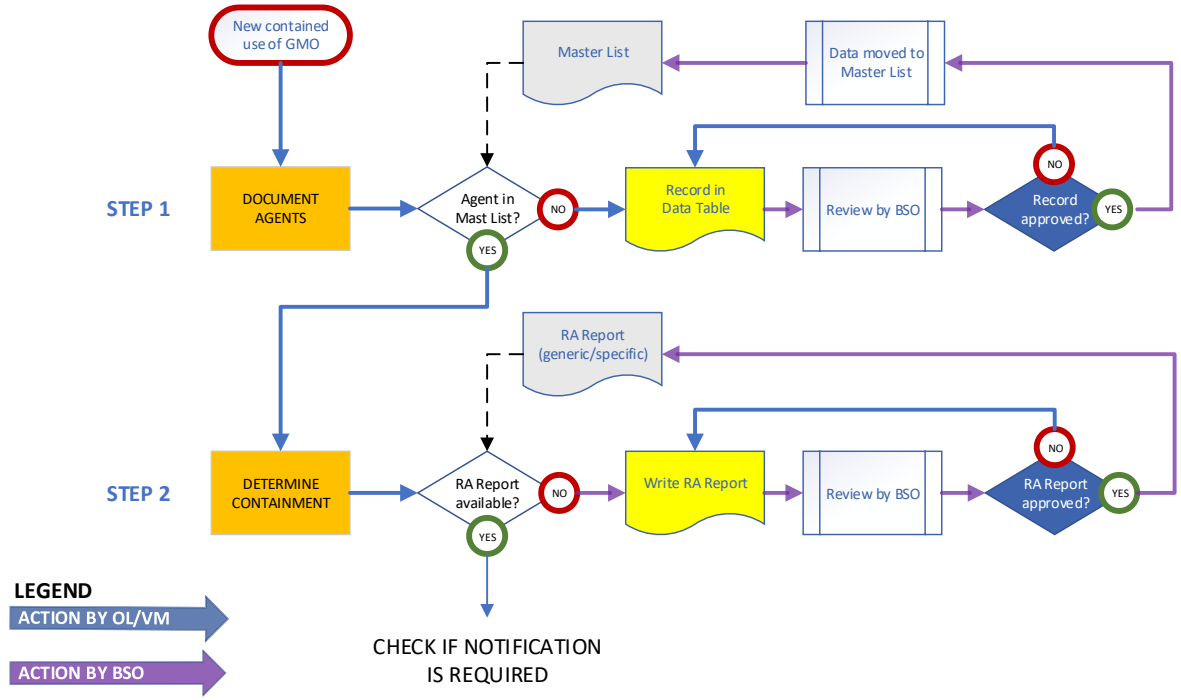


Figure 4A: Contained use of GMO risk assessment procedure at VU.

4.4 Q organisms

containment level also For Quaratine organisms,

5 Administrative Records Keeping

5.1 Use of hazardous biological agents

For activities with hazardous biological agents, the Occupational Safety and Health Decree imposes specific administrative requirements. These are listed in table 5-1. The records must be kept by each department.

Table 5-1: Administrative requirements according to the *Occupational Safety and Health Decree* for use of hazardous biological agents

Requirement	**Reference to articles *Occupational Safety and Health Decree***	**Remarks**
Specific risk assessment	4.85	Assessment of potential exposure of personnel to hazardous biological agents.
List of employees that can be exposed to biological agents of risk group 3 or 4	4.90	For each employee the register must include the performed activities involving biological agents to which he/she has been exposed due to regular activity or by accident. The register must be kept available for a period of at least 10 years after the last known or possible exposure and up to 40 years in specific situations*.
Results of work-related individual employee health surveys	4.91	Results must be kept available for a period of at least 10 years after the last known or possible exposure and up to 40 years in specific situations*.

* In case of long lasting, recurring or latent infections, infections that may occur more than 10 years after exposure, or that may induce serious long-term complications, the records must be kept longer than 10 years, but not longer than 40 years.

5.2 Contained use of GMO

5.2.1 Institutional and departmental administrative records

The administrative requirements according to the GMO Regulation for contained use of GMO activities are listed in table 5-2.

These requirements are also listed in detail in the Audit Questionnaire on contained use of GMO (see Biosafety Resource).

The centralized GMO administrative record keeping is structured as follows:

- **GMO_VU**, a shared Suf drive folder contains lists of biological agents, risk assessment reports, notifications, permits and registers. Furthermore documents referenced in this manual.
- **Incident registration system**, an intranet application with incident records, including biosafety incidents.

The local GMO record keeping must be kept at departmental level and should contain at the very least the required features that are not included in the central GMO administrative record keeping. A critical feature of the local GMO administrative record keeping is the inventory of GMO. The GMO Regulation specifies that the following data on GMO must be recorded:

- hosts, including the names of the GMO derived from them;
- vectors, preferably as vector map with legend stating name, total size in base pairs and name, size, function, source and relative position of the constituting parts;
- contained use notification number (IG-number);
- concise description of construction activity;
- date of execution;
- name of researcher and of OL/VM.

Departments must also keep inventories of stored GMO that meet these criteria. However, regarding the temporary storage of GMO that are under development, construction and application records in laboratory journals are also sufficient.

Table 5-2 Administrative requirements for contained use of GMO activities.

INSTITUTIONAL ADMINISTRATIVE RECORD KEEPING		**Regulatory reference**
Safety procedures	Inventory of safety procedures & instructions	GMO Regulation, art. 10.1a
Notifications & licenses	Inventory and archive of active & closed notifications and licenses	GMO Regulation, art. 10.1b, 10.1c, 10.1.d
Risk assessment reports	Archive locations of risk assessment reports	GMO Regulation, art. 10.1e
	BSO-approved risk assessment reports	GMO Decree, art. 2.32.1, 2.53.1, GMO Regulation, art. 10.1h
Contained facilities	Register and plans of contained facilities,	GMO Decree, art. 2.14.2, 2.55, 10.1f
Audit reports	Reports of periodic inspections and audits	GMO Regulation, art. 10.1g
Incidents	Inventory of incidents	GMO Regulation, art.10.1i, 10.2e
Administrative record keeping	Inventory of decentral administrative record keeping	GMO Regulation, art. 10.1f
DEPARTMENTAL ADMINISTRATIVE RECORD KEEPING		**Regulatory reference**
GMO	Construction of GMO	GMO Regulation, art. 10.2a
GMO storage	Inventory of GMO per storage facility	GMO Regulation, art. 10.2b
Staff	Persons authorized to perform activities with GMO	GMO Regulation, art. 10.2c1 up to 10.2c4
	Other persons associated with GMO activities	GMO Regulation, art. 10.2d
Access authorization	Persons authorized to access level III/IV, per facility	GMO Regulation, art. 10.2h
Incidents	Records on loss of containment, deviation from procedures and instructions	GMO Regulation, art. 10.2e
Procedures & instructions	Procedures and work instructions issued by or on behalf of the OL/VM	GMO Regulation, art. 10.2f
Notification numbers	The notification numbers (IG numbers) of the activities performed in the contained facility	GMO Regulation, art. 10.2g
Waste	GMO containing waste: volume, date	GMO Regulation, art. 10.2i, Annex 9

Risk assessments, construction data of GMO and notifications/licenses must be kept available for 20 years after the date the Bureau GGO was notified that activities under the associated notification number (IG-number) were aborted (GMO Regulation, art. 18.9). To meet this requirement, the archive procedure should not be dependent on individual researchers. Departments should set up their record keeping in a transparent way and keep them available for inspection by the BSO. All records must be kept available for inspection by ILT

5.2.2 Administrative record keeping of stored GMO

The GMO Regulation does not specify how to keep the records of GMO, but organizations should establish an adequate policy. At VU, the following GMO administrative record keeping policy applies:

- GMO should be stored in an archived collection as soon as feasible. GMO that will not be included in a collection must be destroyed to prevent accumulation of non-registered GMO;
- For a GMO collection an inventory must be kept, providing the data indicated in par. 5.2.1;
- When multiple collections exist, a list must be kept, specifying their names, physical locations, managers and how and where the inventories are available;
- Vials with GMO, whether or not stored as part of a collection, must be adequately labelled (see par. 7.7).

5.2.3 Administrative record keeping of authorized personnel

Departments must keep an account of individuals that are authorized to work in contained facilities. This record must include:

- A register of authorized individuals, stating their name, worker ID, authorized highest containment level, authorization date, end date of authorization period (when applicable), name of direct manager and name of authorizer;
- Individual training records, including which components of the biosafety introduction program has been passed (see chapter 13 *Education, training and instruction*);
- For each authorized person, a completed and signed Employee Qualification Form (Formulier kwalificatie medewerker) and associated Employee Introduction Checklist (Checklist introductie medewerker).

This registration must be kept using the shared Register of personnel authorized to work with GMO (see “GMO-VU folder”). Training records and employee qualification forms must be archived along with this register.

5.2.4 Register of contained facilities for activities with GMO

The GMO Regulation and the environmental permit requires that a register is kept of the facilities that are being used for contained activities with GMO. This Register of contained facilities (see GMO Administration) is kept by the C-BSO. Laboratory managers must notify the C-BSO of operational changes. Changes must be approved by the C-BSO when any facility clearance involves reassignment. The C-BSO archives the clearance documents of all contained facilities as part of the GMO administrative record keeping.

5.3 Environmental release of GMO: gene therapy studies involving patients

The administrative requirements that the Phytosanitary Regulation requires for handling Quarantine material, patients are presented in table 5-2. All administrative requirements are listed in detail in the Audit Questionnaire on Quarantine Organisms (see GMO-VU folder).

Table 5-3 Administrative requirements for Quarentine organisms

Administrative requirement	Regulatory reference

5.4 Animal by-products

The regulations on animal by-products require that use of these materials is tracked from source to end point. To this end, quite some administrative requirements are imposed, e.g. the amount of animal by-products that are acquired, their origin, where they are stored, their destination and the amount of waste generated. Materials must also be specifically labelled and stored by category. Many of the requirements are very impractical for research institutions, and also not functional, since the chance that in such institutions the materials will flow into the food and feed chain is negligible. Inspectors of the NVWA have shown to have an eye for this situation, although views do differ among individual inspectors. Based on practical experiences, an audit list has been assembled listing operational practices for use of animal by-products for purposes of research, diagnostics and education (see Biosafety Resource). The key practices on record keeping described in this audit list are:

- No detailed account of the nature and volume of individual animal by-products used is required: a more generic record stating the types of animal products with a realistic estimate of the volume used per year for each type suffices. However, the shared Inventory of animal by-products must be recorded (see Biosafety Resource);
- Suppliers of animal by-products must provide a Commercial Document. Departments must archive these documents as long as the animal by-products are present.
- When disposed of as hospital waste (UN 3291), no documentation is required recording the fraction of animal by-products in this waste. However, animal by-products that are shipped to Rendac for removal, e.g. cadavers or parts of larger animals, do require recording of the waste volume and storage facilities. Also, waste containers involved must bear a category 1 label.

5.5 Decontamination methods

The effectiveness of a method used to destruct biological agents must be established. The methods that are applied need approval from the C-BSO. The C-BSO keeps an inventory of the approved methods (see GMO-VU folder).

6 Physical containment

6.1 Contained facilities

6.1.1 Containment levels and categories

To minimize the risk of accidental release of biological agents into the environment and exposure to personnel, the European and Dutch law set technical requirements for buildings and equipment in order to attain a safe level of physical containment. Both the Occupational Safety and Health Decree and the GMO Regulation describe physical containment requirements. These regulations implement requirements imposed by EU Directive 2000/54 and EU Directive 2009/41, respectively. While the former only describes contained laboratories and industrial installations, the latter describes additional categories of physical containment facilities. When the requirements of the GMO Regulation are met, those of the Occupational Safety and Health Decree are met as well. For labs, these requirements are in addition to the guidance on the lay-out of laboratories in general.

There are four levels of physical containment, level 1 being the lowest level. The containment level that must be applied when handling genetically unmodified hazardous biological agents correlates with the risk group of the agent, e.g. handling biological agents of risk group 2 must at least be carried out in a lab of containment level 2 (biosafety level 2, BSL 2), unless the outcome of a risk assessment indicates that a higher level is required. Activities that do not involve direct handling (e.g. culturing or concentrating) of biological agents, but involve risk of exposure to infectious biological agents, must be carried out at BSL2 at minimum.

The containment level necessary for safe use of GMO activities not only depends on the risk group of the agents used, but is also determined by other factors, e.g. the ability to survive in the natural environment. The applicable containment level must be assessed first (see chapter 4 Risk Assessment). An overview of the containment levels and containment categories for laboratories, animal housing facilities and equipment rooms is given in table 6-1.

Table 6-1: Physical containment levels for contained use of GMO activities.

Containment Level	Containment Category			
	Laboratory	Animal ward	Ward for animals in association with microorganisms	Equipment room
I	ML-I	D	DM-I	AP-I
II	ML-II	n.d.	DM-II	n.d.
III	ML-III	n.d.	DM-III	n.d.
IV	ML-IV	n.d.	DM-IV	n.d.

LEGEND: n.d. = not described in the GMO Regulation. The categories shaded green are available at VU.

Storage of GMO in contained facilities is preferred, but storage of containment level I or II GMO is allowed outside of the contained facilities, as long as the storage is located within premises approved for GMO activities as recorded in the environmental permit. The spaces within the premises that are not (yet) occupied by contained facilities are called “Remaining areas within GMO premises (ODG, Overig Deel GGO-gebied). Moreover, storage of waste containing GMO from level I and II facilities is allowed in the ODG-area.

6.1.2 Building requirements

Requirements for facilities where contained use of GMO activities takes place are listed in Annex 9 of the GMO Regulation. Comparable requirements for laboratories designed for activities with hazardous microbiological agents are found in Annex V of EU directive 2000/45. In the GMO-VU folder, checklists are available for the containment levels relevant for VU. These checklists include legally required operating procedures (also see par. 7.2) and indicate which of the building requirements are imposed by law or based on the policy of the institution. The latter include the following:

- ***Door shield***. Contained facilities have a door shield indicating the room number, function, containment level, contact information and pictograms indicating potential hazards, obligations and prohibitions. The template for this shield and the pictograms are available in Kwaliteitsnet. See figure 6A for an example;
- ***Climate control***. Because the use of protective clothing is mandatory, new laboratories must have climate control in order to provide a comfortable work environment.
- ***Door closers***. Entrance doors must be fitted with door closers. Closers must allow for safe passage when carrying materials.
- ***Eye washing station***. Labs must be equipped with an eye washing station, preferably connected to ambient tap water to enable prolonged rinsing. Eye washing bottles are an alternative option.
- ***Hand washing station***. Labs must be equipped with a hand washing station near the main exit with a tap that can be operated without using one's hands. The tap should supply water at ambient temperature to facilitate prolonged washing.
- ***Mechanical room ventilation***. Total air volume of the room must be replaced at least five times per hour.
- ***Cleanability.*** Care must be taken to keep the interior accessible for cleaning.
- ***Water system with check valve***. To eliminate the possibility of microbiological contamination through back flow of tap water, water piping must be fitted with check valves.
- ***No office desks***. Office desks and other facilities typically associated with office functions, e.g. copiers, archives, storage for office supplies, shall not be situated within laboratories, animal wards and equipment rooms.

6.1.3 Facility clearance procedure

Contained facilities and storage places for GMO or GMO waste in the ODG-area must be approved by the C-BSO before they become operational. If the location of these facilities changes, the C-BSO will consult the institutional environmental officer in order to verify whether the changes fit within the scope of the environmental permit. If not, the environmental officer will decide on how to proceed. The C-BSO keeps a register of the contained facilities and storage places, and archives the clearance checklists along with it.

6.2 Biological safety cabinets

There are three classes of biological safety cabinets (BSC). Cabinets of class I feature downward airflow which is filtered by a High Efficiency Particulate Air (HEPA) filter in order to prevent entry of airborne contaminants. Class I cabinets are designed to protect the experiment, not the operator, against microbiological contamination.

BSC of class II and III protect the workspace as well as the operator. The class II BSC features has an open front with a sash window that should be closed when the cabinet is not used. For most activities

with hazardous biological agents, a class II cabinet is appropriate. They exist in several types (see fig. 6B):

- ***Type IIA***, with exhaust air passing a single HEPA filter.
- ***Type IIB1***, with exhaust air passing a double HEPA filter and with partial air recirculation.
- ***Type IIB2***, with exhaust air passing a double HEPA-filter and no air recirculation (total exhaust).

All types may emit the HEPA-filtered exhaust air into the workspace or have the exhaust duct connected to a ventilation duct. When this ventilation duct is part of a mechanical ventilation system, the connection must be a canopy construction (thimble) to guarantee proper functioning of the BSC in case the ventilation system becomes disabled. The thimble guarantees that in such a situation the BSC will emit the HEPA-filtered exhaust air into the room without decreasing the inflow that protects the operator.

A class III BSC has a closed front, which means that manipulations are carried out using the gloves fitted in the front windowpane.

There are several normative references on biosafety cabinets. Cabinets marketed in Europe are most commonly certified according to the EU standard NEN-EN 12469 and the US standard NSF 49. Annex E of the NSF gives guidance on design, construction, performance and field certification, including proper location and work practices (see Biosafety Resource). BSC must be properly maintained to ensure their correct functioning (see chapter 10 *Maintenance*).

Handling hazardous biological agents that have a transmission route by air must take place in a type B class II BSC or in equipment offering an equivalent level of protection, e.g. HEPA-filtered individually ventilated cages (IVC) for animals. Whether the exhaust of a BSC should be connected to a ventilation duct depends on the outcome of a risk assessment. A BSC for handling experimental animals must have its exhaust air connected to a ventilation duct by default to control allergens.

Because the air flow in a BSC is quite delicate, care must be taken not to disturb it. Therefore, position a BSC:

- As far away as possible from room ventilation inlets and outlets;
- ≥ 1.5 m away from an entrance door facing the cabinet;
- ≥ 1.0 m distance from a facing wall and ≥3.0 m distance from a facing cabinet;
- ≥1.5 m space to pass the cabinet.

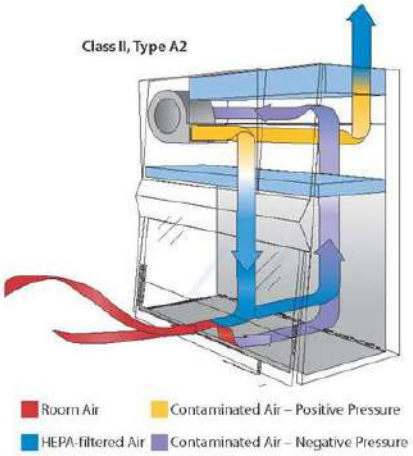
Before a cabinet is to be taken into operation for the first time, and whenever it has been relocated, it must be calibrated by a qualified party. Thereafter, the parameters must be checked annually. The protection parameters are detailed in European standard NEN EN 12469. Critical parameters are:

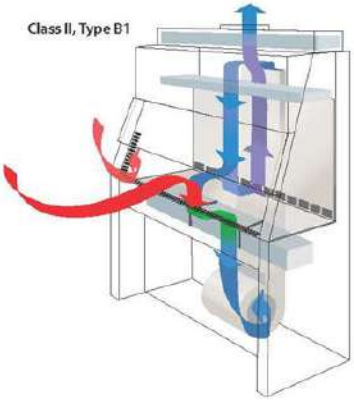
- Mean downflow velocity 0.25 - 0.50 m/s. Optimal value is 0.4 m/s. None of the eight points used to calculate the mean value should deviate >20% of this value;
- Inflow velocity between 0.4 m/s and 1.0 m/s;
- Sound pressure at 1.0 m from the center of the work opening should not exceed 65 dB(A) at a background sound level of ≤55 dB(A).

Airflow Characteristics of Class II Biological Safety Cabinets

Class II, Type A1 and Type A2 Cabinets

- Recirculating systems
- May be vented into the room or to the facility's HVAC system through a canopy exhaust connection
- Remaining air is recirculated to the work area through a HEPA supply filter.
- HEPA-filtered downflow air is a mixture of recirculated and inflow air from a common plenum, and will vary in total volume based on the cabinet design
- Intake air velocity for a Type A1 is a minimum of 75 FPM and Type A2 is a minimum of 100 FPM
- All biologically contaminated ducts and plenums are under negative pressure or surrounded by negative pressure ducts and plenums





Class II, Type B1 Cabinet

- Recirculating system
- Exhausted air is pulled through a dedicated duct and through a HEPA filter (location of the filter varies by manufacturer) before entering a facility's HVAC system
- Must be hard-connected to an exhaust system
- Remaining air is mixed with the inflow air and recirculated to the work area through a HEPA supply filter - in some designs this recirculated air is HEPA filtered to prevent contamination of the cabinet plenums
- Intake air velocity is a minimum of 100 FPM

Class II, Type B2

- Provide no air recirculation within the work area
- Must be hard-connected to a facility's exhaust system
- HEPA filter air is immediately exhausted through a dedicated duct, into the HVAC system (HEPA-filter location varies by manufacturer)
- Room air enters through a blower/motor located near the top of the cabinet (specific location varies by manufacturer) and pushed through a HEPA supply filter into the work area.
- Descending air is pulled through the base of the work area through the perforated front and rear grilles
- Simultaneously, air entering through the front opening is pulled through the perforated front grille
- Intake air velocity is a minimum of 100 FPM

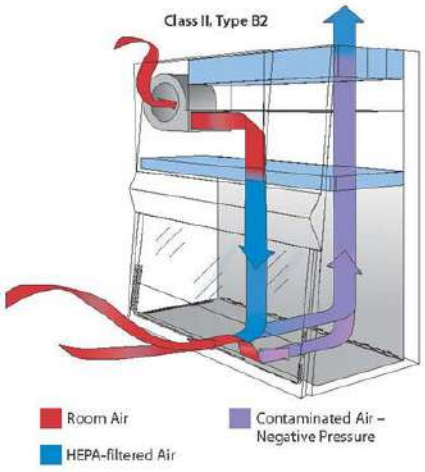


Figure 6B: Overview of Biological Safety Cabinets of class II (from: Baker, 2014)

7 Safe Microbiological Practices

Technical measures like physically contained labs and equipment will fail to prevent exposure to and spreading of hazardous biological agents unless proper working practices are applied by everyone who handles these agents. Appropriate working practices will depend on the nature of the activities that are carried out, and the nature of the biological agents or materials handled. Effective practices proven over time are frequently referred to as Safe Microbiological Practices. Paragraph 7.1 summarizes the general behavioral rules that are of critical importance in preventing exposure to microbiological agents during work. The remaining paragraphs provide additional guidance on selected topics.

7.1 Essentials of Safe Microbiological Practice

Entering a contained facility

- Take off jewelry, watches etc. from hands and wrists before entering a contained facility and store these objects outside of the lab. If earrings might touch the collar of your lab coat, take them off as well. Take care when wearing other items, like necklaces, to ensure that these stay underneath the lab coat;
- Tie long hair back (e.g. using a hair net);
- Do not bring in food, drinks or cosmetics;
- Do not bring in plants or animals that are not associated with experimental work;
- Apply personal protective equipment (PPE) as prescribed (e.g. labcoat, gloves). PPE that must be worn when entering the facility is indicated on the door sign at the entrance. When personal circumstances interfere with the instructions, e.g. when wearing a brace, consult the lab manager or Local BSO on how to proceed;
- Properly cover non-intact skin that can be exposed to biological agents or materials with a bandage that is impervious to liquid. Renew bandages when visibly contaminated.

While working in a contained facility

- Keep doors and windows (in case these can be opened) closed;
- Avoid contact between your hands and your face (mouth, nose, eyes);
- Organize your work in a way that minimizes the need to move around, especially when working in a biosafety cabinet;
- Wear safety goggles or a face mask when there is risk of splashes;
- Wear appropriate gloves that fit well when there is a risk of direct contact with biological material;
- When microbiologically contaminated aerosol can be generated, perform manipulations gently and work in a biological safety cabinet of at least class IIA (see par. 7.7).
- Avoid at all times swallowing biological material, e.g. never pipette by mouth;
- Avoid the use of sharps, but when necessary, discard them into a sharps container;
- Do not re-cap needles of syringes etc., but discard them directly into a sharps container;
- Never leave biological material unattended;
- Properly label materials (see par. 7.7);
- Always clean and disinfect your workplace every time you leave it;
- Keep the lab tidy and clean (see par. 7.3).

Handling incidental contamination

- Decontaminate (potentially) exposed body parts immediately by ample washing or, if indicated, by first applying an effective disinfectant;
- Treat microbiologically contaminated wounds according to the injury protocol (see chapter 12 Incident Management);
- Decontaminate and remove all spills immediately no matter what the size of the spill is;
- Act according to the incident management procedure (see chapter 12 Incident Management).

Leaving a contained facility

- Wash or disinfect your hands before leaving the facility (see par. 7.4). Only use disinfectant when indicated;
- Leave work clothing inside the contained facility;
- If you know or suspect your work clothing has become contaminated, immediately replace it. Place the contaminated clothing in an appropriate container for decontamination and cleaning (e.g. in an autoclave bin) or discard it as hospital waste.

Questions?

- In case of questions on hazards, risks or on how to control them, turn to a colleague for help! If necessary, contact the lab manager or one of the other responsible people listed on the door sign at the lab entrance (e.g. ABV and C-BSO).

7.2 Regulatory operating procedures for contained use of GMO

In Annex 5 Part I and Part II the GMO Regulation imposes regulatory operating procedures that must be adhered to for each containment category. The instructions referred to in Part II are specified in Annex 9 of the GMO Regulation. The C-BSO provides checklists (see Biosafety Resource) listing these requirements, as well as the building requirements that apply to the contained facility (see also chapter 6, Physical containment).

7.3 Cleaning microbiological and cell culture laboratories

7.3.1 Organization and nature of the cleaning activity

The lab manager is responsible for ensuring that the workplace is kept clean and tidy. The manager documents which cleaning activities must be carried out, including their frequency, and who will perform the tasks. In establishing this cleaning program, table 7-1 shall be used as guidance.

The lab manager organizes the activities of the cleaning program that are not carried out by the contracted cleaning service company. For cleaning activities that are to be carried out once a year, a “cleaning event” can be organized. The lab manager takes care to provide adequate cleaning materials when these are not provided by the cleaning service company.

Table 7-1: Guidance on cleaning activities in microbiological and cell culture laboratories at ML-I and ML-II levels.

Activity	Performance	Frequency indication*
Wiping the floor	Wipe with anti-static cloth. Dispose of dirt and used wipes in the waste bin for hospital waste (SZA).	2x/week
Disinfecting the floor	Mop with approved disinfectant. Dispose of used material in the waste bin for hospital waste (SZA), or autoclave and wash before reuse.	1x/week
Cleaning of sinks and accessories	Clean sink, counter, tap, soap and towel dispenser with microfiber cloth. Refill dispensers.	5x/week (every working day)
Cleaning of work surfaces, shelves, interiors of cupboards and drawers, etc.	Wipe with microfiber cloth or with water and soap.	According to need
Cleaning of (cupboard) doors, drawer fronts, handles, etc.	Wipe with microfiber cloth	1x/week
Cleaning of water baths	Empty reservoir, clean with water, refill with fresh demi water. Add biocide if necessary.	1x/week
Cleaning fridges and freezers	Clean exterior and interior with water and soap. De-ice.	1x/year, remove ice when necessary
Cleaning other equipment	Clean with water and soap or with other suitable detergent. Clean according to user/maintenance instruction of equipment.	1x/year
Disposal of microbiological waste	Close full waste bins (fill up to 75% max.) and store at waste station. Autoclave waste collected in autoclave bins. Disinfect outside of all bins before transporting outside the lab.	1x/week, full bins daily
Change of lab coats	Deposit used lab coats for washing in designated bin. Autoclave lab coats from ML-II labs first.	1x/2 months, more frequent when needed

* The frequency refers to laboratories of containment level 1 and 2. The actual frequency will depend on the intensity the lab is being used. Labs of higher containment require a dedicated schedule based on the specific activities performed in these facilities.

For keeping general areas clean, FCO contracts cleaning service companies. The following rules apply to employees of these companies with respect to cleaning level 1 and 2 microbiological and cell culture labs:

- Only persons that have attended a safety class on cleaning contained laboratories and who have received technical instruction about which cleaning activities to carry out and how to perform them are authorized to perform the cleaning. The instructions will be repeated yearly. Cleaning personnel will receive a cleaning instruction card summarizing how to work in the labs.

- Cleaners must put on a dedicated lab coat when they enter the lab. This coat must be visually clean, and is to be refreshed according to the lab coat management schedule of the lab they are working in. Lab coats for cleaners must be marked to distinguish them from other lab coats.
- During cleaning, appropriate disposable gloves are to be worn.
- The cleaning trolley must stay outside of the lab.
- Objects that have not been cleared by the user are not to be cleaned.

7.3.2 Tasks and responsibilities

Tasks and responsibilities associated with housekeeping and cleaning of labs are listed in the table below along with the responsible party.

Table 7-2: Tasks and responsibilities with respect to ML-I/II lab cleaning.

Task/Responsibility	Responsibility of:
Instruct cleaners of the cleaning service company on working in ML-I/II laboratories (safety instruction)	BSO
Register cleaners of the cleaning service company that are authorized to clean ML-I/II labs	BSO
Supply a listing of the cleaning activities in ML-I/II labs that are included in the contract with cleaning service company	BSO
Document the cleaning program of a lab, assign cleaning tasks to lab users	Lab manager
Take care to provide the materials needed to carry out the cleaning program	Lab manager
Introduce new cleaners to the laboratories they are going to clean	Lab manager
Instruct cleaners of the cleaning service company on which cleaning activities to carry out and how to perform these (technical instruction)	Supervisor of cleaning service company
Only schedule cleaners for cleaning ML-I/II labs that are authorized for this task	Supervisor of cleaning service company
Introduce cleaners to the lab managers and departmental experts on biosafety of the labs they are going to clean	Supervisor of cleaning service company
Provide a cleaning instruction card to the cleaners	Supervisor of cleaning service company
Manage the contract with the cleaning service company	Staff member of Facilitair Bedrijf / Facilitaire Campus Organisatie

7.4 Hand cleaning

Keeping your hands clean is a critical microbiological control measure. Therefore, wash your hands:

- Whenever they have become visually dirty;
- When you leave the toilet.
- When leaving a contained facility.

In most cases, thoroughly washing your hands using warm water and mild soap suffices. Only disinfect your hands when standard operating procedures require you to do so. The correct procedures for washing and disinfecting hands are described below (see also Kwaliteitsnet ID 065001). Treat your hands with nourishing cream after cleaning to prevent the skin from drying out because of frequent cleaning.

Good hand washing practice is as follows:

- Open the tap (using elbow, wrist or hands-free) wide enough to have ample amount of water flowing, wait until the water temperature is comfortable;
- Wet your hands and wrists, then apply soap from the dispenser;
- Rub hands using the technique shown in figure 7A to reach all parts, including the wrists. Rub hands thoroughly for at least 10 seconds;
- Rinse hands thoroughly, dry with a disposable towel;
- Close tap using elbow, wrist or hands-free or if needed turn knob with disposable towel;
- Dispose of used towel in waste bin.

Good hand disinfection practice is as follows:

- Press the dispenser with hand disinfectant with your wrist and take enough disinfectant to leave your hands moist for at least 30 sec.;
- Rub hands using the technique shown in figure 7A to reach all parts, including the wrists. Then rub both wrists;
- Rub hands thoroughly until dry, but at least for 30 sec.

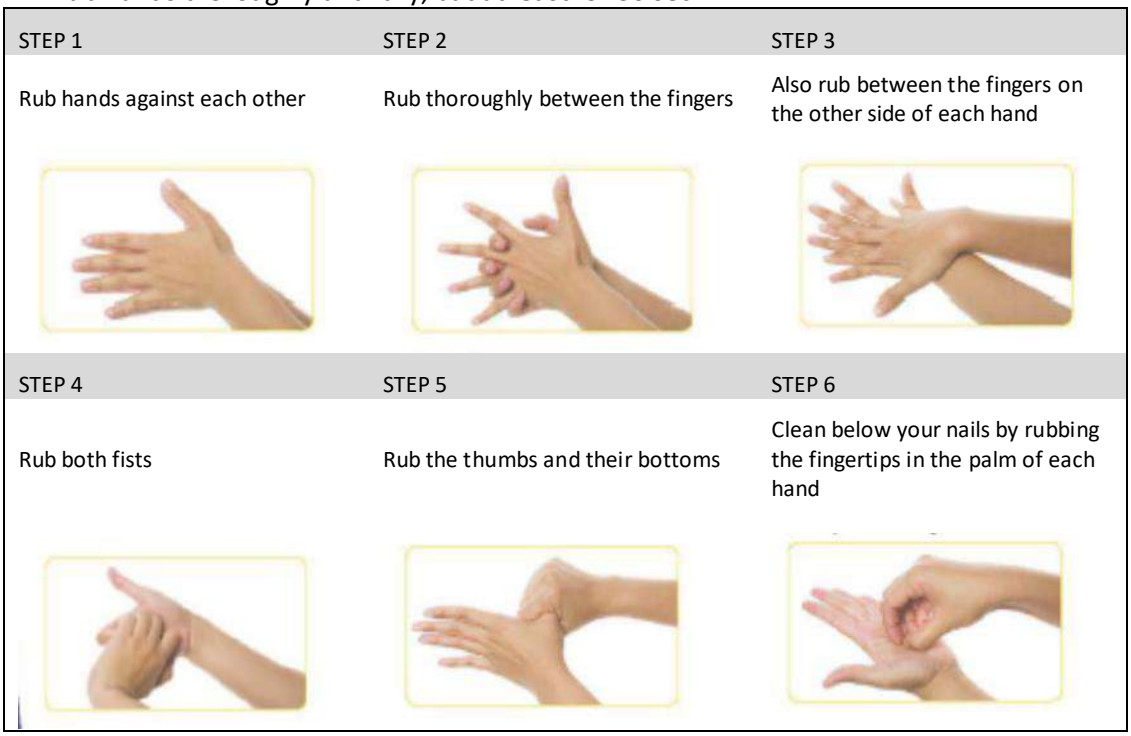


Figure 7A: Good hand cleaning technique.

7.5 Lab coats

Anybody who enters a microbiological or cell culture lab must put on protective clothing. In most cases this will be a lab coat. The choice of the protective clothing must be based on the outcome of the risk

assessment and must be approved by the BSO. Protective clothing should always meet the institutional safety requirements that are relevant for the work that will be carried out (e.g. fire and chemical resistance).

For microbiological and cell culture activities at containment level I (ML-I, ML-II, D-I, DM-I and DM-II), a regular lab coat or working suit will generally be appropriate. However with certain activities in a ML-II lab, using a sleeve cover will improve the safety

7.6 Control of identity and purity of biological agents and DNA/RNA

The GMO Regulation requires that the biological agents and donor sequences used are properly identified and free of contamination. There are many ways to verify identity and purity of these entities, and which method is appropriate depends on the nature of the agent that is to be assessed. It is the responsibility of the OL/VM to apply only the agents that have been included in the risk assessment and to guarantee their purity. This might need verification of identity and purity of biological agents and/or DNA/RNA sequences. The decision on whether or not verification of identity and/or purity should be carried out, must be made in accordance with to the policy given below.

- Obtain biological agents and DNA/RNA sequences from a party with a certified quality control system and that provides an identity certificate and lot number of the acquired material. Keep records for all collections including identity certificate, lot number, supplier and date of purchase;
- Label agents according to the requirements in 7.7.1;
- Handle and store agents in accordance with standard operating procedures designed to minimize the chance of contamination and misidentification. An example of an appropriate procedure is the use of master cell banks (MCB) and working cell banka (WCB). Only WCB-vials are used for experimental work. When no WCB vials are left, new vials are generated from MCB vials. When no MCB vials are left, a new batch is ordered from the supplier to make new MCB vials;
- Record in lab journals etc. which agents are used;
- Whenever there are doubts about identity or purity of a sample, destroy it or verify its identity and/or purity.

No verification is required when all of the criteria are met. It is beyond the scope of this manual to give guidance on how to assess identity and purity of biological agents and DNA/RNA sequences. If according to the policy verification is required, the OL/VM must decide on the method and frequency. Verification data must be included in the administrative records for future use.

7.7 Storage of biological materials

7.7.1 Storage of biological agents

Adequate labeling of vials containing biological agents, including GMO, is of great importance in order to secure their identity and link them to records/lab journals containing information on source, safety data, and construction data (in case of GMO). The minimal labeling requirements differ for vials with biological agents that are part of a collection and for material under development (see Table 7-3). The data listed in this table must be shown on the physical label or must be part of the data read-out in case other techniques are used to mark vials, e.g. bar codes or QR codes*.

* NOTE: Relabeling of materials is not required. Departments are encouraged to update the labeling of collections whenever possible.

Table 7-3: Minimal labeling requirements for vials containing GMO.

LABEL DATA	MATERIAL IN COLLECTION	MATERIAL IN PROGESS
Unique inventory ID	mandatory	n.a.
Preparation date	mandatory	mandatory
Name owner	optional	mandatory
Name biological agent/material	mandatory	mandatory
Abbreviation “GMO” or “GGO”	mandatory (when GMO)	mandatory (when GMO)
Containment Level	mandatory	optional
Biohazard sign	mandatory (when > level I)	optional

Storage of agents, whether in progress or in a collection, must meet the following conditions:

- Store in closed, leak-tight and robust vials. When stored outside of a contained lab (in ODG), then storage must be in double containers;
- Disinfect the outside of vials before storage;
- Storage facilities, e.g. fridges, biofreezers and cryo containers, used to store biological agents of risk group 2 or higher, or GMO handled at containment level II or higher, must show a biohazard sign;
- Whenever possible, store hazardous agents and GMO in dedicated compartments in case other materials are stored in the same storage facility.

7.7.2 Storage of animal by-products

- When all animal by-products used are classified as category 1, then these materials are allowed to be stored in a shared facility;
- Animal by-products must be stored separately from other materials, but there is no need to store them in a dedicated facility (e.g. a refrigerator or freezer) if a storage unit has a compartment (e.g. drawer, shelf, box) that is dedicated to storage of animal by-products;
- Packaging or vials containing animal by-products must bear the phrase “for research or diagnostics” unless stored in a designated space or facility that is clearly labeled including category classification.

Labels to identify animal by-product storage spaces are available for download (see “GMO-VU” folder).

Figure 7B: Labels for animal by-products of Category 1, 2 and 3

7.8 Usage of a biological safety cabinet

Biological safety cabinets (BSC) of class IIA and IIB are widely used to protect the operator, the product, and the experimental space from microbiologically contaminated aerosol (see also par. 6.2). For BSC to be effective, proper working practices must be followed.

Preparing to work in a class II BSC

- Turn the cabinet on and wait until the airflow indicator signals that the downflow and inflow have stabilized.
- Decontaminate the work surface of the compartment using 70% ethanol, with a contact time of 30 sec. ;
- Collect all materials needed for your work in the workspace of the cabinet and wipe the outside surfaces with an appropriate disinfectant;
- Put on gloves (when indicated);
- Take care not to obstruct the inward and downward airflow:
- Minimize the amount of materials to be placed in the BSC;
- Do not block the ventilation grids;
- Put large objects on stands to enable airflow to pass underneath;
- Place objects away from the sides and the back panel.
- Make sure that enough waste bags are available inside the cabinet to collect all waste.

Working in the class II BSC

- Take care not to disturb the air flow balance while working, by moving slowly;
- Burners will destabilize the airflow, and shouldn't be used because of this. If required, use one without ignition flame, position the burner at the back of the cabinet, and always use it as briefly as possible;
- Colleagues must take care not to pass cabinets closely while in use.

Finishing working in a class II BSC

- Close the waste bag(s), and wipe the outside of the bag(s) with appropriate disinfectant;
- Wipe the outside of all materials that you want to take out of the BSC with appropriate disinfectant;
- Disinfect the work compartment (worksurface, sides and back panel);
- Place new waste bag(s) in the cabinet;
- Take off gloves (if worn) and discard them into the newly installed waste bag;
- You now may take your hands out of the BSC workspace. Put on new gloves and remove all disinfected materials from the cabinet;
- Remove the disinfected waste bag(s) from the cabinet and dispose of it as hospital waste;
- Let the BSC run for at least 5 minutes before closing the sash window and switching it into standby or off;
- If required, register use of the cabinet in its log book.

7.9 Usage of centrifuges

Centrifuges are a potential source of contamination because tubes can break or leak and centrifugal airflow can easily lead to the unnoticed spread of agents. To control this source of potential contamination, act according to the following guidance:

- For centrifuging biological agents of risk group 2 that can be transmitted by air, and for centrifuging agents of risk group 3 or 4, use of a contained centrifuge is mandatory. This is a centrifuge with a closed rotor or buckets that should be loaded and closed in a BSC, and after centrifuging be opened for unloading in a BSC;
- Use tubes that will withstand the G-forces that will be generated according to the specifications, that neatly fit into the buckets and that are made of material resistant to the contents (e.g. solvents);
- Do not use tubes that show crazing or cracks;
- Verify that the O-rings that seal the caps of the bucket and the lid of the rotor fit well and are in good shape;
- To prevent leakage while running, never overfill tubes;
- When using fixed angle rotors, let aerosol settle for 10 minutes before opening the centrifuge. Waiting time is not required in case of swing-out rotors used in combination with individually capped buckets or when unloading a rotor or buckets in a BSC;
- When opening a centrifuge, check for leaks. If leakage has occurred, close the centrifuge immediately and consult your ABV on how to proceed safely. If possible, unload the centrifuge in a BSC;
- Keep rotors, buckets and the inner space of the centrifuge clean. Disinfect when required, e.g. after leakage.

8 Disinfection

8.1 General methodological requirements

Disinfection and destruction of biological agents are essential processes to control or eliminate (potential) microbiological contamination. To guarantee their effectiveness, disinfection and destruction treatments must meet the following criteria:

- Effectiveness of the method must be proven and documented (a validated method). When a reduction of log 6 is proven, the method is considered effective. Methods applied must be approved by the C-BSO;
- When chemical disinfectants (biocides) are used, the product must be approved for marketing by the Cgtb and the product must be used according to the labeled instructions;
- Equipment, like autoclaves or neutralization tanks, must be maintained to guarantee proper functioning. Performance criteria, maintenance activities and maintenance frequency must be documented.
- On equipment that needs periodic servicing, the dates of the most recent maintenance and the next scheduled maintenance must be clearly indicated;
- When using equipment for disinfection or destruction, parameters must be logged for each run to ensure proper functioning. Logged data must be archived until the disinfected or destructed materials have been processed as waste or have passed the next level of decontamination treatment.

The C-BSO keeps an inventory of approved methods of decontamination (see “GMO-VU” folder).

8.2 Decontamination of lab coats

Under normal operational conditions, protective clothing worn in facilities of containment level I does not need to be disinfected, e.g. autoclaving is not necessary. Decontamination by regular washing is sufficient. However, disinfection by autoclaving is required when clothing is known or suspected to be contaminated with biological agents.

Protective clothing worn in facilities of containment level II must be disinfected by autoclaving before cleaning by laundry service. Disposable coats do not need to be autoclaved before being disposed of as hospital waste.

All protective clothing worn in facilities of containment level III, including disposables, must be autoclaved before washing or disposal.

Used protective clothing must be kept in a closed, robust container until it is washed or autoclaved. When used in level II or III facilities, clothing awaiting sterilization must be in closed autoclavable bags within the contained facility. These bags must be transported to the autoclave in a way that prevents rupturing.

Departments must document the frequency of changing protective clothing and provide a rationale for the frequency chosen.

8.3 Decontamination of surfaces

Surfaces that are (potentially) microbiologically contaminated, but not with an infectious agent, can be cleaned with warm water and mild soap. In case of contamination with hazardous micro-organisms, an approved disinfectant must be used. Concentration, contact time and method of application must be in compliance with the labeled instructions (see par. 2.9).

Be aware that on dirty surfaces, disinfectants may be less effective, depending on the active substance used. Also, some active substances are unstable, which means that care should be taken not to apply working solutions of disinfectants that have passed their shelf life. For example, solutions of dichloroisocyanurate must be not older than 1 day when stored at room temperature or 1 week when stored at 4° C.

The GMO Regulation requires that work surfaces must be disinfected routinely when the work has finished. Always remove spills of biological agents and materials immediately and disinfect the (potentially) contaminated area. For large or hazardous spills, see chapter 12 Incident management.

8.4 Decontamination of liquids

When decontaminating liquids, care must be taken to meet the effective end concentration as stated in the labeled instruction. Disinfectants based on active chlorine must be made fresh, because the inactivation potency of the active ingredient diminishes after it has been prepared. Dispose of diluted disinfectants according to the instruction label: sink disposal is not always allowed.

8.5 Decontamination of skin

Frequently clean your hands thoroughly with warm water and soap while carrying out microbiological activities (see par. 7.4 for hand washing technique). Only use disinfectants when needed or indicated by the standard operating procedure. When disinfection is required, use a moisturized hand sanitizer.

8.6 Decontamination of water baths

Water baths can be a major source of microbial contamination. The best method to control microbiological contamination is periodic heating at 60^0 C for two hours once a day and renewing the water once a week. In case biocides are used, take care to use a product approved by the Cgtb in accordance with the instructions on the label.

8.7 Decontamination using ultraviolet radiation

Ultraviolet radiation (wavelength ~254 nm) is not a preferred sanitation method. It should only be used as an additional treatment to decontaminate objects, e.g. to keep work surfaces of a BSC that have been chemically disinfected sterile by overnight exposure to UV. This policy is in effect because the effectiveness of UV decontamination can be easily diminished by various factors, can deteriorate materials, and is hazardous to unprotected eyes and skin. When using UV radiation for disinfection purposes, note the following:

- UV is only effective for disinfection of air, surfaces and thin layer liquids;
- UV does not eliminate spores;
- Surfaces must be directly exposed to UV: concealed surfaces will not be disinfected;
- The effectiveness of UV radiation decreases rapidly with distance;
- The presence of dirt strongly diminishes UV effectiveness: treated surfaces must be clean;
- UV radiation is a health hazard to skin and eyes. As a consequence, use of UV is never allowed when anyone is present in the lab;
- UV causes colors to fade, and promotes the deterioration of some materials.

9 Transport

Regulations on the transport of dangerous goods are extensive, detailed and vary depending on the nature of the goods. The ADR/VLG constitutes the core of the regulations on shipping hazardous substances, including biological agents. The BVF Platform has published a comprehensive and practical guide on shipping biological materials (see GMO-VU folder). In this chapter, the main requirements for shipping hazardous biological agents on-site and off-site by road, air and mail are described.

Waste containing hazardous biological agents, GMO and animal by-products must also be shipped in accordance with the ADR/VLG. See chapter 11 for details on waste shipment.

9.1 On site transport

Transport of hazardous biological agents on site must be done in a safe way. However, there are only regulatory requirements on the internal transport of GMO, which must comply with article 3 and annex 1 of the GMO Regulation. The institutional policy is to apply the same requirements when transporting hazardous biological agents that are non-GMO.

The GMO regulations aim to achieve transportation methods that minimize the risk of spills or (environmental) contamination:

- Use closed, break-resistant, leak-proof primary containment when transporting micro-organisms and cells. Disinfect the exterior of the containment and place into a robust, leak-tight secondary transport container and close tightly. Disinfect exterior of secondary transport container before taking it outside the contained facility;
- Use closed, break-resistant and leakproof bins or cages to transport small animals. Cages to transport animals between the AARC and VU facilities are supplied by the AARC and these transports must be carried out by authorized persons. See the AARC website for more details (url in Annex 2);
- For transportation of animals associated with micro-organisms, use closed, break-resistant and leakproof containment designed to prevent the release of the associated micro-organisms. Disinfect the exterior of the container before bringing it outside the contained facility.

Transport of waste containing biological agents must also comply with these rules.

9.2 Transport by road

Shipping of biological samples like hazardous biological agents and genetically modified organisms by road must comply with the requirements of the ADR/VLG. Some biological materials are exempt from the ADR:

- Biological materials that do not contain active pathogens or infectious substances;
- Biological agents that do not cause disease in human or animals, these include biological agents that may carry pathogens or toxins in concentrations equal to the natural environment and/or biological agents unlikely to contain disease causing agents;
- Human blood and blood components or products intended for transfusion or transplantation;
- Human tissues and organs collected for transplantation;
- Decontaminated biological waste.

Table 9-1 summarizes the main requirements for shipping biological samples. The combination of hazardous (infectious) and non-hazardous agents in a single package is not allowed. Detailed instructions on shipping diagnostic materials in compliance with ADR is available in the GMO-VU folder

Table 9-1. Summary of ADR requirements for shipping biological agents by road.

Material	**ADR class**	**Packaging Instruction**	**UN number**	**Notes**
GMO, containment level I or II	n.a.	n.a.	n.a.	GMO that need to be handled at containment level I or II, e.g. ML-I or ML-II, are not considered dangerous substances in the ADR.
GMO, containment level III or IV *and* non-infectious/non-toxic	9	P904	3245	When packaged as substance of category B other requirements stated in the ADR do not apply.
Substance of category A hazardous to humans	6.2	P620	2814	Agents of category A may cause permanent impairment, life-threatening or fatal disease in human or animals. ADR has a list of species. Ask BSO when species is not on this list.
Substance of category A hazardous to animals	6.2	P620	2900	See above.
Substance of category B	6.2	P650	3373	Agents of category B are hazardous agents that do not qualify for category A. Examples are (diagnostic) samples of blood or urine.
Animal by-products	6.2	P620 or P650	2814, 2900 or 3372	See paragraph 9.5 and table 9-2 for details.

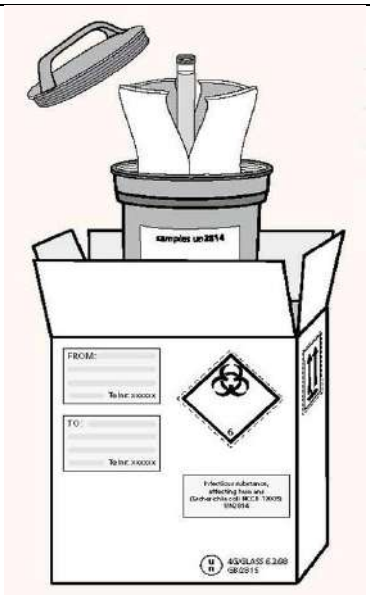
	***Inner layer*** Leak-tight primary receptacle surrounded by enough absorbent material to contain its content. ***Middle layer*** Leak-tight secondary container. ***Outer layer*** Outer layer appropriate for type of shipment. Package may contain multiple primary receptacles. Packaging instructions P620 and P650 are identical, but P620 requires that UN-certified packaging is used. Guidance on shipping biological materials is available in the Biosafety Resource.

Figure 9A: Triple-layered packaging according to P620/P650 (source: Extern Transport van Biologische Materialen, 2012).

The packaging instructions P620, P621, P650 and P904 are triple-layered (see figure 9A). Transport services that carry dangerous goods by road need no accreditation, but the driver must have an ADR certificate. Various operators offer this service, e.g. Biologistic Services, DHL, World Courier and SDX. World Courier and SDX have proven to be reliable providers for shipping temperature sensitive samples.

Shipments of UN 2814 and UN 2900 substances must be accompanied by a shipping document that contains the following information:

- UN identification number;
- ADR shipping name of the substance as referred to in the dangerous goods list, with the technical name added where required;
- Substance class;
- Number of packages;
- Description of the content(s);
- Total amount of the hazardous substances by volume or weight;
- Name and address of sender and receiver;
- Name and address of a person responsible for the shipment;
- Packaging category, when applicable.

In the Netherlands, the lay-out of the shipping document to transport animal by-products is not regulated (vorm-vrij). Therefore, the legally required commercial transport documentation is suitable for the biological hazard shipping document involving UN 2814 and UN 2900 substances, as long as all of the information stated above is included.

9.3 Transport by air

An organization that wants to ship dangerous substances by air must be accredited by ILT with the so-called A-status. VU does not has this status, so it must arrange air shipments through external organizations that have been granted an E-status and comply with all of the requirements imposed by

the ICAO Technical Instructions and the IATA Dangerous Goods Regulations. The E-status company assumes the entire public liability for the shipment, and is responsible for (final) packaging, labeling and documentation. E-status companies are listed on the website of ILT. Currently there are four companies operational in The Netherlands: DGM Services, Global DG Solutions, Special Cargo Services and SGS Nederland. There is an exemption for shipments of category B biological agents (UN 3373) and GMO (UN 3245): there is no need for an A- or E-license when these substances are packaged according to packaging instructions PI650 (which equals P650 of ADR) or PI959 (which equals P904 of ADR).

9.4 Transport by mail

PostNL accepts diagnostic samples, category B biological substances (UN 3373) and GMO when packed and labeled in compliance with ADR packaging instruction P650. Packages approved by PostNL must be used. They can be ordered from suppliers like Minigrip, Carepack, Transposafe, and Klinipath.

9.5 Shipping and importing animal by-products

9.5.1 Shipping animal by-products

Animal by-products must be shipped in compliance with the ADR regulations and the EU regulations 1069/2009/EC and 142/2011/EC on animal by-products. Below is a summary of the requirements. Extensive guidance is provided in a publication on the BVF Platform on the use of animal by-products in research, diagnostics and education (see Biosafety Resource).

Depending on the nature of the material, one of the following ADR rules applies:

- ***Animal by-products that (may) contain pathogens listed in the ADR as Category A***. If this concerns a microorganism that can affect humans, shipments must comply with the requirements set for UN 2814. In case of microorganisms that affect animals only, shipments must comply with the requirements set for UN 2900.
- ***Other animal by-products***. The remaining animal by-products must be shipped in compliance with the ADR requirements set for UN 3373. The majority of animal by-products will belong to this identification number.

Regulation 142/2011/EC imposes two additional requirements for animal by-products that are shipped for the purpose of research or diagnostics (see also table 9-2):

- ***Labeling***. The outer package must bear the phrase “For research and diagnostics”;
- ***Documentation***. Any shipment of animal by-products must be accompanied by a commercial document;
- ***Shipper***. The shipper must be registered as shipper for the category of the animal by-product that will be shipped. Animal by-products that will be used for research or diagnostics belong to Category 1.

Table 9-2. Summary of requirements for shipping animal by-products.

Identification number	Substance class	Packing Instruction	Labeling text	Other requirements
UN 3373	6.2	P 650	*Biological substance category B* and *For research and diagnostics*	Documents: Letter of Authority (= EU shipping declaration) when shipped by courier, packing list (advised), commercial document*. Shipper: Own transport allowed, but registration as shipper at NVWA required. Mail within The Netherlands is allowed when PostNL-approved packaging is used.
UN 2900	6.2	P 620	*Infectious substance, affecting animals only* and *For research and diagnostics*	Documents: Letter of Authority, packing list, shipping bill (vrachtbrief), safety instruction, commercial document*. Shipper: Registered at NVWA, driver with ADR certificate. Mailing not allowed
UN 2814	6.2	P 620	*Infectious substance, affecting humans* and *For research and diagnostics*	See UN 2900 above.

* As mentioned in par 9.2.1, the commercial transportation documentation can substitute for the Letter of Authority describing biological hazards when all required information is provided.

9.5.2 Importing animal by-products into the EU

Animal by-products are frequently imported into the EU for research or diagnostic purposes. The following requirements and exemptions apply:

- ***Import permit***. For category 1 and 2 materials, the importing party must possess an import permit from the NVWA following EU regulation 1029/2009 that restricts import of these categories of animal by-products. VU holds a permit to import animal by-products for purposes of research and diagnostics (see GMO-VU folder). It requires that the materials enter the EU at Amsterdam Schiphol Airport. Each shipment must be pre-announced at the Customs Desk Schiphol Cargo Center using the Common Veterinary Entry Document (CVED, gemeenschappelijk document van binnenkomst, GDB). The VU will be billed by the NVWA for verifying the pre-announced shipment. The CVED can be downloaded in Dutch and English from the GMO-VU folder. Also, instructions how to fill out the CVED/GBD is available on this site.
- ***No approved country origin required***. By law, import of animal by-products into the EU is restricted to sources that have been approved by the NVWA. Animal by-products imported for the purpose of research or diagnostics are exempt from this restriction.
- ***No Health Certificate required***. By law, import into the EU of animal by-products must be accompanied by a health certificate issued by an accredited veterinarian for each shipment. If none of the certificates provided in annex XV of EU directory 142/2011 applies, the shipment must have a commercial document. Animal by-products imported for the purpose of research or diagnostics, however, do not need a health certificate: only a commercial document is required. Transport within the Netherlands after EU-import only requires a commercial shipping document. An example of a commercial shipping document (vorm-vrij) and the updated EU commercial document can be downloaded from the Biosafety Resource.
- ***No Letter of Authority (EU shipping declaration) required***. By law, shipments of animal by-products to another EU Member State must be accompanied by an EU shipping declaration. Shipments of animal by-products that are to be used for research, diagnostics or education are exempt from this requirement.

See for more information on handling animal by-products, including import into the EU, the guidance document issues by the BVF Platform (see GMO-VU folder).

10 Maintenance

10.1 General guidance

Maintenance, e.g. of equipment, in contained facilities requires preparation to prevent exposure of personnel to biological agents and the accidental spread of biological agents into the workplace and/or environment.

Act according to the following guidance:

- Report malfunctioning alarms, equipment, etc. to the equipment manager, lab manager or ABV.
- Determine the precautionary measures needed for safe inspection, maintenance or repair, e..g. the need for decontamination and personal protection equipment. Document the hazards and the associated control measures on a Biosafety Clearance Declaration (see Biosafety Resource);
- No other work should be carried out in the lab during maintenance activities, unless pre-approved by the ABV or lab manager;
- After the (bio)safety measures have been completed, the workplace and/or the equipment must be cleared by the ABV before maintenance/repair activities can start. The Institutional and risk assessment procedures must be followed and clearance status should be documented using the appropriate form. If none is applicable, use the *Equipment Release* Form (see Biosafety Resource);
- Accompany the service engineer(s) that will carry out the maintenance or repair;
- When the repair or maintenance is complete, release the object by completing the Biosafety Clearance Declaration, and communicate that the object is in operation again.

10.2 Biological safety cabinet

BSC must be properly maintained to ensure their correct functioning. Maintenance must be carried out in compliance with the instructions of the supplier. Cabinets must be serviced once a year to check if the protection parameters (inflow, HEPA-filter integrity) still meet the normative criteria. The due date for retesting must be indicated on the cabinet and the test reports must be kept available for inspection.

Departments must take care to change prefilters frequently. Clean prefilters will decrease the chance of microbiological contamination of the work compartment and will extend the lifespan of the HEPA filters.

Before the HEPA-filters are replaced, the BSC must be disinfected by a validated method using vaporized hydrogen peroxide (VHP). This service is offered by several service companies. Proper disinfection of a BSC is the responsibility of the user, timely maintenance is the responsibility of the owner. For disposal of HEPA filters, see chapter 11, *Waste*.

10.3 Autoclave

Autoclaves used with hazardous biological agents must be serviced once every year. This service should be performed by an accredited party. Servicing must include verification of pressure, temperature and the parameters of pre-installed programs. Maintenance records must be kept available for inspection.

10.4 Contained facilities

When contained facilities, like laboratories and animal wards, require extensive maintenance or restructuring, they must be taken out of operation. Precautions taken for safe clearance must be recorded on the Biosafety Clearance Declaration (see Biosafety Resource). The form must be signed by the C-BSO. When a facility is ready for operation again, the clearance procedure for new contained facilities must be followed (see par. 6.1.3).

10.5 Vacuum systems

Vacuum systems in microbiological facilities must be protected with high efficiency particulate air (HEPA) filters and liquid disinfectant traps. This can be done by installing a suction system consisting of a collection flask, an overflow bottle and a HEPA filter in line with the vacuum system (see figure 10-1). The collection and overflow flasks must contain disinfectant. Both flasks must be robust to withstand pressure, must be surrounded by metal gauze to shield personnel in case of implosion, and must be placed in a spill tray to contain leaks. Care must be taken to empty the bottles in time and to replace the filter before indicated due date. These points of control must be included in a lab maintenance list.

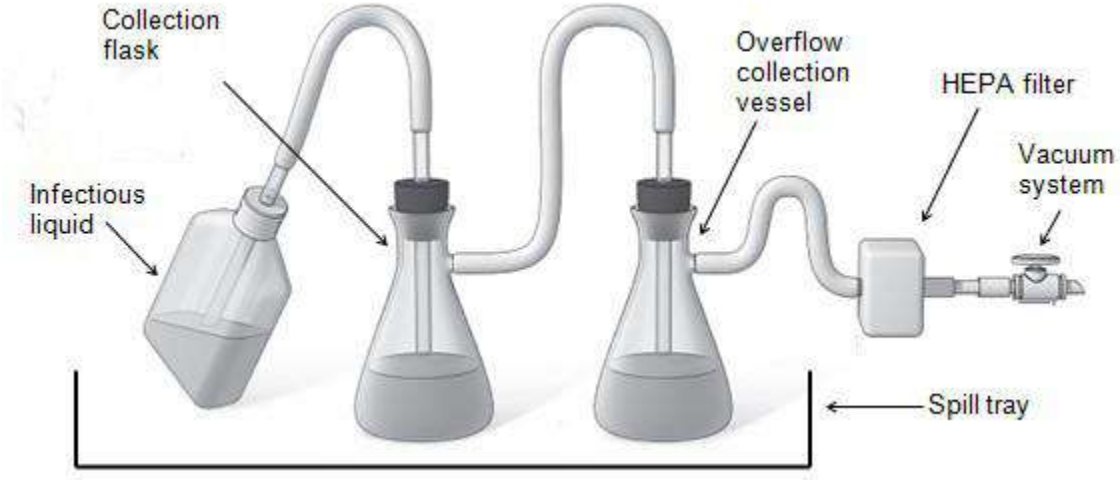


Figure 10-1: HEPA filtered overflow set to prevent microbiological contamination of vacuum systems.

11 Waste

11.1 Biological waste policy

Biological waste must be processed in accordance with the following policy:

- Safe and environmentally sound disposal following the institutional requirements in the VU Waste Manual.
- Biological waste should be disposed as hospital waste. Other ways of disposal may be applicable;
- When inactivation of biological agents prior to disposal is required, autoclave the waste. If autoclaving is not an option, use an approved and validated method for inactivation (see par. 8.1);
- Document the inactivation of biological agents, e.g. log autoclave runs, and archive records for six months to keep the records available until the treated waste is finally eliminated.

Inactivated microbiological waste that does not contain other hazardous components can be delivered to a contracted receiver of inactivated waste along with an optional Declaration of Microbiological Inactivation of Waste (see Biosafety Resource).
Microbiological waste containing microorganisms of risk class 3 or 4 or agents listed on the quarantine lists of animal or plant pathogens must be inactivated on-site and must be disposed as hospital waste even after this treatment. When inactivation of these agents is not feasible, a permit according to EU directory 2008/61/EC must be obtained from the NVWA in order to ship the waste for incineration.

11.2 Waste decontamination by autoclaving

As for all autoclave treatments, the program used for waste destruction must be validated, the equipment must be maintained to operate properly, the user must be trained in its operation, and each run must be recorded to monitor proper processing.

Waste for autoclaving must be collected in stainless steel containers and lined with plastic bags that are permeable for steam. Liquid waste must be placed in glassware in these containers. Lids of containers that are not designed to admit vacuum and steam entry should be loosely closed when placed in the autoclave.

11.3 Waste streams for biological waste

The following waste streams described in the Waste Manual are relevant for biologically hazardous waste (see figure 11A):

- Animal waste;
- Chemical waste;
- Hospital waste;
- Radiological waste;
- Regular waste (non-hazardous).

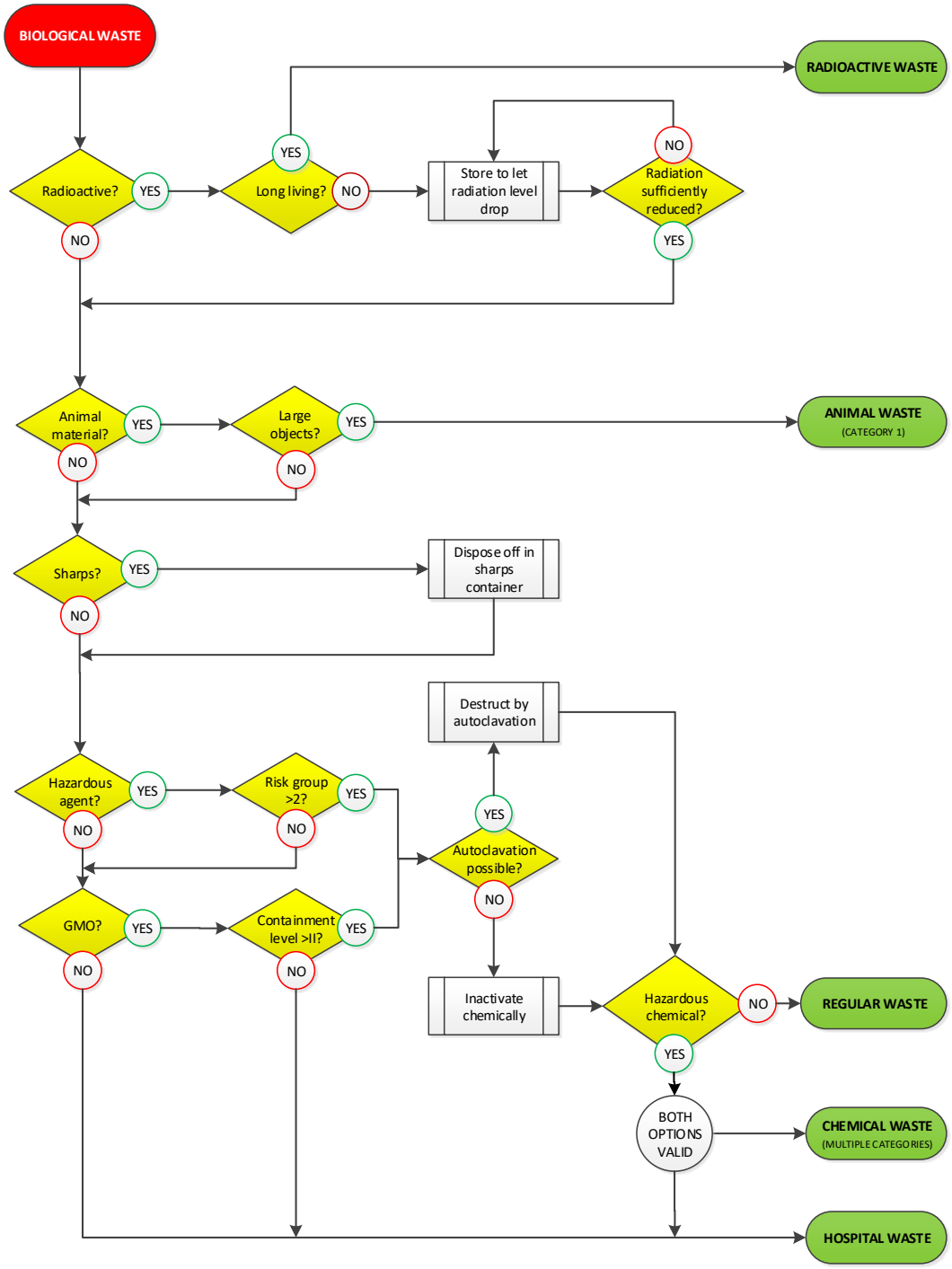


Figure 11A. Overview of handling biological waste. See Waste Manual for details.

At times there may be a need to initiate dedicated waste streams for extraordinary materials, like disposal of large used HEPA filters. Contact Facility Services on how to proceed in such instances.

11.3.1 Hospital waste

To keep waste logistics simple, at VU (micro)biological waste is shipped as hospital waste without prior inactivation whenever possible. It is shipped as ADR class 6.2 substance with UN 3291 and the waste containers comply with packaging instruction P620. Because of this policy, the regulations on alternative practices are not described in this chapter.

Hospital waste is the common name for waste that is generated from human or animal health care activities. *Specific* hospital waste (specifiek ziekenhuisafval, SZA) is a subcategory that may contain infectious waste, non-infectious body parts or organs, and/or cytotoxic or cytostatic substances that originates from human healthcare activities. At VU the abbreviation SZA is however used in a broader sense because this waste stream is also used to trash other materials that might pose biohazards or that may raise public concerns, like animal by-products and GMO that must be handled at containment level I or II.

Hospital waste must be collected and shipped in UN-certified, robust, leak- and airtight containers that meet the criteria of ADR Packaging Instruction P621. Hospital waste from VU is shipped by an approved shipper to ZAVIN (Ziekenhuis Afval Verwerkings Installatie Nederland) for incineration.

This waste stream does not require on-site inactivation. As a consequence it is not fit to dispose off biological agents of risk class 3 and 4 or GMO that must be handled at containment level III or IV.

The following work instructions apply for SZA:

- Only use the containers described in the Waste Manual. These materials meet the regulatory requirements (figure 11B);
- Dispose of sharps in special sharps containers (figure 11C), and deposit these in a hospital waste container;
- Dispose of liquids in closed vials of relatively small volume, deposit these in a hospital waste container, add enough absorbent to hold at least the amount of liquid equivalent with the volume of the largest vial that has been disposed of in the container;
- Close the lid firmly when the container is 75% full. Never overfill containers: 75% full = full. Because the containers are stacked manually during transportation, the maximum weight should not exceed 15 kg;
- Decontaminate the exterior of the container with an appropriate disinfectant before taking it outside the laboratory;
- Apply an ADR-label showing hazardous substance class 6 and UN number 3291 on the side of the container (figure 11D) if this is not yet present on the container.
  The ADR applies multiple UN numbers, depending on the nature of the biological waste (see Waste Manual for details). However, to keep logistics simple, it has been agreed with the waste handler that all SZA containers will be labelled with UN 3291 (unspecified hospital waste). This practice is approved by ILT also for waste with GMO originating from containment level I or II.
- Fill out the general tracking label (figure 11E left) and stick it on the grip of the lid. This label enables the logistic service to contact the supplier of the waste in case of any questions;
- When the container contains GMO, also fill out GMO on tracking label as well. (figure 11E right). By applying this label, the regulatory requirement to mark GMO-containing waste is met;

- Do not leave waste unattended outside the lab. Deposit full containers in a waste station, do not keep them in the lab.

FCO takes care that waste is cleared from the waste stations frequently. Collected SZA containers are stored in the institutional waste compound in ADR-approved transport containers, awaiting shipment for incineration. FCO keeps a record of the amount of hospital waste that is shipped for destruction. The amount of GMO waste in total SZA is not recorded separately. Therefore, departments must keep a record of the number of GMO-containing hospital waste containers.

11.3.2 Animal by-products

11.3.2.1 Waste from experimental animals

Waste from experimental animals is considered to be an animal by-product classification category 1 and disposal must comply with the requirements for labeling, packaging, documentation, shipping and destruction as dictated in EU Regulation 2009/49 on animal by-products. Containers with category 1 material must bear the label shown in figure 11F, unless shipped as hospital waste. Disposal of animal remains as hospital waste is allowed as long as the waste does not consist of animal remains only and the logistics of the hospital waste stream are up to the task. The hospital waste stream is e.g. not fit for disposal of animal remains that are too large to fit in a regular hospital waste container and/or waste that must be kept refrigerated during storage and transportation.

Figure 11c: Label for waste of animal by-products of Category 1.

- ***Animal remains***. Animal remains are allowed to be disposed of as hospital waste. Containers with such remains must be kept refrigerated when short term waste storage is necessary before collection by the Facility Services. Remains that are too large to fit in a hopsital waste container must be shipped to Rendac for incinerartion as animal by-product waste of category 1. This waste must be collected in dedicated containers that are kept cool or frozen until offered for incineration. Storage is limited to one week at 4^{o}C and to two months when stored at minus 18^{o}C. Shipping has to be done by a certified transporter. Since this type of waste is currently only generated within the AARC facility of the VU, waste handling is carried out according to the institutional procedure of the VU.
- ***Faeces, urine and bedding***. Materials that are (potentially) contaminated with infectious agents of risk group 1 or 2 and/or with GMO handled at containment level I or II, must be disposed of as hospital waste. When (potentially) contaminated with biological agents of risk group 3 or 4 and/or GMO handled at containment level III or IV, decontamination by autoclaving before processing as hospital waste is compulsory.
- ***Laboratory reagents***. Waste consisting of animal by-products that qualify as laboratory reagents must be incinerated, e.g. by disposal as SZA. In rare circumstances, such materials may be disposed of via the sewer system (down the sink) when highly diluted. However, dilution of this waste for the sole purpose of sink disposal is prohibited.

11.3.3 Radiological waste

The processing of biological waste that is radioactive depends on the nuclear chemical nature and decay of radiation activity. Waste containing short-lived nuclides is stored until radiation levels have dropped sufficiently for incineration as hospital waste or regular waste, depending on its composition. Waste containing long-lived nuclides is sent for long-term storage in shielded facilities to COVRA in Vlissingen (Zeeland).

11.3.4 Chemical waste

Chemical waste consists of chemicals harmful for humans and the environment. Waste collection and processing will depend on the chemical composition and quantity. Consult the Waste Manual for details. Microbiological contamination of chemical waste will complicate its disposal. The following situations may apply to chemical waste containing hazardous biological agents, GMO and/or animal by-products:

- ***Microbiologically not inactivated, no containment level 3 or 4 biological agents***. This waste mixture must be processed as hospital waste, animal by-product waste or radiological waste, whatever is the most appropriate waste stream;
- ***Microbiologically inactivated, no containment level 3 or 4 biological agents***. This waste should be processed as chemical waste. Disposal as hospital waste is still an appropriate option, since both will result in incineration of the material;
- ***Waste from a containment level 3 facility (e.g. ML-III, BSL-3*)**. On-site microbiological inactivation is compulsory. After this treatment, the waste still needs to be disposed of as hospital waste. In case microbiological inactivation is not feasible, the waste must be disposed of as special infectious waste, and a permit to ship it off-site from the NVWA is required (see par. 11.1).

11.3.5 Regular waste

Regular waste is the category for non-hazardous waste. Biological waste that contained infectious biological agents of risk group 1 or 2, or GMO that must be handled at containment level I or II, is allowed to be disposed of as regular waste after it has been autoclaved/sterilized. It must be labeled with the EURAL (Europese afvalstoffenlijst) waste code 180104 meaning “waste not subject to special requirements in order to prevent infection, non-hazardous". The waste code is marked on the document Declaration of Microbiological Inactivation of Waste (see GMO-VU folder) and must including a printout of the critical parameters that show that decontamination has been effectively accomplished. Whenever this disposal route is expected to pose sanitary problems, e.g. due to decomposition or public concern, the waste should be disposed of as hospital waste.

11.4 Disposal of HEPA filters

HEPA-filters are used to eliminate microorganisms and viruses from air. Therefore, before disposal, such filters must be disinfected in situ with a validated method using vaporized hydrogen peroxide. Be aware that disinfected HEPA filters are only microbiologically decontaminated, consequently appropriate disposal will depend on whether the filters may contain hazardous chemicals, like cytotoxins. The following options are available to discard fumigated HEPA filters:

- When only microbiologically contaminated:
- After validated disinfection: regular waste;
- When not disinfected: hospital waste;
- When (also) contaminated with hazardous chemicals;
- After validated disinfection: chemical waste;
- When not disinfected: hospital waste.

Disposal of HEPA filters as hospital waste or chemical waste requires a special arrangement with the waste handling service party because these filters will probably not fit in the regular hospital waste containers.

12 Incident management

12.1 Incident categories

Various categories of incidents that may present biological risks can be discriminated:

- ***Biologically unsafe situation***: A situation that has the potential to develop into a biosafety incident, accident, calamity or near miss. Nothing went wrong yet.
- ***Biosafety near miss***: A situation that could have resulted in a biological incident, accident or calamity. Something went wrong, but nothing really happened.
- ***Biosafety incident***: An occasion that (probably) has resulted in exposure of persons to hazardous biological agents and/or an emission of GMO into the workplace.
- ***Biosafety accident***: A biosafety incident with personal injury.
- ***Biosafety calamity***: A biosafety incident that resulted or might result in emission into the environment of a hazardous biological agent or a GMO, serious health risks to the community, serious environmental risks, public concern, severe financial damage or that threatens business continuity.

12.2 First Response

To mobilize assistance in case of incidents, dial the appropriate emergency number indicated on the wall chart:

- ***General emergency***: 22222 or 020-59822222 (contact Meld- en Bedieningscentrum);

12.3 Incident reporting

Biosafety incidents, accidents and calamities must be reported immediately to the lab manager, ABV and C-BSO. In case the biosafety incident occurs in a workspace where experimental animals are present, also notify the Experimental Animal Coordinator. The BSO and Experimental Animal Coordinator will assist in deciding on how to follow-up the incident. The BSO will also inform accountable management, initiate a root cause analysis in cooperation with the relevant stakeholders and will report the outcome of this analysis to accountable management.

Register incidents, accidents and calamities in the institutional incident registration system (see Annex 2) within 24 hours after their occurrence. Preferably also register near misses and unsafe situations to help to create awareness and enable decision making on preventive actions. Anyone who has an IT account can use the incident reporting application. Consult the ABV when you want to report an occasion to ensure that correct and clear information is entered.

The intranet home page provides guidance on how to act in case of large scale or severe incidents: see under “Environment” (Emergencies). It gives access to various documents, including the institutional emergency plan. This guidance aims to prevent escalation of the incident and to resolve it as quickly and efficiently as possible. The remainder of this chapter focuses on incident management issues that are relevant when a biological safety incident has occurred and will detail the guidance provided on the intranet.

Incidents that involve biological agents of risk group 2, 3 or 4 must be reported to the Works Council. Incidents involving biological agents of risk group 3 or 4 must immediately be reported to the Management Council. These notifications are done by or on behalf of the Faculty Board

12.4 Notification of severe incidents to the authorities

Three types of incidents must be reported to the authorities:

- ***Health and safety***. Incidents that result in hospitalization, (potential) disability, or death must be reported according to the Occupational Safety and Health Act.
- ***Emission of GMO.*** Incidents that result in accidental release into the environment of GMO must be reported according to the GMO Decree. The guidance given by Bureau GGO and ILT on when to notify GMO releases is given in table 12-1.
- ***Gene therapy.*** Unexpected, unplanned and unwanted occurrences during gene therapy studies that may pose an increased health or environmental risk.

Table 12-1: Criteria for notifying GMO incidents.

Containment Level	**Incident category**		
	A	B	C & D
I or II	ABV	C-BSO	C-BSO and ILT
III or IV	C-BSO	C-BSO and ILT	C-BSO and ILT
Category description			
A	Small scale release into work space that can be cleaned by employees themselves.		
B	Release into work space of a volume large enough so there is a risk of release outside of the contained facility, e.g. spreading due to personal contamination.		
C	Realistic probability of release of GMO into the environment because of a loss of containment of the facility, e.g. in case of nearby fire, broken windows, loss of facility atmospheric under pressure or contaminated personnel that left the facility.		
D	Factual emission of GMO into the environment, e.g. through sink disposal, air emission or a contaminated person that left the facility.		

Notifying incidents to the authorities is the responsibility of the Management Council and will be done on its behalf by:

- Health and safety incidents: notification done by or on behalf of the Faculty Boards;
- Contained use of GMO incidents: notification done by the C-BSO;
- Incidents with Q organisms: notification done by the C-BSO

12.5 Treatment of wounds and splashes

Treat any wound, like cuts, bruises, and punctures, as well as mucous membranes that might have been contaminated by hazardous biological agents or GMO as follows:

- ***Intact skin***: Clean intact skin using disinfecting hand gel. Take care to include potentially exposed skin underneath contaminated clothing.

- ***Wounds***, including self-inoculation: Bleed thoroughly: massage the wound while rinsing it with warm water. Apply disinfectant: chlorine hexidine or povidone iodine. Apply ethanol 70% only when no other disinfectants are readily available, since this substance will hinder healing.
- ***Mucous membranes*** (eyes, nose): Flush with ample warm water. Rinse eyes for 15 minutes using eye wash station. In case of use of an eye rinsing flask: after first rinse, continue rinsing elsewhere using eye wash station as soon as possible (a.s.a.p.) and keep eyes open while rinsing.

After such incidents, c consult a.s.a.p. a medical doctor at spoedeisende Eerste Hulp (020-4434101 or *986862, location: W0.G.201). In case of self-inoculations, this consultation is mandatory. The medical doctor will decide whether or not a medical intervention and follow up is needed after self-inoculation. To enable proper follow-up of these incidents, always record the material involved when reporting the incident in the institutional incident management system (see par. 12.2) and whenever possible keep a sample of the material involved.

12.6 Spill resolution

The standard operating procedures (SOP) below give guidance on how to effectively and safely handle spills. They aim to remove and sanitize (potential) microbiological contamination caused by spills. The SOP provide instructions to minimizes the chance of personal exposure and to limit spread of the contamination. The SOP are applicable to relatively small spills, i.e. a spill that can be cleaned up by the individual that caused the spill or in cooperation with a few colleagues. When more assistance or help from outside the department is required to handle the spill, contact the C-BSO (see table 1-1) to help decide the best way to manage the spill.

12.6.1 General strategy for spill resolution

When dealing with a spill, act according to the following general strategy:

- Avoid inhalation of microbiologically contaminated aerosol;
- In case of eye splashes, rinse eyes first, then disinfect exposed body parts;
- Ask for assistance, in case of large or complicated spills;
- Clean-up spill area;
- Dispose of contaminated materials as hospital waste or autoclave;
- Record and report a spill affecting a larger or complicated area as biosafety incident according to incident category.

In general, the following materials are needed for spill clean-up. If these materials are not readily available during normal operation, they should be assembled and kept available as a spill kit:

- Disinfectant(s) effective for the spilled biological agent/material and suitable for the surfaces to be treated. See guidance in table 12-2 in general disinfectants. Use other disinfectants when indicated in your protocols;
- Eye rinsing flasks, if no eye wash station is available in the room;
- Disinfecting skin gel: Sterilium GEL Pure (N14408, 85% ethanol, and skin protecting additives). Dose: apply 3 ml two times to keep skin wet during a total contact time of 1.5 minutes;
- Absorbing material, e.g. tissues, paper towels, granules (korrels);

- Personal protection: Lab coat, gloves, liquid repellent shoe covers or waterproof boots/clogs, safety goggles, hair net, FFP2 nose-mouth mask;
- Forceps, squeegee (or other appropriate device), dustpan, sharps container, autoclavable bags;
- Warning sign: "Do Not Enter/Use – Spill" warning sign.

Table 12-2 General disinfectants.

Biological agent	Spill area	Active ingredient	Brand	Approval number	Dose	Contact time
Microorganisms	≤0.5 m^2	isopropyl ethanol	Klercide 70/30 Denatured Ethanol	N14031	70%, use 40 ml/m^2	≥5 min.
	>0.5 m^2	chlorine*	Suma Tab D4	N7321	1000 ppm, (1 tablet in 5L water)	≥5 min.
Viruses	all	Pentapotassium bis(peroxymonosulphate) bis(sulphate)	Virkon S	N13676	1% (100g in 10L water) for labs, 4% for animal wards	10 min. (labs), 30 min. (animal wards)

* Chlorine solution must be prepared freshly. Chlorine is corrosive, therefore rinse spill area thoroughly with water after disinfection.

12.6.2 Spill clean-up inside a biosafety cabinet

- Leave BSC running;
- Place warning sign on BSC;
- Put on lab coat and gloves, if not yet wearing;
- Remove contaminated objects from spill:
  - ***Waste***: dispose of as hospital waste according regular BSC working procedure: collect in bag inside cabinet, decontaminate outside of bag, dispose bag in hospital waste container;
  - ***Material/equipment to be kept for reuse***: wipe exterior thoroughly with disinfectant. If possible, autoclave materials before reuse.
- Remove sharps, e.g. broken glassware, using forceps only, never directly with your fingers;
- Remove tiny sharp remains, like glass splinters, using damp cottonwool;
- Disinfect and remove spilled material:
  - ***Small volume*** (can be absorbed by a single tissue): absorb spilled material with absorbent pad and dispose, apply/wipe disinfectant on the spill surface taking care to cover the whole surface. After appropriate contact time, dispose in BSC waste bag;
  - ***Larger volumes***: cover the spill with absorbent material, gently apply disinfectant by pouring in circles beginning at the outside of the spill, and dispose of absorbent after the minimum contact time. Repeat this procedure towards the center of the spill until all of the liquid has been removed. Wipe spill area with disinfectant one final time.

- If the spill has spread beyond the work surface, e.g. on grilles, inside seams, or into catch basin under the work surface, take out decontaminated equipment and materials, disassemble the BSC, and disinfect all potentially contaminated parts. Autoclave parts if possible;
- Wipe down all interior surfaces of the BSC with disinfectant in case there is any suspicion that the spill could have contaminated these areas. Always do this when a larger volume was spilled;
- Properly dispose of contaminated materials;
- Let BSC run for 10 minutes after cleanup;
- Remove warning sign to clear BSC for use;
- Record incident in BSC logbook (if in use);
- Record and report spills of larger volumes inside a BSC as biosafety incident assigning the appropriate category (see par. 12.1).

12.6.3 Spill clean-up spill in a room, biological agent without airborne transmission route

- Notify other personnel in the room;
- When exposed:
  - Take off contaminated clothing, put it in an autoclavable bag or dispose of in a hospital waste container;
  - Disinfect contaminated body parts. If needed, first rinse eyes, then disinfect skin. See par. 12.4 for details.
- Put up warning signs up at all entrances to the room;
- Inform lab manager, contact ABV if you need assistance;
- If needed, mark the spill area, e.g. using red-white ribbon;
- Prepare to re-enter room: put on clean lab coat (if not yet on), shoe covers or waterproof boots/clogs, safety goggles, and gloves.
- Disinfect spill area:
- Pick up contaminated clothing from floor, put in an autoclavable bag or dispose of as hospital waste;
- Cover spill area with appropriate absorbing material;
- Gently pour disinfectant on absorbing material, circling from the outer rim of the spill inwards. Do not splash disinfectant;
- Allow for appropriate contact time (see Clean-up materials) before removing absorbing material;
- Meanwhile, disinfect all objects around the spill area that might have been contaminated due to the spillage. Use appropriate methods: wipe surfaces, soak objects, autoclave and/or dispose of as hospital waste;
- Clean-up spill area:
- Pick up absorbing materials, dispose of in hospital waste container;
- Pick up any sharps, including broken glass, with forceps and place in sharps container. Never touch sharps directly. Dispose of sharps container in hospital waste container;
- If needed, absorb remaining spilled liquid with appropriate absorbent. Dispose of material in hospital waste container. Repeat until all “free” liquid has been removed;
- Use squeegee (or other appropriate device) and dustpan to collect shards (e.g. of broken glass). Disinfect squeegee and dust pan;
- Wipe spill area and adjacent surface with paper towels soaked in disinfectant. Dispose of towels in hospital waste container;

- Mop spill area with water, and wipe or let dry.
- Leave spill area:
- Take off foot covers, discard them in hospital waste container;
  When wearing waterproof boots/clogs: put on a fresh pair, wipe outside of worn boots/clogs with disinfectant;
- Wipe floor surface in neighborhood of spill area that has been walked on with foot covers/waterproof boots/clogs with disinfectant;
- Proceed to entrance:
- Take off goggles. Wipe them with disinfectant, then rinse with water;
- Take off gloves, put them in the hospital waste container;
- Disinfect hands and wrists with skin disinfectant;
- Wait until mopped spill area has dried, then leave room;
- Remove warning signs from entrance(s), notify lab manager and colleagues that spill has been removed;
- Record and report spill as a biosafety accident of appropriate category (see par. 12.1).

12.6.4 Spill clean-up in a room, airborne biological agent

- Notify other personnel in the room;
- If unexposed: Leave room as soon as possible
- When exposed:
- Put on breathing protection (FFP2) as soon as possible;
- Take off contaminated clothing, put it on the floor;
- Disinfect contaminated body parts. If needed, first rinse eyes, then disinfect skin. See par. 12.4 for details.
- Leave the room (continue eye rinsing when needed);
- Post warning signs on all entrances of the room;
- Notify lab manager, and ABV immediately. Do not reenter the lab until given permission from ABV!
- Let aerosols settle for 30 minutes before re-entering the room to clean-up spill;
- Prepare to re-enter room: put on a clean lab coat, shoe covers/waterproof boots/clogs, safety goggles, gloves, and a FFP2 nose-mouth mask*. Then enter room. Only persons needed for clean-up should enter room.
  * NOTE: As an alternative to using a FFP2 mask, one could request the help of a First Responder trained in the use of a pressurized air purifying respirator.
- Disinfect spill area:
- Pick up contaminated clothing from floor, put in an autoclavable bag or dispose of as hospital waste;
- Cover spill area with appropriate absorbing material;
- Gently pour disinfectant on absorbing material, circling from outer rim of the spill inwards. Do not splash the disinfectant;
- Allow for appropriate contact time (see Clean-up materials) before cleaning up area.
- Meanwhile, disinfect all objects around spill area that might be contaminated due to the spillage. Use appropriate methods: wipe surfaces, soak objects, autoclave and/or dispose of as hospital waste.
- Clean-up spill area:
- Pick up absorbing materials, dispose of in hospital waste container;

- Pick up any sharps, including broken glass, with forceps and place in sharps container. Never touch sharps directly. Dispose of sharps container in a sharps box or in a hospital waste container;
- If needed, absorb remaining spilled liquid with appropriate absorbent. Dispose of material in hospital waste container. Repeat until all “free” liquid has been removed;
- Use squeegee (or other appropriate device) and dustpan to collect shards (e.g. of broken glass). Disinfect squeegee and dust pan;
- Wipe spill area and adjacent surface with paper towels soaked in disinfectant. Dispose of towels in hospital waste container;
- Mop spill area with water, and wipe or let dry.
- Leave spill area:
- Take off foot covers, put them in hospital waste bin. When wearing waterproof boots/clogs: put on a fresh pair of waterproof boots/clogs and wipe outside of worn waterproof boots/clogs with disinfectant;
- Wipe floor surface in neighborhood of spill area that has been walked on while wearing foot covers/waterproof boots/clogs with disinfectant.
- Leave room:
- Proceed to entrance;
- Take off goggles. Wipe them with disinfectant, then rinse with water;
- Take off FFP2 mask, put it in a hospital waste container. When a pressurized air purifying respirator has been used, disinfect outside before taking it out of the room;
- Take off gloves, put them in hospital waste container;
- Disinfect hands and wrists with skin disinfectant;
- Wash face with water and mild soap;
- Wait until mopped spill area has dried, then leave room.
- Remove warning signs from entrance(s), notify lab manager,, ABV and colleagues that spill has been cleaned-up;
- Record and report spill as a biosafety accident of appropriate category (see par. 12.1);
- Consult medical doctor, at least when exposure to biological agent is known or expected.

12.7 Guidance on emergency assistance in contained facilities

This paragraph outlines the general instructions for entry and exit of contained facilities in emergency situations. See the Institutional Emergency Plan (intranet) for details. Situations that are considered to be an emergency include:

- Accidents with victims that need medical assistance;
- Incidents that may result in a life threatening situation or in severe damage to inventory or building.

Under normal operational conditions access to containede facilities is restricted to authorized individuals wearing protective clothing or other personal protection appropriate for the containment level. In case of emergency however, access to contained facilities by emergency personnel is allowed without or with reduced personal protective equipment (PPE) when:

- There is no risk of exposure to biological agents hazardous to human health;
- The duration of being in the facility is minimized, e.g. by moving and treating victims that are not microbiologically contaminated outside the facility as soon as possible;

- Sufficient precautions are taken to minimize the chance of the release of microbiological agents or animals from the facilities.

12.7.1 Labs and equipment rooms of containment level I and II

In facilities of containment level I (BSL1, ML-I, AP-I, D-I, DM-I) no infectious biological agents are handled. In facilities of containment level II (BSL2, ML-II, DM-II) biological agents that might be hazardous to human health or the environment can be present. Therefore level II facilities show the biohazard sign at their entrance. The potential presence of hazardous biological agents in level II facilities makes it necessary to take some precautions to control potential exposure and spread of agents when providing first aid or other emergency assistance in Level II facilities.

See figure 12A for a chart showing the critical decisions when providing First Aid in Level I and II facilities. The following instructions apply (additional measures for Level II are printed in italics):

- Anybody not instrumental in the process of emergency assistance should leave the facility;
- Individuals providing emergency assistance, like First Responders, can safely enter Level I facilities without the use of protective clothing and personal protective equipment.

Level I

- When **no fire**, put on protective clothing (lab coat) and gloves. Inquire if breathing protection (FFP2) should be worn. In DM-II always wear breathing protection, also when the need for it is unknown;
- When **fire**, call for firefighters. They must wear breathing protection and gloves.
- Preferably use foam or carbon dioxide to extinguish fire;
- Animals are allowed to be evacuated in closed cages. Act according to the instructions given by the experimental animal coordinator.

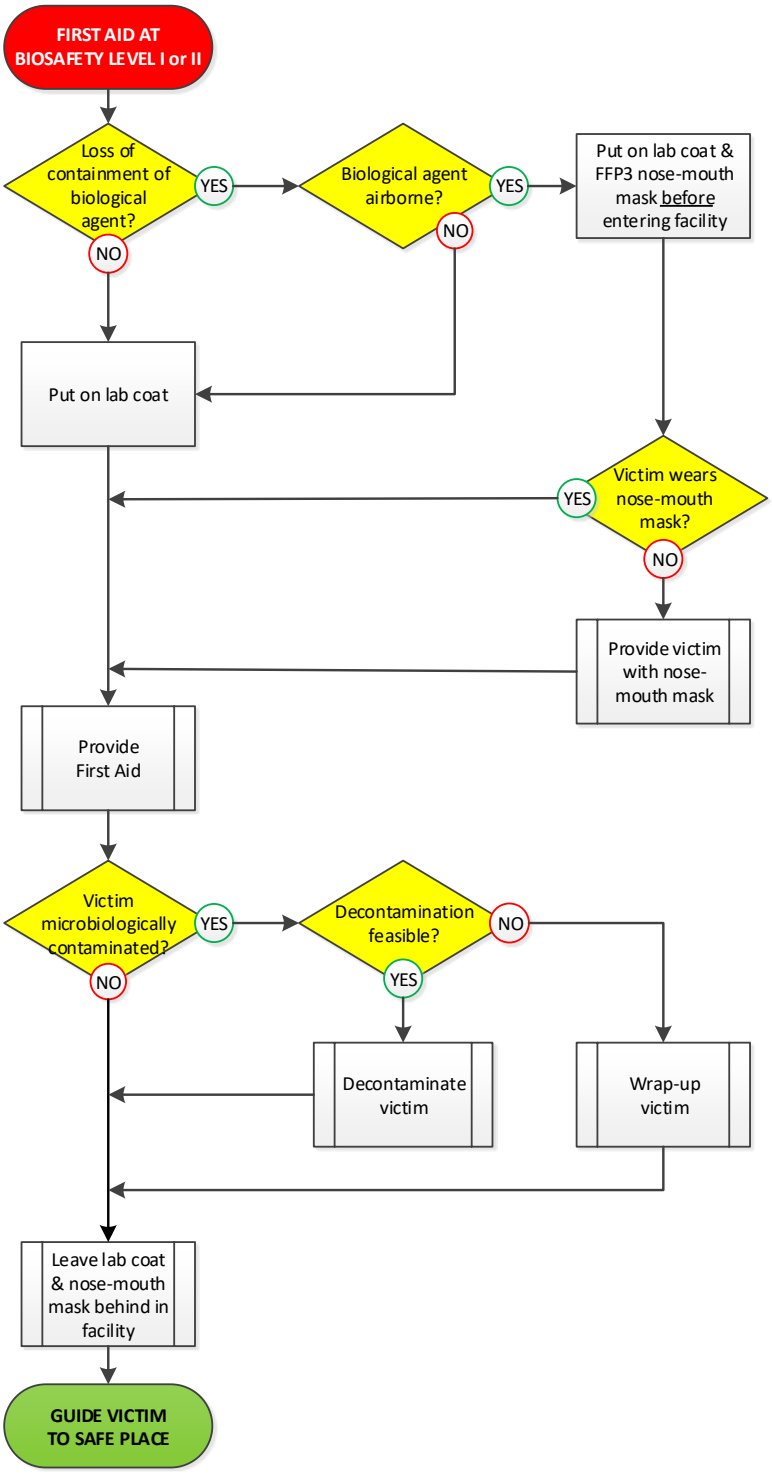


Figure 12A: Critical biosafety decisions when providing First Aid in a Level I or II facility.

Level II

- Animals are allowed to be evacuated to other rooms only when housed in closed cages **with filtertops**.
- Take precautions to prevent the spread of GMO while leaving the facility: e.g. beware of contamination of footwear, clothing (including the victim) and objects. Leave all (potentially) biologically contaminated materials behind. These materials must afterwards be disposed of as hospital waste or decontaminated using an appropriate disinfectant or autoclave program;
- Wash your hands when leaving the facility;
- The facility has to be cleared for use by the lab manager, ABV or C-BSO after verification that the workplace is safe.

12.7.2 Labs of containment level III

In facilities of containment level III (BSL3, ML-III), infectious biological agents and GMO that might be very hazardous to human health or the environment are handled. These facilities show the biohazard sign at their entrance. To control the risk of exposure and spread of the hazardous biological agents when providing first aid or other emergency assistance in Level III facilities, *tailored control measures* are needed. Which measures to take will depend on the specific biologicals agents that are handled. Therefore the Level III lab unit in the O|2 building has a tailored emergency instruction. Nevertheless, some general safety precautions can be outlined for containment level III facilities (see also figure 12B):

- Protective full-body suits and pressurized air breathing protection equipment is compulsory. Only First Responders qualified to use this equipment are allowed to enter;
- Minimize the number of individuals that enter the facility, however at least two responders should enter together at the same time;
- Take precautions to prevent spreading of biological agents when leaving the facility: e.g. beware of contamination of clogs/overshoes, clothing and objects. Follow all guidance given by the lab manager, ABV and/or BSO when leaving. Minimize all traffic directly outside of the facility. Leave all (potentially) biologically contaminated materials behind. These materials must afterwards be disposed as hospital waste or decontaminated using an appropriate disinfectant or autoclave program;
- Spray everything that cannot be left behind amply with an appropriate disinfectant before leaving the facility. If needed, wrap-up (potentially) biologically contaminated individuals, materials and equipment for transport. Upon arrival at the destination, act according to the guidance given by the lab manager, ABV and/or BSO.
- Everyone who may have been microbiologically exposed as a result of the incident must consult the medical doctor to determine post-exposure follow-up;
- The C-BSO must determine that the workplace is safe before work can be resumed.

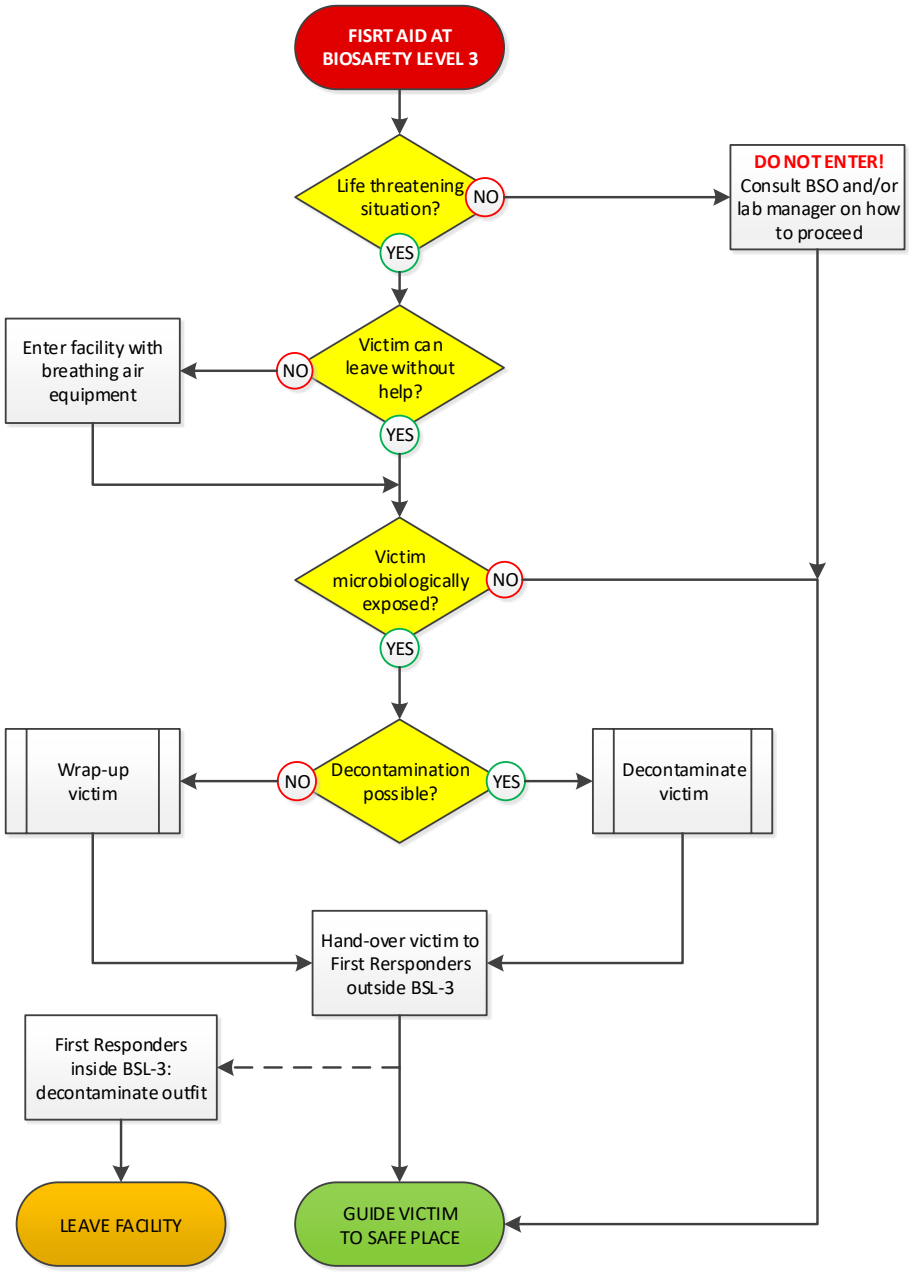


Figure 12B: Critical biosafety decisions when providing First Aid in a Level III facility.

13 Authorization of personnel

13.1 Biosafety Training Program

Anybody who plans to work with biological agents and/or biological material at the VU must attend a Biosafety Training Program. A biosafety program addresses:

- The biological hazards associated with the work and those due to other activities carried out in the same workplace;
- The organization of biorisk management within VU, including the role of OL/VM, ABV and C-BSO;
- The relevant institutional and departmental biorisk management procedures and standard operating procedures/work instructions;
- Correct use of contained equipment, e.g. biosafety cabinet, contained centrifuge, autoclave;
- Biosafety techniques, including the proper use of personal protective equipment (e.g. lab coats, gloves), aseptic hand washing, disinfection of work surfaces, materials and equipment and safe waste disposal.

The elements of the Biosafety Program that are relevant for an individual should be specified on the *Employee Introduction Checklist* (see par. 13.2).

13.2 Qualification criteria and authorization procedure

Working in contained facilities requires authorization. Access is restricted unless the individual is accompanied by an authorized employee (e.g. in case of service engineers or visitors) or the individual is working under the direct supervision of an authorized mentor (e.g. new employees, students). In case of students and short-term temporary workers (assignment <6 months), mentoring should cover the full working period.

Authorization is the responsibility of an employee's supervisor. In the case of working with GMO, authorization is the responsibility of the OL/VM.

Departments should document authorization criteria specific for their facilities based on the general criteria listed in table 13-1. Completion of the Biosafety Training Program (see par. 13.1) is a mandatory prerequisite for any employee who is to handle biological materials and/or biological agents in a contained facility. The Biosafety Training Program must be completed within one month from starting supervised work within a contained facility of level I or level II. Failure to do so will result in denied access. Work in level III, *including supervised work*, requires as a prerequisite at least 12 months of experience at level II.

Table 13-1. General qualification criteria and limitations for working in contained facilities.

Containment level	Qualifications	Limitations
Level I & II	• Relevant professional education • Passed Biosafety Training Program	Students under supervision of experienced mentor. The intensity of supervision can decrease when working experience increases.
Level III	• Relevant professional education • Passed Biosafety Training Program • 12 months working experience at level II	Personnel with long term contracts (>6 months) only, no students.

Authorization is to be recorded on the Employee Authorization Form (Formulier autorisatie medewerker, see GMO-VU folder). This form has two annexes:

- The Employee Introduction Checklist (Checklist introductie medewerker) specifies the Biosafety Training Program elements for the employee and is used to record the completion dates;
- On the Employee Declaration of Consent (Eigen Verklaring) the trainee declares that he/she will act according to the safety instructions.

Departments must keep a register of authorized persons in the table that is available in the GMO Administration, and the aforementioned documents must be archived along with it to comply with the GMO administrative record keeping/ record keeping regulations (see par. 5.2.3, Administrative record keeping of authorized Personnel).

14 Compliance Audits and Inspections

The primary purpose of compliance audits and inspections is to help the organization to adequately implement and adhere to regulatory and institutional requirements. Biosafety audits focus on compliance with the biosafety and biosecurity requirements described in this manual. Compliance audits and inspections are carried out by internal as well as external parties.

External audits and inspections are executed by:

- ILT performs audits to check the compliance with the GMO Decree and the GMO Regulation as well as with the Occupational Safety and Health Act and Occupational Safety and Health Decree;
- The Environmental Authority North Sea Canal Territory, on behalf of the municipality of Amsterdam, determines whether the facilities for contained use of GMO meet the requirements in the GMO regulation and the environmental permit;

Internal audits and inspections are executed by:

- The OL/VM, who are responsible for daily operations and compliance;
- The ABV, who have the task to oversee adherence to standard operating procedures for contained use of GMO in facilities under their supervision, in most cases a specific department;
- Lab supervisors, who have the task to maintain adherence to standard procedures and work instructions in their facilities and to keep facilities and equipment in good condition;
- The C-BSO, who performs audits on contained use of GMO, and reports findings to the heads of departments, OL/VM and ABV

The BSO keeps available audit questionnaires to assess compliance with the requirements on contained use of GMO and those on handling Q organisms.

Annexes

Annex 1 Referenced documents

Chapter	Title	Referenced document	Location*
2	The regulatory framework	Contained Use of GMO Audit Questionnaire	GMO-VU
		Gene Therapy Audit Questionnaire	GMO-VU
		Audit list on use of animal by-products	GMO-VU
		Code of Conduct on Biosecurity	GMO-VU
		Checklist Biosecurity	GMO-VU
3	Notification and licensing	Permission for use of animal by-products for research, diagnostics and education	GMO-VU
		Inventory of animal by-products	GMO-VU
4	Risk assessment	Master lists and data collection tables of hosts, vectors, animals and donor sequences	GMO-VU
		GMO Risk Assessments	GMO-VU
5	Record Keeping	Register of personnel working with GMO	GMO-VU
		Register of contained facilities	GMO-VU
		Inventory of biocides	GMO-VU
6	Physical containment	Checklist BSL 2, 3 and 4	GMO-VU
		Checklists ML-I, ML-II, ML-III, D-I, DM-I, DM-II, AP-I, ODG storage, ODG waste	GMO-VU
		NSF 49:2018. Biosafety Cabinets: Design, Construction, Performance and Field Certification, Annex E	GMO-VU
7	Safe Microbiological Practices	Labels for animal by-products of category 1, 2 and 3	GMO-VU
		065001, Handhygiëne: handenwasinstructie	GMO-VU
9	Transport	Extern transport van biologische materialen, BVF Platform 2012	GMO-VU
		Europese overeenkomst voor het internationale vervoer van gevaarlijke goederen over de weg (ADR), bijlagen, 2017	GMO-VU
		Shipping biological materials in compliance with P620/P650	GMO-VU
		Packaging Instructions P620, P621, P650/PI650, P904/PI959	GMO-VU
		EU commercial document for shipping animal by-products	GMO-VU

		Commercial document for shipping animal by-products within The Netherlands	GMO-VU
		Permit to import DBP into the EU	GMO-VU
		Common Veterinary Document of Entry (in Dutch & English)	GMO-VU
		Guidance on use of the Common Veterinary Document of Entry (in Dutch)	GMO-VU
		Gebruik van dierlijke bijproducten voor onderzoek, diagnose en onderwijs, BVF Platform 2019	GMO-VU
10	Maintenance	Equipment Clearance Form	GMO-VU
11	Waste	Commission Directive 2008/61/EC of 17 June 2008 establishing the conditions under which certain harmful organisms, plants, plant products and other objects listed in Annexes I to V to Council Directive 2000/29/EC may be introduced into or moved within the Community or certain protected zones thereof, for trial or scientific purposes and for work on varietal selections	GMO-VU
		Declaration of Microbiological Decontamination of Waste	GMO-VU
		Label for waste of animal by-products of Category 1	GMO-VU
		041561, Afvalstoffen: handboek VU	KN
		044003, Stralingshygiëne: Algemene voorschriften (Oranje Boek)	KN
12	Incident management		GMO-VU
13	Authorization of personnel	Employee Authorization Form	GMO-VU
14	Audits and Inspections	Audit questionnaire for contained use of GMO activities	GMO-VU
		Audit questionnaire for handling Q organisms	GMO-VU

***** GMO-VU= GMO-VU folder on surfdrive. See Annex 2 for url's.

Annex 2 References for internet and intranet sites

Information	Link to website
Amsterdam Animal Research Center (AARC)	https://aarc.vu.nl
Bureau Biosecurity	www.bureaubiosecurity.nl
Bureau GGO	www.ggo-vergunningverlening.nl
Dutch legislation	www.overheid.nl
European legislation	www.eur-lex.europa.eu
GMO Administration (Surfdrive folder)	https://surfdrive.nl
Incident registration system (intranet)	
Joint Research Centre of the EU	http://gmoinfo.jrc.ec.europa.eu
The Human Environment and Transport Inspectorate (ILT)	www.ilent.nl