

A close-up photograph of several clear plastic microcentrifuge tubes with blue caps, arranged in a white rack. The tubes have blue markings and some have handwritten labels like 'BD' and numbers. The background is slightly blurred, showing more of the rack and laboratory equipment.

# SAMPLING FEASIBILITY STUDY OF PATHOGENIC ORGANISMS GENETICALLY MODIFIED OR NOT IN CONTAINED USE ACTIVITIES

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**Biosafety and Biotechnology | April 2010 | Brussels, Belgium**  
Nr Royal Library: D/2010/2505/19

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**The project is financially supported by**

**The Flemish Agency for Care and Health**



**Toezicht Volksgezondheid (ToVo), Vlaams Agentschap Zorg en Gezondheid**



## Table of contents

### **I. SAMPLING FEASIBILITY STUDY**

#### **1. Introduction**

#### **2. Risk assessment**

2.1. Characteristics of the organism

2.2. Characteristics of the activity

2.2.1. Clinical biology laboratories

2.2.2. Fundamental Research and Research and Development

2.2.3. Large-scale production activity

2.3. Containment level

2.4. Waste treatment, transport and storage

#### **3. Sampling in practice**

3.1. Belgian Regional Decree provisions

3.2. Sampling preparation

3.2.1. Locations inside the laboratory

3.2.2. Locations inside the facility

3.2.3. Performing sampling

#### **4. Interpretation of results**

#### **5. Conclusion**

### **II. SAMPLING SHEETS**

1. *Legionella pneumophila*

2. *Mycobacterium tuberculosis*

3. Bacteria producing toxins

3.1. *Staphylococcus aureus*

3.2. *Escherichia coli* O157:H7

4. Adenoviruses and adenoviral vectors

5. Animal and human cell cultures

### **III. REGULATIONS**

### **IV. REFERENCES**

### **V. LEXICON**

## I. SAMPLING FEASIBILITY STUDY

### 1. Introduction

The contained use of genetically modified (micro-) organisms (GMOs) and/or pathogens is regulated in Belgium at the regional level and is based on the implementation of European Directive 2009/41/EC (repealing Directive 98/81/EC) and the enforcement of subsidiary EC Decisions. These Community measures ask for Member States to regulate the contained use of genetically modified micro-organisms in order to minimize their potential negative effects on human health and the environment.

Although the EU regulatory framework only covers genetically modified micro-organisms (GMMs), the scope of the Belgian regional legislations has been extended to genetically modified organisms and pathogenic organisms for humans, animal and plants. The three Regions (Flemish Region, Walloon Region and Brussels-Capital Region) have implemented the above-mentioned EU legislation as a component of their Environmental laws for classified installations. All activities in laboratories, animal facilities, greenhouses, hospital rooms and large-scale production facilities involving genetically modified and/or pathogenic organisms are subject to a preliminary written authorization from the regional competent authorities on the basis of a specific notification and decision procedure. These regulations also provide for the application of an appropriate containment and other protective measures corresponding to the risk class of the contained use in order to keep workplace and environmental exposure to any GMO and/or pathogen to the lowest reasonably practicable level and in order to ensure a high level of safety.

Since these regulations include inspections and other control measures, inspection has been organized at the regional level. More specifically in the Flemish Region, inspection is carried out by two public services: the Flemish Agency for Care and Health of the Public Health Surveillance, whose main concern is protection of public health, and the Department of Environment, Nature and Energy, focusing on the environment. As part of their mission, both inspection services may perform sampling in order to verify whether the implemented containment and the practices of work are adequate and sufficient to offer maximal protection of human health and environment from biological risks. Art 62 §9 of Vlarem I gives a detailed description of the sampling procedure to be followed by the inspectors (Besluit van de Vlaamse regering van 6 februari 2004).

**The aim of this study is to discern situations where sampling may be feasible and relevant. Both will be determined by the probability of subsisting biological risks arising from a contained use activity. Notwithstanding biological risks may harm the environment, this study will primarily focus on the feasibility of sampling in situations**

**where subsisting biological risks may present a danger for public health. Therefore this report serves as a guideline for inspectors to i) evaluate the potential biological risks for human health ii) consider technical and practical aspects in order to perform sampling in the best conditions and iii) highlight issues encountered during the interpretation of sampling results .**

Ideally, final results should be evaluated as regards to the direct causal relationship with the contained use activity, as sampling could reveal the presence of organisms that do not directly result from the activity. The aim of sampling analysis is to demonstrate that a room contamination by a particular organism is the direct consequence of inadequate practices or/and containment measures in the laboratory, potentially placing population and environment at risk. **Inspection of a facility and sampling should then play the function of a warning in view to (re)-establish the adequate conditions of biosafety.**

Since the present study is mainly focused on public health, only sampling with respect to contained use of pathogenic micro-organisms will be considered, whether they are genetically modified or not. The report will be focused on the feasibility of a sampling in a facility and its immediate surroundings where micro-organisms are cultured, stored, destroyed or disposed off.

General sampling strategies are briefly described at the end of the report for facilities where activities currently use common pathogens. It is mandatory to entrust the analytical part of the work (detection, identification and if necessary quantification) to recognized reference laboratories (Ducoffre, 2007).

## 2. Risk assessment

A biological risk assessment is performed by the user and is evaluated during the authorization procedure of the contained use activity. This risk assessment should be taken into account by the inspectors as it contains important information with regards to the biological risks for human health and environment.

The risk assessment addresses the following issues:

- The characteristics of the handled organisms;
- The characteristics of the activity;
- The applied containment level, the work practices and available safety equipments.

### 2.1. Characteristics of the organism

The biological risk that may arise while manipulating a pathogenic organism is partially determined by the intrinsic characteristics of this organism. The biological hazards will depend on the extent and seriousness of the disease caused by the organism and the capacity to transmit the disease to the population.

To evaluate the biological risk of an infectious organism, it is recommended to consider:

- **The pathogenicity and the virulence** that will define the ability to cause a disease and the ability to multiply in the host.

In case the micro-organism is a pathogenic organism that provokes a severe disease to a human being, sampling must be considered. If the micro-organism manipulated in the laboratory does not cause a disease, sampling is not relevant; the micro-organism do not represent a danger for public health.

- **The route(s) of infection and the transmission mode** indicate the ways a pathogenic organism takes to infect and to spread through population (and environment).

The infectious organism may be transmitted by air, by direct contact from person to person or by indirect contact with contaminated material (fomites). A pathogenic organism may also be transmitted by several of these ways. It enters the organism by ingestion, inhalation, through mucous membranes and/or through damaged skin (Table 1).

Pathogenic organism	Portal of entry (related to manipulation)
Tinea Fungi	Contact with skin
Influenza virus Measles virus	Inhalation of infectious aerosols
<i>Plasmodium falciparum</i> Yellow fever virus Human Immunodeficiency virus Hepatitis B virus	By inoculation (pricks with needles or insects) or through skin lesions
<i>Staphylococcus spp.</i>	Through injuries or skin lesions
<i>Chlamydia trachomatis</i>	Contact with eyes
<i>Salmonella spp.</i> <i>E. coli O157</i> Poliovirus	By ingestion of infectious material

Table 1: Portal of entry of some pathogenic organisms.

In a laboratory, some manipulations involve the generation of infectious aerosols. An infectious micro-organism represents a particular danger when it is aerosolized and can, by this way, enter easily the human organism by inhalation or ingestion. Aerosols are able to travel long distances and can deposit far away from the initial point of generation. They can stay suspended in the air for long periods and cross and escape from the containment by simple personnel movements. Personnel may also be directly exposed to infectious aerosols in the facility in case they do not wear adequate personal protection equipment (PPE). Aerosol contamination is difficult to contain unless strict laboratory practices and appropriate biosafety equipment are put in place.

- **The viability outside the host** indicates the capacity of the micro-organism to persist in the environment.

In the framework of this study, inspection of a contained use facility will only focus on living pathogens. Some infectious organisms do not persist outside their host. In this case, sampling will not reveal the presence of viable pathogens and will therefore not be performed. Other cases are able to persist in environmental conditions for longer periods (Table 2). Note that for some infectious organisms, persistence in the environment is unknown.

In case it is known that the micro-organism may survive into the environment, inspectors should consider sampling suitable and applicable. Spores such as those from *Bacillus anthracis* are primarily a survival mechanism and are adapted for dispersal and surviving for extended periods of time in unfavorable conditions. Another case is that from prions which are proteins classified as non-conventional infectious organisms. They are extremely stable and resistant to standard inactivation protocols, making disposal of these

particles particularly difficult (Qin *et al.*, 2006). Laboratories handling sporulating micro-organisms and prions must proceed to a well-adapted inactivation and decontamination procedures.

Organisms	Environment Matrix	Persistence
<i>Legionella pneumophila</i>	Water Air	Months No persistence
<i>Mycobacterium tuberculosis</i>	Air Dust Paper Sputum	Hours 3 – 4 months 105 days 6 – 8 months
<i>Staphylococcus aureus</i>	Skin Glass Floor Carcasses, organs	30 min to 38 days 46 hours < 7 days 42 days
HIV	Air - desiccated conditions Water Blood	No persistence No persistence Several days
<i>Bacillus anthracis</i> – spores	Air, Soil, Wool, Skins and hides, Milk Filter paper (dried) Pond water	Decades  10 years 41 years 2 years
Adenovirus	Surfaces	3 – 8 weeks
<i>Chlamydia psittaci</i>	Infected fluid from eggs Bird droppings Bird feed Glass Straw	52 hours a few days 2 months 15 days 20 days
SARS-Coronavirus	Faeces and urine Open plastic surface Animals Environment	one to two days 48 hours several days to weeks several days

Table 2: Estimation of the persistence of some micro-organisms in different matrices outside their host(s) (adapted from MSDS-sheets of the Public Health Agency of Canada and from Herman *et al.*, 2004)

➤ **Other factors** contribute to the biological risk of a microorganism.

Among these factors is the host range of the infectious organism. In the framework of this study, attention will specifically be focused on laboratories manipulating organisms that infect humans. The absence of a prophylaxis or treatment for the disease caused by the pathogenic organism and a low infectious dose of this organism, are other factors that increase the biological risk of the micro-organism.

Based on their intrinsic characteristics, micro-organisms have been classified into biological risk classes. Belgium has issued its own official classification. It is part of the reference lists for risk classification of micro-organisms published in the Regional Decrees regulating contained use activities involving pathogens and/or genetically modified organisms (Belgian Biosafety Server, BBS(1)). Four biological risk classes are established going from organisms



presenting a negligible risk for man health (class of risk 1) to organisms provoking a serious hazard for human being and for which no effective prophylaxis or treatment is available (class of risk 4). In Belgium, none of the contained use activities involve class of risk 4 infectious organisms for humans but some of them involve class of risk 4 pathogens for animals.

In conclusion, the characteristics of the manipulated organism(s) such as the pathogenicity, the virulence, the way(s) of transmission and the persistence in the environment should be considered as it will determine the relevancy of performing sampling. The relevancy will increase with the seriousness of biological hazards for immuno-competent individuals. This means that a pathogenic organisms needs to persist in the environment, it should be virulent and it should find an appropriate portal of entry in its susceptible host. Noteworthy, these characteristics are supposed to be evaluated within the biological risk assessment of the activity and are considered in the biosafety dossier as part of the authorization procedure.

## 2.2. Characteristics of the activity

Activities involving manipulation of GM or/and pathogenic organisms are conducted in facilities such as laboratories, animal facilities, green houses, hospital rooms and production plants. Depending on the type of activity, the environmental and human exposure to the manipulated organism can increase considerably. The specificity of an activity is determined by its objectives, the type of biological organisms that are used, the processes achieved and the scales and volumes of handled biological material. These specificities characterize the type and the probability of exposure and, combined to the biological hazards associated to the manipulated organism, define the class risk of the activity. Relevant information concerning the characteristics of the activity is available in the risk assessment part of the biosafety dossier provided by the user.

Three main types of activities handling GM organisms and pathogens can be distinguished:

- The clinical biology activity principally performed in human and animal diagnosis laboratories, and laboratories for quality control of environment, food/feed or drugs;
- The fundamental research and the research and development (R&D) fields performed by universities, governmental institutions, private companies;
- The production activity essentially found in pharmaceutical and biotechnology companies.

In all activities, the handled organism may escape from the containment by airflows in case of airborne pathogens, by contaminated waste and by the (contaminated) worker itself. In this last case, the source of direct contamination of the workers is most of the time similar to all activities and arises from "incidents" with infectious material: inhalation of aerosols, parenteral inoculation (needle prick) and contact with damaged skin or mucous membranes (Table 3).

Portal of entry	Incidents in microbiology work practices
Ingestion	Splashes or droplets from infectious material and contact with mouth and nose
Inoculation	Needle pricks Cuts with sharp instruments Bites and scratches from manipulated animals or insects
Skin and mucosa contamination	Splashes in eyes, nose, mouth, skin Contamination of surfaces and equipment
Inhalation	Aerosols generating procedures

Table 3: Accidental contamination modes linked to laboratory practices with infectious organisms.

### 2.2.1. Clinical biology laboratories

The diagnostic activity is predominantly represented by clinical laboratories. The aim of a microbiology laboratory is to detect, identify and characterize infectious organisms in human samples. Lab workers manipulate human specimen that consist of fluids, tissues, organs, faeces and that are suspected to contain a specific pathogen(s) but sometimes also unknown pathogens. The handled micro-organisms belong to class of risk 1, 2 or 3 and can be airborne and persist into the environment. In this domain of activity, no GMOs are analyzed or manipulated. Most standard microbiological techniques include but are not limited to direct examination, culture, staining, identification and antibiotic resistance testing.

In the clinical samples to be analysed in the laboratory, the pathogenic organism is generally present in small quantities, so they have to be multiplied or amplified in order to be identified. However, caution is needed because these samples may contain high quantities of the pathogenic organism or they may contain enough organisms to expose the environment or/and the worker to the biological risk.

Quality control laboratories aim at detecting pathogenic organisms in samples coming from the environment, food, water or medicines. Similar procedures as those used in the diagnostic laboratory are performed on this type of samples.

Reference laboratory activities are usually dedicated to the further characterization of the infectious organism. This often implies the culturing of organism in higher volumes and/or concentrations, increasing the biological risk for lab workers.

Noteworthy, in this type of activities the infectious organism may stay unidentified during the first tests while workers and facility might be frequently exposed. Specific measures should be

in place to prevent a contamination in such situations, the first measure consisting in strict compliance with Good Microbiological Practice (GMP).

Finally, it has been reported that the diagnostic activity in microbiology presents a particular elevated number of laboratory acquired infections (LAI) among personnel (Baron and Miller, 2008). The study evaluated the situation in hospitals, academic institutions and reference laboratories.

#### 2.2.2. Fundamental Research and Research and Development

Activities of fundamental research and Research and Development (R&D) involving manipulations of GMOs and/or pathogenic organisms are diversified in Belgium. Although research and R&D activities have different scopes, both involve similar type of manipulations in terms of scale and technical and biological material used.

Both types of activities may study biochemical ways, the cycle of infection, the production of a toxin and/or host intoxication, with the aim for R&D, to finally develop new diagnosis and treatment strategies in humans for example. These activities may also involve the study of gene functions that play a role in normal and abnormal physiological processes in humans by the use of GM micro-organisms such as bacteria, virus and yeasts that are exploited as tools in these studies. In this respect, eukaryotic cells such as cell lines and primary cells, GM or not, are frequently manipulated.

The research activity presents particularities that may lead to situations with increased probability of contamination:

- Some activities generate organism form which the genetic material has been altered. The genetic modification may increase the biological risk of the receptor organism;
- Manipulation of laboratory animals in challenge experiments or for animal inoculations with pathogenic organisms are of high risk for personnel due to the generation of aerosols, risk of bites and scratches or shedding after various incubation periods;
- In the case of universities or high schools, a contamination risk resides in the practice work of students that may be not sufficiently trained to the GLP.

#### 2.2.3. Large-scale production activity

The aim is generally to produce recombinant proteins in large quantities by the use of a genetically modified organism (antigens, vaccine, drugs). Production is essentially performed by biotechnology and pharmaceutical companies.

This type of activity is characterized by high volumes of culture and high concentrations of infectious organism that need special infrastructure and material (fermentors, bioreactors,

killtanks). Depending on the characteristic of the micro-organism, an accident in such a facility resulting in the break of the containment barriers could present serious biological risks for the surrounding population and environment. However, the biotech companies have high requirements of quality and they function under quality systems for product safety. Companies must control extremely well the contamination degree and the quality of the product, the aim being to sell high quality products free of pathogenic organisms. They install containment measures in the production room on one hand to avoid the entrance of any biological organism and, on the other side to avoid any escape outside the facility containment or any worker contamination. They combine Good Manufacturing Practices (GMP) and Biosafety measures including Emergency plans to minimize public health and environmental consequences in case an unintentional release of pathogens.

### 2.3. The containment level

Based on the biological risk of the infectious organism and the type of contained use activity, an activity risk classification increasing from level 1 to 4 has been established that results in the assignment of facility containment levels. Room design and technical characteristics, the safety equipment, work practices and waste management define the containment level (Belgian Biosafety Server (2), Fleming and Hunt, 2000). The maximal containment level adopted in Belgium is the level 3. Level 3 facilities including laboratories, animal houses and large-scale production plants are currently used in hospitals, universities, state institutions and private companies (Do Thi *et al.*, 2009).

Irrespective of the adopted containment level, the application of good microbiology practices is one of the basic requirements for personnel working with GMOs or pathogens. It is also mandatory to use decontamination and inactivation procedures adapted to the specific organism(s) manipulated in the facility in order to decontaminate surfaces, waste or reusable material.

To illustrate the necessity to adapt the decontamination procedure to the micro-organism manipulated in a facility, Table 4 gives an overview of some pathogen resistances to disinfectants commonly used in laboratories.


	<p>Less sensitive</p> <p>Prions (CJD, BSE)</p> <p>Coccidia (<i>Cryptosporidium</i>)</p> <p>Bacterial spores (<i>Bacillus</i>, <i>C. difficile</i>)</p> <p>Mycobacteria (<i>M. tuberculosis</i>, <i>M. avium</i>)</p> <p>Cysts (<i>Giardia</i>)</p> <p>Non-enveloped small virus (Poliovirus, adenovirus)</p> <p>Trophoïtes (<i>Acanthamoeba</i>)</p> <p>Gram negative bacteria non-sporulating (<i>Pseudomonas</i>, <i>Providencia</i>)</p> <p>Yeast (<i>Candida</i>, <i>Aspergillus</i>)</p> <p>Non-enveloped virus (enterovirus, adenovirus)</p> <p>More sensitive</p>
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Table 4: Relative scale of pathogen resistance to common disinfectants  
(based on McDonnell and Russell, 1999; Berghmans *et al.* 2006).

Laboratory procedures, including instructions to follow in case of incident or accident with infectious material, must be written and personnel have to be regularly trained to biosafety measures. In high containment level facilities the elementary measures are supplemented by more complex and adapted work practices as well as technical systems in function of the risk class of the activity.

Measures taken in a containment level 3 facility are stringent because of the high risk. The probability of escape of the infectious organism from such a facility is hampered by the implementation of negative air pressure, HEPA-filtration of the exhaust air and the capacity to render the laboratory sealable to allow fumigation. However, these features proper to L3 facilities are designed to protect environment and do not ensure additional personal protection to the worker operating within the L3. The worker should be aware of the fact that the high probability of exposure to pathogenic organisms still remains for him and he should wear adequate personal protection equipment even when all open manipulations are conducted under a class II biological safety cabinet (BSC).

During an inspection, the control of the state, the quality and the suitability of the installation and appliances as well as the observance of the safety measures for a specific activity, is essential. Sampling should therefore be considered in case the containment level is not adapted to the activity or not respected.

## 2.4. Waste treatment, transport and storage

Waste generated in a facility in which GMOs and pathogens are manipulated is potentially dangerous and needs a particular management until its final elimination. To protect public health and environment, it is crucial to ensure the containment of infectious organisms from the beginning to the end of the contained use process. Waste management and elimination are regulated at the European level by Directives 91/156/CEE, 91/689/CEE and specifically for waste from GMMs activities, the Directive 2009/41/EC, which has been implemented in Belgium in the Regional decrees. These decrees have in addition included specific regulations on waste imposing rules for storage, incineration and even for collection by an certified company. The waste management in any facility includes identification, registration, conditioning, internal transport and storage of waste until its evacuation by a specialized company that will proceed to incineration.

In the framework of the present report, sampling could be useful to check:

- Validation of waste inactivation procedures specific to the GMOs and pathogens manipulated in the facility,
- Adequate containment for biologically contaminated fluids, solids and sharps;
- Adequate internal transport in closed boxes (whose external surfaces have been decontaminated);
- Storage rooms that are made inaccessible for unauthorized persons, which allow air circulation and that must be decontaminated regularly.

Obviously, particular attention should be paid to waste management in a containment level 3 facility. Same consideration must be taken for production plants where large volumes of waste are treated and inactivation procedures and equipment are specific to that kind of activity.

## **3. Sampling in practice**

### 3.1. Belgian Regional Decree provisions

Regional Decrees in Belgium have foreseen and defined the general dispositions to perform a sampling including the number of samples, the registration procedure, the process timing and monitoring. A sampling form must be fulfilled during the control inspection in order to register all relevant information concerning the taking of samples in the facility.

The Flemish decree foresees that each sample should be composed of two identical parts, one intended for the analysis for inspection and the other for a counter-expertise in case the user asks for it (Besluit van de Vlaamse regering van 6 februari 2004, art 62 §9). Each part has to be immediately stored under identical conditions that will maintain biologic and genetic stability of the pathogenic organism. The sample intended for inspection is sent to a reference laboratory, which will perform the detection analysis. The number and size of samples will depend on their availability and on the nature of the manipulations that will be performed.

Only organisms manipulated in the framework of the contained use of the inspected laboratory should be subject to sampling. Inspector should refer to those infectious organisms that are mentioned in the laboratory register, in publications issued from the laboratory or from the biosafety dossier. Within the framework of the GMOs and pathogens contained use legislation, only living organisms should be considered. It means that detection of molecules like nucleic acids, allergens, toxins should not be taken into account as such for the purpose of sampling but may be used as tools for final identification of an initially detected micro-organism.

It's worth mentioning that the inspector is allowed to ask the laboratory for all the necessary material to perform sampling and the user must provide the specific microbiological and/or molecular detection methods for the manipulated organisms.

### 3.2. Sampling preparation

Preparative work prior to sampling consists in collecting adequate sampling material, relevant documentation and identifying appropriate questions that could be addressed in relation with the contained use. Prior the inspection visit, it is recommended to define the objectives of sampling by i) determining the biological risks specific to this facility, ii) identifying the type of organisms that are aimed to be sampled, iii) determining the number, the volume and types of samples, iv) considering locations in the facility that will be sampled (room, surface, instrument,...).

The identification of specific hazards associated to the use of the pathogenic organism(s) is achieved by performing the risk assessment as detailed previously in this report. It considers important biological aspects of the organism as well as particularities of the activity, the level of containment of the facility and the work practices in place. By operating in this way, the inspector will be prepared to perform a sampling in the best conditions (see also Norm ISO 14698-1:2003).

### 3.2.1. Locations inside the laboratory

When the inspector has considered that the activity may represent a risk for public health and he has decided to perform a sampling, the choice of sampling location will be crucial for obtaining significant results.

As mentioned above and except in case of accident, the predominant way of contamination occurs during manipulations that generate infectious aerosols and splashes. Under these conditions, workers may be directly at risk of contamination and it is also possible that the nearest outside environment could be contaminated as well.

In a working area, the following manipulations are known to generate aerosols:

- The use of blenders, sonicators and grinders. These devices are routinely used for sample homogenization;
- The use of centrifuges. Centrifugation is a commonly used separation step in several analysis methods;
- Re-suspending dehydrated or pelleted pathogenic organisms;
- Blowing out pipettes and syringes;
- Using a hot wire loop to pick up a colony;
- Opening of ampoules, tubes and bottles containing infectious liquids.

These manipulations are the most common in laboratories but the present list is obviously not exhaustive.

Taking into account these manipulations, the inspector should consider performing sampling in, out and around locations and equipments where infectious aerosols and splashes could be generated:

- Laminar flows, hoods, biosafety cabinets;
- Vacuum pipelines;
- Incubators and humidifiers;
- Centrifuges;
- Microscopes.

And in their immediate surroundings, including:

- Floors;
- Walls;
- Benches;
- Door handles;
- Lab clothes.

Commonly used devices or objects in laboratories such as computers, phones, fridges, freezers, autoclaves, cabinets but also lab books and pens are frequently handled by workers and may therefore become contaminated as well. Sampling their surfaces may identify a



weakness in the containment, the biosafety procedures and/or the work practices in place for that activity.

Different environmental matrices may host and act as a reservoir for infectious organisms. Handled micro-organisms could survive for long periods after their dispersion and deposition (Table 5) in these conditions. In a laboratory, bacteria, fungi and viruses that are mainly dispersed through aerosols will principally be attached to dust particles or within biofilms after deposition. Dust and biofilm are particularly complex matrices meaning that they may contain many different types of materials and organisms: virus, bacteria, fungi, and protozoa. Dust in common human environments (homes, offices) contains human skin cells, small amounts of plant pollen, human and animal hairs, textile fibers, paper fibers, minerals from outdoor soil, and many other materials which may be found in the local environment. A biofilm is an aggregate of micro-organisms in which cells are stuck to each other and sometimes also to a surface. These adherent cells are frequently embedded within a self-produced matrix of extracellular polymeric substance. Biofilms can contain many different types of micro-organism, bacteria, archaea, protozoa, fungi and algae.

As a consequence, sampling these complex matrices may present difficulties in the culture step, the separation, the detection and identification of the infectious organism(s) originating from the specific contained use activity.

<b>Environmental matrix</b>	<b>Abundance of organisms</b>	<b>Complexity</b>
Air	Low	Very low
Surfaces	Low-medium	Low
Clothes	Low	Very low
Sewage	High	High
Biofilm	High	Very high
Dust	High	High
Soil	High	Very high

Table 5: Environmental matrices hosting infectious organisms and some of their properties  
(Based on experimental findings of Bagutti, unpublished data)

It's worth pointing out that the reception of samples\* in laboratories and particularly in a clinical laboratory is a critical location for contamination. Samples that are sent to the laboratory have been packed and transported in certain conditions that are not controlled by the lab personnel. Personnel have to receive, register and unpack samples. Sample package could be contaminated and this situation potentially exposes the lab worker to infectious

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\* *the reception of samples does not fall within the scope of contained use activities, despite the fact that such an activity deals with biosafety concerns.*

organisms. Because this probability is not negligible, the application of Good Laboratory Practice (GLP) at least and the work inside a BSC in addition should diminish the risk at the reception step.

### 3.2.2. Locations inside the facility

Attention should also be drawn on biohazard waste management as waste represents a potential way of escape of the pathogenic organism(s) outside the facility. The waste course through the facility, from its initial production in the laboratory until its final elimination by a specialized company, may give indications for sampling locations. The aim is to control the containment, the inactivation procedure, the internal transport and storage of solid and liquid waste. These include the surfaces of the waste containers as well as locations in the waste storage room: control of outside waste container surfaces, floor and walls and door handles of the room.

Some facilities present in addition specificities that can allow other ways of escape of a pathogenic organism outside the containment.

- In an animal house-room, the material in- and outflow: cages, water bottles, feeders, waste boxes, litter...;
- In a large scale plant: the bioreactors and the kill tanks;
- In a room hosting device(s) (such as the flow cytometry apparatus, a confocal microscope) used by different operators manipulating several different pathogenic organisms: the device itself, registers, any common material at place.

### 3.2.3. Performing sampling

In practice, it is essential that sampling must be performed in sterile conditions with sterile material. It means that inspectors should wear adequate PPE that could involve the use of a lab coat, gloves, an adapted respiratory mask or faceshield depending on the risk of the activity (Pauwels *et al.*, 2008). Inspectors should therefore foresee to perform negative controls in parallel to check any contamination of all the material used for sampling. Until analysis, taken samples must be kept in appropriate conditions to maintain the microorganism viability and genetic stability.

Resources used for this part of the report have been reached essentially in specialized microbiology companies such as EmLab (<http://www.emlab.com/app/main/Welcome.po>) and EMSL ([http://www.emsl.com/Index.cfm?nav=Sampling\\_Guides](http://www.emsl.com/Index.cfm?nav=Sampling_Guides)).

In function of the type of matrices to sample as well as the type of surfaces to sample, sampling is performed by means of:

- Petrifilm AC (Aerobic Count) plate to enumerate total aerobic bacterial population on surfaces;

- Swabs and Lethen broth tubes for irregular surfaces, biofilms, moisture;
- RODAC plates (Replicate Organism Detection and Counting) for flat surface sampling;
- Air pumping for air sampling (impact sampler or impingement sampler);
- Tapes for direct microscope examination.

This list is not exhaustive. It may be necessary to add a neutralizing agent such as the sodium thiosulfate in order to neutralize biocides.

The viability of the organism is determined by its ability to grow when cultured in optimal conditions. The organism will be identified by culture and by classic microbiological techniques or the use of molecular biology tools. **It's worth mentioning that culturing is a sensitive method but it requires stringent conditions for maintaining the viability of the microorganism prior and during culturing. On the contrary, DNA material is relatively stable and remains detectable for a longer time period, but DNA-based detection on its own will not give information on the viability of the microorganism.**

In the case several different pathogenic organisms are routinely tested and manipulated within a laboratory it could be useful to start the procedure with a large screening using a medium allowing culture of several types of organisms such as the TSA (TryptoneSoya Agar) that supports general microbial colonies and the SDA (Sabouraud Dextrose Agar) that supports yeast and fungal colonies. In a following step, a specific medium will be selected for replication and identification of a given pathogenic organism. Inversely, when one single pathogenic organism is manipulated in a facility such as a production plant, inspectors may choose the specific material and culture medium allowing multiplication of this organism.

#### **4. Interpretation of results**

Analysis of samples taken in the inspected facilities may show positive or negative results in one or more locations of a facility. Generally, sampling will be performed because a risk assessment of the activity has pointed out a potential hazard and/or a weakness in applied biosafety measures. The sampling test will reveal first a qualitative situation in a specific location. However, the interpretation of what will be revealed must be taken with caution due to:

- The possible low quantity of the pathogenic organism in a contamination (traces);
- the difficulty of conservation of some micro-organisms (before being tested);

- the limit of detection of a given method (LOD);
- the technical and physical limits of the sample collection and the detection methods. The detection techniques present most of the time specific troubleshooting. For example, the presence of inhibitors of DNA amplification may interfere with the PCR;
- the natural presence of commensal micro-organisms or naturally occurring micro-organisms in the environment that may interfere in the interpretation of results.

These factors may give rise to false negative result in which case sampling will not lead to a representative evaluation of the biological risks prevailing in a facility.

On another side, some micro-organisms, whose presence does not arise from the activity of contained use, are growing in the facility environment and may be detected by sampling. To establish a limit of “natural” contamination, some laboratories determine baselines of contamination that are considered as reference levels which can not be exceeded without interfering in the activity. Assuming that below this limit, the risk for human health is acceptable, these baselines, if available, may be useful to inspectors to interpret their sampling results and to determine whether there is an increased hazard for human health. When these baselines are not available, there are sometimes norms or rates adopted by national health institutions that may give indications for acceptable basic contamination levels (ISO, AFNOR, WHO). In addition, these levels may be different according the facility and the activity. Taking and comparing samples from different locations of the same facility may also reveal spots where levels of pathogenic organisms are higher and could represent increased risks for human health in case of exposure.

## 5. Conclusion

Belgian Regional Decrees on the contained use of micro-organisms and genetically modified micro-organisms provide a framework for inspections to control the adequacy and efficiency of containment measures and work practices in facilities where contained use is performed. **This report focuses on the relevance of performing a sampling during such an inspection taking into account on the one hand the potential hazard represented by the contained use activity for the public health and on the other hand, the practical and technical challenges that an inspector responsible for sampling should face.**

Prior to sampling in a facility, the inspector should analyse and have a critical view on the biological risk assessment of the activity that has been performed by the user and evaluated during the authorization procedure. Sampling will be considered relevant in case the

pathogenic organism and the type of activity present a risk for the public health and/or the environment, including the worker health. The containment measures in place, work practices and waste management should therefore be evaluated with regards to the type of activity (e.g. generation of infectious aerosols) and the characteristics of the pathogen itself (e.g. airborne pathogens, persistence outside the host or the laboratory conditions).

The representativeness of the samples will depend on the sampling locations inside the facility and the sampling method. Sampling consists in taking portions of different matrices in different locations of the facility and in testing these samples for the presence of specific pathogenic organism(s) handled within the scope of the contained use activity. Detection will only be focused on living organisms. Sample storage and transport need particular attention because both procedures have to preserve viability and genetic stability of the pathogenic organism. Finally, the detection method should be able to detect low numbers of the specific micro-organism.

The Regional Decrees do not define the type of analysis to perform: should it be a quantitative or semi-quantitative analysis? Should it allow a statistic analysis or is it a simple qualitative result? Is it enough to give an appreciation of the biological hazard induced by the activity? **In the framework of this report and as a preliminary study, only a qualitative approach has been discussed.** Literature on the most appropriate approach to control containment measures is scarce and much more research data and analysis are needed in order to be able to analyze sampling results in a quantitative way. The acquisition of more data regarding different parameters in different type of facilities in Belgium (e.g. baselines) will help to decide whether sampling will be relevant in a specific facility and will constitute a support to interpret sampling results. For the time being, the decision to perform sampling and the interpretation of results still rely on case-by-case approach.

## II. SAMPLING SHEETS

The following sampling sheets aim to illustrate sampling by practical examples in order to bring to light the questions and the difficulties that could be encountered in a specific facility. After a brief risk assessment of a given contained use activity handling pathogenic organisms and an analysis of the pertinence of sampling for such facility, a procedure of sampling for the specific pathogenic organism will be described. General resources used for this part of the report have been selected essentially in national or international public websites and reports: the material safety data sheets of the Public Health Agency of Canada, the Environmental Health and Safety documents of the University of Iowa, the US Food and Drug Administration as well as the CDC fact sheets and reports, the CEN and ISO methods and norms and resources from the WHO.

The interpretation of the possible results of sampling of a specific pathogenic organism will be discussed. The interpretation of negative results merits particular attention due to the technical challenges during sampling and detection.

It is always mandatory for the inspector to work in sterile conditions and it is recommended to use adapted PPE such as a respiratory mask, a lab coat and gloves. Negative controls must always be foreseen to check sterility of the sampling material used by the inspector.

Two identical samples of a same location must be taken, one for direct analysis, the other for the counter-expertise if requested. Where possible, taking a third additional sample in the same location is recommended to consolidate obtained results. If necessary, the visited laboratory could be asked to transmit the specific detection method and/or identification method of the micro-organism manipulated in the laboratory.

## 1. *Legionella pneumophila*

*Legionella pneumophila* is a flagellated Gram negative bacterium, aerobic and polymorph. It is generally found in biofilms into natural and artificial aqueous media such as creeks, ponds, swimming pools, hot water systems, air-conditioning cooling towers, evaporative condensers, respiratory therapy devices, hot and cold water taps, showers. This infectious organism is also suspected to colonize mud. *L. pneumophila* develops freely in fresh water or may develop in association with amoeba or some protozoan. It may survive for months in tap water and even in distilled water. Stagnation of water is favourable to rapid multiplication with optimal temperature 35 – 45°C. To date, it has never been detected in seawater or dried soil.

### **Biological risk assessment:**

*L. pneumophila* is a risk class 2 micro-organism. It causes principally an acute pneumonia in infected persons. The infectious dose is unknown. The human being and unicellular eukaryotes (amoeba) are the hosts of this bacterium. There is no reported case of animal infection. It is transmitted by inhalation of infected aerosols and by ingestion of contaminated water. There is no direct transmission from person to person. An antibiotherapy is available to treat legionellosis. Note that in hospitals, this organism may represent a particular danger because of the possible nosocomial infection.

### **Activity:**

In Belgium, detection and characterisation of *Legionella pneumophila* is performed regularly in diagnostic laboratories for food quality control and for water and environment quality control.

R&D laboratories aiming the molecular characterization of *L. pneumophila* operate in Belgium as well as production laboratories aiming the development and commercialization of diagnostic tests for *L. pneumophila*.

The major danger in a laboratory handling this bacterium is associated with the generation of aerosols containing *L. pneumophila* during manipulations and the subsequent inhalation of infectious particles. *Legionella pneumophila* may also survive and multiply after deposition on surfaces that harbour adequate conditions for its multiplication such as a pre-formed biofilm or humid surfaces thereby presenting risks of ingestion through indirect contact of the mouth with infected material (Miskowski, 2007).

### **Containment level and decontamination:**

The manipulation of *L. pneumophila* for diagnostic, R&D and production laboratories needs a containment of level 2 with the use of a class I or II biosafety cabinet (BSC) when the manipulations involve opening of sample tubes or vessels containing the bacteria.

To decontaminate surfaces potentially contaminated with *L. pneumophila*, sodium hypochlorite 1 %, ethanol 70 %, glutaraldehyde or formaldehyde could be used. These chemicals are also used to inactivate liquid waste contaminated with *Legionella*. To inactivate infectious waste and material, the use of moist heat (121° C for 15 minutes at least) or dry heat (160-170°C for 1 hour at least) are sufficient.

### **Sampling**

All facilities where this infectious organism is manipulated, stored or disposed of should be considered for sampling. More precisely, unintended contamination due to sample manipulation or deposition of droplets or infectious aerosols should be focused. Humid and dark surfaces are more likely to be contaminated because *L. pneumophila* does not survive under desiccated conditions and under UV light. Sampling could also be focused on the collection of biofilms in which the bacteria may survive and multiply. Sampling the facility water canalisation is less relevant as any positive result will be difficult to relate to the contained use activity in the laboratory.

Therefore, laboratory locations of sampling interest could be:

- Sinks and taps;
- Frequently used solutions;
- Any device using water such as vacuum pumps and incubators, inside and outside surfaces of the device, especially where contact with hands is possible;
- Outside surfaces and around waste containers for solid waste, for sharps and liquid waste.

Secondary locations, with low probability to find the micro-organism alive, could be sampled:

- Bench surfaces;
- Microscopes;
- Biosafety cabinets;
- Autoclave;
- And their surrounding floor.

Techniques for *L. pneumophila* sampling must allow viability and identification of the micro-organism. Depending on the type of matrix, one could use:

- Swabs for biofilms to place directly in sterile water in a bottle or to roll directly out an agar culture plate;
- Rodac plates for surfaces sampling;
- Sterile bottles for liquid samples.



Sample transport to laboratories for analysis must be rapid, within 24 hours and between 6 and 18°C. The sample must be kept at 4°C or -20°C minimum if time is extended up to 24 to 48 hours. For water or liquid samples it could be required to add sodium thiosulfate (preservative and chlorine neutralizing agent) and leave air space in the bottle (*L. pneumophila* is an aerobic bacterium).

Typically a charcoal yeast extract (CYE) agar or buffered charcoal yeast extract (BCYE) agar is used for culture of this organism. Incubation is at 35-37°C for 3 to 10 days in a moist atmosphere. Wait 10 days of culture to discard presence of *L. pneumophila*. Molecular methods using nucleic acid techniques (such as PCR) as well as the Direct Fluorescent Antibody (DFA) method are routinely used as confirmatory tests. It can be important to further characterising the strain of *L. pneumophila* in order to demonstrate the relationship between sampling results and a given strain manipulated in the laboratory. This is performed by Sequence Based Typing molecular technique.

#### ***Expected results and discussion***

In case the culture reveals positive results for *L. pneumophila* in one or more locations, the strain obtained in sampling must be checked and compared with the one manipulated in the facility. If it is the same strain, the question will remain as what's the acceptable amount of bacteria present in the facility which do not represent a hazard for people (workers and population) and environment. The infectious dose of *L. pneumophila* is unknown. However, while there is no international consensual *Legionella* limit established for safe water, the WHO has proposed a level of 1000 UFC/L of this micro organism as the limit above which action of decontamination has to be taken to avoid risks for public health. This limit could be re-evaluated depending on the localisation of the inspected facility. For example, in France, acceptable concentrations of *Legionella pneumophila* in sanitizer water differ in public facilities and in health care institutions.

## **2. *Mycobacterium tuberculosis***

*Mycobacterium tuberculosis* is a Gram-positive rod, non-sporulating and non-motile bacterium. It is acid-resistant, obligated aerobe and characterized by lack of pigmentation on solid agar. *M. tuberculosis*, along with *M. bovis*, *M. africanum*, and *M. microti* all cause the disease known as tuberculosis (TB) and are members of the tuberculosis species complex. *M. tuberculosis* infects primarily humans, cattle, primates and rodents and may stay latent for years (Rubin, 1991).

Tuberculosis remains a major public health problem worldwide, killing nearly 2 million people each year (WHO). *M. tuberculosis* belongs to the top 10 of laboratory-acquired infections (Harding and Byers, 2000). It is noted that many of these acquired infections could not be associated to a lab incident such as a needle prick, pointing out that the *M. tuberculosis* principal route of transmission is via aerosols.

### ***Biological risk assessment:***

*M. tuberculosis* is a risk class 3 micro-organism. The airborne route is the predominant means of transmission to humans. The bacteria are carried as airborne particles (droplet nuclei) that can remain airborne for minutes to hours. The portal entry into the host is the lung. The most common clinical manifestation of *M. tuberculosis* infection is the pulmonary disease. In the laboratory, transmission modes are principally by inhalation of infectious aerosols, accidental inoculation, direct contact with mucous membranes and by ingestion of infected material. Direct contact with infected animals or tissues of infected animals is also of high risk of transmission. The infectious dose is approximately 10 bacilli by inhalation which is very low taking into account that a human sputum sample may contain millions of bacilli (Gilchrist and Fleming, 2000; fact sheets, University Iowa).

Outside its hosts, *M. tuberculosis* may survive for long periods: more than 100 days in dust, around 50 days in carcasses, more than 6 months in sputum (MSDS, Public health Agency of Canada).

Once the disease is diagnosed in a human being, a combination of antimicrobial drugs must be administered to be effective against multi-drug resistant strains. These multi-drug resistant tuberculosis strains are emerging worldwide and become a public health problem.

### ***Activity:***

In the majority of clinical laboratories, the diagnostic activity of human samples susceptible to contain *M. tuberculosis* complex is limited to primo-identification. Specific detection methods such as the smear examination and primary cultures are carried out on clinical specimens. As most clinical laboratories do not have the required containment level facility allowing further

characterization of *M. tuberculosis* (see below), clinical specimens or/and positive primary cultures must be sent to a reference laboratory for strain typing and to test the specific antimicrobial susceptibility.

In R&D, manipulation of *M. tuberculosis* is performed to search for and develop new anti-bacteria molecules, to elucidate immunity response to infection or to set-up new tests to be used for diagnostics. To perform this type of research, amplification of the bacteria by secondary cultures is needed to get enough infectious material.

***Containment level and decontamination:***

In all activities manipulating *M. tuberculosis*, appropriate practices and precautions have to be taken to minimize the production of infectious aerosols and contamination of the laboratory and the worker.

Level 2 facilities and equipment are required for clinical diagnostic activity in general. In case of primo-identification of *M. tuberculosis*, additional work practices are required such as the use of a class I or II biosafety cabinet (BSC) for every manipulation involving opening of tubes or vessels containing the bacteria, the use of aerosol-free buckets in the centrifuge ("safety cups") to avoid any fluid spill, and appropriate *in situ* waste inactivation (Herman *et al.*, 2006).

Level 3 work practices, equipment and facilities are required for the amplification and manipulation of *M. tuberculosis* secondary cultures. Work practices include that rotors, buckets and tubes should be opened in a BSC and mechanical homogenizing (vortexing, grinding, blending) of samples should be performed in a BSC. Appropriate systems of respiratory protection with HEPA filtration should be worn when aerosols cannot be safely contained or for the handling of positive cultures in the BSC. FFP2 filter level mask should be worn (CEN, 2001; NIOSH, 1999).

Infected rodents (mice, rats, rabbits) can be housed in level 2 containment as far as they are maintained in isolators. However, level 3 work practices should be adopted. Manipulations involving opening of cages should be realized in class I or II BSC. Cages, litters and carcasses should be autoclaved and/or incinerated before disposal. Non-human primates infected with strains of the *M. tuberculosis* complex should be handled using standard precautions in level 3 animal facilities, equipment and work practices. Samples from infected animals should be handled using standard precautions in level 2 facilities and level 3 work practices.

*M. tuberculosis* presents high intrinsic resistance to disinfection and fumigation processes and possesses effective defense mechanisms against oxidative stress (Grare *et al.*, 2008). Disinfection requires longer contact times for most disinfectants to be effective.

*M. tuberculosis* is sensitive to moist heat (121° C for at least 15 min), ionizing radiations and UV light. It is recommended to validate inactivation methods used on *M. tuberculosis* suspension before removal from BSL-3 laboratory.

### **Sampling**

It has to be pointed out that most of the clinical laboratories identify a few *M. tuberculosis* positive samples per year. Inspectors should take this situation in consideration and should be informed about when the last positive sample has been detected in order to evaluate the relevance of sampling in such conditions. Inversely, in L3 reference laboratories and in research facilities, samples and cultures of *M. tuberculosis* are handled on a daily basis. To prove effective protection of workers and population against this high pathogenic organism, it may be useful to perform sampling in the most exposed locations of the level 3 laboratory.

As mentioned before *M. tuberculosis* is particularly resistant and may persist for long periods outside a host. In addition, this pathogen may be spread over a long distance by aerosols and may deposit far from where it was initially manipulated. These two factors suggest that *M. tuberculosis* might be able to contaminate any location in any facility where this pathogen is manipulated, stored or disposed off. However, the highest concentration is likely to be found at:

- Reception room and/or table;
- Waste disposals, their external walls
- Laboratory air;
- BSC surfaces, internal and external walls and floor around;
- Centrifuges, internal and external surfaces and the surrounding floor;
- Refrigerators, internal and external surfaces and door handle;
- Microscopes and their surroundings;
- Benches;

Secondary locations may be:

- Reagents and reagent containers (bottles) surfaces;
- All material susceptible to have been used to manipulate specimens, cultures containing *M. tuberculosis*;
- Automated devices;
- Airlocks;
- Waste storage rooms: wall, floors, door handle. However, in a L3 laboratory, waste should be autoclaved before storage.

Sampling may be performed with:

- Swabs to place immediately in sterile water;
- Opened plate with Lowenstein Jensen culture medium placed for a period of time on surfaces nearby operational devices or benches;
- Rodac plate for flat surfaces;
- Search of airborne *M. tuberculosis* particles by air pumping:
  - with PTFE (polytetrafluoroethylene) filter (pores of 1µm), rate of 4L/min. (NIOSH Manual of Analytical Methods, 4<sup>th</sup> ed, 1998)
  - with membrane filter made of polycarbonate (pores of 0,01 to 20µm), rate of 2 L/min. (Mastorides *et al.*, 1999)
  - with membrane filter made of polycarbonate (uniform pore size of 0,4µm), rate of 22 L/min. (Chen and Li, 2005)

Membrane filters are removed from the membrane holder and stored at -70°C until analysis or in sterile water for immediate analysis.

Note: Time of pumping will depend on particle concentration which it is assumed to be very low. It is recommended to pump during 8 hours at least.

Samples must be decontaminated before performing any culture or test in order to eliminate other micro-organisms that may be present in the sample and may hide the presence of *M. tuberculosis*. Culture and detection may be carried out by several ways:

- on solid Lowenstein Jensen medium or on Middelbrook medium at 35°C with an examination once a week during 8 weeks;
- in the MB/BacT broth system (automated) with monitoring continuously for 8 weeks;
- with BACTEC system in which radio-labeled palmitate is metabolized by the bacteria to produce radioactive CO<sub>2</sub>. This test requires 6-16 days, a shorter time for detection than the other detection systems and may be more adequate for inspection purposes.
- By performing a Ziehl-Neelsen staining (for detection of all *Mycobacteria*)
- By air sampling to be used in combination with molecular biology techniques for identification such as PCR and Real Time-PCR.
- The molecular biology techniques are high sensitive and allow detection of low concentration of bacteria. They are also relatively rapid and more adequate for inspection purposes. PCR generally target a sequence of the multicopy IS6110 gene for the specific detection of *M. tuberculosis* (Wilson *et al.*, 1993; Desjardin *et al.*, 1998).

***Expected Results and discussion:***

Technical difficulties include the low growth of *M. tuberculosis* compared to other micro-organisms and the presence of a bacterial flora in the taken sample that will overgrowth in culture plates and mask the possible presence of *M. tuberculosis*. On the other hand, in swabs samples and in air samples, concentration of bacilli could very low. Thus, the probability of false negative samples is high. Bacilli detection will require the use of high sensitive molecular biology methods which are based on detection of DNA (Bagutti, 2009, unpublished data). However, without a culture, viability of *M. tuberculosis* will not be checked.

The results of sampling in facilities manipulating *M. tuberculosis* must show no traces of viable bacterium in any location because of the high biological risk this pathogenic organism represents for humans. It has to be pointed that just 10 bacilli per inhalation are enough to infect a human being. A positive sample result will reveal a weakness in the biosafety containment measures and work practices. Location(s) where the positive sample will be found may give indications on which biosafety measures failed and should be revised.

### 3. Bacteria producing toxins

Toxins are effective and specific poisons produced by living organisms such as bacteria, fungi, algae and plants. Bacterial toxins have been defined as soluble substances that alter the normal metabolism of host cells with deleterious effects on the host (Schlessinger and Schaechter, 1993). Indeed, major symptoms associated with disease caused by toxin producing organisms are related to the biological effects of their toxins (Schmitt *et al.*, 1999). Some bacteria produce just one toxin while others produce several toxins with distinct modes of action. The best example is *Staphylococcus aureus* that produces up to 25 different toxins. Typical and well-known toxin producing bacteria include *Corynebacterium diphtheriae* producing diphtheria, *Clostridium tetani* for tetanus, *Bordetella pertussis* producing whooping cough, *Escherichia coli* producing bloody diarrhea and haemolytic uremic syndrome, *Staphylococcus aureus* with its toxic-shock syndrome toxin.

Bacterial toxins can be subdivided into endotoxins, which are not secreted but are structural components of the bacterium, and exotoxins, which are extracellular proteins that are generally secreted. LPS (lipopolysaccharides) are endotoxins found in the outer membrane of various Gram negative bacteria that induce a strong response from animal immune systems. Exotoxins are divided into classes depending on their mode of action on the host-cell: binding to the host cell surface (*S. aureus* enterotoxins), pore forming (*S. aureus*  $\alpha$ -toxin) or binding to host cell surface and translocation into the cell (*E. coli* O157:H7 Shiga toxin).

In the present sampling feasibility sheet, two representative toxin producing bacteria are chosen and analyzed to determine relevance of sampling: *Staphylococcus aureus* and *Escherichia coli* O157: H7. They represent emerging micro-organisms that may be harmful for public health and are also frequently manipulated in diagnostic laboratories. However, even though toxins are the principal cause of pathogenicity and detection tests for some of these toxins are rapid and accurate, specific and sensitive, sampling must focus on the detection of living micro-organisms (Teel *et al.*, 2007; Lina *et al.*, 1998).

### 3.1 *Staphylococcus aureus*

*Staphylococcus aureus* are Gram-positive spherical cocci usually disposed in clusters. *S. aureus* is normally found in the human flora: it colonizes mainly the nasal passages, but it may be found in many other anatomical locations, including the skin, oral cavity and gastrointestinal tract. *S. aureus* strains produce exotoxins such as the enterotoxins, mainly responsible of food poisoning, the  $\alpha$ -toxin, the toxic shock syndrome toxin (TSST-1) and exfoliative toxins A and B, responsible of skin lesions (PHAC).

Since several decades, a methicillin-resistant *S. aureus* (MRSA) has emerged worldwide in hospital environments and is today a major cause of hospital acquired (nosocomial) infection (CDC, 2008). Actually, MRSA strains present multi-resistance to antibiotics. In addition, MRSA strains have recently emerged outside the hospital becoming known as community associated- MRSA (CA-MRSA) or superbug strains, which now account for the majority of staphylococcal infections (Todar, 2008). CA-MRSA strains display enhanced virulence, spread more rapidly and cause much more severe illness than traditional Hospital Acquired-MRSA (HA-MRSA) infections. This is thought to be due to toxins carried by CA-MRSA strains (Tristan *et al.*, 2007).

#### **Biological risk assessment:**

*Staphylococcus aureus* is a class risk 2 micro-organism. The preferred host of the bacterium is the human being and to a lesser extent, warm-blooded animals. *S. aureus* may cause a variety of suppurative infections and toxinoses. It causes superficial skin and soft tissue lesions but also more serious infections such as pneumonia and meningitis, and deep-seated infections, such as osteomyelitis and endocarditis.

The human respiratory tract, the skin and superficial wounds are common sources of *S. aureus*. The bacterium is transmitted by contact (direct and indirect) with nasal carriers (30-40% of population) or with draining lesions or purulent discharges. It can spread from person-to-person. The infectious dose varies greatly with the strains.

Outside its host, *S. aureus* has the capacity to survive in carcasses, organs, and meat products for up to 1 month. It may survive for one week on the floor, on coins, on polyester and polyethylene plastic and for several hours in glass or in the sunlight. *S. aureus* may survive in the human skin from 30 minutes to 38 days (Public Health Agency of Canada, Neely and Maley, 2000).

*S. aureus* is susceptible to many common disinfectants such as a 1% sodium hypochlorite solution, iodine/alcohol solutions, glutaraldehyde, formaldehyde but the bacterium shows



resistance to quaternary ammonium compounds. The micro-organism is destroyed by heat: moist heat at 121° C for at least 15 minutes and dry heat at 160-170° C for at least 1 hour. One should keep in mind that enterotoxins are heat resistant and stable at boiling temperature. As mentioned above, many strains are multi-resistant to antibiotics and are of increasing health importance. Vancomycin resistant (VRSA) strains are increasingly reported (Tenover *et al.*, 2001). There is no vaccine available but several experimental vaccines and drugs are under development and tested in clinical trials.

**Activity:**

Clinical laboratories regularly analyze human and hospital environment samples for the detection and antibiogram determination of *Staphylococcus aureus*. Several quality laboratories also test samples from food, water, pharmaceutical products to control the presence of *S. aureus*. Another important sector of activity manipulating *S. aureus* is the research and development in universities and pharmaceutical companies. The aim of these studies is to understand mechanisms involved in the virulence and the resistance of this micro-organism.

**Containment level:**

In the laboratory, the primary hazards for workers manipulating *S. aureus* samples are the injuries caused by contaminated sharp instruments and the contact with damaged skin. Ingestion, aerosol inhalation and direct contact with infected material represent also major ways of lab infections. According to the Canadian Public Health Agency, 29 cases of laboratory-acquired infection with *S. aureus* have been reported. More recently, several cases of laboratory acquired MRSA infection have been reported and published (Gosbell *et al.*, 2003; Wagenvoort *et al.*, 2006). Containment level 2 practices, equipment and facilities are required for activities involving *S. aureus* cultures and potentially infectious materials. A laboratory coat must be worn as well as gloves when skin contact is unavoidable. It is recommended to perform manipulations susceptible to generate infectious aerosols under a class II BSC. Hand washing must be frequent and take place at least just after handling infectious materials and before leaving the laboratory.

**Sampling:**

To evaluate the relevance of sampling in a laboratory manipulating *Staphylococcus aureus*, it is first important to look to the type of activity and the location of the laboratory. A clinical laboratory situated in a hospital for example could be considered relevant for sampling because of the frequent tests performed in it for *S. aureus* and the proximity of particular susceptible patients. However, in all facilities, it has to be kept in mind that an accidentally contaminated laboratory worker may extend the infection to members of its family and friends by person-to-person transmission. In the case of MRSA strains, this could represent a serious hazard for public health.

In a facility, the inspector should appreciate the hazard of the activity as well as the actual state, quality and efficiency of the equipment, work practices and waste management in place. Unintended contamination due to sample manipulation may come from aerosols generation, from poor or non-adapted decontamination of surfaces and material or from poor waste management.

Locations of interest:

- BSC surfaces, internal and external walls, floor around;
- Centrifuges inside and outside surfaces, surrounding floor;
- Refrigerators, internal and external surfaces, door handles;
- Incubators, internal and external surfaces, door handles, water from bath;
- Microscopes and their surroundings;
- Benches;
- Reagent bottles surfaces and reagents themselves;
- Automated devices surfaces;
- Waste containers, external surfaces;
- Waste storage room, floor, walls, door handle.

Techniques for *S. aureus* sampling must allow viability and identification of the micro-organism. Depending on the type of matrix, one could use:

- Swabs or gauze strips for biofilm samples and for heterogeneous surfaces, to place directly in sterile water or in an adapted broth such as the TSB broth (Trypsicase soy broth). Swabs could be rolled directly out an agar culture plate;
- Rodac plates for homogeneous surfaces sampling filled with sterile Dey-Engley neutralizing agar that neutralizes all commonly employed disinfectants.

Sample transport to laboratory for analysis must be rapid. Sample has to be kept at 4°C until analysis.

The classic detection and identification procedure is performed in two steps:

- o Culture plates are directly incubated for 45 - 48 h at 35°C and *S. aureus* are recognized by their typical appearance;
- o The coagulase test and a Gram staining confirmed the identification of *S. aureus*.

A more rapid alternative to this test is the latex agglutination test.

In the present case, an antibiogram must be performed in order to reveal the resistance developed by *S. aureus* strain in the sample.

If the starting sample is supposed to contain low concentrations of the bacteria, the first step should consist in an inoculation of TSB tubes (Trypsicase soy broth) 10% NaCl 1% sodium pyruvate with the sample, followed by an incubation of these tubes at 35°C during 48±2h. Then, Baird-Parker agar culture plates (selective *S. aureus* culture) are inoculated with samples from broth tubes showing turbidity (bacteria growth).

Ancillary tests exist (Bennett and Lancette, 2001):

Catalase test

Anaerobic utilization of glucose

Anaerobic utilization of mannitol

Thermostable nuclease production

Different kits exist that allow for faster detection and identification of *Staphylococcus aureus*, for example the RAPID'Staph test (from Bio-Rad), the Petrifilm Staph Express Count plate (from 3M).

**Expected results and discussion:**

*S. aureus* is a common bacterium that can be found on healthy individuals. It is possible that sampling results reveal a low level of facility contamination with *S. aureus*. Some laboratories have established a baseline contamination of the laboratory that must not be exceeded in order to avoid interferences with their analysis. It is easy in these conditions to compare sampling results with this baseline level. If such baselines are not established, the contamination of the facility with *S. aureus* may be assessed by choosing different locations inside and outside the facility for sampling and to compare the results of the analysis.

The level of “acceptable” contamination will depend on the site of the activity and on the type of bacteria strain. For example, a diagnostic laboratory in a hospital environment, where a nosocomial infection is extremely dangerous for patients, and in case of multi-resistant strain manipulations, the best situation in such a laboratory is that no traces of this strain bacterium should be found in any sampling location.

### 3.2 *Escherichia coli* O157:H7

*Escherichia coli* is a Gram negative rod bacterium which is motile and aerobic. *E. coli* is a normal inhabitant of the intestines of all animals including humans. A minority of *E. coli* strains is capable of causing human illness by several different mechanisms. *E. coli* serotype O157:H7 is a rare variety of *E. coli* that produces large quantities of one or more potent toxins that cause severe damage to the lining of the intestine. These toxins are closely related or identical to the toxin produced by *Shigella dysenteriae*, the Shiga toxin (Stx). Stx is a cytotoxin that inhibits protein synthesis (see Melton-Celsa and O'Brien, 2000; Nataro and Kaper, 1998 for review).

*E. coli* serotype O157:H7 is the most common enterohemorrhagic *Escherichia coli* (EHEC) and is also called Verotoxin producing *Escherichia coli* (VTEC) or Shiga toxin producing *Escherichia coli* (STEC). Other EHEC serogroups causing illness exist (*E. coli* O26, O111 and O103) but they cause less severe disease than *E. coli* serotype O157:H7.

#### **Risk assessment and decontamination:**

The enterohemorrhagic *Escherichia coli* O157:H7 is a class risk 3 toxin-producing micro-organism. The bacterium provokes hemorrhagic colitis, an intestinal disease accompanied by cramps and abdominal pain, followed by bloody diarrhea. It affects all ages but higher death rates occur in elderly and young people. Complications of the disease threatening life include hemolytic uremic syndrome (HUS) and the thrombocytopenic purpura (TTP).

Its host range encompasses animals such as piglets, calves, cattle and humans. Modes of transmission of the bacteria include the ingestion of contaminated food (undercooked hamburger meat, unpasteurized milk), fecal-oral transmission, person-to-person transmission (extremely high), contact with infected animals (sheep, goats, pigs, poultry, calves, cattle) and wastes. The infectious dose is estimated to be low i.e. approximately 100 organisms per ingestion. The bacterium is not thought to be airborne, however, a recent outbreak report in the United States suggests that the bacterium was carried in the air and by this way, infected people participating in a county fair (Varma *et al.*, 2003). In laboratory settings the airborne route is to be considered for *E. coli* O157:H7.

*E. coli* O157:H7 survives for months outside its host: in hamburger meat, cream, raw milk, unpasteurized fruit juices, in slurry systems (Kudva *et al.*, 1998). It survives well for months in contaminated faeces and soil as well as in water (Wang and Doyle, 1998). *E. coli* O157:H7 is also found to be resistant at refrigeration temperatures, at low pH and may survive for long time in biofilms.

*Escherichia coli* O157:H7 is sensitive to a wide spectrum of antibiotics. However, antibiotics are not used to treat *E. coli* O157:H7 infection except in very severe cases because they may increase the risk of HUS complication. There is no available vaccine.

To inactivate and decontaminate surfaces contaminated with the bacterium many common disinfectants may be used such as 1% sodium hypochlorite, 70% ethanol, phenolics, glutaraldehyde, iodines and formaldehyde. It is heat sensitive and is inactivated by moist heat (121° C for at least 15 min) and dry heat (160-170° C for at least 1 hour).

**Activity:**

In Belgium, contained use of *E. coli* O157:H7 occurs in clinical laboratories handling human samples for diagnostic as well as in quality control laboratories for environment, food and water sample analysis. Other facilities maintain the micro-organism in a collection and send samples to laboratories on their demand. Research activities investigate this bacterium with the aim to develop vaccines, to improve diagnostic methods and to improve and optimize methods of food conservation.

**Containment level:**

There are several reported cases of laboratory-acquired infections with *E. coli* (Booth and Rowe, 1993; Burnens *et al.*, 1993; Rao *et al.*, 1996; Spina *et al.*, 2005). The primary hazard in a laboratory is ingestion of infected material but attention should be drawn to avoid aerosol generation and inhalation. Containment level 2 facilities and equipment are required in a laboratory handling this bacterium. However, due to the very low infectious dose and the powerful Stx toxin produced by *E. coli* O157:H7, containment level 3 work practices are required. These measures include, in addition to the general good microbiology practices,

- The use of gloves for any manipulation at risk;
- The frequent hand washing, in a sink with hand-free faucets, with an automatic soap distributor as well as single-use towels;
- To avoid the formation of micro-droplets;
- To disinfect work surfaces and any used material before and after each operation;
- To establish written procedures for *E. coli* O157:H7 manipulations and decontamination.

It is also recommended to wear a lab coat closing in the back and tightening wrists. It is recommended in addition to perform any manipulations susceptible to generate aerosols under a class I BSC at least.

**Sampling:**

As already mentioned, *E. coli* O157:H7 is particularly resistant and may stay alive for long periods of time outside its host. Moreover, the bacterium is characterized by a low infectious dose. These bacterium characteristics imply that facilities where this infectious organism is

manipulated, stored or disposed of should be considered for sampling. Locations of interest for sampling could be:

- Reception room and/or table;
- Waste containers, external surfaces and lid;
- Waste storage rooms, walls and floor, door handles;
- BSC surfaces, internal and external walls, floor around;
- Centrifuges inside and outside surfaces, surrounding floor;
- Refrigerators inside and outside surfaces, door handle;
- Microscopes and their surroundings;
- Benches;
- Sinks and taps;
- Any device using water such as vacuum pump, incubators, water bath.
- Reagent bottles surfaces and reagents themselves;
- All disposable material susceptible to have been used to manipulate specimens or cultures;
- Automated devices.

Techniques for *E. coli* O157:H7 sampling must allow viability and identification of the micro-organism. Depending on the type of matrix, one could use:

- Cotton swabs for heterogeneous surfaces and biofilms to place directly in sterile water or in an adapted broth (modified TSB broth with novobiocin plus casaminoacids);
- Rodac plates for smooth surface sampling;
- Sterile bottles for water or liquid sampling.

Sample transport to the reference laboratory for analysis must be rapid. The sample has to be kept at 4°C until its analysis.

Different methods of specific detection of *E. coli* O157:H7 exist and are recognized by the national food safety authorities of Belgium (NAFCS) and international norm organizations (AFNOR, ISO). Some of them (VIDAS ECO, horizontal method for detection of *E. coli* O157) rely on the following principles:

- (1) Enrichment in modified tryptic soy broth supplemented with novobiocin (mTSBn),
- (2) Separation and concentration of *E. coli* O157:H7 by means of immunologic techniques,
- (3) Isolation by subculture onto two selective indicative isolation media (cefixime potassium supplemented Sorbitol MacConkey (SMAC) agar as mandatory isolation media and the other isolation media is free of choice), and
- (4) Confirmation of the *E. coli* O157:H7 by identification of O157 and H7 antigens (by ELISA) or by identification of specific DNA sequences (by PCR).

Other methods of detection are based on the use of the selective culture media combining chromogenic substrates and biochemical indicators (RAPID'E.coli O157:H7) or on direct PCR amplification (iQ-Check *E. coli* O157:H7, BAX® *E. coli* O157:H7).

***Expected results and discussion:***

Regarding the risk assessment described above on activities involving *E. coli* O157:H7, this bacterium should not be found in the facility environment. So, the results of sampling in facilities manipulating *E. coli* O157:H7 should be negative in any location. A positive result will reveal a weakness in the biosafety containment measures and in work practices in place. The location where the positive sample will be taken could give indications on which measures have to be revised.

#### 4. Adenoviruses and adenoviral vectors

Adenoviruses are non-enveloped, linear double-stranded DNA viruses comprising over 40 serotypes. They are a common cause of upper and lower respiratory tract infections, illness known as a "cold." Adenoviruses are also implicated in conjunctivitis and gastro-enteritis.

Adenoviral vectors are viruses that are specifically used to introduce exogenous DNA into host cells. They have a high cloning capacity and can infect a wide variety of cell types. Laboratory-made recombinant adenoviral vectors are most of the time derived from the type 5 adenovirus. Specific DNA region deletions render the virus incapable of autonomously reproducing itself and make the virus more susceptible to the human immune defense system. Foreign genes can be inserted in place of the deleted genes. The recombinant vector can be produced to very high titers in cell lines such as Human Embryonic Kidney (HEK) 293 which express the missing Ad5 genome.

##### ***Biological risk assessment:***

Wild type Adenoviruses are risk class 2 micro-organisms. The epidemiology of adenovirus indicates that it is found worldwide with high occurrences in children under the age of 5 years. The mode of transmission of adenovirus is by direct oral contact or aerosolized droplet exposure to the mucous membrane and by fecal-oral route. Waterborne transmission has been occasionally reported.

Replication-defective adenoviral vector are minimum risk class 2 micro-organisms (Belgian Biosafety Server (3)). It is however important to properly understand the nature of the inserted gene and the characteristics of the final recombinant adenovirus. The following parameters should be considered to evaluate the biological risk of the GMM (Belgian Biosafety Server (3)).

- The effects of the inserted sequences:
  - o The level of expression of an inserted gene, which depends on the host cell type and the transcription regulators;
  - o The biological activity of the expressed protein itself, which may increase the risk in case it is related to cell proliferation, apoptosis mechanisms, is a toxin or simply may be of unknown function.
- The effects of the genetic modification on phenotypic characteristics of the adenovirus:
  - o The modification of virus tissue tropism, changing the host range;
  - o The alteration of the infectivity and pathogenicity.

Finally, when evaluating the biological risk of the new recombinant adenovirus, the possibility of accidental generation of replication-competent adenoviruses by recombination (with DNA



sequences of the wild type virus or from 293 cells) or complementation (with DNA sequences from cell lines such as HeLa cells or from other viruses such as the Epstein-Barr virus) has to be considered.

There are no specific antiviral drugs or vaccination currently available against adenoviruses. In addition, adenoviruses are unusually robust, stable against chemical or physical agents and to adverse pH conditions, allowing for prolonged survival periods outside the body (3 to 8 weeks on environmental surfaces at ambient temperature (Public Health Agency of Canada)). Even after treatment with ether or chloroform, they can still be infective.

**Activities and Containment level:**

In Belgium, the R&D and pharmaceutical sectors are the principal sectors involved in contained uses of adenoviruses and adenoviral vectors. The aim is the development and construction of adenoviral vectors as tools for studies on gene function in gene disorders and diseases, on anti-tumor immunologic responses, for gene therapy or for anti-tumor vaccination. Activities involve *in vitro* cell cultures, or *in vivo* studies with animals and also on human beings in the framework of clinical trials.

In a laboratory manipulating adenoviruses and adenoviral vectors, major ways of contamination could be:

- Ingestion of infected material;
- Droplet exposure of the mucous membrane (eyes, nose, mouth);
- Aerosol inhalation;
- Accidental injection.

Manipulation of wild type adenoviruses and their derivative recombinant vectors need a containment level 2 as a minimum, even if the recombinant vectors are replication defective (see guidance of the Harvard University, 2000; UTHHSC guidelines). Special attention should be paid to the possible generation of aerosols from both procedures and waste materials. So, in addition to the laboratory containment and practices of level 2, manipulation of the viruses should be performed under a class II BSC. In case that the biological risk of the adenoviral vector is increased by the nature of the inserted gene, the containment level is raised to level 3. All administrations and manipulations of animals should be performed under the BSC. When performing administrations involving concentrated materials to human beings, negatively pressured room is recommended.

**Sampling:**

Sampling a facility where adenoviruses and adenoviral vectors are manipulated is relevant when high volumes and/or high virus titers are handled, for example in production of adenovirus. In case of adenoviral vectors manipulation, sampling is indeed relevant if the risk

assessment has demonstrated that the modification and the use of the vector may represent a serious hazard for human health, as described above.

In such facilities, the production of infectious aerosols is the most common way of contamination and sampling should therefore focus first on places where the activity could generate aerosols:

- the centrifuge(s), internal and external walls;
- the benches;
- the biosafety cabinet(s), internal and external surfaces.

It is always relevant to control waste management with taking samples in:

- the waste disposals, the external surfaces;
- the waste storage rooms, walls, floor, door handles;

Swab is the preferred method for sampling. It should be placed in a viral transport medium such as the Richards viral transport medium. It should be immediately analyzed for virus detection. If this could not be achieved, maintain the sample at 4°C until analysis.

Wild type adenoviruses and adenoviral vectors detection can be based on the observation of cytopathogenic effects on human cells in culture, in combination with their identification by a molecular technique such as PCR or an immunoassay. By this combination, virus infectivity and identification will be highlighted. PCR or real-time PCR are sensitive and specific methods for detection of adenovirus DNA. Several commercial kits exist that allow for the specific adenovirus serotype detection or for detection of universal serotype sequences.

Specific adenoviral vector detection, which is in use in the inspected laboratory, could be performed by PCR or real-time PCR. For this purpose, the inspected laboratory may be asked to provide with primers (and probe) sequences in order to detect the specific inserted gene or the specific region link between the insert and the vector.

***Expected results and discussion:***

Adenoviruses are relatively common micro-organisms circulating among population. This means that a positive result of a sampling in a facility manipulating such viruses will not be easily related to its activity. In addition, adenovirus does not induce severe disease in healthy individuals. Thus, sampling facilities manipulating this type of virus could not be relevant except if the facility is situated in a sensitive area such as a health care institute. On the contrary, in the case of manipulations of adenoviral vectors that carry an insert increasing the risk for public health, the facility sampling is relevant.

It has to be remembered that the viability and infectiousness of the viral vectors on cell cultures before their specific identification is essential. Indeed, while the major advantage of detection by PCR is the specific identification of low concentrations of sequences of interest, the method will not allow to discern the origin of these detected sequences. The sequences may derive from the inactivated virus or from a plasmid used for its construction, cases in which there is no immediate risk for the human health.

## 5. Animal and human cell cultures

Today, several applications in the field of biotechnology, medicinal and veterinary sciences, are supported by animal and human cell cultures. They are used as substrates for the production of viral vaccines and other biological and pharmaceutical products, for diagnostic testing and as *in vitro* models for fundamental research. Cell cultures are manipulated as an intermediate tool to understand intra and inter cellular responses occurring in normal and pathogenic situations.

In the framework of a facility inspection, it may be complex to determine the health hazard represented by cell culture. Not only inherent properties of the cells should be evaluated, but an identification of the biological risks associated to the biological organisms these cells may harbor needs to be performed as well. We present here some important parameters to take into account by the inspector in order to establish the relevancy to perform a sampling in such a facility.

### **Risk assessment:**

Animal and human cell cultures become of biosafety concern for public health and the environment mainly when they support the survival and multiplication of pathogenic organisms, either by deliberate or by inadvertent contamination of cells with pathogens. Similarly, the genetic modification of cells from an animal or human source doesn't represent in itself a risk for human health and environment as far as the modification is performed by non-viral vectors and the culture is guaranteed to be free of pathogens.

In the case of deliberate infection of animal cell cultures, the inspector should consider the intrinsic properties of the pathogen and its biological risk class as well as the type of manipulation to determine the risk class of the cell culture. This is also true for genetically modified cell cultures that support the production of recombinant or wild type viruses. The inspector should consider in addition the possibility of integration, replication and expression of the foreign genetic material in cells in the case of viral vectors production.

In the case of an inadvertent contamination of the cell cultures, an array of micro-organisms, that could be potentially hazardous for human health, may be involved and includes bacteria, viruses, fungi, parasites, mycoplasmas (Ryan, 2002). The sources of such contaminating agents are diverse and may derive from the cells themselves or from the lab material, the lab personnel, the culture media and other medium components. Note that animal cell cultures that are not intentionally infected with pathogens or that are not genetically modified fall beyond the scope of the regulatory provisions.

**Activity:**

Handling cell cultures harboring pathogenic organisms may constitute a source of contamination of the facility and manipulations may increase the exposure of cells to adventitious pathogens. For example, changing or altering cell culture conditions may have consequences for the susceptibility of cultured cells to pathogens such as viruses. Handling cells outside the BSC, for a flow cytometry analysis for example will increase the probability of exposure of cells to inadvertent infection with pathogenic organism. If inadvertent infection occurs, the facility may become contaminated as well and expose the lab personnel to biological risks.

**Containment levels:**

Fig. 1 presents a schematic guidance to determine the appropriate containment level when manipulating cell cultures (Pauwels *et al.*, 2007).

In the case of an activity that implies the use of cell cultures infected deliberately with a pathogenic organism, the assignment of the containment level will depend on the containment of this specific pathogenic organism.

The containment level necessary to guarantee safety of human health and environment when genetically modified cell cultures are manipulated in a facility will depend on the genetic material introduced and the resulting genetically modified cells. When the genetic modification does not increase the biological risk of the cells, the class of risk of the activity is not changed.

Cells of class of risk 1 may be manipulated in a containment level 1 facility except for primary cells from human or primate origin, which will minimally require a containment level 2. This is essentially due to the fact that these cells may inadvertently become infected with human pathogens. A class II BSC will be helpful for all opened phase operations with cell cultures, at least to protect cells from adventitious contamination.

A particular attention should be paid to the inactivation procedure applied to cell cultures that are intentionally or unintentionally infected by pathogenic organisms. Indeed some pathogens may still represent a biological hazard even if their host cells are not viable anymore (e.g. production of viral particles, viruses).

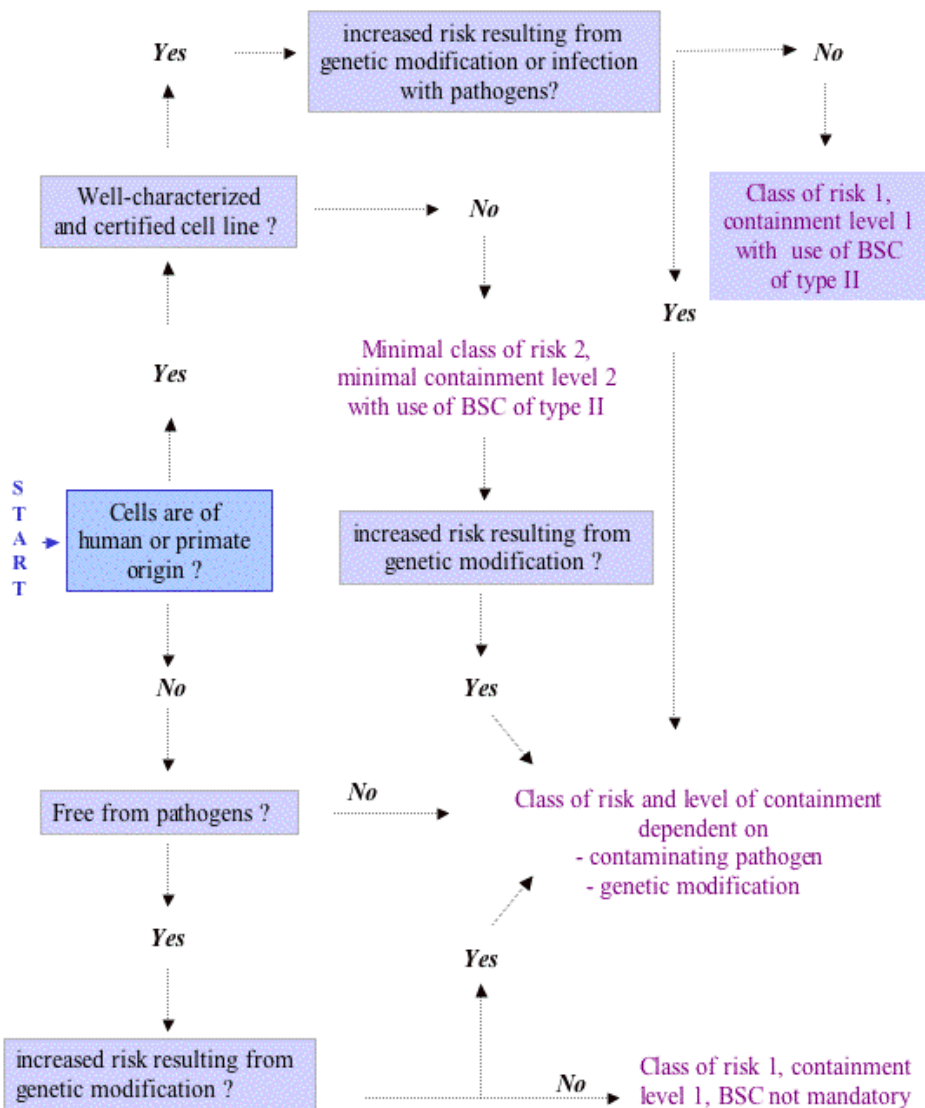


Figure 1: Guidance for the assignment of the containment level to cell cultures (Pauwels *et al.*, 2007).

**Sampling:**

Animal and human cell cultures have a restricted capacity to survive into the environment. Hence, sampling will not be based on the detection of animal cells but on the detection of pathogenic organisms that inadvertently or deliberately infect the cell cultures. However, taking samples of cells that are cultured at the time of the inspector visit is helpful to determine the relationship between the contained use activity (cell culture) and the facility contaminating micro-organism.

In the case of animal or human cell cultures, genetically modified or not, but deliberately infected by a pathogenic organism belonging to at least risk group 2, the sampling may be performed and the methodology and the detection method must be adapted to this particular pathogenic organism.

In the case of animal or human cell cultures that are not intentionally infected, the relevance to perform a sampling will depend on the good practices of the laboratory to manage and manipulate cell cultures but also on the existence, the frequency and the completeness of a check program for contaminants in the visited laboratory. The inspector should always pay a particular attention in a facility manipulating human or primate primary cells (from tissues, organs, blood). The best detection methodology will be a combination of methods to scan the diversity of pathogenic organisms susceptible to contaminate the cell type in culture. The most common pathogenic organisms that may inadvertently infect cells in culture are:

1. Bacteria and fungi: detection will be based on the use of different bacteriological growth media (blood agar, thioglycollate broth, yeast broth, Sabouraud's medium for fungi,...) for the same sample in at least duplicates and incubate each duplicate at different temperatures (from 26 to 37°C) for 14 days at least. This method will cover a wide spectrum of possible contaminants.
2. Mycoplasma, the most common cell culture contaminants and some species belong to risk group 2 for human. Detection will use the combination of
  - Direct test using the culture method in Mycoplasma pig agar plates and Mycoplasma pig agar broth and incubations in aerobic and anaerobic conditions for 20 days at least.
  - PCR to detect specific Mycoplasma DNA.
3. Viruses: contaminating viruses of humans tissues and cells cultures may include HIV, HBV, Epstein-Barr, herpes viruses, retroviruses but it has to be kept in mind that many viruses have a broad host range and can cross species barriers. The best methodology will screen for several human pathogenic viruses. Methods include
  - Observation of cytopathic effects on different indicator cell lines (RhMK, MRC-5, A549) after incubation for days or weeks (culture allows also to multiply viral material which could be used for more tests);
  - Detection of viral protein through immunofluorescence;
  - Detection of specific nucleic acid sequences by PCR (Reverse transcriptase PCR for retroviruses).

Locations to sample:

Sampling should be performed in locations that are susceptible for contamination by cell cultures deliberately or unintentionally infected. These places include:

- BSC, internal and external surfaces;
- Incubators, including the water from bath;
- Centrifuges, inside and outside surfaces;
- Water baths;
- Pipette disposal trays and buckets;
- Waste containers, external surfaces.

How to perform sampling:

Several samples of the same location should be taken if possible in order to perform the different detection tests necessary to cover the largest array of possible contaminating micro-organisms.

Samples should be taken on surfaces through opened Petri dishes, Rodac plates and swabs. Samples may include liquids that have to be recovered in sterile bottles. It could be useful to collect also samples of cell cultures themselves and the medium supporting the cultures.

***Expected results and discussion:***

As already mentioned, in the framework of the Regional Decrees on contained use of GM and pathogenic micro-organisms, only cell cultures deliberately infected with a pathogenic organism or genetically modified should be considered. So cell cultures unintentionally contaminated fall out of the scope. However, biosafety measures should be taken when cell cultures are manipulated in a facility to insure inadvertent contamination of the cell and protect personnel's health against inadvertent biological risks. If the detected pathogen is not the object of the contained use activity and is coming from an unintentional contamination of cells, sampling would prove that the contamination has occurred because of a weakness in quality control of the activity as well as a weakness in the biosafety containment.

In any case, sampling will rely on the detection of pathogenic organisms in laboratory locations where cell cultures are generally manipulated or disposed of. In addition, inspectors should take samples of the cell cultures themselves for testing the presence of the same pathogenic organism. Results from both types of samples (laboratory surface and cell culture) must corroborate the presence of the same pathogen in order to prove that laboratory contaminations demonstrated by sampling is the consequence of the cell culture activity.



### III. REGULATIONS

Arrêté du Gouvernement Wallon du 4 juillet 2002 déterminant les conditions sectorielles relatives aux utilisations confinées d'organismes génétiquement modifiés ou pathogènes. (MB 21.09.2002, p. 41711)

Besluit van 8 november 2001 van de Brusselse Hoofdstedelijke Regering betreffende het ingeperkt gebruik van genetisch gemodificeerde en/of pathogene organismen en betreffende de indeling van de betrokken installaties. (BS 26.10.2002, bz. 7209)

Besluit van de Vlaamse regering van 6 februari 2004 tot wijziging van het besluit van de Vlaamse regering van 6 februari 1991 houdende vaststelling van het Vlaams reglement betreffende de milieuvergunning, en van het besluit van de Vlaamse regering van 1 juni 1995 houdende algemene en sectorale bepalingen inzake milieuhygiëne. (BS 01.04.2004, bz. 18281)

Council Directive 91/156/CEE of 18 March 1991 on waste, amending Directive 75/442/CEE. (OJ L.78, 26.3.1991, p. 32)

Council Directive 91/689/EEC of 12 December 1991 on hazardous waste. (OJ L.377, 3.12.1991, p. 0020-0027)

Directive 2009/41/EC of the European Parliament and of the Council of 6 May 2009 on the contained use of genetically modified micro-organisms repealing Directive 98/81/EC. (OJ L.125, 21.5.2009, p. 75)

### IV. REFERENCES

Bagutti C., Alt M., Vogel G., Hächler H., Vögeli U. and Herrmann A.. Using real-time "in culture" PCR for the detection of dangerous microorganisms in suspicious samples. From the State Laboratory of the Canton Basel City. Unpublished data.

Bagutti C.. Biosafety in laboratories of safety level 3: results of a sampling campaign in TB laboratories 2008/2009. Joint campaign. From the State Laboratory of the Canton Basel City. Unpublished data.

Baron E.J. and Miller J.M.. Bacterial and fungal infections among diagnostic laboratory workers: evaluating the risks. *Diagnostic Microbiology and Infectious Disease*. 2008; 60: 241-246.

The Belgian Biosafety Server (1): Belgian classifications for micro-organisms based on their biological risks. Accessed online, May 2010 :

<http://www.biosafety.be/RA/Class/ClassBEL.html>

The Belgian Biosafety Server (2): Containment criteria and other protective measures. Accessed online, May 2010 :

<http://www.biosafety.be/CU/refdocs/InperkNL.html>

<http://www.biosafety.be/CU/refdocs/ConfinFR.html>

The Belgian Biosafety Server (3): Risks associated to the use of viral vectors, inserts or cell cultures. Accessed online, May 2010 :

<http://www.biosafety.be/CU/Annexes/virNL.html>;

<http://www.biosafety.be/CU/Annexes/virFR.html>

Bennett R.W. and Lancette G.A.. *Staphylococcus aureus*. Bacteriological analytical manual, 8<sup>th</sup> edition, Revision A. 2001, Chapter 12. The US Food and Drug Administration.

Berghmans L., Pauwels K., Van Vaerenbergh B. *et al.*. Biological waste treatment and inactivation methods. Royal Library of Belgium. 2006, D/2006/2505/33. Accessed online, May 2010 :

[http://www.biosafety.be/CU/PDF/Doc\\_biologisch\\_besmet\\_afval.pdf](http://www.biosafety.be/CU/PDF/Doc_biologisch_besmet_afval.pdf)

[http://www.biosafety.be/CU/PDF/DechetsBiol\\_SBB\\_2006\\_2505\\_33.pdf](http://www.biosafety.be/CU/PDF/DechetsBiol_SBB_2006_2505_33.pdf)

Booth L. and Rowe B.. Possible occupational acquisition of *Escherichia coli* O157. *Lancet*. 1993, 342: 1298-1299.

Burnens A.P., Binden R., Kaempf L. *et al.*. A case of laboratory-acquired infection with *Escherichia coli* O157:H7. *International Journal of Medical Microbiology, Virology, Parasitology and Infectious Diseases*. 1993, 279: 512-517;

CEN EN 149: 2001 norm: Respiratory protective devices - Filtering half masks to protect against particles - Requirements, testing, marking. European Committee for Standardization, 2001.

Centres for Disease Control and Prevention (CDC), Division of healthcare quality promotion. Environmental management of Staph and MRSA in community settings. July 2008.

Accessed online, May 2010 :

[http://www.cdc.gov/ncidod/dhqp/ar\\_mrsa\\_Enviro\\_Manage.html](http://www.cdc.gov/ncidod/dhqp/ar_mrsa_Enviro_Manage.html)

Chen P. and Li C.. Quantification of airborne *Mycobacterium tuberculosis* in health care setting using real-time qPCR coupled to an air-sampling filter method. *Aerosol Science and Technology*. 2005, 39 (4): 371-376.

Desjardin L.E., Chen Y., Perkins M.D., Teixeira L., Cave M.D. and Eisenach K.D.. Comparison of the ABI 7700 System (Taqman) and competitive PCR for quantification of IS6110 DNA in sputum during treatment of tuberculosis. *Journal of Clinical Microbiology*. 1998, 36: 1964-1968.

Do Thi C.D., Van Vaerenbergh B., Herman P. *et al.*. Les installations de haut niveau de confinement en Belgique. Rapport pour la période 1995-2008. Service Biosécurité et Biotechnologie, Institut Scientifique de Santé Publique. Bibliothèque Royal de Belgique. 2009, D/2009/2505/40. Accessed online, May 2010 :

[http://www.biosafety.be/CU/PDF/Rapport\\_WIV\\_NL\\_D\\_2009\\_2505\\_40.pdf](http://www.biosafety.be/CU/PDF/Rapport_WIV_NL_D_2009_2505_40.pdf)

[http://www.biosafety.be/CU/PDF/Rapport\\_ISP\\_FR\\_D\\_2009\\_2505\\_40.pdf](http://www.biosafety.be/CU/PDF/Rapport_ISP_FR_D_2009_2505_40.pdf)

Ducoffre G.. Laboratoires de référence : leur rôle dans le cadre du Réseau des Laboratoires Vigies. Section Epidémiologie, Institut Scientifique de Santé Publique. Bibliothèque Royal de Belgique. 2007, D/2007/2505/04. Accessed online, May 2010 :

<http://www.iph.fgov.be/epidemio/epifr/plabfr/reflab06.pdf>

EN ISO norm 14698-1: 2003: Cleanrooms and associated controlled environments – Biocontamination control – Part 1: General principles and methods.

Gilchrist M.J. and Fleming D.O.. Biosafety precautions for *Mycobacterium tuberculosis* and other airborne pathogens. In: Fleming D.O., Hunt D.L. editors, *Biological Safety, principles and practices* 2000. 3<sup>rd</sup> edition, Washington, American Society for Microbiology Press: 209-219.

Gosbell I.B., Mercer J.L., Neville S.A.. Laboratory-acquired EMRSA-15 infection. *Journal of Hospital Infection*. 2003, 54: 324-325.

Grare M., Dailloux M., Simon L., Dimajo P., and Laurain C.. Efficacy of Dry Mist of Hydrogen Peroxide (DMHP) against *Mycobacterium tuberculosis* and use of DMHP for Routine Decontamination of Biosafety Level 3 Laboratories. *Journal of Clinical Microbiology*. 2008 ; 46 (9): 2955–2958.

Harding A.L. & Brandt Byers K.. Epidemiology of Laboratory-associated infections. In: Fleming D.O., Hunt D.L. editors, *Biological Safety, principles and practices*. 2000. 3<sup>rd</sup> edition, Washington, American Society for Microbiology Press: 35-54.

Harvard University. Environmental Health and Safety Program. Guidance on commonly used viral vectors. 2000, March, Annex III. Part 2B. Accessed online, May 2010 :  
<http://www.hms.harvard.edu/orsp/coms/BiosafetyResources/Oxford-Physiology-Viral-Vector-discussion.pdf>

Herman P., Verlinden Y., Breyer D. *et al.* Biosafety risk assessment of the severe acute respiratory syndrome (SARS) coronavirus and containment measures for the diagnostic and research laboratories. *Applied Biosafety Journal of the American Biological Safety Association*. 2004, 9 (3): 128-142.

Herman P., Fauville-Dufaux M., Breyer D. *et al.* Biosafety recommendations for the contained use of *Mycobacterium tuberculosis* complex isolates in industrialized countries. Royal Library of Belgium. 2006, D/2006/2505/22. . Accessed online, May 2010 :  
[http://www.biosafety.be/CU/PDF/Mtub\\_Final\\_DL.pdf](http://www.biosafety.be/CU/PDF/Mtub_Final_DL.pdf)

Kudva I.T., Blanch K., and Hovde C.J.. Analysis of *Escherichia coli* O157:H7 survival in ovine or bovine manure and manure slurry. *Applied and Environmental Microbiology*. 1998, 64 (9): 3166-3174.

Lina G., Cozon G., Ferrandiz J. *et al.* Detection of staphylococcal superantigenic toxins by a CD69-specific cytofluorimetric assay measuring T-Cell activation. *Journal of Clinical Microbiology*. 1998, 36 (4): 1042-1045.

McDonnell G. and Russell A.D.. Antiseptics and Disinfectants: Activity, Action, and Resistance. *Clinical Microbiology Reviews*. 1999, 12 (1): 147–179.

Mastorides S.M., Oehler R.L., Greene J.N., *et al.* The detection of airborne *Mycobacterium tuberculosis* using micropore membrane air sampling and polymerase chain reaction. *CHEST Journal*. 1999, 115: 19-25.

Melton-Celsa A.R. and O'Brien A.D.. Bacterial Protein Toxins, Chapter 17: Shiga Toxins of *Shigella dysenteriae* and *Escherichia coli*. Editors M. Aepfelbacher, K. Aktories, Ingo Just. Springer 2000.

Miskowski D.. An overview of *Legionella* analyses. 2007 EMSL Analytical.

Accessed online, May 2010 :

<http://www.legionellatesting.com/pdf/Paper.pdf>

Nataro J. P. and Kaper J. B.. Diarrheagenic *Escherichia coli*. Clinical Microbiology Reviews. 1998, 11 (1): 142-201.

National Institute for Occupational Safety and Health. TB respiratory protection program in health care facilities, Administrator's guide. DHHS (NIOSH) 1999; Publication No. 99-143. p. 1-103.

Accessed online, May 2010 : <http://www.cdc.gov/niosh/docs/99%2D143/>

National Institute for Occupational Safety and Health. *Mycobacterium tuberculosis*, airborne: method 0900, Issue 1. NIOSH Manual of analytical methods (NMAM) Fourth Edition. 1998.

Neely A.N. and Maley M.P.. Survival of enterococci and staphylococci on hospital fabrics and plastic. Journal of Clinical Microbiology. 2000, 38 (2): 724-726.

Pauwels K., Herman P., Van Vaerenbergh B., Do Thi C.D. *et al.*. Animal cell cultures: risk assessment and biosafety recommendations. Applied Biosafety Journal of the American Biological Safety Association. 2007, 12 (1): 26-38.

Pauwels K., Coppens F., Verheust C. *et al.*. Respiratory protection (contained use of GMOs and/or pathogens). Royal Library of Belgium. 2008, D/2008/2505/01.

Accessed online, May 2010 :

[http://www.biosafety.be/CU/PDF/Protec\\_Resp\\_ISP\\_08\\_2505\\_01.pdf](http://www.biosafety.be/CU/PDF/Protec_Resp_ISP_08_2505_01.pdf);

[http://www.biosafety.be/CU/PDF/Ademhalingsbescherm\\_WIV\\_07\\_2505\\_64.pdf](http://www.biosafety.be/CU/PDF/Ademhalingsbescherm_WIV_07_2505_64.pdf)

Pike R.M.. Laboratory-associated infections: summary and analysis of 3921 cases. Health Laboratory Science. 1976, 13: 105-114.

Public Health Agency of Canada. Material Safety Data Sheets (MSDS) for Infectious Substances. Accessed online, May 2010 :

<http://www.phac-aspc.gc.ca/msds-ftss/index-eng.php>

Qin K., O'Donnell M. and Zhao R.. Doppel: more rival than double to prion. Neuroscience. 2006, 141 (1): 1-8.

Rao G.G., Saunders B.P. and Masterton R.G.. Laboratory acquired verotoxin producing *Escherichia coli* (VTEC) infection. Letter. Journal of Hospital Infection. 1996, 33: 228-230.

Ryan J.. Understanding and managing cell culture contamination, Technical bulletin. Corning Life Sciences, 2002.

Rubin J.. Disinfection, sterilization and preservation. Mycobacterial disinfection and control. 1991. Chapter 23, 4<sup>th</sup> Ed. Seymour S. Black. ISBN: 0-8121-1364-0.

Schlessinger D. and Schaechter M.. Bacterial toxins; mechanisms of microbial disease. Schaechter M., Medoff G., Eisenstein B.I. Editors. 2<sup>nd</sup> edition, Baltimore: Williams and Wilkins 1993: 162-75.

Schmitt C.K., Meysick K.C. and O'Brien A.D.. Bacterial toxins: friends or foes? Emerging Infectious Diseases. 1999, 5 (2): 224-234.

Spina N., Zansky S., Dumas N. and Kondracki S.. Four laboratory-associated cases of infection with *Escherichia coli* O157:H7. Journal of Clinical Microbiology. 2005, 43 (6): 2938-2939.

Teel L.D., Daly J.A., Jerris R.C. *et al.*. Rapid detection of Shiga toxin-producing *Escherichia coli* by optical immunoassay. Journal of Clinical Microbiology. 2007, 45 (10): 3377-3380.

Tenover F.C., Biddle J.W. and Lancaster M.V.. Increasing resistance to vancomycin and other glycopeptides in *Staphylococcus aureus*. Emerging Infectious Diseases. 2001, 7 (2).

Todar K.. *Staphylococcus aureus* and Staphylococcal disease. 2008. Online Textbook of bacteriology. Accessed online, May 2010 :

<http://www.textbookofbacteriology.net/staph.html>

Tristan A., Ferry T., Durand G. *et al.*. Virulence determinants in community and hospital methicillin - resistant *Staphylococcus aureus*. Journal of Hospital Infection. 2007, 65 (S2) : 105-109.

The University of Iowa, Health Protection Office: Fact sheets. Accessed online, May 2010 :

[https://research.uiowa.edu/ehs/edocs/labs/biological\\_safety](https://research.uiowa.edu/ehs/edocs/labs/biological_safety)

The US Food and Drug Administration, Food borne pathogenic micro-organisms and natural toxins handbook: *Escherichia coli* O157/H7. Accessed online, May 2010 :

<http://www.fda.gov/Food/FoodSafety/FoodborneIllness/FoodborneIllnessFoodbornePathogensNaturalToxins/BadBugBook/ucm071284.htm>

The University of Texas at Houston Health Science Center. Guidelines for the Safe Handling of Adenoviral Vectors In Laboratory, Animal and Human Experiments.

Accessed online, May 2010 :

<http://www.uth.tmc.edu/safety/biosafety/adenoviral.pdf>

Varma J.K., Greene K.D., Reller M.E. *et al.*. An outbreak of *Escherichia coli* O157 infection following exposure to a contaminated building. *Journal of the American Medical Association*. 2003, 290 (20): 2709.

Wagenvoort J.H., De Brauwier EIGB, Gronenschild J.M., Toenbreker H.M., Bonnemayers G.P., Bilkert-Mooiman M.A.. Laboratory-acquired methicillin-resistant *Staphylococcus aureus* (**MRSA**) in two microbiology laboratory technicians. *European Journal of Clinical Microbiology & Infectious Diseases*. 2006, 25 (7): 470-472.

Wang G., Doyle M.P.. Survival of enterohemorrhagic *Escherichia coli* O157:H7 in water. *Journal of Food Protection*. 1998, 61(6): 662-7.

Wilson S.M., McNerney R., Voller A., *et al.* Progress toward a simplified polymerase chain reaction and its application to diagnosis of tuberculosis. *Journal of Clinical Microbiology* 1993, 31: 776-782.

World Health Organization. Laboratory Biosafety Manual, 3rd Edition, Geneva. 2004. WHO/CDS/CSR/LYO/2004.11.

## V. LEXICON

**Accident:** any incident during the contained use that leads to an important and unintentional release of GMOs/GMMs or pathogenic organisms and that may present a potential danger for public health and/or the environment.

**Aerosol:** a colloidal suspension of liquid or solid particles dispersed in a gas (usually air). Microbial aerosols are suspensions of particles in the air consisting partially or wholly of micro-organisms. They may remain suspended in the air for a long period of time, some retaining and others losing infectivity or virulence.

**Airborne micro-organism:** a micro-organism that is transmitted by air and may infect his host by this way.

**Autoclave:** a device to expose items to steam at a high pressure in order to inactivate waste or material and/or equipments or to render re-usable material sterile.

**Biological Safety cabinet (BSC):** device that are intended to offer protection to the user and the environment (which will include other people in the laboratory) from the aerosol hazards arising from the handling of infected and other hazardous biological material. There are three types (or classes) of BSC, which differ significantly in design and mode of operation (Class I, Class II and Class III cabinets). The exhaust system of BSC is equipped with HEPA filters.

**Biosafety:** safety for human health and the environment, including protection of biodiversity, in the use of GMO or GMM and in the contained use of pathogens for humans, animals and plants (from the Cooperation Agreement for Biosafety in Belgium).

**Contained use:** any activity in which micro-organisms are genetically modified or in which such GMMs and/or pathogens are cultured, stored, transported, destroyed, disposed or used in any other way, and for which specific containment measures are used to limit their contact with the general population and the environment.

**Containment measures:** Set of technical characteristics and safety equipment of a laboratory as well as work practices and waste management in order to avoid escape and contact of the manipulated micro-organisms with the outside environment and infection of personnel inside the laboratory. Air filtration with HEPA filters, inactivation of waste, use of specific clothes for the activity are examples of containment measures.



**Decontamination:** in the present context, it is the reduction by disinfection or sterilization of a biological contamination to an acceptable level which does not present a risk anymore.

**Disinfectant:** a chemical agent (or physical) that inactivates vegetative bacteria, fungi and viruses, but not necessarily spores in specific conditions. Disinfectants are usually applied to inanimate surfaces or objects.

**Disinfection:** a physical or chemical means of inactivating micro-organisms (but not necessarily spores) on surfaces, intact skins or mucous membranes.

**Droplet:** airborne particle consisting primarily of liquid. Droplets are usually generated by talking, sneezing or coughing and while some settle out almost immediately, many dry to become droplet nuclei which add significant numbers of micro-organisms into the air.

**Dust:** technically, a solid particle generated by mechanical action, present as an airborne contaminant.

**Fomites:** Definition1: inanimate carriers or materials that act as an intermediate carriers of microbial contamination. Animal hair or bedding's are fomites, but contaminated tools used to assemble a sterile spacecraft could be fomites too. Definition 2: inanimate objects that carry infectious biological organisms. Fomites are one of the most common way by which kids get sick. Droplets transmission, foeco-oral transmission or contact transmission often do so by means of fomites.

**Genetically Modified (Micro-)Organism (GMM/GMO):** a micro-organism in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination.

**HEPA filter or High Efficiency Particulate Air filter:** filter retaining 99,97 % of particles with a 0,3  $\mu\text{m}$  diameter in the air. The HEPA filter does not filter gases, chemical vapors or odors. HEPA filters have also the capacity to retain particles with a greater or smaller diameter than 0,3  $\mu\text{m}$ .

**Incident:** event that could cause damages. An example is when a culture flask of pathogenic micro-organisms is accidentally dropped but is rapidly controlled by application of the internal emergency plan (cleaning and disinfection) and has not cause any contamination to the personnel.

**Infectious dose:** number of pathogenic micro-organisms required to cause an infection of the host.

**Infectious organism:** an organism capable of invading a susceptible host, undergoing replication and causing an adverse host reaction commonly referred to as a disease.

**Ingestion:** taking a substance into the body through the mouth as food, drink, or medication, or doing so unknowingly from contact with contaminated hands, smoking materials, etc.

**Inhalation:** the breathing in of an airborne substance such as a gas, fume, mist, vapour, dust or aerosol.

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

**Pathogenicity:** refers to the ability of an organism to cause disease (ie, harm the host).

**Personal Protective Equipment (PPE):** any device or clothing worn by the worker to protect against hazards in the environment. Examples are respirators, gloves and goggles.

**Reservoir:** (of infectious organisms): any person, animal, arthropod, plant, soil or substance (or combination of these) in which an infectious organism normally lives and multiplies, on which it depends primarily for survival and where it reproduces itself in such a manner that it can be transmitted to susceptible host.

**Sterilization:** a process that kills and/or removes all classes of micro-organisms and spores. Sterilization may be not total, it consists in a reduction of 6 logarithms the initial concentration of micro-organisms.

**Transmission of pathogenic organisms:** any mechanism by which an pathogenic organism is spread from a source or reservoir to a person (or animal). These mechanisms are as follows:

- Direct transmission: direct and essentially immediate transfer of pathogenic organisms to a receptive portal of entry through which human or animal infection may take place.
- Indirect transmission: vehicle-borne and vector-borne (mechanical or biological).

**Viable particle:** a discrete physical particle that contains one or more viable micro-organisms.

**Virulence:** the degree of pathogenicity of a pathogenic organism, indicated by case-fatality rates and/or the ability of the organism to multiply, invade and damage tissues of the host.

