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Laboratory-Acquired Infections in Flanders (2007-2012)

An online survey

Biosafety and Biotechnology Unit

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GLOSSARY

For the purpose of this document:

Accident

Accident means any incident involving a significant and unintended release of genetically modified and/or pathogenic (micro-)organisms in the course of their contained use which could present an immediate or delayed hazard to human health or the environment.

Bio-incident

Bio-incidents are defined as all irregularities that occur while handling biological agents. They can be caused by human errors or technical failure.

Biological agents

All types of (micro-)organisms, including those which have been genetically modified, cell cultures and parasites which may be able to provoke any infection, allergy or toxicity.

Biological laboratory

A facility within which microorganisms, their components or their derivatives are collected handled and/or stored. Biological laboratories include clinical laboratories, research facilities, animal research facilities, diagnostic facilities, regional and/national reference centres, public health laboratories, research centres (academic, pharmaceutical, environmental, etc.) and production facilities (manufacturers of vaccines, pharmaceuticals, large scale GMOs, etc) for human, veterinary and agricultural purposes.

Biosafety (Belgian definition)

Biosafety is defined as safety for human health and the environment, including the protection of biodiversity, during the use of genetically modified organisms or micro-organisms, and during the contained use of pathogenic organisms for humans.

Contained Use:

Contained use means any operation (activity) in which micro-organisms are genetically modified or in which genetically modified and/or pathogenic micro-organisms are cultured, stored, used, transported, destroyed or used in any other way, and for which specific containment measures are used to limit their contact with, and to provide a high level of safety for the general population and the environment.

Hazard

A danger or source of danger; the potential to cause harm.

Laboratory-acquired infections (LAI):

The term laboratory-acquired infections (LAIs) refers to all direct or indirect human infections with or without the onset of symptoms following exposure to pathogens in the laboratory.

LAI:

see laboratory-acquired infections

Micro-organism

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

1. INTRODUCTION:

PURPOSE OF THE STUDY

On request of the Flemish Agency for Care and Health, Public Health Surveillance[1], the Biosafety and Biotechnology Unit (SBB), which belongs to the Scientific Institute of Public Health, developed a survey in the interest of mapping and evaluating the risk for "laboratory-acquired infections" (LAIs) related to bio-incidents with pathogenic organisms (genetically modified or not) in Flanders over the last 5 years (2007-2012). This timeframe was chosen in order to connect this survey report to a similar survey that was conducted by Ghent University in Flanders over the period 2001 to 2006 (1).

Bio-incidents are defined as all irregularities that occur while handling pathogenic organisms. They can be caused by human errors or technical failure.

The term "laboratory-acquired infections" or LAIs refers to all direct or indirect human infections with or without the onset of symptoms following exposure to pathogenic organisms in a biological laboratory[2].

According to the Belgian legislation on the protection of workers against biological agents at work[3], any accident[4] or incident which may have resulted in the release of biological agents[5] and which can cause an infection or serious illness in humans has to be notified to the authorities. However, while a certain number of papers on LAIs in the US and Europe has been published in scientific literature, very few data are available regarding their incidence in Belgium.

The aim of this survey was to gather information on bio-incidents and LAIs in biological laboratories[6] in the Flemish region and to gain insight into the possible underlying causes in order to provide biosafety officers, prevention officers and occupational health practitioners with tools that can enhance biological safety in the laboratory.

[1] Toezicht Volksgezondheid ; http://www.zorg-en-gezondheid.be/over-ons/contacteer-ons/
[2] see glossary
[3] Royal Decree of April 29, 1999 (Belgian Official Journal of 07.10.1999 - p. 37917) amending the Royal Decision of August 4, 1996 concerning the protection of workers from risks related to exposure to biological agents at work (Belgian Official Journal of 01.10.1996 - p. 25285)
[4] see glossary
[5] see glossary
[6] see glossary

METHODOLOGY

In this study, 124 private companies or public institutions with notified contained use activities[7] had been contacted by e-mail to answer questions of an online survey about LAIs. This survey was designed for the biosafety officers, prevention officers and occupational health practitioners and is hereafter called "survey 1". The mailing list was established using the data available in the database of the SBB that contains all notified or authorized contained use activities in Belgium since 1994.

Using this database, it was also possible to select a number of private companies (n=4) and public institutions (n=6) that are active in diagnostic activities (n= 5) and R&D sector (n=5), based on the work they perform with biological agents that are often mentioned in the scientific literature about LAIs. These institutions & companies received an invitation for their personnel to answer an online survey about LAIs. This "personnel–oriented survey" is hereafter called "survey 2".

Both surveys were circulated online using Limesurvey 2.0, a free online web survey tool, and were carried out in an anonymous way. The survey was available in Dutch and English and was made accessible for 4 months. On average every 2 weeks a reminder e-mail was sent to the institutions that had not completed the survey or did not respond to the invitation. In total ~50 questions and sub questions were addressed to each respondent, consisting of single-answer questions and multi-answer questions, most of the questions were mandatory, see URL: http://www.biosafety.be/CU/LAI/Intro_LAI.html.

The invitation (e-mail) for survey 1 provided a web link (URL) and a unique token which granted access to the survey. The invitation was sent to the biosafety officer with the request to also forward the invitation to the prevention officer and the occupational health practitioner. For survey 2, it was decided not to contact the personnel directly. The invited institutions were asked in advance whether they wanted to cooperate. In practice an invitation e-mail with link (URL) including a unique token was also sent to the biosafety officer with the request to forward it to the personnel involved in relevant contained use activities.

In addition, several services for prevention and protection at work[8], the "Fund for Occupational Disease"[9], the "Fund for Occupational Incidents"[10], the "Federal Public Service Employment, Labour and Social Dialogue, Well-being of workers"[11] and the Flemish Agency for Care and Health, Infectious Diseases[12] had been asked to provide additional data.

[7] see glossary
[8] IDEWE, PROVIKMO, SECUREX
[9] Fonds voor arbeidsziekten; http://www.fmp-fbz.fgov.be/web/index.php
[10] Fonds voor arbeidsongevallen; http://www.faofat.fgov.be/
[11] Federale Overheidsdienst Werkgelegenheid, Arbeid en Sociaal Overleg, Welzijn op het werk; http://www.werk.belgie.be/welzijn_op_het_werk.aspx
[12] Vlaams Agentschap Zorg en Gezondheid ; http://www.zorg-en-gezondheid.be/Home/

2. RESPONSE

CATEGORIZATION OF PARTICIPANTS: TYPE OF INSTALLATIONS AND ACTIVITIES

Table 1 summarizes the type of institutions that were contacted in the 5 provinces of the Flemish Region. 68 of the 124 (~55%) invited institutions completed the survey. The participation rate of R&D institutions was over ~62% and 10% higher compared to institutions that mainly carry out diagnostic activities (~52%). On the other hand, ~70% of the public institutions participated compared to ~52% of the private companies. 116 people with one of more functions in the institutions responded to survey 1. In total 69 biosafety officers, 35 prevention officers and 15 occupational health practitioners participated, some of them (27) were also involved in other functions in the institution, such as lab responsible or manager.

Table 1: participation rate for survey 1

		West Flanders	East Flanders	Antwerp	Flemish Brabant	Limburg	**Total***
Number of Invitations	Diagnostic Institutions**	13	22	23	12	7	**77**
	R&D institutions***	7	16	20	15	2	**60**
	Private companies	16	27	29	18	7	**97**
	Public institutions	3	8	10	4	2	**27**
	Total	**19**	**35**	**39**	**22**	**9**	**124**
Participation rate (%)	Diagnostic Institutions**	46%	55%	57%	42%	57%	**52%**
	R&D institutions***	57%	69%	60%	67%	0%	**62%**
	Private companies	37%	52%	59%	56%	43%	**52%**
	Public institutions	100%	75%	60%	75%	50%	**70%**

* Some institutions (N=13) do diagnostics and R&D; ** "Diagnostic" includes also quality control;
*** "R&D" includes also (large scale) production

As the surveys were anonymous, the identity of the contacted institutions in survey 2 is not communicated. In total 432 employees who are possibly exposed to biological agents at work were invited to participate in survey 2. 219 of them (51%) responded to the invitation. Table 2 summarizes the participation rate of the contacted personnel in the different types of institutions. There was no significant difference in the participation rate between R&D and diagnostic institutions or between private companies and public institutions. Although it was asked to contact also students and animal care takers, only 2 students and 3 veterinarians answered (reason unknown). In total, 134 lab technicians (61%), 75 researchers (34%), 9 staff members (4%), 3 veterinarians (1%), 2 students (1%), 3 dispatchers (1%) and 12 others (5%) answered to survey 2.

Table 2: participation rate for survey 2

	Number of Invitations	Participation rate (%) (Min - Max)
Diagnostic Institutions	312	50% (37,5%-70%)
Research and Development Institutions	120	53% (32,5%-100%)
Private companies	282	49% (37,5% - 73%)
Public institutions	150	54% (32,5% - 100%)
Total	432	

Figure 1 shows the course of the responses of the participants to online survey 1 and 2 (4 months duration). Whereas both response curves show a steady increase as a function of time, a quick increase can be observed for survey 1 at each reminder in comparison to survey 2. This is probably due to the fact that the personnel could not be contacted directly (see figure 1).

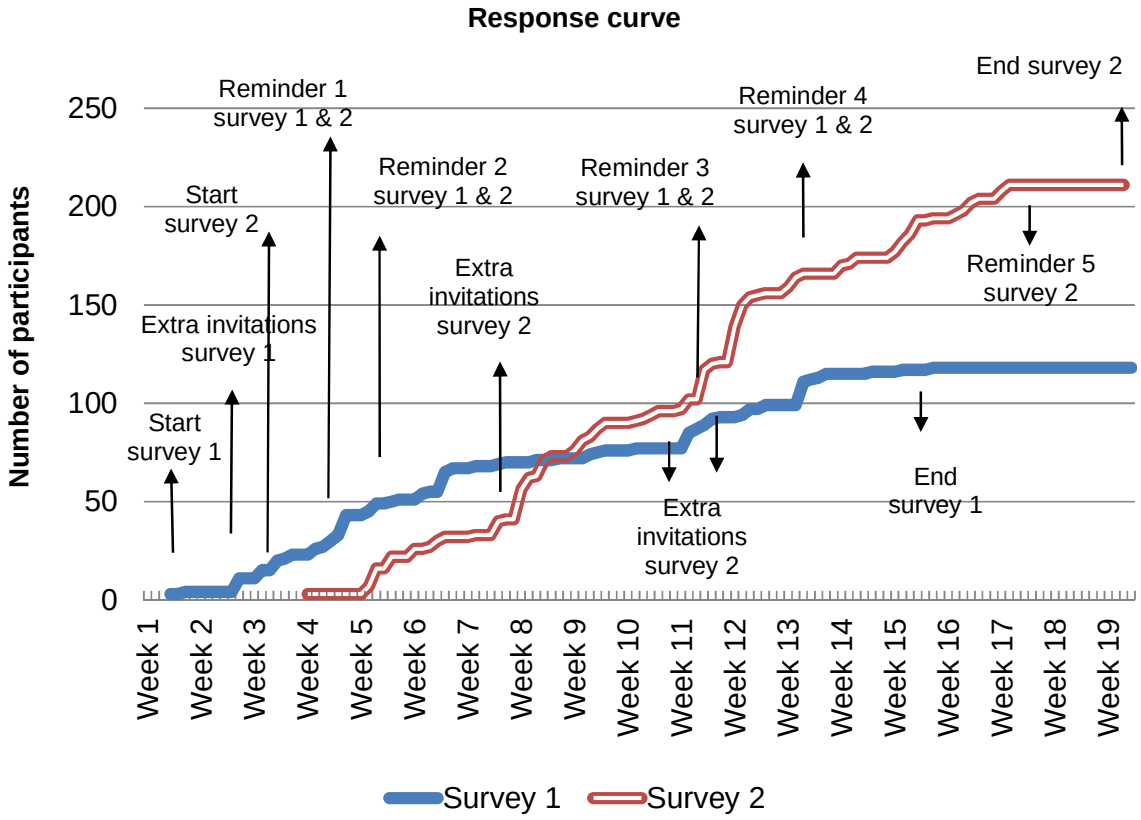


Figure 1: Response curve in function of time

Type of facility/installation

To identify the work environment of people involved in contained use activities in Flanders, we analysed the average containment level of the authorized contained use activities of the last 4 years. The proportion of the different types of installations, as stipulated in the Belgian legislation on contained use[13] (laboratories of containment level 1-3 (L1-L3), animal facilities of containment level 1-3 (A1-A3), greenhouses of containment level 1-2 (G1-2) or others) is shown in figure 2.

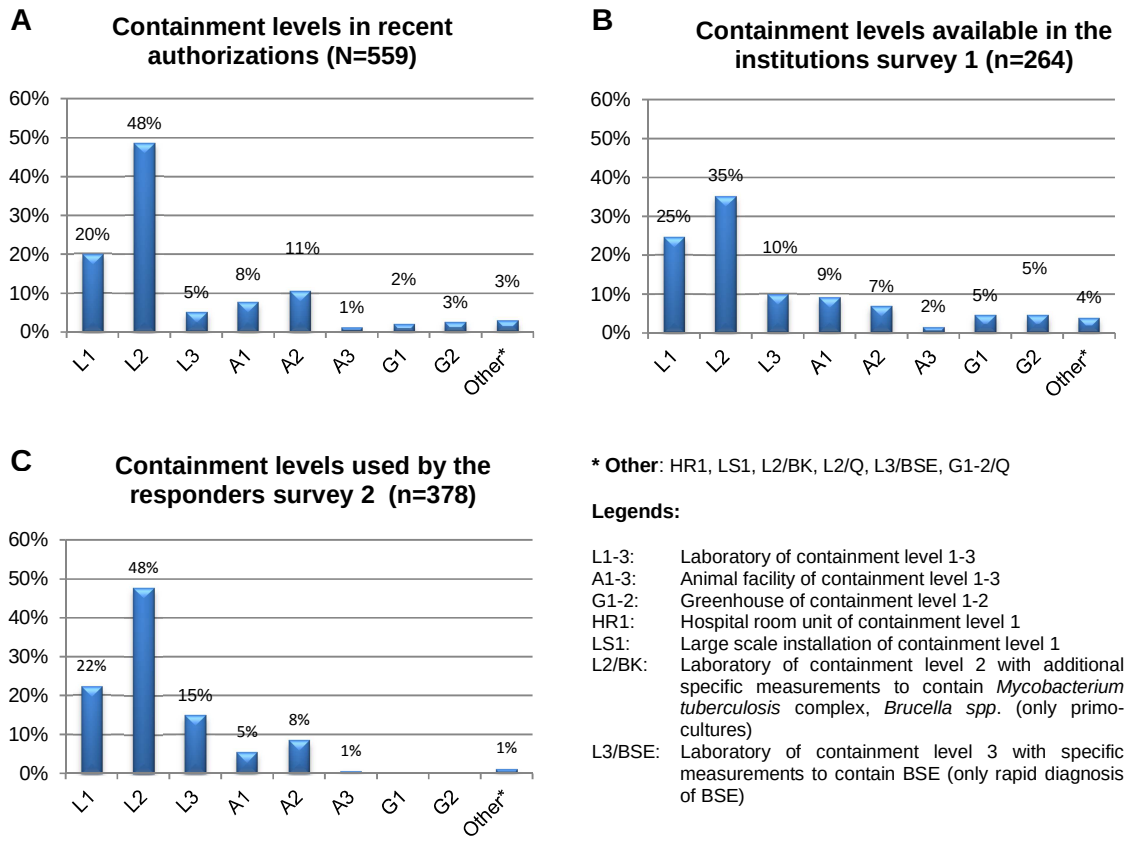


Figure 2: Containment levels in recent authorizations in Flanders (A), Containment levels available in the institutions according the respondents of survey 1 (B) and Containment levels used by the surveyed personnel, survey 2 (C).

According to the requested level of containment (figure2.A) it seems that the majority of the contained use activities in Flanders is carried out in laboratories of containment level 2 (~50%).

[13] The **contained use** of genetically modified micro-organisms (GMMs) or organisms (GMOs) and/or pathogens is regulated in Belgium at the **regional level** and is based on the implementation of **European Directive 90/219/EEC & 98/81/CE to** regional Decrees (respectively in November 2001 for the Brussels Region, in July 2002 for the Walloon Region, and in February 2004 for the Flemish Region). Directives 90/219/EEC and 98/81/EC have since been replaced by Directive 2009/41/EC, which consolidated Directive 90/219/EEC and subsequent amendments 94/51/EC, 98/81/EC and Council Decision 2001/204/EC

Table 3: Participation rate for survey 1 & 2 in different sectors

	Participation rate (%) survey 1 (n=116)	Participation rate (%) survey 2 (n=219)
(Bio)medical (Human)	74%	61%
Veterinary (Animals)	9%	39%
Plant research and diagnosis (Plants)	17%	1%

In comparing the participation rates of survey 1 and 2 with regard to different sectors related to work with human, animal and plant pathogens (see table 3), both surveys showed the highest rate for the bio(medical) sector. (74% and 61% respectively). For survey 1, this was followed by plant research and diagnosis (17%) and veterinary medicine (9%). For survey 2 this order was reversed, the veterinary sector showed a participation rate of 39%, while the plant sector accounted for less than 1%. This is due to the fact that institutions for plant research and diagnosis were not invited to participate in survey 2, since the risk for LAIs was presumed to be rather low in the field of agrobiotechnology.

Types of activities

To measure the risk for the personnel using biological agents, it is crucial to characterize the type of activity carried out with the biological agents as it determines the risk of exposure.

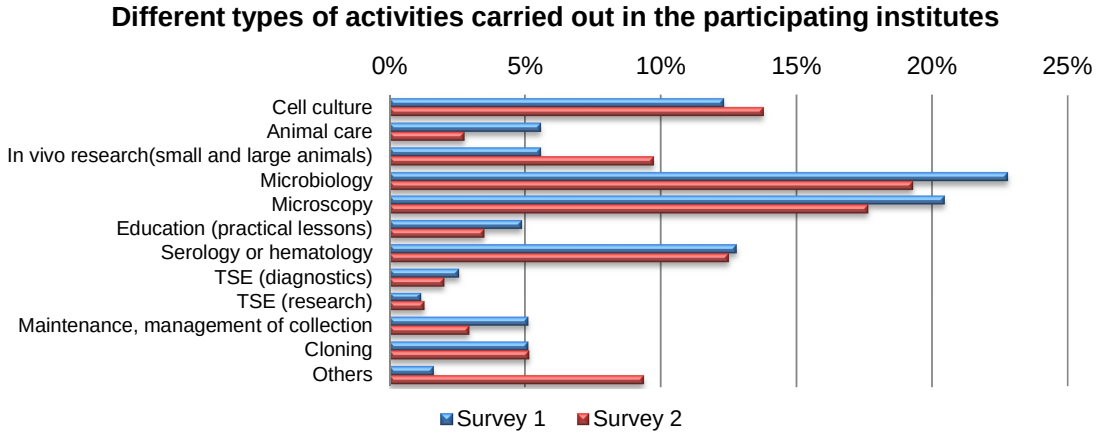


Figure 3: Comparison of the different types of activities carried out by the respondents in survey 1 & 2 *(TSE: Transmissible spongiform encephalopathy)*

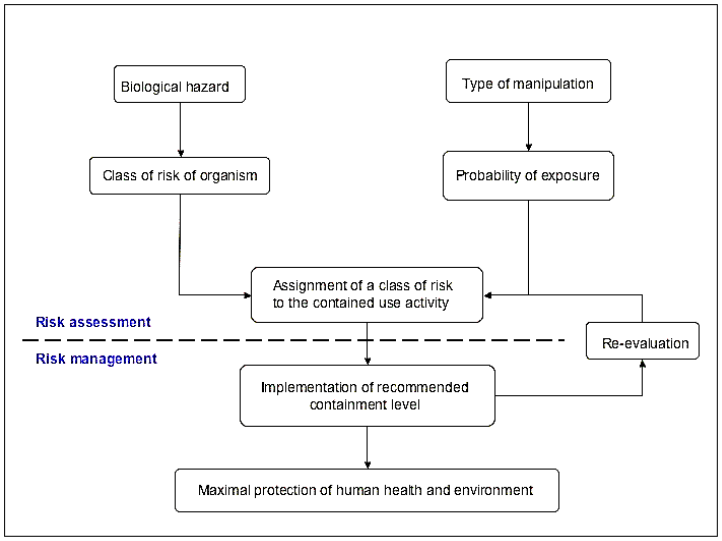
Figure 3 shows clear similarities between both survey groups with regard to the most common types of activities. Microbiology, and more particularly microscopy, cell culture and serology/hematology seem to be the activities that are carried out mostly in the surveyed institutions in Flanders.

In general, the distribution of type of installations in survey 1 is quite similar to the requested containment levels in the Flemish authorizations (figure 2A), suggesting that survey 1 is representative for Flanders. Remarkably, similar patterns are observed in survey 2 for types of activities and installations (figure 2B&C and 3), although survey 2 constituted of personnel from 10 deliberately chosen institutions.

3. RISK ASSESSMENT AND RISK MANAGEMENT

Biological risk assessment is a process that considers the identification, the probability of occurrence and the severity of a potential negative effect on human health or the environment associated with a specific use of a GMO or a pathogen. A known risk will therefore lead to the implementation of appropriate management measures.

For the risk assessment & management of 'contained use' activities, five successive steps are distinguished:



1.Identification of biological hazards

2.Determination of the class of risk of the genetically modified or pathogenic organism

3.Consideration of the type of activity in terms of probability of exposure to potential biological hazards

4.Assignment of a class of risk to the contained use activity

5.Implementation of recommended containment level (Risk Management)

Figure 4 : Biological risk assessment and management

BIOLOGICAL RISKS IN THE LABORATORY

Any employee who is exposed to infectious biological agents on the workplace (laboratory, animal facility, large scale production facility) is prone to (primary) infections (LAIs - laboratory acquired infection). In this context it is important to note that the transmission of a pathogen in the laboratory can happen by other modes than those usually occurring in daily life. This can be illustrated by considering the manipulation of typical blood-borne pathogens such as HIV or Hepatitis B viruses, naturally transmitted by percutaneous or mucosal exposure to infected blood or other body fluids. In the laboratory, an infection can occur via cutting injuries and through contact of the mucous membranes with aerosols that contain high titres of the virus. Another example is the manipulation of parasites such as *Plasmodium falciparum*, or *Trypanosoma gambiense*. These parasites are usually transmitted by an insect vector. In a (research) lab however, a lab worker could become infected by needle stick injury or by aerosol / droplet exposure of the mucous membranes of the eyes, nose, or mouth.

It is worth mentioning that LAIs can also result in transmission of the pathogen to people outside the lab, when the infected laboratory worker infects relatives or other people he comes in contact with, also called secondary infection or transmission (see chapter 'LAIs in Flanders').

Generally speaking, in a laboratory setting contamination can take place through 4 different ways:

- **inhalation** (e.g. aerosols);
- **percutaneous inoculation** (needle stick injuries, cuts or abrasions from contaminated items and animal bites and scratches);
- **contact** with mucous membranes (eyes, mouth, nose) through contaminated hands, after touching surfaces, infectious droplets, aerosols and splashes etc.;
- **ingestion** (mouth pipetting, mouth contact with contaminated material via fingers or gloves), droplets, splashes etc.

This means that, considering the characteristics of the used biological agent (pathogenicity, infectious dose, viability outside the host) and its mode of transmission, certain manipulations involve higher risks than others. Typical manipulations that may generate higher risks are *in vivo* pathogen injection to animals or manipulations generating infectious splashes or aerosols such as vortexing, centrifuging or clearing tips or pipettes.

To evaluate this key aspect in biosafety, a general question was asked in both surveys 1 and 2.
The respondents were asked what they perceived as the activity with the highest risk that is carried out in the institution (survey 1) or performed by themselves (survey 2). Injecting mice with lentiviral vector was given as an example to orientate the respondents and show them which type of answer they were expected to give.
To score the respondents'methodology of risk assessment, the answers were evaluated against the three main elements of the risk assessment methodology described above (biological agent, type of activity and mode of transmission). The results for both surveys are summarized in table 4.

Table 4: Evaluation of risk assessment of the participants

Risk assessment	**Survey 1 (n=117)**	**Survey 2 (n=178)**
Adequate (focusing on organism, type of activity, transmission)	9%	13%
Partial (focusing on only 2 risk assessment elements)	37%	15%
Incomplete (focusing on only one risk assessment element)	42%	54%
Answer is out of (biosafety) scope, e.g chemical risks	12%	17%

We received many and very different answers. The majority of answers (40-55 %) were rather general although a minority (8-13%) spontaneously took into consideration all three main elements of the risk assessment methodology.

Application of containment measures: the respondents' viewpoint

The assessment of biological risks is based on an empirical basis, following awareness of the risk posed by the handling of biological agents, and must cover reasonably foreseeable situations (spill, accidents). Behind this awareness there exists a practical part that aims to minimize this risk, which is called the risk management. Risk management regards the implementation of different biosafety measures (technical requirements, specific equipment, work practices and other protective measures) to protect human health and environment (containment level) that can be re-evaluated at all times.

The respondents of survey 1 were asked to evaluate the compliance of their work conditions with some specific biosafety measures that can be applied in a laboratory or animal facility of containment level 2 and 3 (figure 5).

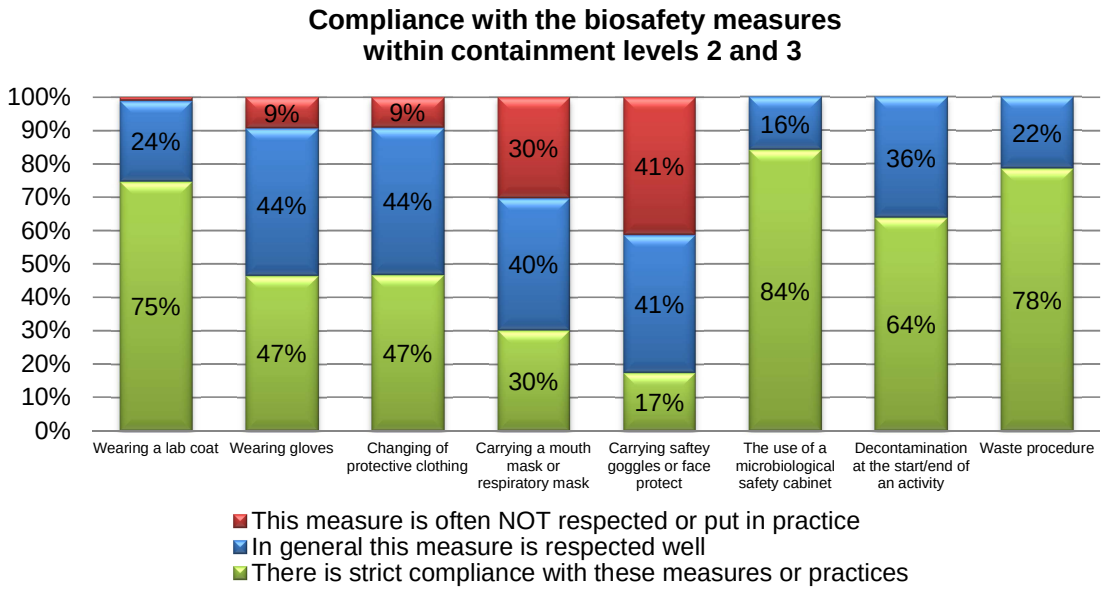


Figure 5:Compliance with biosafety measures in a laboratory or animal facility of containment level 2 or 3

In general, this chart suggests there is less compliance when more personal protection equipment has to be adopted to ensure (bio)safety. Where 75% of the respondents to survey 1 judge that a general protective measure such as wearing a lab coat is respected strictly, only 46,5%, 30 % and 17,5% of the respondents also judged this to be the case for wearing gloves, carrying masks (mouth and respiratory protections) and safety goggles respectively.

Appropriate gloves are an important protective barrier when contact with potentially contaminated samples, surfaces or equipment can occur. However, figure 5 might suggest that the practice of wearing them is not always respected the way it should be. The same conclusion can be drawn when it comes to face and eye protection, which should be used when there is a risk of infectious droplets or splashes. The use of a biosafety cabinet (BSC) and the procedures for decontamination and waste management show a higher level of compliance (approximately 84 – 64 - 78% respectively).

BIO-INCIDENTS

In order to better assess whether or not wearing adequate personal protection plays an important role in preventing LAIs, it is important to map the routes of exposures and bio-incidents. Possible causes of LAIs are non-compliance with biosafety measures (e.g. inadequate decontamination or poor hygiene), ignorance (e.g. unknown transmission routes, *e.g.* spores of mycobacteria (2)) and bio-incidents due to human errors (*e.g.* splashes, aerosols, needle sticks or cuts with sharps, animal scratches and bites) or technical failure (equipment or infrastructure failure). Therefore it is interesting to know the types of bio-incidents that occur frequently in Flanders. Hence, the respondents of both surveys were asked which types of bio-incident occurred in their facility within the last 5 years (see figure 6a).

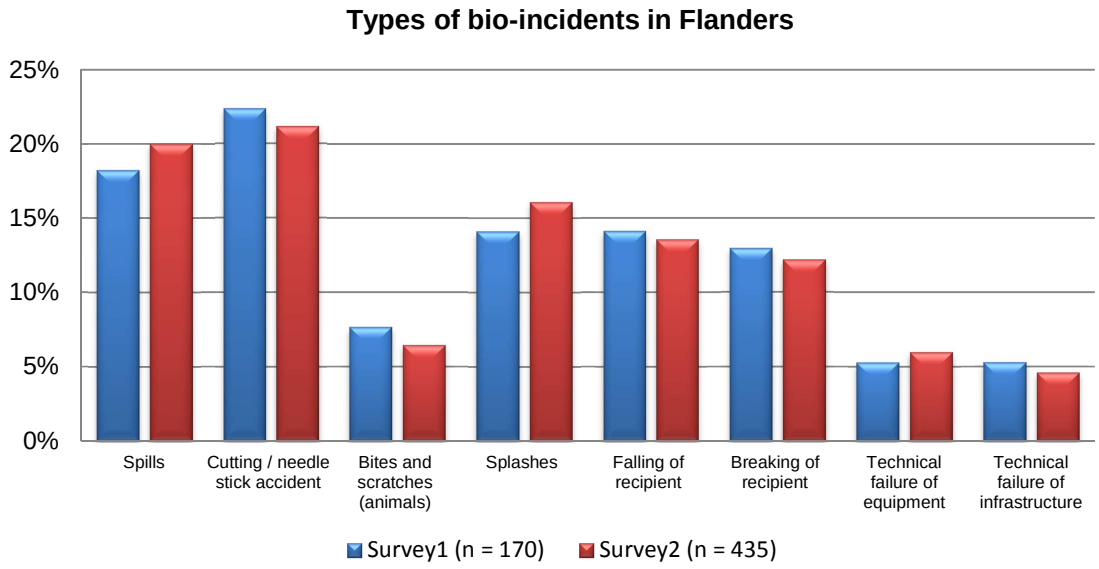


Figure 6a: Different types of bio-incidents over the last 5 years in Flanders (%)

In total, survey 1 reported 170 types of bio-incident while survey 2 reported 435 types of bio-incident. Although asked, many respondents did not give exact numbers, but rather indicated “daily” or “monthly” incidents, and several others did not specify any frequency. This makes exact quantifications of the type of bio-incidents difficult. Therefore figure 6a probably shows a slightly distorted picture of the reality. However, extrapolating the data of survey 1 of the cases with exact quantification (38% of the cases) gives an idea of the amount of different bio-incidents in Flanders in one year as shown in figure 6b.

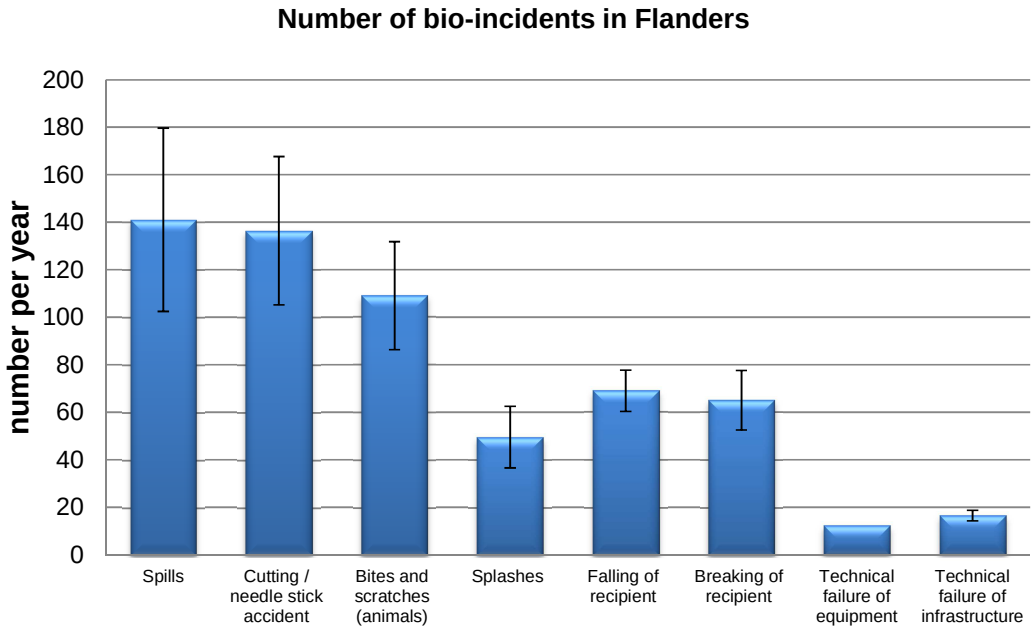


Figure 6b: Number of bio-incidents in Flanders on a yearly basis (extrapolation)

Spills and cutting/needle stick accidents remain the most frequently reported bio-incidents followed by animal bites and scratches, falling and breaking of and splashes.

Regarding this information, it appears that the biggest part of bio-incidents is related to human errors (95%). A small share (only 5%) of bio-incidents is the result of a technical failure (equipment or infrastructure, respectively 12 and 17 +/- 2 incidents a year).

In the context of risk management it could be useful to look at the respondents' perception of underlying causes of bio-incidents. The respondents of both surveys were asked to rate (from 1 = totally disagree to 10 = totally agree) the importance of possible underlying causes of a bio-incident, see figure 7.

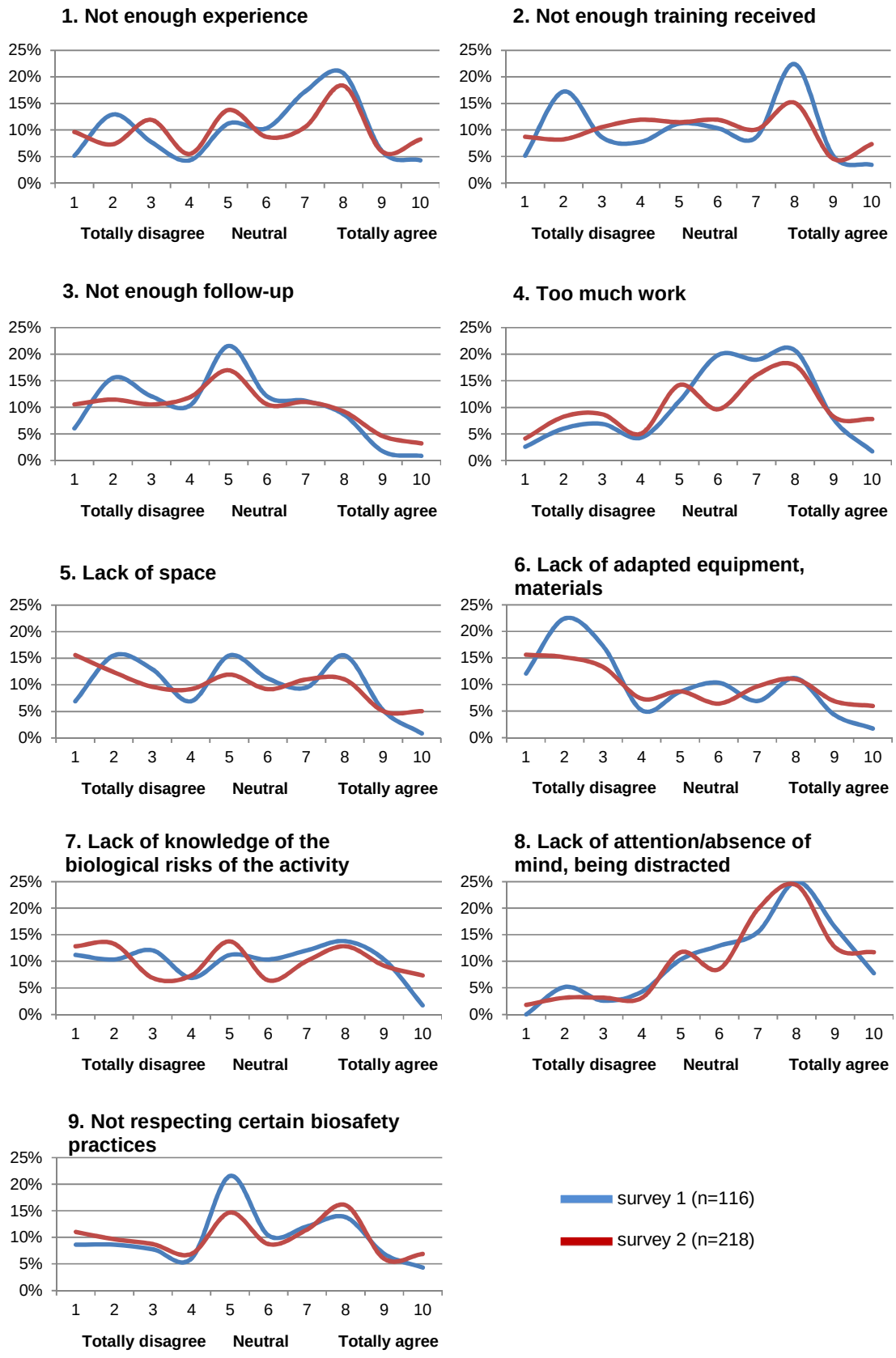


Figure 7: Overview of the perception of the respondents as regards the rationale of bio-incidents.

These underlying causes of bio-incidents can be divided in 3 different groups:

1. structural causes (lack of space or lack of adapted equipment);
2. occupational and human related causes (work-related stress, too much work load, lack of attention);
3. supervision and training related causes (lack of experience, no appropriate training or follow-up…).

Nevertheless, the situation is not always clear-cut: a bio-incident could be the result of different factors interacting with each other. For example, a lack of space could induce work-related stress which can trigger a lack of attention, which in turn can lead to a bio-incident, eventually resulting in a laboratory acquired infection. The same may occur when a certain task becomes too repetitive, resulting in boredom or weariness which can then lead to distraction etc…

According to the perception of the respondents, a lack of experience in the lab (figure 7.1) and the occupational and human factors that may come with the job, such as high workload (figure 7.4), absent-mindedness or distraction (figure 7.8) lead to more bio-incidents. Factors like the lack of appropriate training (figure 7.2), a lack of knowledge (figure 7.7) or respect of certain biosafety practices (figure 7.9) have no conclusive pro or contra. Accordingly, the follow-up of the personnel (figure 7.3), the lack of space (figure 7.5) and well adapted equipment or materials (figure 7.6) seem not really problematic.

Also, there seems to be no important differences in perception between the two groups of respondents (survey 1 and survey 2) except for the question related to training (figure 7.2) which shows a clear peak on 2 (totally disagree) in survey 1 and not in survey 2. Further detailed analysis of this statement showed that the general opinions on this statement in both surveys are similar. Noteworthy, the clear peak accounts mainly for the opinion of the prevention officer and occupational health practitioners. Figure 7bis indicates that a small majority (43%) of the biosafety officers have the feeling that more training is needed to avoid bio-incidents.

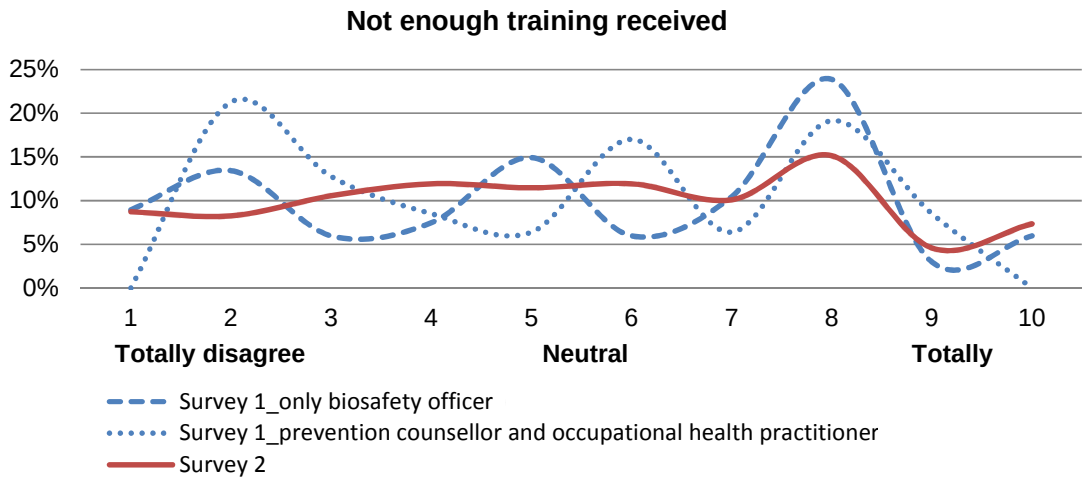


Figure 7bis: Detailed analysis of the statement "not enough training received" as a possible underlying cause of a bio-incident in survey 1

4. LABORATORY-ACQUIRED INFECTIONS

WORLDWIDE

Laboratory-acquired infections (LAIs), also called occupational illness or laboratory-associated infections are not new phenomena (3). Epidemiological reviewing of LAIs had a slow start. The first publication of a LAI was published in 1898 by Riesman reporting an infection with *Corynebacterium diphtheriae* (Diphteria) via mouth pipetting. The first survey about LAIs was carried out in 1915 (4). The largest published LAI survey was conducted in 1976 by Pike and Sulkin, who reported 3921 cases due to 159 different agents (5). From this report it appeared that 10 biologic agents accounted for more than 50% of the cases, listed in table 5. Many publications on LAIs refer to these 'top 10' organisms, but other surveys that had been carried out after Pike's surveys or that took place in another geographical context give us a different picture (see table 6).

Table 5: Ten most frequently reported laboratory-acquired infections worldwide[$] (5)

Biologic agent	Risk class[14]	Number of LAI cases (%)
Brucella spp.	3	423 (11%)
Coxiella burnetii	3	278 (7%)
Salmonella thyphi	3*	256 (6,5%)
Hepatitis B, C and D viruses	3*	234 (6%)
Francisella tularensis	3	225 (6%)
Mycobacterium tuberculosis complex	3	176 (4,5%)
Trycophyton mentagrophytes	2	161 (4%)
Venezuelan equine encephalitis virus	3	141 (4%)
Rickettsia bacteria	3	124 (3%)
Chlamydia psittaci (avian)	3	116 (3%)

[$] mainly US; 2465 of the 3921 cases occurred in the United States
3* : class of risk 3 infectious agents that are normally not airborne pathogens.

Remarkably, the organisms in the "top 10" (table 5) mainly belong to biological risk class 3 for humans/animals. Infections with organisms of risk class 2 often result in a mild disease and may evolve even without obvious clinical manifestation, meaning these infections can remain unnoticed. Also LAIs are sometimes difficult to identify as such. Therefore, one could assume that not all LAIs were known and there might be as well a substantial underrepresentation of risk class 2 organisms in the table above. Also, this table is completed with data available in different publications, and is certainly non exhaustive for several reasons. One could assume that a certain number of LAIs still remains not notified, not reported, not diagnosed and therefore unknown.

Currently, many laboratory infections cases are reported worldwide, with most of the reports describing only one specific case while others are more general. A more recent study surveyed laboratories in the UK in the period 1994–1995 and reported that tuberculosis and gastrointestinal laboratory infections predominated (e.g. shigellosis or salmonellosis) (6, 7). Another LAI survey from the UK showed a predominance of gastrointestinal infections, with most of them having occurred in microbiology laboratories (8). According to Sewell (2000), the most common organisms causing LAIs

[14] Classes of biological risk are given for human and are based on the Belgian classifications of micro-organisms

were *Shigella* and *Salmonella* spp., *Escherichia coli* 0157:H7, *Francisella tularensis*, *Brucella* spp., *Mycobacterium. tuberculosis*, Hepatitis B virus (HBV), Hepatitis C virus (HCV), Human immunodeficiency virus (HIV), and the dimorphic fungi (9). The survey of Baron & Miller (2008) identified the bacteria *Shigella,* followed by *Brucella*, *Salmonella*, and *Staphylococcus aureus* as the main causes of LAIs (10). Singh (2009) identified from previous LAI surveys that *Brucella* spp, *Shigella* spp, *Salmonella* spp, *Mycobacterium tuberculosis,* and *Neisseria meningitidis* are the most common agents involved in LAIs. Bloodborne pathogens Hepatitis B virus, Hepatitis C virus, and HIV) account for the majority of the reported viral infections and dimorphic fungi are responsible for the greatest number of fungal infections (10, 11).

Besides these published general LAI surveys, there are at least 57 described reports or more specific LAI surveys to be found in the literature worldwide via publications, reports, or by means of alerting systems (e.g. Promedmail). 47 of these reports were selected for further analysis. In total, 309 laboratory-acquired infections are analysed, see table 6 and annex 1 (analysis).

Table 6: Recent laboratory-acquired infection (LAIs) worldwide: organism, risk class, number of cases (summary)

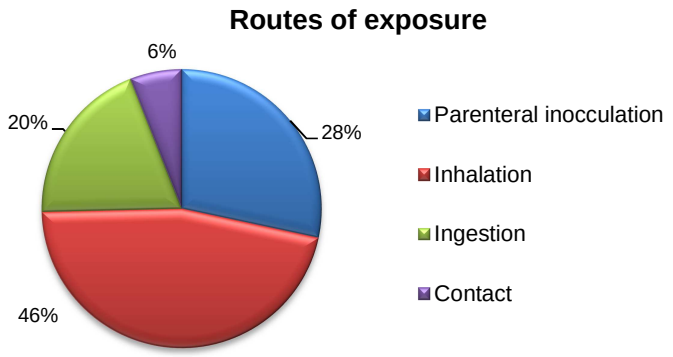
Biologic agent	Risk class[15]	Number of LAI cases (%)
Salmonella bacteria	2	130 (42%)
Brucella bacteria	3	123 (40%)
Neisseria meningitidis	2	11 (4%)
Vaccinia virus	2	11 (4%)
Francisella tularensis	3	6 (2%)
Filovirus (Ebola virus and Marburg virus)	4	5 (2%)
Escherichia coli (O157:H7)	3*T	4 (1%)
Mycobacterium bacteria	2-3	4 (1%)
Staphylococcus areus	2	3 (1%)
Bacillus anthracis and *Bacillus cereus*	2-3	2 (1%)
Burkholderia pseudomallei and *Burkholderia mallei*	3	2 (1%)
Clostridium difficele	2	2 (1%)
Chlamydophila psittaci (avian strain)	3	1 (~0%)
Cowpox virus	2	1 (~0%)
Dengue virus	3	1 (~0%)
Leptospirosis bacteria	2	1 (~0%)
SARS	3	1 (~0%)
Shigella sonnei	2	1 (~0%)

(*) : Pathogens of risk 3 that may present a limited risk of infection for humans and animals because they are not normally infectious by the airborne route; T : Toxin production.

Table 6 suggests that LAIs are not limited to the pathogens mentioned in table 5 and that also *Salmonella* species, *Neisseria meningitidis*, Ebola virus, West Nile virus and Vaccinia virus can be added to the list. Possible reasons are different methodology/method of analysis (literature analysis versus survey data analysis), geographical focus (worldwide versus mainly US), the re-emergence of 'old' pathogens or the discovery of new pathogens with a potential high risk of pandemics (e.g. SARS coronavirus, avian influenza viruses, West Nile virus, Ebola virus).

[15] Classes of biological risk are given for human and are based on the Belgian classifications of micro-organisms

It appears from the review of these published reports that the majority of LAI cases reported came from surveys conducted in microbiological laboratories. Nevertheless LAIs happen in laboratories as well as in animal facilities, R&D or production installations. Interestingly, although the precise route of exposure remains unknown (45%) or is poorly defined (6%), the analysis of the available information revealed that the main routes of exposure are inhalation (46%), parenteral inoculation (28%), ingestion (19%) and contact (6%), see chart figure 8. The majority of the infections were caused by not respecting biosafety measures (73%) followed by bio-incidents (24%) due to human errors (e.g. spill accidents, needle stick incidents,…). Ignorance and bio-incident due to technical failure are far less important as cause of LAI, see figure 8.



Causes of LAIs

A. No compliance (73%)

B. Ignorance (2%)

C. Bio-incidents (24%)

C1. Technical cause (8%)

C2. Human cause (93%)

Figure 8: Recent laboratory-acquired infections (LAIs) worldwide (2000-2012): routes of exposure (chart) & causes of LAIs (based on recent literature)

Although LAIs still exist today, several studies suggest a gradual decline in the number of LAIs during the last 50 years (5, 11). Possible reasons for this apparent decrease could be:

1. an increased awareness in the scientific community and the adoption of several biosafety legislations;
2. an increased attention for improved work practices and preventive measures (e.g. the use of gloves, vaccination, prohibiting of mouth pipetting, avoiding “sniffing” of cultures and re-capping of used needles);
3. improvements in laboratory design (L3-laboratories with negative air pressure, use of biosafety equipment like microbiological safety cabinets, sealed centrifuges etc….);
4. creation of professional biosafety organisations that actively (started to) promote biosafety as a scientific discipline and identify the need of biosafety professionals and lab workers (e.g. American Biological Safety Association (ABSA, 1984); European Biosafety Association (EBSA, 1996), Asia-Pacific Biosafety Association (A-PBA, 2005). More particularly in Belgium there is the Belgian Biosafety Professionals (BBP) created in 2006 which is the Belgian section of EBSA.
5. the legal requirement to appoint a “biosafety officer”

LAIs IN FLANDERS

In Flanders 3 cases have been published since 2000. They describe laboratory acquired infections with *Mycobacterium kansasii* (2005), *Shigella sonnei* (2006) and *Chlamydophila psittaci* (2009) (12-14). In 2006, a first survey focusing on bio-incidents was carried out (1) on request of the Flemish environmental agency "Vlaamse Milieumaatschappij (VMM)". A questionnaire was sent to numerous private and public laboratories (n=137). Despite a response rate of 49%, only 2 LAIs were identified caused by the bacteria *Brucella melitensis* and *Listeria monocytogenes*.

In our present survey we observed a response rate of 51%, which corresponds to 335 respondents (see earlier in this document). In total 47 respondents reported 89 LAIs that happened within the last 5 years in Flanders (2007-2012). Caution should be taken when interpreting these results because a particular LAI could have been mentioned more than once as several employees working in the same institutions participated in the same survey. So we assumed that different people could have mentioned the same LAI cases. These were filtered out by comparing the answers that were given to other questions linked to each LAI case in order to remain with unique cases only. This resulted in 52 (survey 1: 14; survey 2: 38) distinct LAIs that had been caused by 15 different pathogenic organisms (survey 1: 7 different pathogens; survey 2: 12), see table 7.

Table 7: Summary table of laboratory acquired infection reported in survey 1 and survey 2.

Organism	**Risk class**[16]	**Survey 1**	**Survey 2**	**Total**
		Number of LAI cases		
Salmonella bacteria *(*)*	2-3	2-3	10-11	13 (25%)
Mycobacterium turberculosis complex (*)	3	0	7	7 (13,5%)
Trypanosoma brucei gambiense	2	1	4	5 (10%)
Shigella bacteria ($)	2-3	4	0	4 (8%)
Brucella bacteria (*)	3	1	2	3 (6%)
Mycoplasma	2	0	2	2 (4%)
Herpes virus	2	0	2	2 (4%)
Trichophyton verrucosum	2	0	2	2 (4%)
Parvovirus B19	2	1	0	1 (2%)
HIV	3	0	1	1 (2%)
Dermatophyte	?	0	1	1 (2%)
Campylobacter	2	0-1	0-1	1 (2%)
Avian Influenza (*)	2	0	1	1 (2%)
BCG (Bacillus Calmette Guérin)	2	1	0	1 (2%)
Toxoplasma gondii	2	0	1	1 (2%)
Unknown		3	4	7 (13,5%)

*The pathogens marked with an asterisk cause a disease mentioned in the list of notifiable infectious diseases as defined by the 19 June 2009 Ministerial Order laying down the list of notifiable infections

$Only notifiable when it concerns a collective outbreak (not individual cases)

[16] Risk classes for humans as based on the Belgian risk classifications of micro-organisms, http://www.biosafety.be/RA/Class/ClassBEL.html

Bacterial infections predominate with 31 cases (60%), followed by infections with parasites (6 cases, 11,5%), viral infections (5 cases, 10%) and 3 fungal infections (6%). Infections with enteric pathogens (*Salmonella* and *Shigella*) seem to be the most frequently reported laboratory-associated (bacterial) infections in Flanders with 17 reported cases. In contrast with the very first top 10 list of pathogens (table 5), table 7 lists many organisms that belong to risk class 2. In that respect the list shows more similarities with the recent literature (salmonellosis, tuberculosis, shigellosis, dermatomycosis and brucellosis). The many reported *Trypanosoma brucei gambiense* infections may seem atypical for Belgium, since this tropical pathogen is not endemic in Belgium. This relatively high number of LAIs with tropical pathogens could be related to Belgium's colonial past and (historical) involvement in research on tropical diseases and participation in several international projects.
Eventually, it has to be mentioned that the origin of 55% of the infections remain unknown. Another remarkable observation is that more than 70% among the affected employees work in diagnostics, see table 8.

Table 8: Sector of the participating institution where at least one LAI was reported (n= 12)

	Number	Percentage
Research and Development	4	21%
Diagnostics and Quality Control	14	74%
Education	1	5%

The next figures summarize the responses to questions concerning the circumstances in which the LAIs occurred (see figure 9). Figures 9.A to F report the results of the answers to the following questions in the survey:

A. Who was infected?;

B. Where did the infection happen?;

C. In which context did the infection happen?;

D. Which type of incident was involved in the infection?;

E. Was there transmission to another person?;

F. Has it been proven that the infection was work related?

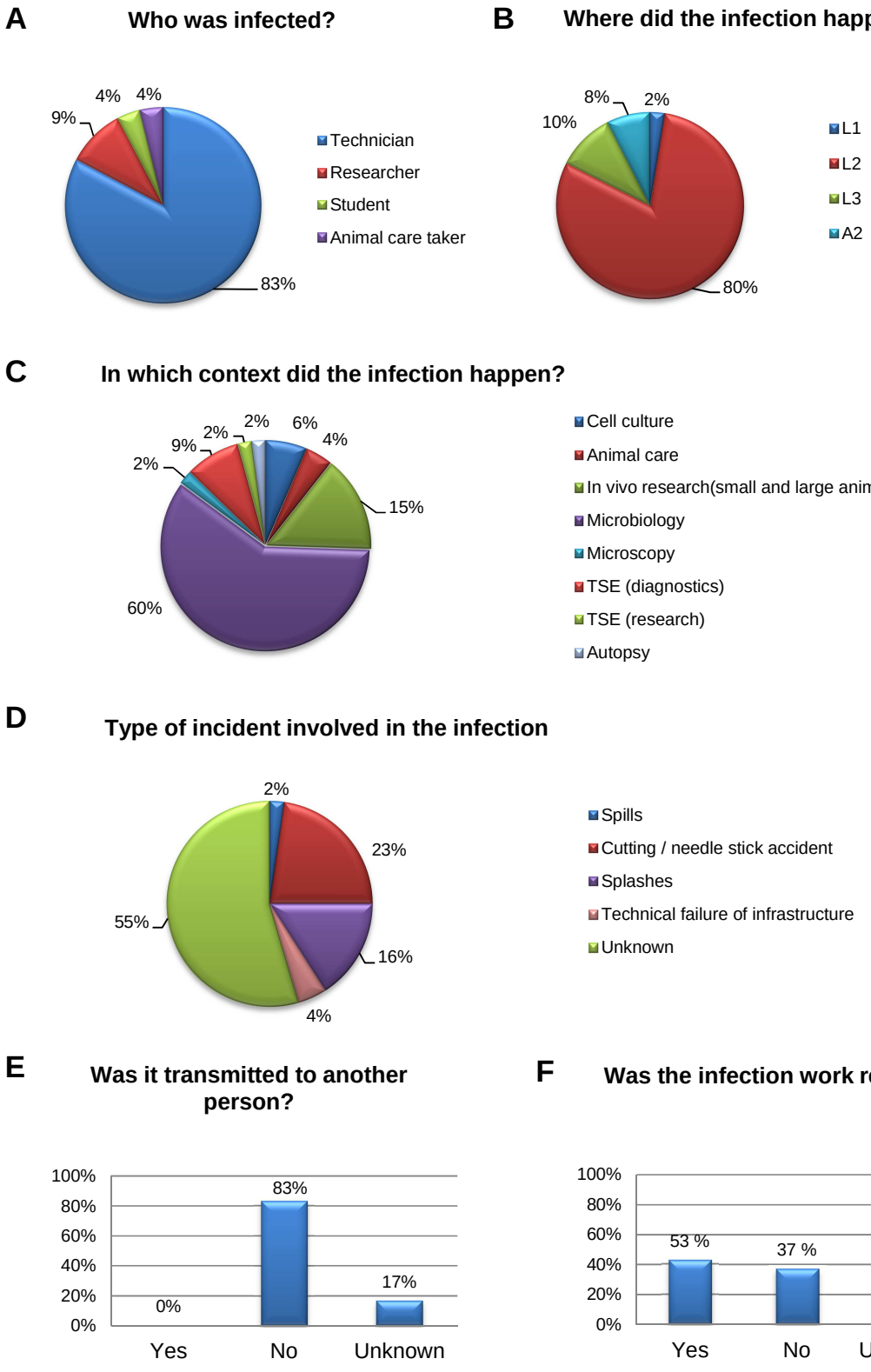


Figure 9: summary of the responses to questions concerning the circumstances in which the LAIs occurred

In the majority of the cases (83%), a technician was infected whereas in only 9% of the cases the infected person was a researcher. One should bear in mind the fact that there are more laboratory technicians than researchers (as mentioned earlier) and that they are probably more exposed to biological agents as well (in terms of time, number of manipulations, frequency and routine).
A majority (60%) of the described LAIs occurred in the context of microbiology activities, followed by *in vivo* research and animal care (19%), Transmissible spongiform encephalopathies (TSEs) diagnostics and research (11%). It has to be mentioned that the presence of TSE research in our results is likely due to a wrong interpretation of the question by the respondent because infections are reported with organisms that are not supposed to be manipulated in this context (2 times *Salmonella spp*., *Trichopyton verrucosum* and *Trypanosoma brucei gambiense* and 1 unknown).

Another observation is that a majority (60%) of the LAIs occurred in a laboratory of containment level 2 (and not in containment level 3). This is probably due to a higher number of facilities of containment level 2 in Flanders compared to containment level 3 facilities (see figure 2). Also, some LAIs (6 in total) caused by risk class 3 organisms were reported to have originated from activities in L2 laboratories. Perhaps this can be explained by the fact that, in Flemish laboratories, primo-isolation[17] of *Mycobacterium tuberculosis* and *Brucella spp.* can be performed in L2 laboratories with L3 work practices.

An important observation is that only 50% of the LAIs were actually proven to have originated in the laboratory and that in 55 % of the reported LAIs, the actual cause of the infection remains unknown. When the cause of the LAI was known, it was usually due to human error (90%), mainly by splashes, needle sticks and/or cutting accidents. Technical failures accounted for approximately 9,52 % of the cases. Looking at the underlying causes of LAIs with one of the three major groups of pathogens often involved in LAIs, namely enterobacteria (such as *Salmonella spp*., *Shigella spp*. and *Campylobacter spp*.), airborne pathogens (such as *Mycobacterium spp*. and *Brucella spp*.) and bloodborne pathogens (such as HIV, *Toxoplasma spp*. and *Trypanasoma spp*.), it appears that for bloodborne pathogens the bio-incident is clearly indicated, while for enterobacteria and airborne pathogens more than 66% of the causes of infections remain unknown, see figure 10.

[17] Analysis of *M. tuberculosis* is limited to primo-isolation from clinical specimens (i.e. primary culture, microscope examination of smears from clinical specimen, nucleic acids amplification, histological examination)

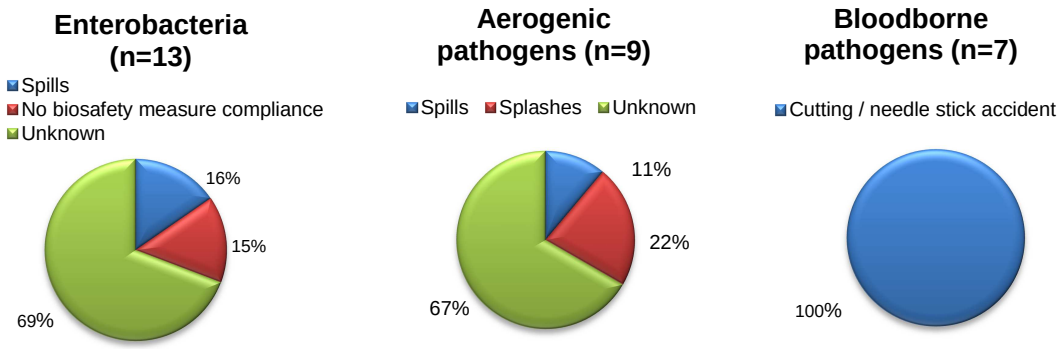


Figure 10: Detailed analysis of underlying causes of LAIs with three major groups of pathogens often involved in LAIs, namely enterobacteria, airborne and bloodborne pathogens

In survey 1, disability periods were mentioned for 11 of the 14 reported LAIs. They corresponded with 69 days of disability. This suggests an average of 6 +/- 2 days of disability per LAI. Moreover, the risk of secondary transmission (person-to-person) from the infected person is quite real (13), demonstrating the potential risk of LAIs to the public health. Fortunately, no person-to-person transmission has been mentioned in both surveys.

5. DISCUSSION

Five years after the first survey on LAIs (laboratory acquired infections) in Flanders (1), the SBB realised a new, similar investigation with a focus on the period 2007-2012. A relatively high participation was observed as approximately 55% (survey 1[18]) and 51% (survey 2 [19]) of the contacted people responded to the two surveys. This allows to draw a representative picture of the occurrence of laboratory acquired infections in Flemish laboratories. However, it is important to note that this report is the result of analysis of survey data, which are subject to bias. Nevertheless, the high number of duplicates in the answers to additional questions linked to the reported LAIs supports the reliability of the reported cases. Many findings of this survey correspond to what is described in the literature and case reports on LAIs worldwide.

The surveys identified 52 LAIs, caused by 15 different organisms (see table 7). They consisted of 31 bacterial, 6 parasitic, 5 viral and 3 fungal infections. 29 out of 52 cases (56%) belong to the three major groups of pathogens often involved in LAIs, namely enterobacteria, airborne pathogens and bloodborne pathogens (see figure 10). Remarkable are the observed differences between the LAIs reported in survey 1 and 2. While the institutions in survey 2 also participated in survey 1, the respondents in survey 1 mentioned only 14 LAIs compared to 38 LAIs in survey 2. Moreover survey 2 mentioned 8 organisms that do not appear in survey 1.

In order to link the survey results to official data, the main organizations that are involved in occupational health were contacted and asked for information, since laboratory acquired infections have to be considered occupational diseases.

These organizations are:

(1) the Federal Public Service Employment, Labour and Social Dialogue;
(2) the Fund for Occupational Diseases;
(3) the Fund for Occupational Incidents;
(4) the Flemish Agency for Care and Health, Infectious Disease;
(5) external and independent (neutral) services for prevention and protection on the work floor, *e.g.* Securex, Provikmo, Idewe.

The majority of these organizations were not able to provide adequate data on the occurrence of LAIs. There were no uniformly kept records either due to privacy reasons or the lack of a proper database. Nevertheless, the Fund for Occupational Incidents was able to provide an anonymous list of 700 registered cases of incidents with biological material in Belgium (for the period 2008-2011). The majority of the cases related to hospital acquired infections and only 4 of these incidents appeared to be the result of a bio-incident in laboratory setting (2 spill incidents, 1 inhalation incident and 1 needle

[18] survey for the attention of the biosafety officers, prevention officers and occupational health practitioners
[19] survey for the attention of the personnel

stick incident). The Fund for Occupational Diseases was able to provide anonymous data that were relevant to the interpretation of our survey as 25 LAIs recorded in Belgium in the period 1995-2011 had been officially recognized as occupational diseases, see table 9.

Table 9: Infectious diseases among laboratory personnel, recognized by the Fund for Occupational Diseases (1995-2010)

Year of submission	Language of submission	Infection/Disease	Micro-organism	Risk class[20]
1995	Dutch	Salmonellosis	*Salmonella bacteria*	2
1997	Dutch	Tuberculosis	*Mycobacterium tuberculosis*	3
1998	Dutch	Salmonellosis	*Salmonella bacteria*	2
1999	Dutch	Hepatitis	Hepatitis C virus	3(*)
1999	Dutch	Tuberculosis	*Mycobacterium tuberculosis*	3
1999	French	HIV	HIV-virus	3(*)
2000	French	Mycoplasmosis	*Mycoplasmose bacteria*	2
2000	French	Meningitis	*Neisseria menigtidis*	2
2001	Dutch	Salmonellosis	*Salmonella bacteria*	2
2001	Dutch	Cytomegaly	Cytomegalo virus	2
2002	Dutch	Hepatitis	Hepatitits B + C virus	3(*)
2003	Dutch	Shigella gastro-enteritis	*Shigella sonnei*	2
2004	Dutch	Tuberculosis	*Mycobacterium tuberculosis*	3
2004	French	Tuberculosis	*Mycobacterium tuberculosis*	3
2004	Dutch	Mononucleosis	Epstein-Barr virus	2
2004	Dutch	Brucellosis	*Brucella melitensis*	3
2004	French	Tuberculosis	*Mycobacterium tuberculosis*	3
2005	Dutch	Salmonellosis	*Salmonella bacteria*	2
2005	French	Tuberculosis	*Mycobacterium tuberculosis*	3
2005	Dutch	Tuberculosis	*Mycobacterium tuberculosis*	3
2006	French	Salmonellosis	*Salmonella bacteria*	2
2007	Dutch	Mononucleosis	Epstein-Barr virus	2
2007	Dutch	Mononucleosis	Epstein-Barr virus	2
2008	French	Tuberculosis	*Mycobacterium tuberculosis*	3
2010	French	Tuberculosis	*Mycobacterium tuberculosis*	3

*The pathogens marked with an asterisk cause a disease mentioned in the list of notifiable infectious diseases as defined by the 19 June 2009 Ministerial Order laying down the list of notifiable infections

Surprisingly, none of the 52 LAIs mentioned in either survey 1 or 2 are to be found in this list. Also, for the period 2007-2012 which corresponds with the surveyed time interval 2 cases of infection with Epstein-Barr virus and 2 cases of infection with *Mycobacterium tuberculosis* have been reported to the Fund for Occupational Diseases. Considering the language of the notification (Dutch), we can assume that with regard to LAIs in Flanders only 2 Epstein-Barr virus cases have been notified. However, no Epstein- Barr virus caused LAI has been mentioned by the respondents in our survey, unless these cases were reported for the Brussels Capital Region.

[20] Classes of biological risk are given for human and are based on the Belgian classifications of micro-organisms

Despite the occurrence of several LAIs in Flanders (data in our survey could suggest approximately 10 cases a year), there seems to be no systematic reporting neither to the Fund for Occupational Diseases nor to the Fund for Occupational Incidents.

In Belgium, the Royal Decree of April 29, 1999, amending the Royal Decree of August 4, 1996[21] concerning the protection of workers from risks related to exposure to biological agents at work, requires the notification of incidents. These have to be notified to the regional offices of Federal Public Service Employment, Labour and Social Dialogue. However, as to date, no such notification has yet been done.

Perhaps, this can partly be explained by the fact that this reporting procedure remains a rather unknown and thus ignored legal obligation. The overall results of survey 1 suggest that approximately one third of the respondents were familiar with the Royal Decree at the time of the survey. When we look at the institutions with reported LAI cases, (survey 1), only one of the nine institutions was acquainted with this Royal Decision. In contrast to this, the institution that claimed to know this Royal Decree described an infection which had not been reported to the Federal Public Service Employment, Labour and Social Dialogue.

Another finding is a clear difference between the answers of the biosafety officer, the prevention officer and the occupational health practitioner (survey 1). Not one specific LAI case was mentioned more than once, although people from the same institution responded to the same questions. In other words, 10 LAIs were mentioned by biosafety officers, whereas only 2 LAIs were mentioned by prevention officers and 2 by occupational health practitioners.

All the above suggests a lack of an adequate integrated system to ensure the follow-up and evaluation of LAIs. When it comes to LAIs, communication, reporting and notification are not evident, nor internally (between colleagues of the same institution) nor externally (to the public services mentioned above). In the literature a lack of clear communication and reporting is also a recurrent factor in many LAI cases. One might suggest that reporting and describing LAI cases gives the opportunity to evaluate and optimize the risk management measures in order to help avoiding infections in the future (15, 16).

The first people to communicate bio-incidents are, of course, the personnel working in the labs. Survey 1 and 2 revealed that, although 83,62% of the institutions report an internal procedure for dealing with a bio-incident, only in 65% of the cases bio-incidents are spontaneously notified by the personnel. This means that in 35% of the cases this does not happen. Apparently, fear or shame in having to report a bio-incident to superiors or colleagues plays a role in 24 % of the cases. When a bio-incident is not notified, 76% of the bio-incident were judged not severe enough to report and that

[21] see glossary

mitigation actions were considered adequate to cope with the incident. Some respondents of survey 1 (~10%) indicate the administrative burden to be a barrier.

When the incident is spontaneously notified, it is usually first told to the lab responsible (75-85%). In 55-75% of the cases the biosafety officer will be informed, and in 50-65% the occupational health practitioner. Colleagues are only informed in 30-45% of the cases. This suggests there is a certain hierarchy that is relatively well respected when the bio–incident is notified spontaneously.

Spills represent the majority of bio-incidents. 86 % of the institutions (survey 1) mentioned specific procedures to clean up a biological spill, 79 % of the respondents to survey 2 knew about such a procedure and approximately 60% of the institutions (survey 1) made a spill kit available[22]. The majority (2/3) of these kits are assembled in-house, others have been purchased entirely as a complete ready-to-use kit. In spite of these good intentions, only 44 % of the respondents to survey 2 confirmed the existence of a specific training for dealing with biological spills. However, a good knowledge of the risks and cleaning procedures when a bio-incident is happened is critical. This requires a complete risk assessment, taking into account multiple factors such as the characteristics of the biological agent itself (its risk class, mode of transmission, infectious dose, survival outside the host) and the circumstances of the bio-incident (type, volume, localisation, ..), and appropriate decontamination and inactivation methods.

It was mentioned above that quite often a bio-incident is handled by the personnel without notifying it to superiors or other colleagues. The question remains whether the personnel is actually able to perform a suitable risk assessment to judge the incident as “not being severe enough” as this was mentioned above. We realize that the limit between minor and major bio-incidents is certainly not easy to define, as it depends on multiple factors (see above). Also, according to the answers given concerning risk perception, more than half of the respondents (~55%) is not fully familiar with risk assessment (see table 4) and approximately 38% of the personnel feel they are not adequately trained in biosafety (figure 7.2). In addition to the above, figure 7 bis suggests that a small majority of the biosafety officers agrees that the personnel should receive more training in order to avoid bio-incidents.

Remarkably, in ~55% of the reported LAI cases the type of incident prior the infection is unknown (see figure 9D). In case of enterobacteria and airborne pathogens the number of unknown causes were even higher (>65% of the cases) (see figure 10), hence it is reasonable to assume that this type of pathogens are more often involved in unnoticed infections.

[22] This is only a legal obligation in large scale facilities.

When the cause of the LAI is known, human error accounts for 90% of the underlying causes, cutting accidents and splashes representing the majority of causes in survey 1 and 2. It seems that in 30% of the cases, incautious/careless handling is at the root of LAIs. Absent-mindedness accounts for more than 50% (see figure 6.8). Other parameters that seem to play a role are work-related stress, a high workload and the repetitive nature of certain manipulations. Technical failures are apparently less common (~10%).

Given the fact that bio-incidents and LAIs are not always avoidable, biosafety measures are implemented to protect against exposure to biological agents. Nevertheless, a certain decline in compliance was observed when specific measures become more stringent (figure 5) and a lack of compliance with biosafety measures was identified by the respondents as an important factor causing bio-incidents (figure 7.9). This is also confirmed by data from the literature (figure 8).
Surveys 1 and 2 show that, in general, LAIs and bio-incidents were observed in institutions with a large number of relatively complex activities that show a high diversity in available infrastructure and harbour different containment levels (see annex 2). One might suggest that this complexity is also reflected in the adopted stringent biosafety measures. Hence the importance of good compliance. An additional question could be whether the requested biosafety measures are well adapted in all cases.

Unfortunately, the survey data do not allow us to draw detailed conclusions on the risk assessment and management in Flemish laboratories or institutions. This again shows the importance of communication and reporting of incidents, as this can perhaps initiate a re-evaluation of biosafety measures.

6. CONCLUSION & RECOMMENDATIONS

The findings and facts of the online survey (survey 1 and 2) are discussed in the previous section. In this part, we conclude and give some recommendations in order to limit the risk associated with bio-incidents and the occurrence of LAIs. Although the current study on laboratory acquired infections (LAIs) is only the second one of this type in Flanders and this type of studies does not provide substantiated data as they have been collected online, the results of these online surveys give a general idea of the safety culture and LAI perception in Flemish laboratories. Besides recommending a strict compliance with the required biosafety measures in order to prevent laboratory-acquired infections, the online survey made it possible to draw the following additional conclusions, recommendations and opportunities for improvement:

AN IMPORTANT PROPORTION OF THE IDENTIFIED LAIS HAS UNKNOWN CAUSES

We observed that an important proportion of the identified LAIs has unknown causes (see figure 8, 9D and 10). It can be assumed there exists a certain baseline of LAI cases with a cause/origin that is difficult to identify: because the presence of (unintentionally spread) pathogenic organisms cannot be easily visualized, the personnel is often not aware of a contamination and risks an infection. Some micro-organisms represent a higher risk of infection because of their specific characteristics (low infectious dose, aerogenic spread and high survival outside the host). Also, incubation periods can vary between organisms and infected persons (from weeks to months). These facts make it difficult to trace (back) which bio-incident or initial event caused the infection.

Another possible source of unnoticed contamination is an inadequate decontamination at the end of activities or after a bio-incident. It is known that biological aerosols (generated by centrifuging, pipetting, after a spill or break incident) can move around not only by air currents generated by ventilation, but also by resuspension of settled materials. The contaminated / affected area may be greater than expected, possibly leading to only partial cleaning / decontamination, which is followed by unintentional spreading of the pathogen due to the movement of primarily or secondarily contaminated material/lab workers. Moving personnel in a contaminated area prior to incident recognition or as a result of an emergency response to an incident (e.g., emergency operations, mitigation and restoration activities) may also disturb settled material, spread the contamination allowing recirculation of biological materials into the air. It has been estimated that resuspension can extend the risk of infection from biological aerosols for hours and even days beyond an initial event when compared to a situation where particles are allowed to settle without disturbance (17).

On the other hand, 27% of the bio-incidents is judged (by the concerned lab worker) not serious enough to seek advice or to notify and it is believed they can cope with it in an appropriate manner. This is in contradiction to the fact that not everyone considers he or she has received enough training

(see discussion and Figure 7.2). Also, as shown in the answers about risk perception (see discussion, table 4), not everyone is familiar with the risk assessment methodology, suggesting that a certain part of bio-incidents are not adequately handled, leading to a possible increased risk of dissemination, contamination, spreading in the environment and risk on infections.

Furthermore, it is observed that one-fourth of the bio-incidents that are not reported is due to feelings of shame or fear of sanctions. This observed “taboo on mentioning” is detrimental to a proper biological risk management.

Finally, not reporting an incident, or an inadequate decontamination, increases not only the risk of unintentional spreading of the pathogenic agent and unnoticed contamination, but leads to missed opportunities to evaluate the incident, improve the actual situation and avoid similar incidents in the future.

Recommendations :

a) Better internal communication

Ideally, every bio-incident should be communicated through a system of internal reporting within the institution, resulting in a quick response by the people who are in charge of biosafety. These people can in case of a bio-incident provide advice and support to the personnel for adequate decontamination and follow up. Afterwards the incident can be evaluated in order to optimize the prevention policy in the institutions to limit bio-incidents and to avoid LAIs in the future.

When there is a possible risk of infection, it is recommended to have the lab worker followed by an occupational health practitioner. A communication of the follow-up-result to the management and the people in charge of biosafety is recommended and should be beneficial for the prevention policy. Because of the confidentiality between the physician and the patient and since it is observed that there can be feelings of shame or fear of sanctions, it is fundamental to approach those events with respect to the privacy of the individual.

To remain workable, it is important to define / determine in advance the severity of the bio-incident before starting to report the bio-incident or before asking a follow-up by the occupational health practitioner. The severity of a bio-incident depends on multiple factors (see discussion) and should be assessed using the principles of biological risk assessment. The definition of the severity level of the bio-incident can be difficult and should be done on a case-by-case basis. Therefore it is maybe worth to provide a kind of decision tree with relevant examples to help the lab worker to define the severity of a bio-incident.

Furthermore it is also important to always inform (immediately) the direct colleagues (colleagues that can enter the ‘contaminated’ area) about the bio-incident to avoid infections of uninformed colleagues.

B) Courses and practical training in biosafety (general and specific training)

Courses and practical training in biosafety are important tools to contribute to a (bio)safe work environment and are considered a legal obligation in biosafety and occupational health[23]. Based on the survey results there is a general impression that there exists a lack of training but also a lack of knowledge. A lack of knowledge not only with regard to bio-incidents, but also with regard to general aspects of biosafety (see discussion). This suggests that the knowledge of biosafety principles can still be improved in order to enhance awareness of biological risks in the laboratory and to avoid LAIs. The principles of risk assessment are the fundaments of biosafety (practices). The personnel needs to be informed about the risks that are present when working with pathogenic organisms in general (general training). Besides this general training, lab workers involved in manipulation with specific (high) risks should receive a more specific and detailed training. Hence, to be able to manage these risks, the lab workers should be aware of which appropriate personal protection measures need to be taken to protect themselves, their colleagues and the environment. These should also include specific procedures to handle bio-incidents.

Furthermore, the identified lack of communication (see above, A) and the underreporting of bio-incidents due to shame or fear of sanctions should be addressed and discussed during the biosafety trainings. This could improve the internal communication and help overcome the psychological barrier that is associated with the reporting of bio-incidents.

Besides informing on the theoretical aspects of biosafety, there should also be a proper practical training in dealing with bio-incidents (with a focus on risk recognition, decontamination and communication).

C) Evaluation and control of adopted biosafety measures

A recent survey in the US has shown that a lot of written procedures (e.g. good laboratory practices, adequate waste management,...) and extensive biosafety training not always lead to a safer lab (17, 18). To make all these efforts useful it is important to evaluate the effectiveness of all biosafety measures as required in the legislation on biosafety and occupational health. In that respect, there is still progress to be made. Biosafety measures should be assessed for effectively reducing the occurrence of LAIs (for example by avoiding accidental dissemination of pathogens in the work environment). In particular, the validation of performance of disinfectants,..., personal protection (masks, gloves, …) and equipment (*e.g.* biosafety cabinet, autoclave,) is of primary importance. Also, the chosen performance assay should be relevant to the concerned laboratory activities. Finally it is recommended to have trainings and courses evaluated by the participants and also to have the participants evaluated (17, 18)

[23] see glossary

'Good laboratory practices' can be assessed by tracing viable organisms on the workplace. This can be achieved by sampling (swapping) a few specific places in the lab (e.g. centrifuge, control panel of biosafety cabinet or incubators, telephone receiver,...) that are potential hot spots of biohazards if there is no good compliance with biosafety measures.

Where control of biosafety measures reveals a higher potential risk of LAIs (or dissemination), action must be taken to improve biosafety (e.g. better adapted products, procedures, …).

In summary, there exist opportunities for improvement when it comes to communication, handling of bio-incidents, enhancing (practical and theoretical) knowledge on biosafety and raising awareness of the risks of LAIs.

MANY LAIS ARE NOT IMMEDIATELY RECOGNIZED OR REMAIN UNNOTICED

Although it is recommended to have the lab worker followed by an occupational health practitioner when there is a possible risk of infection after an bio-incident, there is still a unknown part of LAIs that remain unnoticed, especially when the LAIs occur asymptomatically, with relatively mild symptoms or symptoms similar to endemic diseases. In case of mild symptoms or symptoms similar to endemic diseases, the infection (of the lab worker) may not be linked to the work in the lab and could lead to a wrong diagnosis by the general practitioner (for example, a lab acquired infection with the bacteria *Francisella tularensis* could be misdiagnosed as a case of influenza (19). This could lead to inappropriate treatment or conclusions, and maybe even result in secondary infections (colleagues, family members,...).

Within this context, personnel exposed to pathogenic organism should receive adequate information about the possible range of symptoms that can occur after a LAI with the pathogen(s) they manipulate.

Recommendations :

a) Providing proper training in better knowledge of the pathogenic properties of the micro-organisms the personnel is working with. This could maybe make the personnel more alert to certain symptoms linked to an infection with the manipulated organism.

b) Provide the employee's personal general practitioner with adequate information on the risks associated with potential occupational exposure to pathogenic organisms on the workplace and expected symptoms. To keep it workable it is important to define which type of activities needs to be considered by the general practitioner.

THERE IS NO SYSTEMATIC REPORTING OF BIO-INCIDENTS WITH RISK OF LAI OR NOTIFIABLE INFECTIOUS DISEASES IN FLANDERS

Although bio-incidents and LAIs happen in Flanders and despite the existence of legal obligations to notify bio-incidents[24] or notifiable infectious diseases[25], there is no systematic reporting of bio-incidents to the regional offices of Federal Public Service Employment, Labour and Social Dialogue (Royal Decision of 29 April 1999) nor of notifiable infectious disease to the Flemish Agency for Care and Health, Infectious Disease Surveillance.

This implies that several cases are unnotified and unknown. The opportunity to evaluate the incident and possibly improve the actual situation to avoid similar incidents in the future is therefore not fully used. It is both in the interest of the involved laboratory and policy-makers to dispose of clear and solid (anonymized) data on the occurrence of LAIs to make a complete evaluation possible and to make it available to the biosafety community.

Recommendations:

a) Emphasizing the existence of legal obligations with respect to notification of bio-incidents and notifiable infectious diseases.

b) Establishing a centralized system, generating data that will identify possible gaps (in risk management, knowledge,...).This novel system could serve for reporting LAIs to the authorities and other interested parties (the biosafety community) in an anonymous way.

c) Also here it is important to define which type of bio-incident needs to be communicated.

[24] Article 75 of Royal Decree of 4 August 1996, amended by the Royal Decree of April 29, concerning the protection of workers from risks related to exposure to biological agents at work

[25] The list of notifiable infectious diseases is defined by the 19 June 2009 Ministerial Order laying down the list of notifiable infections and delegating the authority to appoint civil servant doctors and civil servants. This fits in with the 21 November 2003 Flemish Parliament Act on the preventive health policy and the 19 June 2009 Flemish Government Decree on initiatives to prevent the harmful effects caused by biotic factors from spreading (Article 2).

IMPACT OF OCCUPATIONAL AND HUMAN FACTORS

Besides the high workload as a potential cause for a bio-incident, many respondents (65%) share the feeling that being distracted increases the risk on bio-incidents (see figure 7.4 and 7.8), The feeling of being overloaded (stress) or discomfort can happen at any moment and at any level within an institution. Although this may lead to increased risk of incidents, many institutions impose deadlines and create a high work load. Potential consequences could be an increase in inaccurateness, distraction/absent mindedness and a higher act of neglect. Although these could also be a consequence of highly repetitive work.

Furthermore, it is observed that increased incidents and LAIs can also be associated with institutions that show a high diversity in available infrastructure and harbour different containment levels (see annex 2). One might suggest that this complexity is also reflected within a large number of relatively complex activities with increased biological risk, which require more biosafety measures to be adopted. In the survey it is observed that this increased level of adopted biosafety measurements often leads to a decreasing level of compliance (see figure 5). Moreover, in the literature lack of compliance with biosafety measures is described as one of the most important cause of LAIs (see figure 8). Other possible causes could be created by discomfort when wearing additional personal protective equipment and by an inappropriate risk assessment. These can result in less (strict) compliance.

All the above suggests that there is an opportunity to reduce risk of LAIs when occupational and human aspects of the profession are recognized and appropriate measures are taken to reduce their consequences.

7. NEDERLANDSE SAMENVATTING

LABORATORIUMINFECTIES IN VLAANDEREN: EEN ONLINE BEVRAGING

Vijf jaar na de eerste bevraging over bio-incidenten en LAI's ("Laboratory Acquired Infections" of laboratoriuminfecties) in Vlaanderen (1) heeft de Dienst Bioveiligheid en Biotechnologie (SBB) van het Wetenschappelijk Instituut Volksgezondheid in opdracht van Toezicht Volksgezondheid (Vlaams Agentschap Zorg en Gezondheid) een gelijkaardig onderzoek uitgevoerd met specifieke aandacht voor de periode 2007-2012. Concreet werd de enquête opgesplitst in 2 delen en anoniem verwerkt. In deel 1 werden de bioveiligheidscoördinatoren, arbeidsgeneesheren en preventiecoördinatoren verbonden aan 124 instellingen die een Vlarem vergunde biotechnologische activiteit uitoefenen (enquête 1) bevraagd. In deel 2 werd aan 4 private bedrijven en 6 instituten gevraagd de werknemers (in totaal 432) de enquête te laten invullen. Meer bepaald werden hier 5 instellingen met diagnostische activiteiten geselecteerd en 5 instellingen behorende tot de deelsector 'Onderzoek en Ontwikkeling' (R&D) (enquête 2). De bevraging kan een succes genoemd worden gezien de hoge deelnemingsgraad (respectievelijk 55% en 51%) en de evenwichtige vertegenwoordiging van deze sector in beide enquêtes. De resultaten van de bevraging leidden tot een aantal bevindingen over LAI's in Vlaamse laboratoria voor de periode 2007-2012:

- In totaal werden er 89 laboratoriuminfecties gemeld door 47 respondenten waarvan 52 laboratoriuminfecties met 15 verschillende organismen werden weerhouden als unieke gevallen. Opvallend was dat enquête 1 melding maakt van 14 unieke LAI's, terwijl enquête 2 tot 38 unieke LAI's meldt – dit ondanks de instituten uit deel 2 van de enquête (de werknemers) ook meegenomen waren in deel 1 van de enquête. Tevens is er ook een rapportagediscrepantie binnen de groep van bioveiligheidscoördinatoren, preventieadviseurs en arbeidsgeneesheren (enquête 1).
- Bacteriële infecties domineren (60% - vooral enterobacteriën), gevolgd door infecties met parasieten (11.5%), virale infecties (10%) en schimmelinfecties (6%).
- 70% van de geïnfecteerde werknemers werkt in een diagnostische labo, 83% van hen was laborant, 80% van de infecties was terug te brengen tot een zone met inperkingsniveau L2, 60% gebeurde in de context microbiologie – 15 % in vivo research, in 53% van de cases is de labo-specifieke bron van infectie onbekend.
- 83% van de geïnfecteerde werknemers geeft aan dat de infectie niet werd doorgegeven aan een derde persoon, overdracht mens-op-mens – 17% geeft aan dit niet te weten. En 43% geeft aan dat de infectie bewezen werk gerelateerd is, in 20% van de gevallen is dit niet geweten.
- Van de gekende oorzaken van de aangegeven LAI's was 90% terug te brengen op menselijke fouten.
- De bevraging gaf aan dat het resulterende gemiddelde werkverlet neerkwam op 6 +/- 2 dagen per LAI.

Het groot aantal herhalingen in de antwoorden en de grote gelijkenis met wat wordt beschreven in de internationale literatuur laat ons toe met enige zekerheid te stellen dat deze gerapporteerde LAI's de werkelijkheid benaderen voor Vlaanderen.

In een poging de resultaten van de enquête te linken aan officiële data werd bijkomend de belangrijkste instanties die betrokken zijn bij arbeidsgeneeskunde gecontacteerd (het Fonds voor arbeidsziekten, het Fonds foor arbeidsongevallen, de FOD Werkgelegenheid, Arbeid en Sociaal Overleg, Welzijn op het werk en het VAZG – team infectieziekten). LAI's worden tenslotte over het algemeen beschouwd als beroepsziekten.

Gebaseerd op deze resultaten, en onafhankelijk van standaard voorgeschreven praktijken die in principe reeds vaak wettelijk verplicht zijn, worden de volgende **conclusies en aanbevelingen** opgesteld in het kader van een betere preventie van LAI's:

EEN BELANGRIJK DEEL VAN DE BESCHREVEN LAI'S HEEFT ONBEKENDE OORZAKEN

Meer dan de helft van de LAI's hebben een ongekende oorzaak (55% voor Vlaanderen versus 40% in de literatuur). Er kan worden aangenomen dat er een bepaalde baseline is voor LAI's met ongekende oorzaak omdat de aanwezigheid van (onbedoeld verspreide) pathogene organismen moeilijk waarneembaar is en het personeel zich niet altijd bewust is van een blootstelling aan biologische agentia. Bovendien verhogen sommige karakteristieken van micro-organismen de kans op infecties met onbekende oorzaak, zoals een lage infectieuze dosis, aërogene spreiding, lange incubatietijd en hoge overlevingskans buiten de gastheer. Een andere mogelijke oorzaak van een ongewenste blootstelling aan biologische agentia is na een niet-adequate ontsmetting op het einde van een activiteit of na een bio-incident. Tenslotte is ook opgemerkt dat in 35% van de gevallen het bio-incident niet wordt gemeld. Het niet melden van een incident leidt mogelijks tot onbedoelde verspreiding van mogelijk infectieuze biologische agentia met een risico op infecties bij derden en kan bovendien gezien worden als een gemiste kans om het incident te evalueren ter voorkoming van soortgelijke incidenten in de toekomst.

Volgende elementen kunnen bijdragen tot het vermijden van LAI's met onbekende oorzaak:

- betere interne communicatie na een bio-incident;
- opleidingen en praktische trainingen in bioveiligheid waarbij de nadruk ligt op risico's van bepaalde handelingen met pathogenen in het labo;
- evaluatie en controle van aangepaste bioveiligheidsmaatregelen (uitrusting, decontaminatieprocedures, opleidingen…).

VEEL LAI'S WORDEN NIET DIRECT HERKEND OF BLIJVEN ONOPGEMERKT

Hoewel het is aanbevolen om een (lab)werknemer die betrokken was bij een bio-incident waarbij er risico was op besmetting, op raadpleging te laten komen bij de arbeidsgeneesheer, blijft de infectie mogelijk onopgemerkt wanneer de betrokkene zich niet bewust was van de blootstelling aan infectieuze biologische agentia. Het betreft dan voornamelijk infecties die asymptomatisch verlopen of met relatief milde klachten of met symptomen die vergelijkbaar zijn met endemische ziekten. Bij milde symptomen of symptomen vergelijkbaar met endemische ziekten zal de infectie niet worden gekoppeld aan het werk in het labo met als gevolg een verkeerde diagnose door de huisarts, een ongepaste behandeling, en misschien zelfs verdere verspreiding in de gemeenschap/het leefmilieu.

Volgende elementen kunnen bijdragen tot het voorkomen van LAI's die niet herkend worden of onopgemerkt blijven:

- Adequate training voorzien om kennis van pathogene eigenschappen van micro-organismen te verbreden (inclusief symptoomherkenning). Hierdoor zou het personeel waakzamer worden bij het optreden van bepaalde symptomen die zouden kunnen gelinkt worden aan bepaalde LAI's.

- De arbeidsgeneesheer en de huisdokter(s) van het personeel voorzien van informatie over de mogelijke blootstelling van de patiënt aan pathogene (micro-)organismen op het werk en van de mogelijke symptomen bij infecties. Hierdoor zullen diagnose en behandeling adequater gebeuren en wordt het risico op secundaire transmissie (van persoon op persoon) en verspreiding in de gemeenschap/leefmilieu tot een minimum beperkt.

ER BESTAAT GEEN SYSTEMISCHE RAPPORTERING VAN BIO-INCIDENTEN MET EEN RISICO OP LAI'S OF MELDINGSPLICHTIGE INFECTIEZIEKTES IN VLAANDEREN.

Ondanks het voorkomen van LAI's in de resultaten van onze bevraging en ondanks het bestaan van een wettelijke verplichting wat betreft het melden van bio-incidenten[26] of meldingsplichtige infectieziektes[27], was het onmogelijk deze resultaten in overeenstemming te brengen met de officiële lijsten waar de wettelijk geregelde rapportage en zo mogelijk opvolging van infectieziekten gebeurt. Dit suggereert dat er geen systematische melding is van bio-incidenten en LAI's. Dit is een gemiste kans voor al wie betrokken is bij bioveiligheid om het incident te evalueren en soortgelijke incidenten in de toekomst te voorkomen. Het is dan ook aanbevolen om aan sensibilisatie te doen. Bovendien is de vraag of een online systeem dat gebruikt kan worden voor de (anonieme) melding van laboratoriuminfecties wenselijk is. Oorzaken en context zouden worden beschreven en de melding kan dan bijdragen tot het vermijden van gelijkaardige gevallen in de toekomst.

26 Artikel 75 van het Koninklijk Besluit van 6 augustus 1996 betreffende de bescherming van de werknemers tegen de risico's bij blootstelling aan biologische agentia op het werk
27 http://www.zorg-en-gezondheid.be/Diseases/Infectious-diseases

IMPACT VAN ARBEIDSGERELATEERDE OF MENSELIJKE FACTOREN

Naast een hoge werkdruk als een mogelijke oorzaak voor een bio-incident, delen vele respondenten (65%) het gevoel dat verstrooidheid het risico op bio-incidenten verhoogt. Het gevoel van overbelasting (stress) of ongemak kan gebeuren op elk moment en op elk niveau binnen een inrichting. Hoewel dit kan leiden tot een verhoogd risico op incidenten, blijven vele inrichtingen deadlines opleggen. Naast een te hoge werkdruk, ligt te repetitief werk ook aan de basis van deze problematiek.

Verder wordt opgemerkt dat een toename aan incidenten en LAI's ook kan worden geassocieerd met instellingen die een hoge diversiteit hebben aan activiteiten en infrastructuur. Men zou kunnen veronderstellen dat deze complexiteit ook tot uiting komt in een groot aantal complexe activiteiten die bijkomende bioveiligheidsmaatregelen vereisen. Uit onze bevraging is echter gebleken dat de vereiste aan bijkomende bioveiligheidsmaatregelen vaak leidt tot een verminderd opvolgen van deze bijkomende maatregelen mede door het ongemak dat men ervaart indien men deze opgelegde maatregelen toepast. Het niet opvolgen van de bioveiligheidsmaatregelen staat in de literatuur beschreven als één van de belangrijkste oorzaken van LAI's.

Al het bovenstaande suggereert dat indien het risico op LAI's via deze beroepsmatige of menselijke aspecten erkend wordt en er passende maatregelen worden genomen de kans op bio-incidenten en LAI's kan worden verlaagd.

8. ANNEXES

Annex 1: Summary of the recently reported Laboratory-Acquired Infections since 2000

Annex 2: Analysis of characteristics of institutions with or without reported bio incident or LAI

Annex 1: Summary of the recently reported Laboratory-Acquired Infections since 2000

(based on the list of recent LAIs provided on the Belgian biosafety server (to be found on http://www.biosafety.be/CU/LAI/Recent_LAI.html)

Number	Organism	Organism	# cases	Risk class[28]	Route of exposure	Bio-incident	YEAR of publication	Country	Ref
2	***Bacillus anthracis and Bacillus. cereus***	***Bacillus anthracis***	1	3	Parenteral inoculation	Human error: No compliance with biosafety measures	2002	USA	LINK2
		Bacillus cereus	1	2	Unknown	Unknown	2011	USA	ProMEDmail
124	***Brucella bacteria (Brucella melitensis, Brucella suis)***	***Brucella abortus***	1	3	Inhalation or ingestion	Technical failure : breaking of centrifuge tube	2000	Italy (EU)	(20)
		Brucella bacteria	2	3	Unknown (2)	Human error: Ignorance (2)	2004	USA	(21)
		Brucella bacteria	2	3	Unknown (2)	Human error: No compliance with biosafety measures (2)	2008	USA	LINK11
		Brucella bacteria	3	3	Inhalation? (3)	Unknown (3) (sniffing?)	2008	Turkey	(22)
		Brucella bacteria	1	3	undefined	undefined	2010	Australia	(23)
		Brucella bacteria	1	3	Inhalation	Human error: No compliance with biosafety measures (no use of BSC)	2008	United Arab Emirates	(24)
		Brucella bacteria.	75	3	Inhalation or ingestion???	Human error: No compliance with biosafety measures (60) Unknown (6)	2005	Spain (EU)	(25)
		Brucella melitensis	38	3	Unknown (50%) Inhalation (39%)	Human error No compliance with biosafety measures	2012	Turkey	(26)

[28] Classes of biological risk are given for human and are based on the Belgian classifications of micro-organisms

2	***Burkholderia pseudomallei and Burkholderia mallei***	***Burkholderia pseudomallei and B. mallei***	2	3	Parenteral Inoculation (1) Unknown (1)	Unknown (1) Human error : No compliance with biosafety measures (1) →during spill cleaning	2008	USA en Australia	LINK 13
1	***Chlamydophila psittaci***	***Chlamydophila psittaci (avian strain)***	1	3	Unknown (inhalation?)	Human error: Ignorance	2009	Belgium (EU)	(14)
2	***Clostridium difficile***	***Clostridium difficile***	2	2	Unknown	unknown	2008	Spain (EU) and The Netherlands (EU)	(27)
1	**Cowpox virus**	***Cowpox virus***	1	2	Contact?	Unknown	2011, 2012	USA	ProMEDmail; (28)
1	**Dengue virus**	***Dengue virus***	1	3	Contact	Human error : No compliance with biosafety measures	2011	Australia	(29)
5	**Filovirus (Ebola virus and Marburg virus*)***	***Ebola virus***	2	4	Parenteral inoculation (1) Undefined (1)	Human error: Needle stick or cut incidents	2004	Russia	LINK5
		Ebola virus	1?	4	Parenteral inoculation	Human error: Needle stick or cut incidents	2009	Germany (EU)	ProMEDmail
		Ebola virus	1	4	Parenteral inoculation	Human error: Needle stick or cut incidents	2011	Germany (EU)	(30)
		Marburg virus	2	4	Undefined (2)	Undefined (2)	2004	Russia	LINK5
4	***Escherichia coli (O157:H7)***	***Escherichia coli O157:H7***	4	3(*)T	Inhalation or ingestion	Human error: No compliance with biosafety measures (2) Unknown (2)	2005	USA	(31)
6	***Francisella tularensis***	***Francisella tularensis***	1	3	Inhalation or ingestion	Human error: Ignorance	2002	USA	(32)
		Francisella tularensis	3	3	Unknown	Unknown	2005	USA	LINK6
		Francisella tularensis	1	3	Undefined	Undefined	2009	USA	LINK15
		Francisella tularensis	1	3	Unknown	Unknown	2012	USA	LINK19
1	***Leptospirosis bacteria***	***Leptospirosis bacteria***	1	2	Parenteral inoculation	Human error: Needle stick or cut incidents after breaking tube	2004	India	(33, 34)

4	***Mycobacterium bacteria***	***Mycobacterium kansasii***	1	2	Parenteral inoculation	Human error: Needle stick or cut incidents	2005	Belgium (EU)	(12)
		Mycobacterium tuberculosis	3	3	unknown	Technical failure : leaky aerosol chamber	2005	US States, India, New Zealand, and Northern Ireland (EU)	LINK7
11	***Neisseria meningitidis***	***Neisseria meningitidis***	5	2	Inhalation or ingestion	Human error : No compliance with biosafety measures	2001	UK (EU)	(35)
		Neisseria meningitidis	2	2	Inhalation or ingestion	Human error: No compliance with biosafety measures	2002	USA	LINK1
		Neisseria meningitidis	2	2	Undefined (2)	Undefined (2)	2005	USA	(36)
		Neisseria meningitidis	1	2	Unknown	Human error : No compliance with biosafety measures?	2007	Sweden (EU)	(37)
		Neisseria meningitidis (serogroup A)	1	2	Unknown	Human error : No compliance with biosafety measures?	2007	USA	(38)
130	***Salmonella bacteria***	***Salmonella Serotype enteritidis***	21	2	Unknown	Human error: Spill?	2007	USA	LINK10
		Salmonella typhimurium	109	2			2012	USA	LINK18
1	**SARS**	***SARS***	1	3	Unknown (inhalation)	Human error: Ignorance / cross contamination	2004	Singapore	(39)
1	***Shigella sonnei***	***Shigella sonnei***	1	2	Contact	Human error: No compliance with biosafety measures	2006	Belgium (EU)	LINK9
3	***Staphylococcus aureus***	***Staphylococcus aureus (MRSA)***	2	2	Undefined (2)	Undefined (2)	2006	The Netherlands (EU)	(40)
		Staphylococcus aureus (EMRSA-15)	1	2	Parenteral inoculation	Human error: No compliance with biosafety measures	2003	Australia	(41)
11	**Vaccinia virus**	***Vaccinia virus***	1	2	Unknown	Human error: No compliance with biosafety measures	2006	USA	LINK8
		Vaccinia virus	1	2	Parenteral inoculation	Human error: Needle stick	2003	Brazil	(42)
		Vaccinia virus	1	2	Parenteral inoculation	Human error: No compliance with biosafety measures	2003	Canada	LINK4
		Vaccinia virus	1	2	Parenteral inoculation	Human error: Needle stick or cut incidents	2004	USA	(43)
		Vaccinia virus	5	2	Parenteral inoculation (5)	Human error: Needle stick or cut incidents (5)	2008	USA	LINK12

		Vaccinia virus	1	2	Unknown	Human error: No compliance with biosafety measures (cross contamination)	2009	USA	LINK14
		Vaccinia virus (Recombinant)	1	2?	Contact?	Human error: No compliance with biosafety measures	2003	Germany (EU)	(44)
1	***Vibrio cholerae O1***	***Vibrio cholerae O1***	1	2T	Unknown	Technical failure : overtipping during culturing (spill)	2009	Austria (EU)	(45)
4	**West Nile Virus**	***West Nile Virus***	2	3	Parenteral inoculation	Human error: Needle stick or cut incidents	2002	USA	LINK3
		West Nile virus	1	3	Parenteral inoculation	Human error: Needle stick or cut incidents	2009	South Africa	(46)
		West Nile virus	1	3	Contact	Ignorance / Human error: No compliance with biosafety measures?	2010	South Africa	LINK16
1	***Yersinia pestis (attenuated)***	***Yersinia pestis (attenuated)***	1	2?	Unknown	Unknown	2011	USA	LINK17

LINKs :

LINK1: Centers for Disease Control and Prevention:
http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5107a1.htm
LINK2: Centers for Disease Control and Prevention:
http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5113a4.htm
LINK3: Centers for Disease Control and Prevention:
http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5150a2.htm
LINK4: Public Health Agency of Canada:
http://www.collectionscanada.gc.ca/webarchives/20071214095124/http://www.phac-aspc.gc.ca/publicat/ccdr-rmtc/03vol29/dr2915eb.html
LINK5: http://www.smh.com.au/articles/2004/05/25/1085461754389.html
LINK6: http://www.bphc.org/programs/cib/environmentalhealth/biologicalsafety/forms%20%20documents/tularemia_report_2005.pdf
LINK7: http://www.sunshine-project.org/publications/pr/pr180405.html
LINK8: Centers for Disease Control and Prevention: http://wwwnc.cdc.gov/eid/article/12/1/05-1126_article.htm
LINK9: Tijdschrift voor Geneeskunde:
http://users.telenet.be/dokter.vanschoenbeek.bvba1/07/2007%2014%2006.htm
LINK10:Centers for Disease Control and Prevention:
http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5634a1.htm?s_cid=mm5634a1_e
LINK11:Centers for Disease Control and Prevention:
http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5702a3.htm?s_cid=mm5702a3_e
LINK12:Centers for Disease Control and Prevention:
http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5715a3.htm?s_cid=mm5715a3_e
LINK13:Centers for Disease Control and Prevention: http://wwwnc.cdc.gov/eid/article/14/7/07-1501_article.htm
LINK14:Centers for Disease Control and Prevention:
http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5829a1.htm?s_cid=mm5829a1_e
LINK15: http://news.sciencemag.org/scienceinsider/2009/12/researcher-at-a.html
LINK16:Centers for Disease Control and Prevention: http://wwwnc.cdc.gov/eid/article/16/3/09-1042_article.htm
LINK17:Centers for Disease Control and Prevention:
http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6007a1.htm
LINK18:Centers for Disease Control and Prevention: http://www.cdc.gov/salmonella/typhimurium-laboratory/011712/index.html
LINK19: http://journals.lww.com/infectdis/Abstract/2012/05000/Laboratory_Acquired_Tularemia_Successfully_Treated.13.aspx

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