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Laboratory-Acquired Infections in Belgium (2007-2012)

An online survey

Biosafety and Biotechnology Unit

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GLOSSARY

For the purpose of this document:

Accident

An 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 (LAIs)

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.

LAIs

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:

On request of the Flemish Agency for Care and Health, Department Prevention[1], the Biosafety and Biotechnology Unit (SBB) of the Scientific Institute of Public Health, developed in 2012 a survey in the interest of mapping and evaluating the risk for "laboratory-acquired infections[2]" (LAIs) related to bio-incidents[3] 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 to a similar survey that was conducted by Ghent University in Flanders over the period 2001 to 2006 (1).

The Flemish survey conducted in 2012 showed a high and representative participation and could identify several bottlenecks. The results and analysis of that survey were published on the Belgian Biosafety Server (www.biosafety.be) and received national and international attention. Because of its success, it was decided to extend the survey over the whole of Belgium. This was made possible thanks to the financial support of the Flemish, Walloon and Brussels-Capital Regions (LNE, DGARNE and IBGE-BIM[4]).

PURPOSE OF THE STUDY

The main purpose of both surveys covering Flanders and Belgium was to gather information on bio-incidents and LAIs in biological laboratories[5] to gain insight into the possible underlying causes in order to provide biosafety officers, prevention officers and occupational health practitioners with tools and knowledge that can enhance biological safety in the laboratory. Compared to the previous Flemish survey (2007-2012), the Belgian survey (2007-2012) was also substantively extended with questions related to the incidence of the identified LAIs in Belgium.

[1] Afdeling Preventie: http://www.vlaanderen.be/nl/contact/adressengids/vlaamse-overheid/administratieve-diensten-van-de-vlaamse-overheid/beleidsdomein-welzijn-volksgezondheid-en-gezin/zorg-en-gezondheid/afdeling-preventie ; Former Public Health Surveillance Toezicht Volksgezondheid ; http://www.zorg-en-gezondheid.be/over-ons/contacteer-ons/
[2] see glossary
[3] see glossary
[4] LNE : Departement Leefmilieu, Natuur en Energie ; DGARNE : Direction Générale Agriculture, Ressources naturelles et Environnement ; IBGE-BIM : l'Institut Bruxellois pour la Gestion de l'Environnement - Brussels instituut voor milieubeheer
[5] see glossary

METHODOLOGY

In this study, 206 private companies or public institutions with notified contained use activities[6] have 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 will be hereafter called "survey 1". The mailing list was established using the data available in the database of the SBB that contains all the information and details about the 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=8) and public institutions (n=18) that are active in diagnostic (n=11) and/or R&D sector (n=18) This sections was based on the work they perform with biological agents frequently involved in LAIs. These institutions and companies received an invitation for their personnel to answer an online survey about LAIs. This "personnel–oriented survey" will be 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, French and English and was made accessible for at least 3 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 approximately 50 questions and sub-questions were addressed to each participant, consisting of single answer questions and multi answer questions. Most of the questions were mandatory. More information can be found on URL: http://www.biosafety.be/CU/LAI/Intro_LAI.html.

The invitation (e-mail) for survey 1 provided the respondent 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 has been decided not to contact the personnel directly. Instead, an invitation e-mail with a 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 service providers for prevention and protection at work[7], the "Fund for Occupational Disease"[8], the "Fund for Occupational Incidents"[9], the "Belgian Federal Public Service Employment, Labour and Social Dialogue (well-being at work)"[10], and the regional competent authorities in surveillance of infectious diseases[11] have been asked to provide additional data about notified bio-incidents/infections which took place in biological laboratories.

[6] see glossary
[7] IDEWE, PROVIKMO, SECUREX
[8] Fonds voor arbeidsziekten; http://www.fmp-fbz.fgov.be/web/index.php
[9] Fonds voor arbeidsongevallen; http://www.faofat.fgov.be/
[10] Federale Overheidsdienst Werkgelegenheid, Arbeid en Sociaal Overleg, Welzijn op het werk; http://www.werk.belgie.be/welzijn_op_het_werk.aspx
[11] Flemish Region: http://www.zorg-en-gezondheid.be/meldingsplichtigeinfectieziekten/ ;Brussels-capital Region: http://www.observatbru.be/documents/sante/maladies-transmissibles.xml?lang=nl & http://www.ccc-ggc.irisnet.be/nl/erkende-instellingen/gezondheidzorg/besmettelijke-ziekten; Waloon Region: http://www.sante.cfwb.be/index.php?id=maladiesinfectieuses

2. RESPONSE

CATEGORIZATION OF PARTICIPANTS: TYPE OF INSTALLATIONS AND ACTIVITIES

Table 1 summarizes the types of institutions that were contacted in the 10 Belgian provinces. 119 of the 206 (~58%) invited institutions completed the survey. Of those institutions, 192 people responded to survey 1. In total 110 biosafety officers, 63 prevention officers and 27 occupational health practitioners participated. A number of them (43) were also involved in other functions in the institution, such as researcher, docent, lab responsible or manager.

The participation rates to the survey 1 and 2 are presented in Table 1 and 2 respectively, where the category and location (province) of the responders are mentioned.

Table 1: participation rate for survey 1

		West Flanders	East Flanders	Antwerp	Limburg	Flemish Brabant	Walloon Brabant	Liège	Hainaut	Namur	Luxembourg	Brussels	**Total***
Number of Invitations	Diagnostic institutions**	13	22	23	7	12	6	5	10	5	2	15	**120**
	R&D institutions***	7	16	20	2	15	10	14	13	6	3	20	**126**
	Private companies	16	27	29	7	18	4	5	5	5	0	12	**128**
	Public institutions	3	8	10	2	4	6	10	14	5	4	12	**78**
	Total	**19**	**35**	**39**	**9**	**22**	**10**	**15**	**19**	**10**	**4**	**24**	**206**
Participation rate (%)	Diagnostic institutions**	46	55	57	57	42	100	60	80	40	50	100	**63,5**
	R&D institutions***	57	69	60	0	67	80	36	64	33	67	70	**60**
	Private companies	37	52	59	43	56	100	40	100	40	-	92	**58**
	Public institutions	100	75	60	50	75	67	40	50	40	50	58	**58**

* Some institutions (n=13) do perform diagnostics as well as R&D; ** "Diagnostic" includes also quality control;
*** "R&D" includes also (large scale) production

As discussed above in the methodology, it was also possible to interrogate the personnel of 8 private companies and 18 public institutions that are possibly exposed during diagnostics (n=11) and/or R&D (n=18) to biological agents that are often mentioned in the scientific literature about LAIs.

A total of 873 employees were invited to participate in survey 2. 417 of them (48%) 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 also contact students and animal care takers by forwarding our invitation, only 2 students and 3 veterinarians answered the survey. 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	214	43% (17%-79%)
Research and development institutions	659	55% (10%-100%)
Private companies	180	46% (17% - 83%)
Public institutions	693	46% (13% - 100%)
Total	**873**	

Type of facility/installation

To identify the work environment of people involved in contained use activities in Belgium, we analysed the average containment level of the authorized contained use activities over the last four years (figure 1C). The proportion of the different types of installations, as stipulated in the Belgian legislation on contained use[12] (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 1.

[12] 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 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.

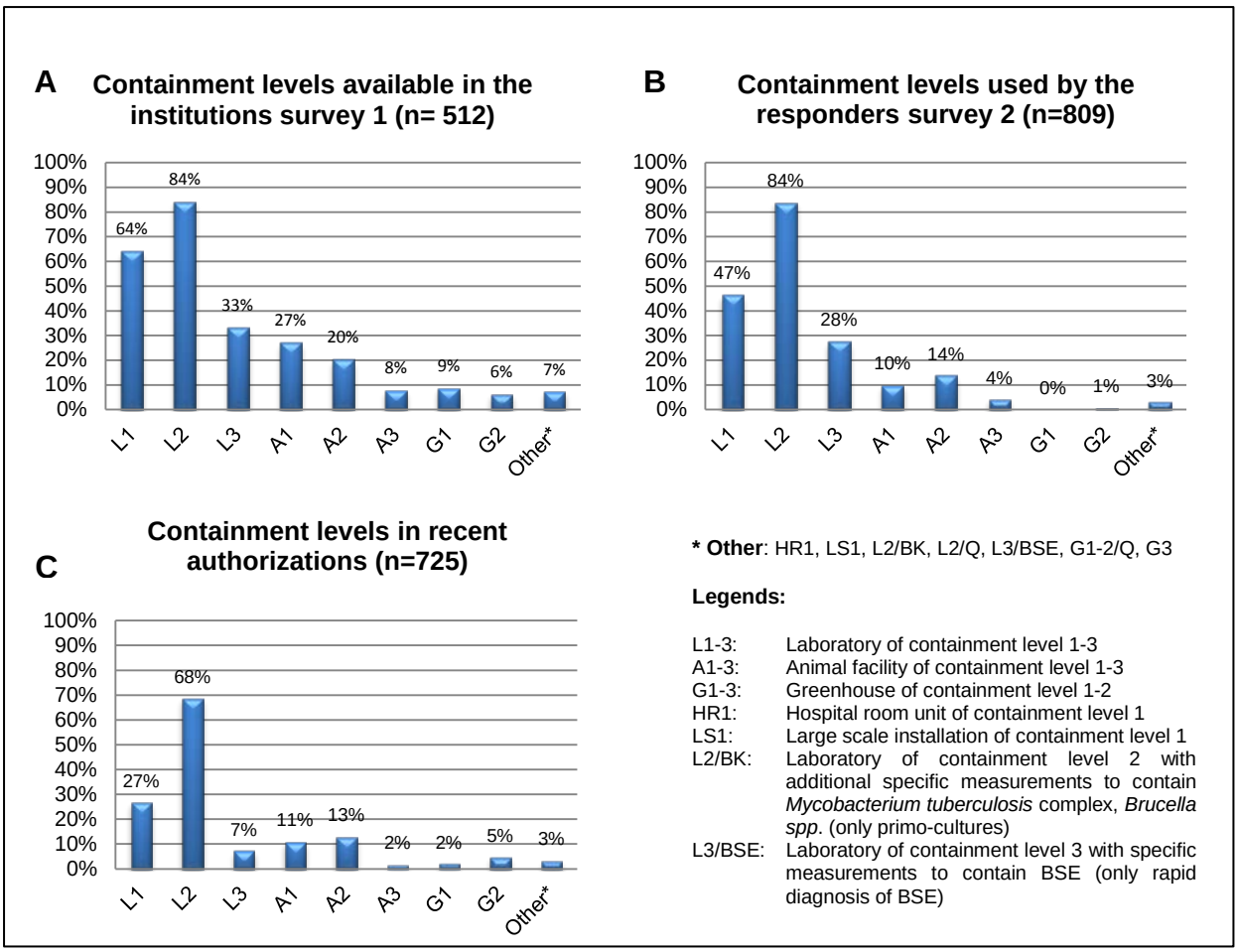


Figure 1: Types of facilities and containment levels available in institutions according the respondents of survey 1 (A); containment levels used by the surveyed personnel of survey 2 (B) and containment levels in recent authorizations in Belgium (2009-2012) (C).

According to recent authorizations in Belgium, the most frequent requested level of containment per authorization in Belgium is mainly containment level 2 (figure 1C).

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

	Participation rate (%) survey 1 (n=192)	Participation rate (%) survey 2 (n=417)
(Bio)medical (Human)	76%	60%
Veterinary (Animals)	9%	39%
Plant research and diagnosis (Plants)	15%	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 (76% and 60% respectively). In survey 1, this was followed by plant research and diagnosis (15%) and veterinary medicine (9%). In survey 2, this order was reversed as 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 was crucial to characterize the type of activity carried out with those biological agents as it determines the risk of exposure.

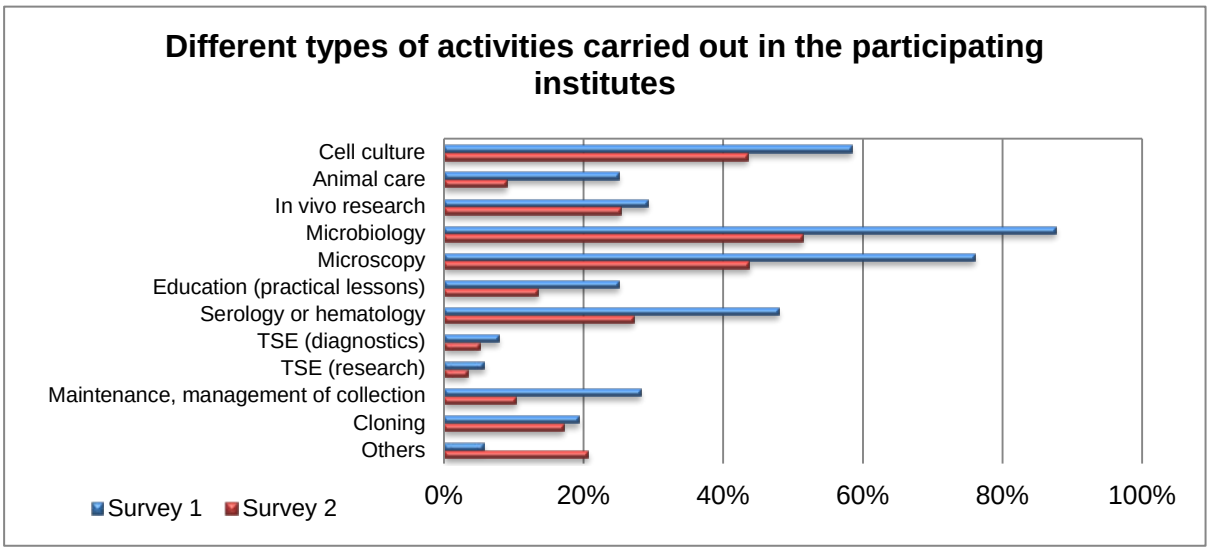


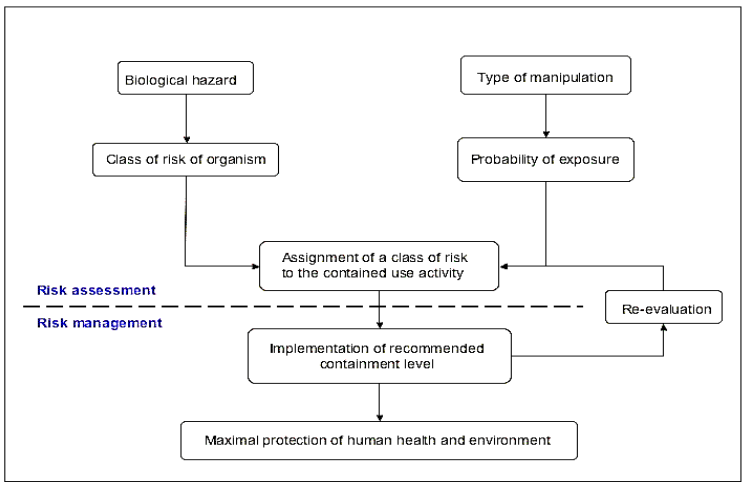
Figure 2: Comparison of the different types of activities carried out by the respondents in survey 1 & 2 (TSE: Transmissible spongiform encephalopathy; Others: dispatching, PCR, microbiology, histology and autopsy)

Figure 2 shows 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 Belgium.

In general, the distribution of the different types of installations in survey 1 is quite similar to the requested containment levels in the Belgian authorizations (figure 1A), suggesting that survey 1 is representative for Belgium. Remarkably, similar patterns are observed in survey 2 for types of activities and installations (figure 1B&C and 2), although survey 2 included personnel from 26 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 genetic modified organism (GMO) or a pathogen. A known risk will therefore lead to the implementation of appropriate management measures. For the risk assessment and management of 'contained use' activities, five successive steps are distinguished, see figure 3.



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 3: 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. 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 bloodborne pathogens, such as human immunodeficiency virus (HIV) or Hepatitis B virus (HBV), which are naturally transmitted by percutaneous or mucosal exposure to infected blood or other body fluids. In the laboratory, an infection with bloodborne pathogens can occur via parenteral inoculation incidents (such as cutting or needle stick incidents) 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) laboratory, 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 laboratory. This is the case when the infected laboratory worker contaminates relatives or other people he comes in contact with. This is called a secondary infection or transmission (see chapter 'LAIs in Belgium').

Generally speaking, in a laboratory setting contamination can take place through four 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, 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 in 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 vectors was given as an example to illustrate which type of answer respondents 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=192)**	**Survey 2 (n=399)**
Adequate (focusing on organism, type of activity, transmission)	14%	13%
Partial (focusing on only 2 risk assessment elements)	52%	32%
Incomplete (focusing on only one risk assessment element)	25%	42%
Answer is out of (biosafety) scope, *e.g.* chemical risks	9%	13%

We received very different answers. The majority of the answers (32%-52%) referred rather to a partial risk assessment, although a minority (13%-14%) spontaneously took into consideration all three main elements of the risk assessment methodology. (In general), this would suggest that biosafety officers, prevention officers and occupational health practitioners are more aware of the biological risks in laboratories compared to the employees, who are actually exposed to the biological agents as a result of their work.

Application of containment measures: the respondents' viewpoint

The assessment of biological risks is based on an empirical basis, following awareness of the risks posed by manipulating biological agents, and must cover quite foreseeable situations (spill, accidents). Behind this awareness, there is a practical part that aims to minimize these risks, which is called 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 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 need to be applied in a laboratory or animal facility of containment level 2 and 3 (figure 4).

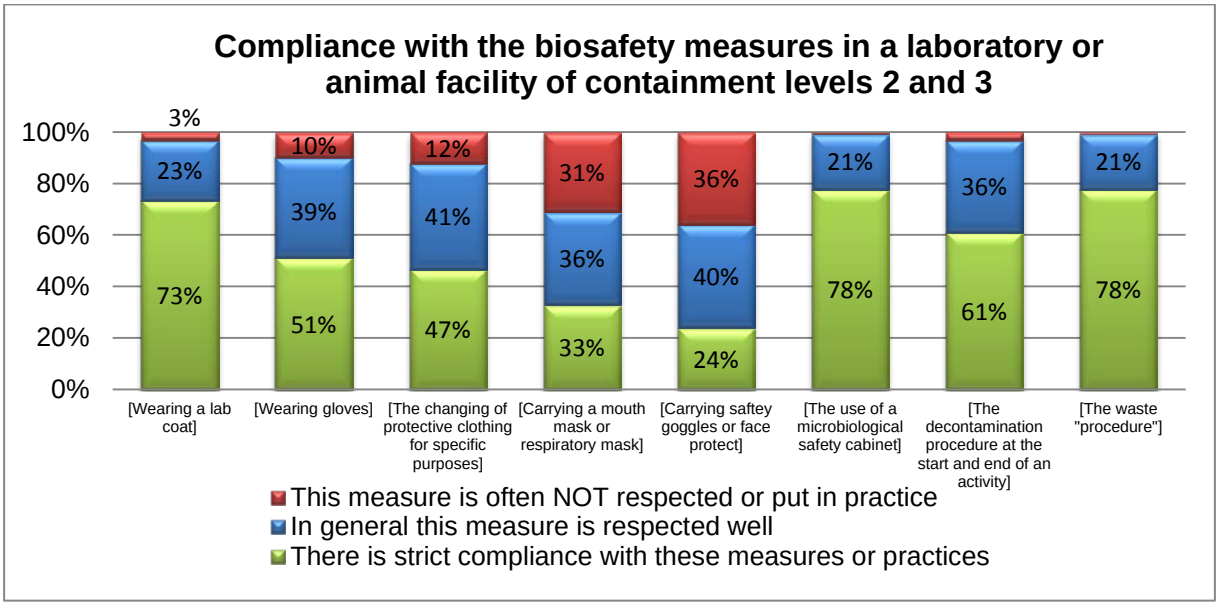


Figure 4: Compliance with biosafety measures in a laboratory or animal facility of containment level 2 or 3

In general, figure 4 suggests there is less compliance when more personal protection equipment has to be adopted to ensure (bio)safety. Where ~75% of the respondents of survey 1 judge that a general protective measure such as wearing a lab coat is respected strictly, only ~50%, ~30 % and ~25% of the respondents also judge this to be the case for wearing gloves, carrying masks (mouth and respiratory protections) and safety goggles respectively.

Appropriate gloves - when properly used - are an important protective barrier when contact with potentially contaminated samples, surfaces or equipment can occur. However, figure 4 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 microbiological safety cabinet (BSC) and the procedures for decontamination and waste management show a higher level of compliance (approximately ~78% - 61% - 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 of biological characteristics (*e.g.* unknown transmission routes, sporulation (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 contained facilities in Belgium. Hence, the respondents of both surveys were asked which types of bio-incidents occurred in their facility within the last 5 years (see figure 5B). Surprisingly, in both surveys only 50% of the participants mentioned one or more incidents during the period 2007-2012. But if we look to the percentage of institutions that have mentioned bio-incidents in survey 2 (~90%), we can assume that 50% is an underestimation and that in almost every institution there happen bio-incidents (figure 5A).

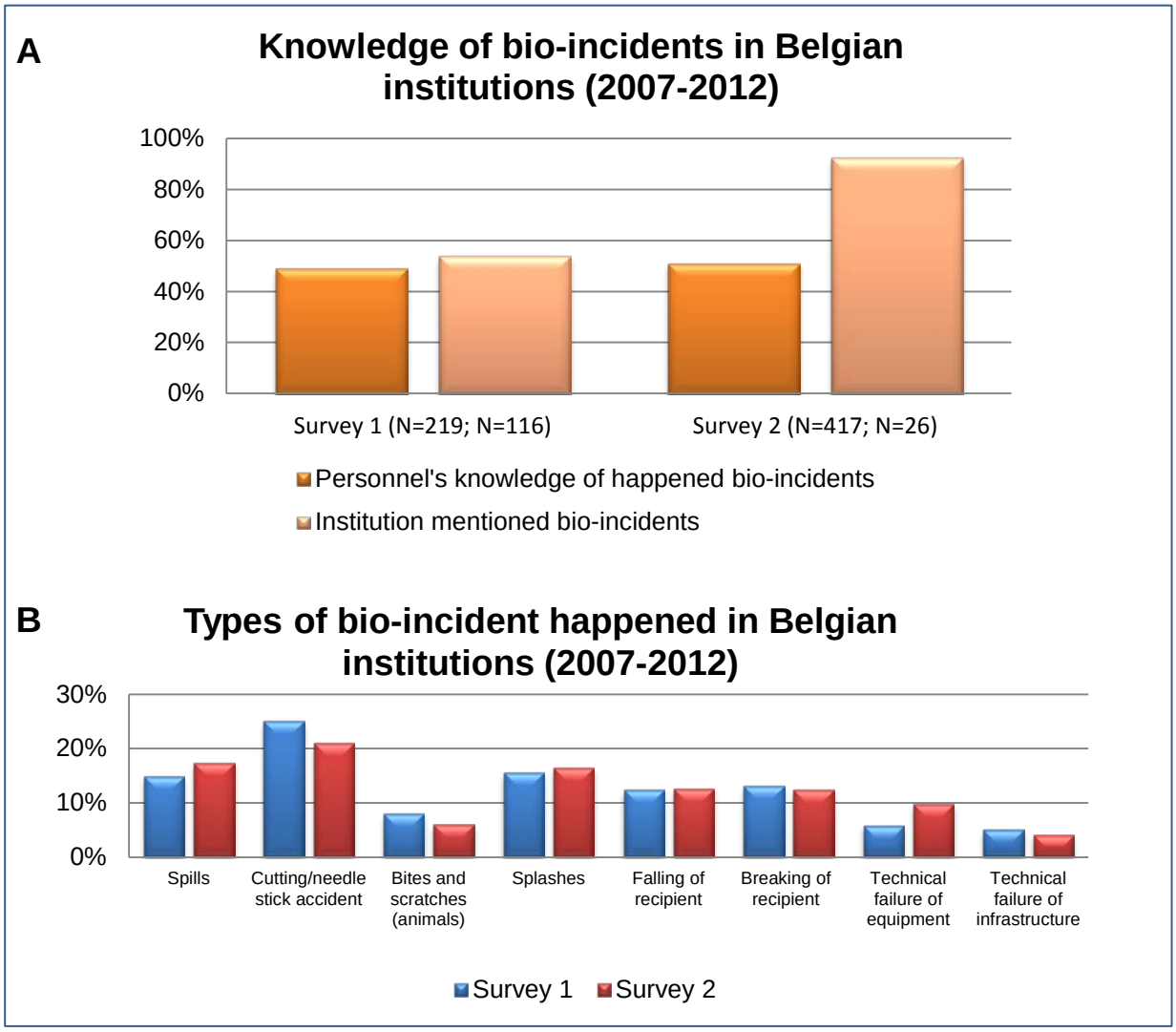


Figure 5: (A) Knowledge of bio-incidents happened in Belgium over the last 5 years (2007-2012) (B) Different types of bio-incidents over the last 5 years in Belgium (%)

In total, survey 1 reported 275 times a certain bio-incident, while survey 2 reported 707 times a certain type 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. Hence figure 5b shows the occurrence/incidence of types of bio-incidents that happened in Belgian institutions between 2007 and 2012. However, extrapolating the data of survey 1 of only the cases with exact quantification (44% of the cases; N=27) gives probably a more realistic view of the amount of different bio-incidents in Belgium over one year, as shown in figure 5c.

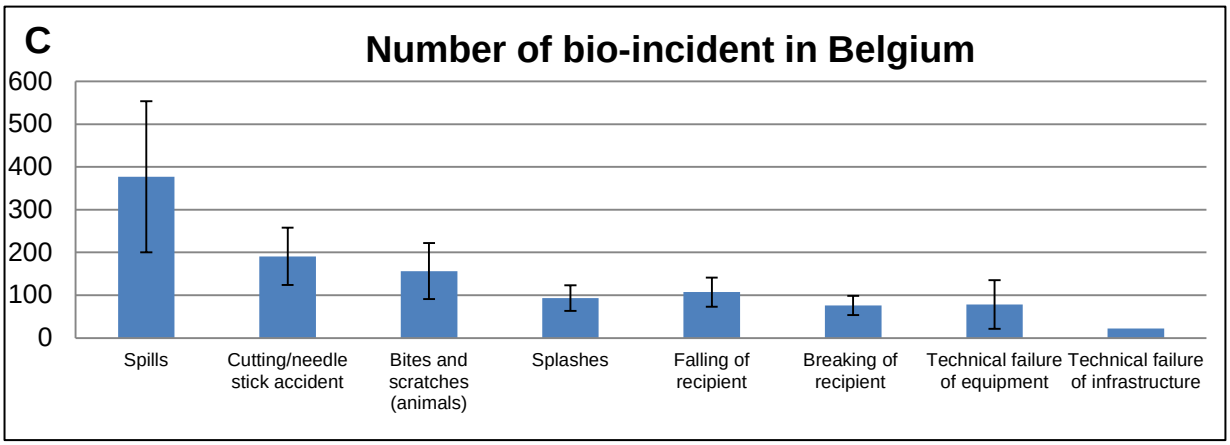


Figure 5C: Number of bio-incidents in Belgium on a yearly basis (extrapolation)

Regarding this information, spills are the most frequently reported bio-incidents, followed by cutting/needle stick incidents, animal bites and scratches. Falling and breaking of a recipient and splashes, or bio-incidents due to technical failure (equipment or infrastructure) occur less frequently. The biggest part of bio-incidents is related to human errors (91%), while only a small number of bio-incidents (9%) is the result of a technical failure.

In the context of risk management, it could be useful to look further 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 6.

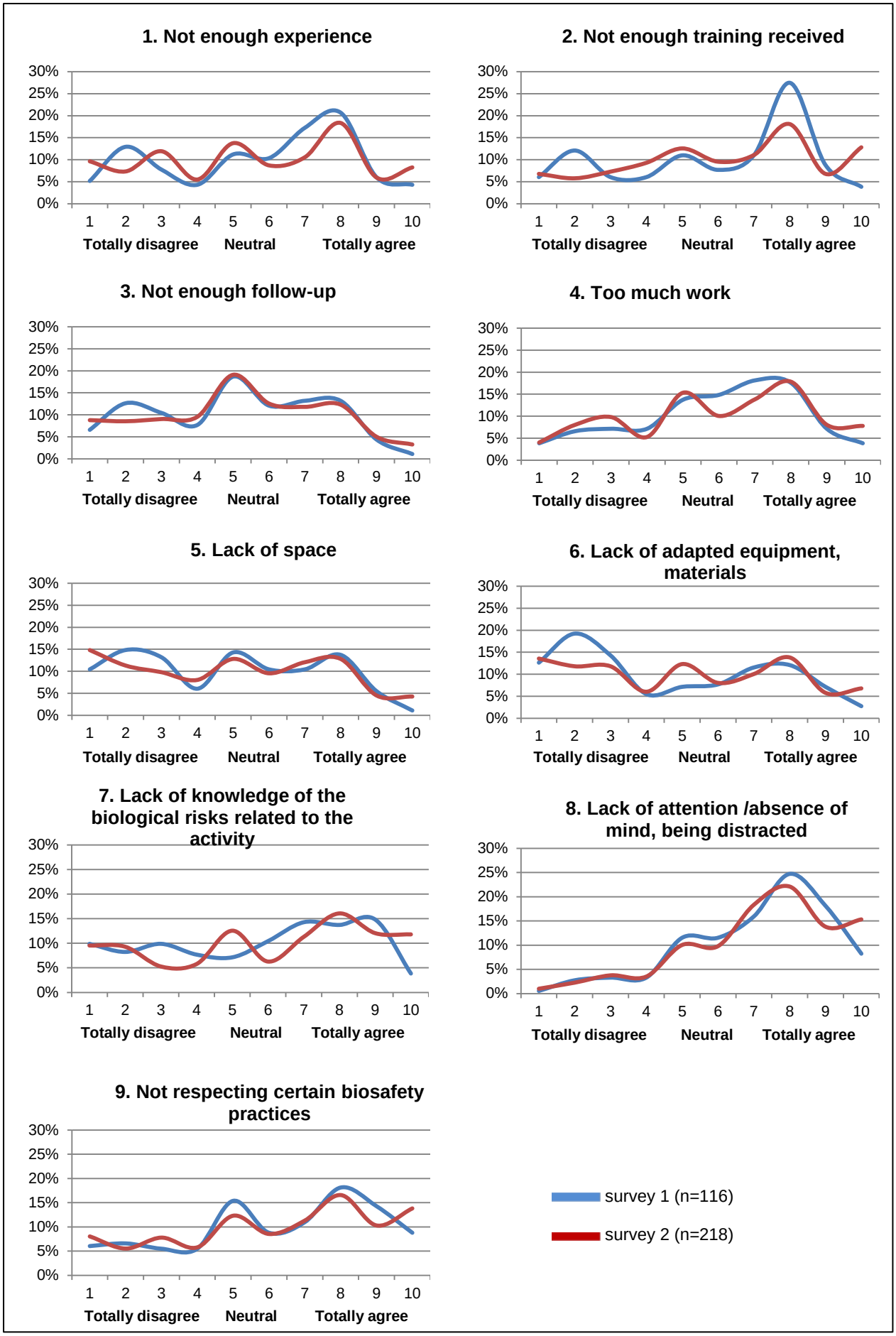


Figure 6: Overview of the perception of the respondents (%) as regards the rationale of bio-incidents.

These underlying causes of bio-incidents can be divided in three 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 and 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 6.1) and the occupational and human factors that may come with the job, such as absent-mindedness (figure 6.8) or non-respect of certain biosafety practices (figure 6.9) lead to more bio-incidents. Factors like the lack of appropriate training (figure 6.2), the follow-up of the personnel (figure 6.3), high workload (figure 6.4), a lack of knowledge (figure 6.7) or non-respect of certain biosafety practices (figure 6.9) have no conclusive pro or contra. Accordingly, the lack of space (figure 6.5) as well as the lack of well adapted equipment or materials (figure 6.6) seem not really problematic.

Also, there seems to be no important differences in perception between the two groups of respondents.

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 (mainly in the US), 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 and Sulkin's surveys (5-7) 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	Class of risk[13]	Number of LAI cases (%)
Brucella spp.	3	423 (11%)
Coxiella burnetii	3	278 (7%)
Salmonella typhi	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 (or risk group 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. Furthermore, 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, reported or diagnosed and therefore unknown.

Currently, many laboratory infection 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) (8, 9). Another LAI survey from the UK showed a predominance of gastrointestinal infections, with most of them having occurred in

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

microbiology laboratories (10). According to Sewell (2000), the most common organisms causing LAIs 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 (11). The survey of Baron & Miller (2008) identified the bacteria *Shigella,* followed by *Brucella*, *Salmonella* and *Staphylococcus aureus* as the main causes of LAIs (12, 12). 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 (12, 13).

Besides these published and 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.* ProMED-mail). 47 of these reports were selected for further review. In total, 309 LAIs are analysed, see table 6 and annex 1 (analysis).

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

Biologic agent	Class of risk[14]	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 difficile	2	2 (1%)
Chlamydophila psittaci (avian strain)	3	1 (<1%)
Cowpox virus	2	1 (<1%)
Dengue virus	3	1 (<1%)
Leptospirosis bacteria	2	1 (<1%)
Severe Acute Respiratory Syndrome (SARS) Coronavirus	3	1 (<1%)
Shigella sonnei	2	1 (<1%)

(*) : Pathogens of risk class 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 methodologies/methods of analysis (literature analysis versus survey data analysis), geographical focus (worldwide versus mainly US), 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).

[14] 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 reported LAI cases came from surveys conducted in microbiological laboratories. Nevertheless, LAIs happen in laboratories as well as in animal facilities, R&D or production facilities. Interestingly, although the precise route of exposure (transmission route) 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 (direct) contact (6%) (see chart figure 7). 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 7).

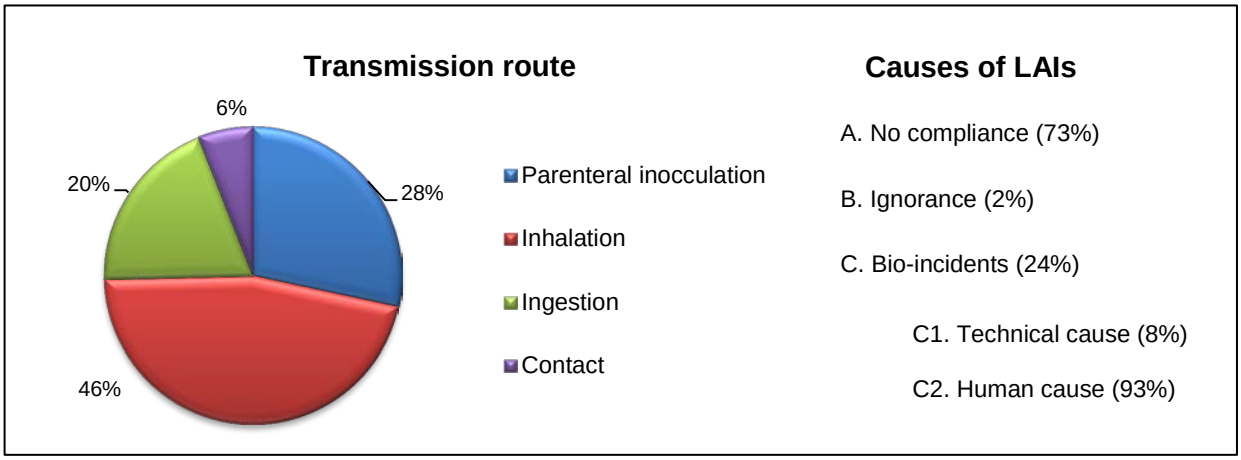


Figure 7: Recent laboratory-acquired infections (LAIs) worldwide (2000-2012): routes of exposure (chart) & causes of LAIs (based on recent literature)

Although LAIs still exist today, several studies suggest a gradual decline in the number of LAIs during the last 50 years (5, 13). Possible reasons for this apparent decrease could be:

1. an increased awareness in the scientific community and the adoption of several biosafety legislations (including workers protection);
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 and safety devices (L3-laboratories with negative air pressure, use of biosafety equipment like BSCs, 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 the Belgian Biosafety Professionals (BBP), an organisation that was created in 2006 as the Belgian section of EBSA.
5. the legal requirement to appoint a “biosafety officer”

LAIs IN BELGIUM

In Belgium, three cases have been published since 2000. They describe laboratory acquired infections with *Mycobacterium kansasii* (2005), *Shigella sonnei* (2006) and *Chlamydophila psittaci* (2009) (14-16). 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 two LAIs were identified and were caused by the bacteria *Brucella melitensis* and *Listeria monocytogenes*.

In the current survey, we observed a response rate of ~50%, which corresponds to 417 respondents. In total 76 respondents reported 140 LAIs that happened within the last five years in Belgium (2007-2012). Caution should be taken when interpreting these results because an infection in a particular context could be misinterpreted as a LAI by the participant (*e.g.* infection of a lab worker during sampling in the field, outside the lab or a natural infection of a lab worker with *Mycobacterium tuberculosis* in a tuberculosis high burden country should not be considered as laboratory-acquired infections) or have been mentioned more than once as several employees working in the same institutions participated in the same survey. So for this study we assumed that different people could have mentioned the same LAI cases. These as well as the presumptive misinterpretations were filtered out. Because the questionnaire was filled in anonymously, the filtering was done 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 75 to 94 distinct LAIs (survey 1: 26; survey 2: 68) that had been caused by 21 different pathogenic organisms (survey 1: 10 different pathogens; survey 2: 18) (see table 7).

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

Organism	Risk class[15]	Survey 1	Survey 2	Min.-Max. Total
		Number of LAI cases		
Salmonella (*)	2-3	4	17	17- 21 (23-22%)
Mycobacterium turberculosis complex (*)	3	3	12	12-15 (16%)
Brucella (*)	3	5	5	6-10 (8-11%)
Trypanosoma brucei gambiense	2	1	5	5-6 (7-6%)
Dermatophyte (Trichophyton verrucosum, Microsporum canis)	2	1	4	4-5 (5-5%)
Shigella ([$])	2-3	4		4 (5-4%)
Coxiella burnetii (*)	2		3	3 (4-3%)
Mycoplasma	2		2	2 (3-2%)
Herpes virus	2		2	2 (3-2%)
Campylobacter	2	2	2	1-2 (1-2%)
Hepatitis B virus	3	1	1	1-2 (1-2%)
Parvovirus B19	2	1		1 (1%)
Human immunodeficiency virus (HIV)	3		1	1 (1%)
Rubella virus	2		1	1 (1%)
Avian Influenza virus (*)	2		1	1 (1%)
BCG bacteria (Bacillus Calmette Guérin)	2	1		1 (1%)
Toxoplasma gondii	2		1	1 (1%)
Bartonella	2-3	1		1 (1%)
Rabies virus (*)	3		1	1 (1%)
Recombinant viral vector	?		1	1 (1%)
Listeria	2		1	1 (1%)
Unknown	?	4	8	8-12 (11-13%)
Total		**26**	**68**	**75-94**

*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)

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, dermatophytosis and brucellosis), see annex 1. 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 (historical) involvement in research on tropical diseases and participation in several international projects as a heritage of its colonial past.

Bacterial infections predominate with 48-60 cases (72-73%), followed by viral infections (9-10 cases 12-13%), infections with parasites (6-7 cases, 9%) and 4 to 5 fungal infections (6%) (see figure 8). Remarkable, 75% of the cases is related to the three major groups of pathogens often involved in LAIs, namely enteric, airborne and bloodborne pathogens. Infections with enteric pathogens (*e.g. Salmonella* spp., *Campylobacter* spp. and *Shigella spp.)* or with bloodborne pathogens (*e.g.* HIV, Brucella spp., Trypanosoma spp., …) seem to be the most frequently reported laboratory-associated

[15] Risk classes for humans as based on the Belgian risk classifications of micro-organisms, http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/index-eng.php

infections in Belgium with respectively 19 to 24 and 20 to 23 reported cases, followed by 10 to 20 cases of infections with airborne pathogens, such as *Mycobacterium tuberculosis* or *Coxiella burnetii* (see figure 8).

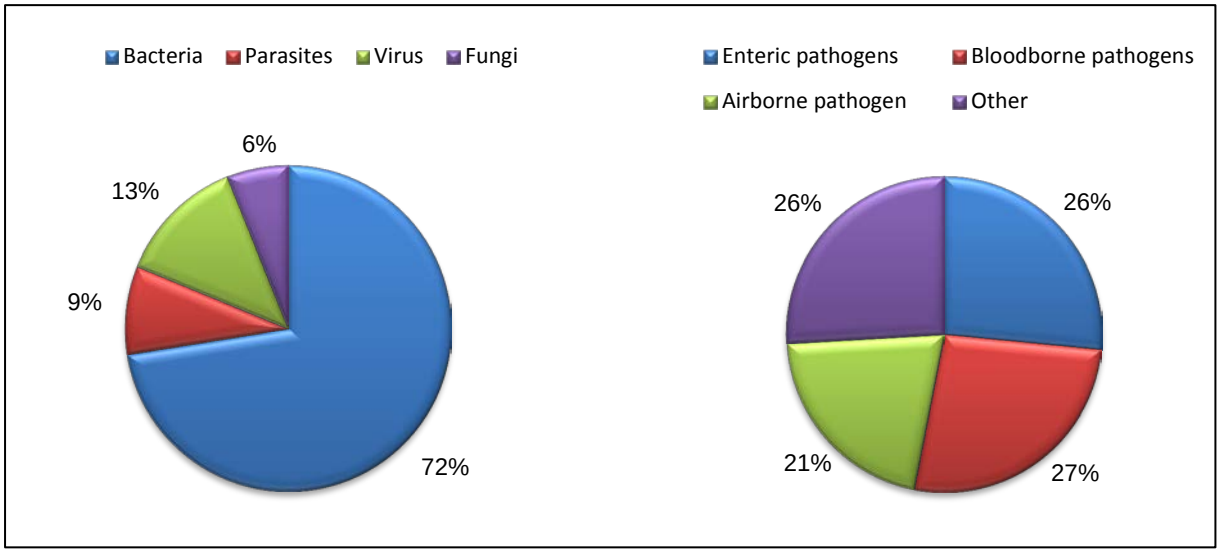


Figure 8: Summary graphs of laboratory acquired infections reported in survey 1 and survey 2.

Another remarkable observation is that in 60% of the cases the way the infection occurred remains unknown, especially in case of airborne and enteric pathogens (see figure 9).

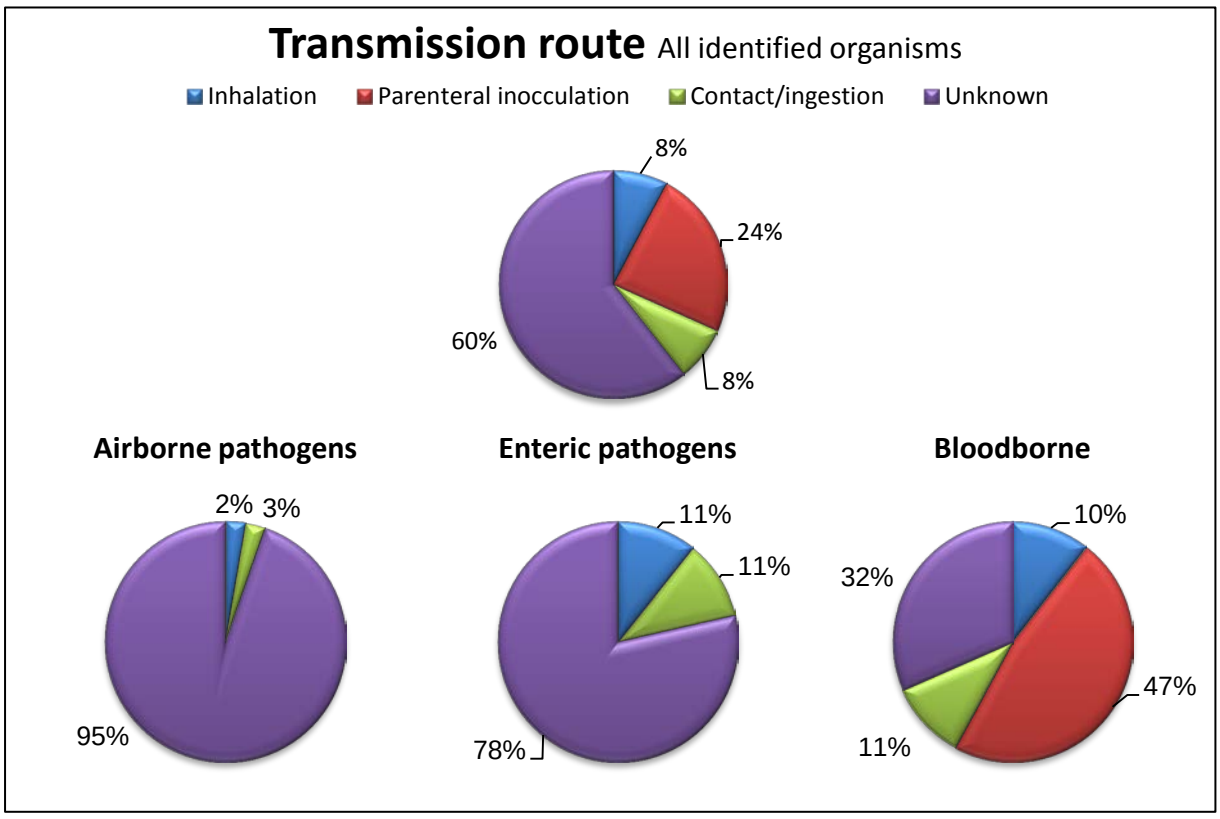


Figure 9: Detailed analysis of transmission routes of the identified LAIs with three major groups of pathogens often involved in LAIs, namely airborne, enteric and bloodborne pathogens

It appears that the origin of infection in the majority of the infection cases with bloodborne pathogens is known (~70%), while for airborne and enteric pathogens, respectively 78% and 95%, the way of how infection occurred could not be indicated.

Next figures summarize the responses to questions concerning the circumstances in which the LAIs occurred (see figure 10). Figures 10.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?

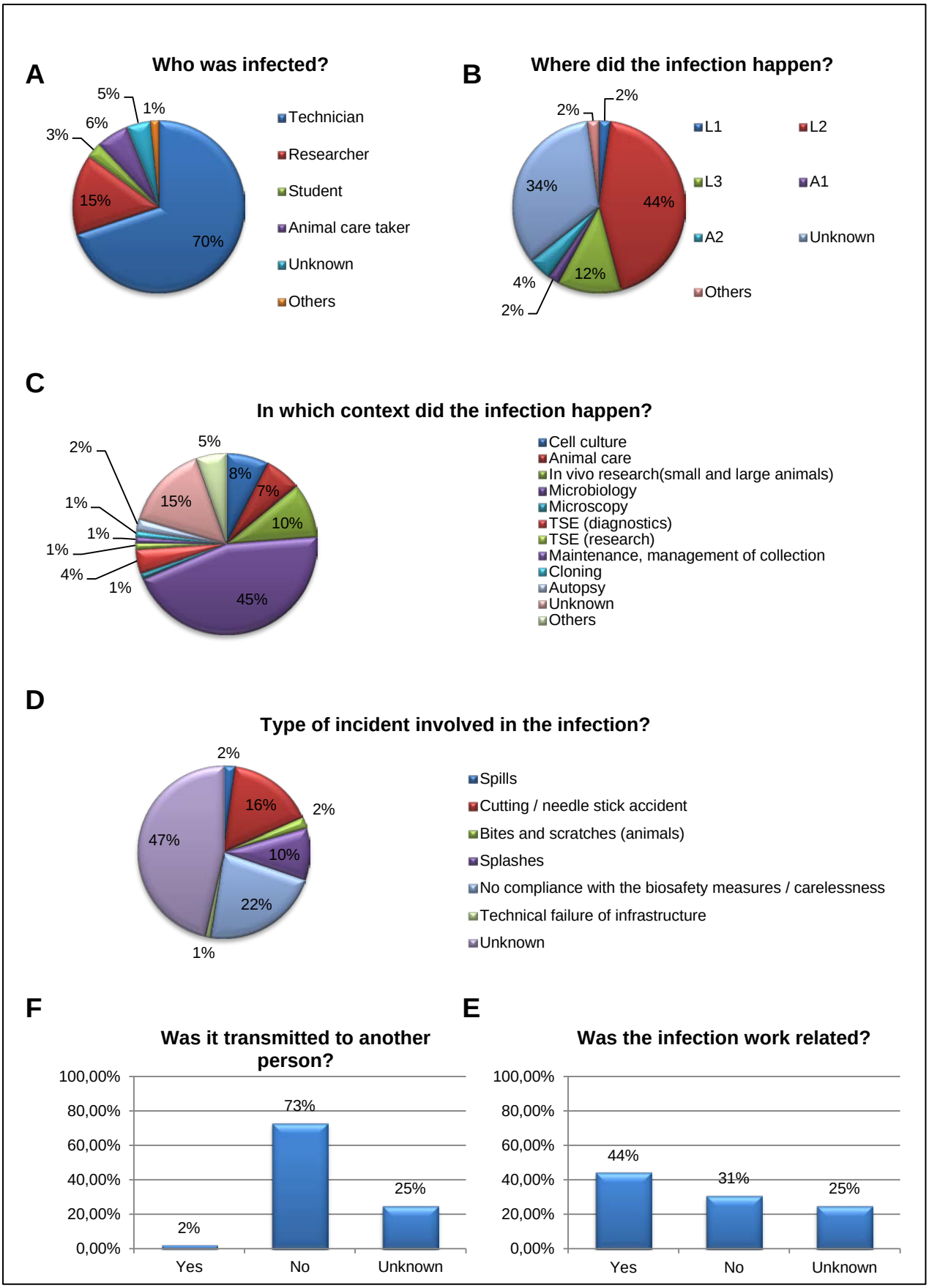


Figure 10: Summary of the responses to questions concerning the circumstances in which the LAIs occurred

In the majority of the cases (70%), a technician was infected whereas in only 15% 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).
In the supplementary survey concerning incidence (see 'LAI incidence in Belgium') we asked for the occupancy in the different types of the facilities. On average, the ratio technician / researcher is 3.5, suggesting a less pronounced difference of 17.5% between technicians and researchers.

A majority (45%) of the described LAIs occurred in the context of microbiology activities, followed by *in vivo* research (10%) and animal care (7%). 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 (two times *Salmonella spp*., *Trichopyton verrucosum* and *Trypanosoma brucei gambiense* and one unknown) (see figure 10C).

Another observation is that a ~45% of the LAIs occurred in a laboratory of containment level 2 (and not in containment level 3) (figure 10B). This is probably due to a higher number of facilities of containment level 2 in Belgium compared to containment level 3 facilities (see figure 1). Some LAIs (nine in total) caused by risk class 3 organisms were reported to have originated from activities in L2 laboratories, which can be explained by the fact that, in Belgian laboratories, primo-isolation[16] of *Mycobacterium tuberculosis* and *Brucella spp.* can be performed in L2 laboratories with L3 work practices.

An important observation is that only 45% of the LAIs were actually proven to have originated in the laboratory and that in 47% 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 (98%), mainly by splashes, needle sticks and/or cutting accidents. Technical failures accounted for approximately 2 % of the cases (one case identified).

In survey 1, disability periods (sick leave) were mentioned for 11 of the 16 reported LAIs. In total 74 days of sick leave were mentioned. This suggests an average of ~7 +/- 2 days of sick leave per LAI.

Although 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, only one case of person-to-person transmission has been mentioned in both surveys. It was a *Brucella* case and although person-to-person transmission of brucellosis is extremely rare, it can occur through direct blood contact with an infected person (sexually or in a clinical setting), congenital (during pregnancy, delivery or via breast milk) (17-19)

[16] 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)

LAI INCIDENCE IN BELGIUM

With the support of the Flemish Agency for Care and Health, Department Prevention, and the Flemish, Walloon and Brussels-capital Regions (LNE, DGARNE & IBGE-BIM[17]) the SBB worked out in 2014 a supplementary survey concerning the incidence of certain LAIs in Belgium.

Incidence is a measure of risk of developing some new conditions within a specified period of time. In this context: the number of LAIs (for a certain organism) during a fixed period of time (R&D) or for a fixed amount of positive diagnostic samples.

To calculate the incidence, the institutions that participated in the initial survey were invited once more to estimate/quantify the time of manipulation of the identified organisms responsible for LAIs in the institution. Although we invited the selected institutions several times to participate, the participation rate was considerable lower than in the first survey in Belgium. In total, we could collect data from only 26 of 118 invited institutions (22%). One possible reason for this low participation rate is the difficulty to answer this question. Not every institution is following up the exposure of the personnel to biological agents. Some institutions considered these data too large for regular update, while others (mainly large institutions) could provide detailed information (see tables 8 and 9).

Table 8: LAI Incidence during R&D in Belgium (2007-2012)

	# LAI / 1000 hours of manipulation			
	Technicians	**Researchers**	**Students**	N-value
Shigella	6.295			3
Salmonella	1.820		0.022	6
Brucella	0.593	0.017		1
Herpes virus	0.367			2
Trypanosoma brucei gambiense		0.353		1
Campylobacter	0.212			2
Avian Influenza virus	0.196			1
Recombinant viral vector		0.006		4

[17] LNE : Departement Leefmilieu, Natuur en Energie ; DGARNE : Direction Générale Agriculture, Ressources naturelles et Environnement ; IBGE-BIM : l'Institut Bruxellois pour la Gestion de l'Environnement - Brussels instituut voor milieubeheer

Table 9: LAI Incidence during diagnostics in Belgium (2007-2012)

	# LAI / 1000 positive samples	N-value
BCG (Bacillus Calmette Guérin)	44.068	1
Mycobacterium tuberculosis	13.916	4
HIV	8.814	3
Salmonella	3.503	8
Shigella	2.988	6
Dermatophyte	1.944	9
Mycoplasma spp.	0.801	2
Campylobacter spp.	0.045	6

Although we cannot expect incidence values of zero, it is expected that the implemented biosafety measures to prevent exposure to hazardous biological material level the differences in the intrinsic biological risk of different organisms during manipulation, hence it is expected that each type of organism has more or less the same incidence number.

An obvious deviation from the mean (incidence number) suggests incompleteness in risk management, *e.g.* no compliance to required biosafety measures, ignorance of biological intrinsic characteristics of the organism, no good laboratory practices & techniques, …

Table 9 shows that diagnostic analysis of *Mycobacterium tuberculosis* has an obvious higher LAI incidence number compared to *e.g. Salmonella* and *Shigella*, suggesting that the risk management during diagnostic analyses of samples of *Mycobacterium tuberculosis* is less well observed compared to diagnostic analysis of *Salmonella* or *Shigella.*

These preliminary data show an interesting path to investigate in the future. When more data become available about time of manipulation or diagnostics of biological agents, it should be possible to provide a complete well substantiated ranking of incidence numbers of each identified LAI, which would allow us to identify obvious deviations from the mean incidence. These outliners might be subject to re-evaluation of risk assessment and management.

BIOSAFETY OFFICERS IN BELGIUM

Next to the questions related to incidence there were some specific questions addressed to the biosafety officers regarding their job. It is the role of a biosafety officer to ensure containment of activities with biological agents in the laboratories and to minimize the likelihood of exposure to biological agents of laboratory workers, other personnel in the building, their families, the community-at-large and the environment. Biosafety officers motivate the personnel exposed to biological agents to follow and adhere to safe laboratory practice recommendations (20). This role cannot be underestimated, hence we explored their employment, the time of service (seniority) in Belgian institutions and its possible impact on the general biosafety culture.

In Belgium, the mean seniority of the questioned biosafety officers is 9 years and 4 months +/- 1 year and 7 months (N=26), while their employment is only 15%+/-2,8% of a full-time equivalent (FTE) (N=25). Furthermore, we attempted to correlate FTE to collected data relating to the general biosafety culture in the institution, the size of the institution or the mandatory tasks of a biosafety officer that are listed in the legislation. This showed us that a significant correlation could only be observed between FTE and the size of the institutions (see figure 11).

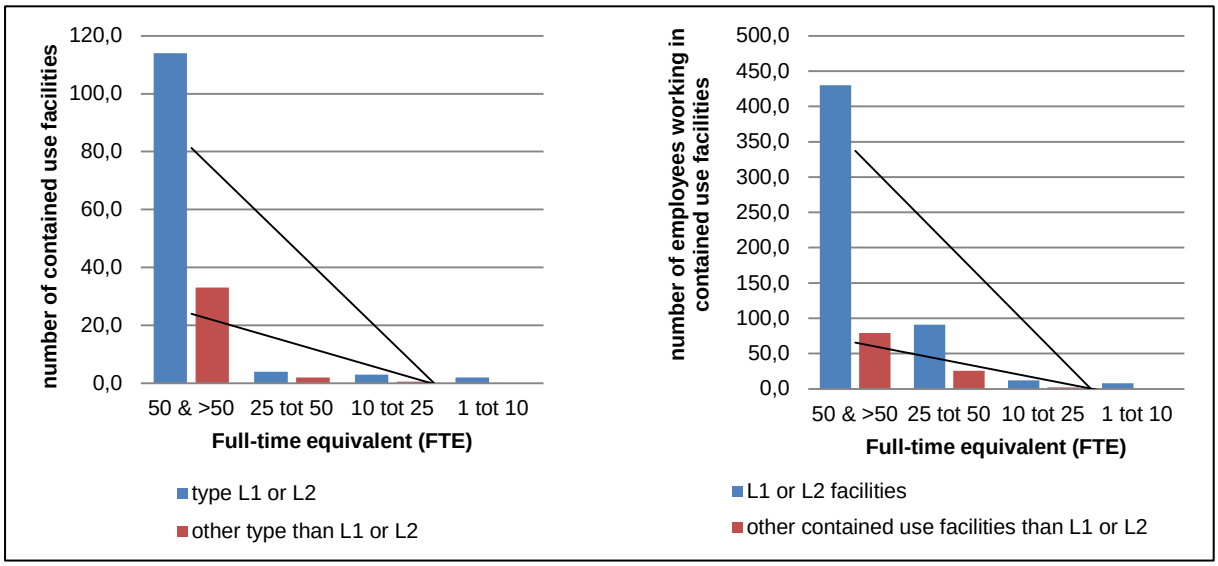


Figure 11: Correlation graphs between full time equivalent (FTE) and number of contained use facilities (left) or number of employees working in contained use facilities (right).

5. DISCUSSION

Five years after the first survey on LAIs (laboratory acquired infections) in Flanders in 2007 (1), the SBB realised a new although similar investigation first in Flanders and finally extended to the whole of Belgium with a focus on the period 2007-2012. A relatively high participation was observed as approximately 55% (survey 1[18]) and 48% (survey 2[19]) of the contacted people responded to both biosafety surveys and indicates the importance of this topic.

This allows to draw a representative picture of the occurrence of LAIs in Belgian laboratories. However, it is important to note that this report is the result of analyses of survey data, which are subject to bias. Nevertheless, many findings of this survey correspond to what is described in the literature and case reports on LAIs and bio-incidents worldwide.

Moreover, the high number of duplicates in the answers to the additional questions linked to the reported LAIs supports the reliability of the reported LAI cases. The surveys identified 75 to 94 LAIs, caused by 22 different organisms (see table 7). They consisted of 48 to 60 bacterial, 9 to 10 viral, 6 to 7 parasitic and 4 to 5 fungal infections. In 8 to 12 cases the organism is unknown. Approximately 74% of the cases belongs to the three major groups of pathogens often involved in LAIs, namely enteric, airborne and bloodborne pathogens (see figure 8). Remarkable are the observed differences between the LAIs reported in survey 1 and 2. While 25 of the 26 institutions in survey 2 also participated in survey 1, the respondents in survey 1 mentioned only 26 LAIs compared to 68 LAIs in survey 2. Moreover, survey 2 mentioned 11 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 LAIs have to be considered as 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 regional competent authorities in surveillance of infectious diseases[20];
(5) external and independent (neutral) services for prevention and protection on the work floor, *e.g.* Securex, Provikmo, Idewe.

[18] survey to the attention of the biosafety officers, prevention officers and occupational health practitioners
[19] survey to the attention of the personnel
[20] Flanders: http://www.zorg-en-gezondheid.be/meldingsplichtigeinfectieziekten/ ;Brussels-capital region: http://www.observatbru.be/documents/sante/maladies-transmissibles.xml?lang=nl & http://www.ccc-ggc.irisnet.be/nl/erkende-instellingen/gezondheidzorg/besmettelijke-ziekten; Waloon region: http://www.sante.cfwb.be/index.php?id=maladiesinfectieuses

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 agents in Belgium (for the period 2008-2011). The majority of the cases was related to hospital acquired infections and only four of these incidents appeared to be the result of a bio-incident in laboratory setting (2 spill incidents, 1 inhalation incident and 1 needle 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 1995-2010 had been officially recognized as occupational diseases (see table 10).

Table 10: 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[21]
1995	Dutch	Salmonellosis	*Salmonella*	2
1997	Dutch	Tuberculosis	*Mycobacterium tuberculosis*	3
1998	Dutch	Salmonellosis	*Salmonella*	2
1999	Dutch	Hepatitis	Hepatitis C virus	3(*)
1999	Dutch	Tuberculosis	*Mycobacterium tuberculosis*	3
1999	French	HIV	HIV	3(*)
2000	French	Mycoplasmosis	*Mycoplasmose*	2
2000	French	Meningitis	*Neisseria menigitidis*	2
2001	Dutch	Salmonellosis	*Salmonella*	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*	2
2005	French	Tuberculosis	*Mycobacterium tuberculosis*	3
2005	Dutch	Tuberculosis	*Mycobacterium tuberculosis*	3
2006	French	Salmonellosis	*Salmonella*	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

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

Surprisingly, for the period 2007-2012, which corresponds with the surveyed time interval, only two cases of infection with Epstein-Barr virus and two cases of infection with *Mycobacterium tuberculosis* have been reported to the Fund for Occupational Diseases. However, no Epstein-Barr virus that caused a LAI has been mentioned by one of the participants, hence only 2 of the 75 to 94 LAIs mentioned in either survey 1 or 2 could be found in this list, namely two cases of tuberculosis.
Despite the occurrence of several LAIs in Belgium (data in our survey could suggest approximately 15 to 20 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[22] concerning the protection of workers from risks related to exposure to biological agents at work, requires the notification of any accident or incident which may have resulted in the release of a biological agent and which can cause serious illness or infection in humans. These have to be notified to the regional offices of the 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 40% 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-fourth of the institutions (n=15) was acquainted with this Royal Decision. Nevertheless, in contrast to this, all four institutions that claimed to know this Royal Decree described one or more LAIs 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, 14 LAIs were mentioned by biosafety officers, whereas 8 LAIs were mentioned by prevention officers and 7 by occupational health practitioners.

All the above mentioned findings suggest 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 (21, 22).

[22] see glossary

The first people to communicate bio-incidents are, of course, the personnel working in the laboratories. Survey 1 and 2 revealed that, although 81% of the institutions report an internal procedure for dealing with a bio-incident, only in 65% of the 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 29 % of the cases (survey 2). When a bio-incident is not notified, 66% of the bio-incidents was judged not severe enough to report and mitigation actions (*e.g.* decontamination) were considered adequate to cope with the incident (survey 2). Some respondents of survey 1 indicated unawareness of the personnel and the administrative burden to be important barriers to report.

When the incident is spontaneously notified, it is usually first told to the lab responsible (83%). In 69% of the cases the biosafety officer will be informed, followed by the occupational health practitioner (57%) and prevention officer (44%). Remarkably, only in 32% of the cases colleagues are informed. 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. 93 % of the institutions (survey 1) mentioned specific procedures to clean up a biological spill, 74 % of the respondents to survey 2 knew about such a procedure and approximately 69% of the institutions (survey 1) made a spill kit available[23]. The majority (~50%) 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 40 % 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 happens 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, almost a half of the respondents (~45%) is not fully familiar with risk assessment (see table 4) and approximately 44% agrees this is a possible reason of bio-incidents (figure 6.7)

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

Remarkably, 60% of the way the infection occurred could not be identified (see figure 9), suggesting that failures of biosafety (procedures or containment) were not noticed or that the knowledge of biological risks during the manipulation has been insufficient, especially in case of enteric and airborne pathogens where the infection route could not be indicated. Hence, it is reasonable to assume that this type of pathogens is more often involved in unnoticed infections, while other LAIs have obvious causes such as needle stick injuries (bloodborne pathogens).

Similarly, in approximately 47% of the reported LAI cases the type of incident prior to the infection is unknown (see figure 10D). When the cause of the LAI is known, human error accounts for 98% of the underlying causes, while technical failures are apparently less common (~2%). It seems that in approximately 40% of the cases, no compliance with biosafety measures and careless handling are at the root of LAIs, while needle stick or cutting and splashes accidents representing the respectively 30% and 18% of causes of the LAIs identified in survey 1 and 2. Important parameters that seem to play a role as underlying cause of an incident are: lack of experience, lack of training, lack of knowledge (awareness) and absent-mindedness (see figure 6).

Given the fact that bio-incidents and LAIs are not always avoidable, biosafety measures are implemented to protect the personnel against exposure to biological agents. Nevertheless, a certain decline in compliance was observed when specific measures become more stringent (figure 4). Moreover, the lack of compliance with biosafety measures was clearly identified by the respondents as an important factor causing bio-incidents (figure 6.9). Also, in the literature the lack of compliance with biosafety measures is described as one of the most important causes of LAIs (see figure 7). General unawareness due to inappropriate risk assessment or inappropriate communication of the risks to the personnel and/or management and/or discomfort when wearing additional personal protective equipment (PPE), such as goggles and masks, are possible causes of this observed non-compliance.

Although each institution is legally required to appoint a biosafety officer, which is expected to motivate the personnel to comply with the required biosafety measures to minimize the likelihood of exposure to biological agents of themselves, their relatives and the community, there is always an institution specific biosafety culture that has grown over the years. Moreover, when the biosafety officer has no full recognition or limited time and resources to build a well substantiate biosafety program, this cannot be established in an optimal way. Hence, the personnel is not always fully aware of all biological occupational risks and is not convinced to comply with all biosafety rules. The facts that 1) on the average only 0.15 FTE biosafety officer is employed, 2) the personnel is not complying with the rules and has the feeling there is not enough training or full biological risk awareness, suggests room for improvement.

Because the majority of LAIs seems to have no identified direct cause (way of infection is unknown), the analysis of the rationale of the LAIs is often complex. A comparative approach of incidence numbers (relative risks) can come towards this problem.

Although the initial participation rate was high, the response on the supplementary survey concerning the incidence number of the identified LAIs was low (~22%). Nevertheless, it was possible to calculate incidence numbers of some LAIs in specific settings, *e.g.* diagnostics, R&D technician or researcher. When going into detail on some calculated incidence numbers with a N-value higher than 4, such as for the organisms *Mycobacterium tuberculosis, Shigella spp* and *Salmonella spp* during diagnostics (see table 9), it has to be mentioned that there is a significantly higher risk for a LAI during diagnostics on *Mycobacterium tuberculosis* samples compared to diagnostics on samples of *Salmonella or Shigella spp*. Although it is obvious that the risk to get infected with *Mycobacterium tuberculosis* is higher than with *Salmonella* or *Shigella bacteria,* due to the intrinsic biological characteristics of *Mycobacterium tuberculosis* (such as the aerogenic transmission route, low infectious dose, …), it is expected that the implemented biosafety measures to prevent infection level out these differences in intrinsic biological risks.

The observed obvious difference in incidence number between *Mycobacterium tuberculosis* and the enterobacteria, *Shigella spp* and *Salmonella spp,* during diagnostics gives evidence of incomplete biological risk assessment or management of *Mycobacterium tuberculosis* compared to *Shigella spp* and *Salmonella spp*. in Belgium. Since we can assume that *Mycobacterium tuberculosis* is a well-known etiologic agent in terms of intrinsic risks, it is reasonable to focus only on the risk management. To manage the biological risks linked to specific manipulations of *Mycobacterium tuberculosis,* different biosafety measures are required, such as (1) wearing gloves; (2) wearing respiratory protection; (3) using a biosafety cabinet (BSC) of class I or higher as primary barrier to minimize exposures to hazardous biological material (mainly infectious aerosols). One of these three required additional measures are often not well respected in Belgium, especially wearing a respiratory protection (see figure 4). Although there seems to be no lack of compliance with the use of a BSC if required to prevent escape of infectious aerosols (~80%, see figure 4), its effectiveness depends on good microbiological practices, which often decline when workload becomes too high or when routine work is performed. Especially, a BSC of class II is more sensitive to this.

This empirical and comparative way of (bio)risk evaluation gives the personnel and management evidence on the effectiveness of taken biosafety measures. Although it is clearly not the only way of (bio)risk evaluation, it is more underpinned and therefore maybe more convincing than the conventional risk assessment, which is mainly based on experience and expert judgment. Hence, collecting data on incidence of LAIs as part of a more “evidence” based biosafety seems to be pivotal in next generation of biorisk management and should be encouraged.

6. CONCLUSION & RECOMMENDATIONS

The findings and facts of the online surveys in Belgium are discussed in the previous section. In this part, we conclude and give some recommendations in order to limit the risks associated with bio-incidents and the occurrence of LAIs. Although this type of studies does provide limited substantiated data as they have been collected online and based on goodwill from the respondents, the results of these online surveys give a general idea of the safety culture and LAI perception in Belgian laboratories and identify some bottlenecks.

INCREASE COMPLIANCE AND BIOLOGICAL RISK AWARENESS VIA TRAINING

As reported in the literature worldwide, a lack of compliance with biosafety measures is identified in this online survey as a possible cause of bio-incidents and LAIs. Discomfort when wearing additional personal protective equipment together with ignorance of the reason to comply with these additional PPE (inappropriate risk assessment or training) are possible causes of this non-compliance.

Besides recommending a strict compliance with the required biosafety measures to prevent LAIs, courses and practical training in biosafety are important tools to contribute to a (bio)safer work environment and are considered as a legal obligation in biosafety and occupational health[24]. Based on the survey results there is a general impression that not only a lack of training exists 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), while the biosafety principles of risk assessment are the fundaments of biosafety (practices) and enhance awareness of biological risks in the laboratory. Hence, the personnel must be able to manage these risks and should be aware of appropriate personal protection measures to be taken to protect themselves, their colleagues, the community and the environment. It is recommended to inform the personnel about these risks, inherent to working with pathogenic organisms in general (general training). Besides this general training, lab workers involved in manipulation with specific (high) risk organisms should receive a more specific and detailed training. These training(s) should also include specific procedures to handle bio-incidents, especially incidents with micro-organisms representing a higher risk of infection, due to intrinsic characteristics increasing the risk such as low infectious dose, aerogenic spread and/or long persistence survival outside the host. This is the case for certain enteric pathogens or airborne pathogens where a possible exposure to infectious aerosols (due to a bio-incident or bad laboratory techniques) poses an important risk.

[24] see glossary

As discussed above, more evidence based biosafety data provide biosafety officers, prevention officers and occupational health practitioners with tools and knowledge to enhance the biological safety culture in the laboratory (to convince personnel and management).

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 9, 10D). It can be assumed that a certain number of LAI cases has a cause/origin that is difficult to identify (see also literature): because the presence of (unintentionally spread of) pathogenic organisms cannot be easily visualized, the personnel is often not aware of a contamination and might be infected. Also, incubation periods depends on the organisms and can vary between 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 inadequate decontamination at the end of the 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 recirculation of settled infectious 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. Personnel moving in a contaminated area prior to incident recognition or as a result of an emergency response to an incident (*e.g.* emergency procedures) may also disturb settled material, spread the contamination by 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 (23).

Moreover, 20% of the bio-incidents is judged (by the concerned lab worker) not serious enough to seek advice or to notify and is believed to 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 6.2). 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 of infection.

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

Obviously, 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.

Therefore, it is recommended to define / determine in advance all possible infection scenarios during manipulation, but also in case of bad laboratory practices and techniques or bio-incidents and to include all these identified infection risks in the initial risk assessment. The probability of infection depends on multiple factors and should be assessed using the principles of biological risk assessment, especially for airborne and enteric pathogens which seems to be largely underestimated and not well assessed. Risk assessment can be challenging 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.

Moreover, every possible 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 and worker's protection. These people can in case of a bio-incident provide advice and support to the personnel for adequate decontamination and follow up. 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 infection of uninformed colleagues.

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

Also, the incident can be evaluated in order to optimize the prevention policy in the institutions to limit bio-incidents and their re-occurrence.

Furthermore, the identified lack of communication 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, beside the increased awareness of biological risks (see above), the internal communication and help to overcome the psychological barrier associated with the reporting of bio-incidents.

Finally, 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).

EVALUATION OF IN HOUSE BIOSAFETY PROGRAM INCREASES EFFECTIVENESS

A recent survey in the US has shown that a lot of written procedures (*e.g.* good laboratory practices, adequate waste management, etc.) and extensive biosafety training not always lead to a safer laboratory (23, 24). To make all these efforts useful it is important to evaluate the effectiveness of all legally required biosafety and worker protection measures . Biosafety measures should be assessed in practice for effectively reducing the occurrence of LAIs (for example by avoiding accidental dissemination of pathogens in the work environment). In particular, the validation of the adequacy of disinfectants, PPE (masks, gloves, goggles, etc.) and safety equipment (*e.g.* BSC, autoclave, HEPA filters, etc.), but also the proper use of these biosafety protective measures is of primary importance.

'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, etc.) that are potential hot spots of biohazards if there is no good compliance with biosafety measures.

Also, it is recommended to have trainings and courses evaluated by the participants, as well as to have the participants evaluated (23, 24)

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, extra training, etc.).

Furthermore, it is observed that increased incidents and LAIs can also be associated with too high workload and absent mindedness (see figure 6.4 and 6.8). The feeling of being overloaded (stress) or feeling discomfort (in case of personal protection equipment) can happen at any moment and at any level within an institution. Potential consequences could be an increase in inaccurateness, distraction and a higher act of neglect (no-compliance). However, the latter could also be a consequence of highly repetitive work. Hence, an additional evaluation on the impact of these occupational and human factors on the (bio)safety program is recommended.

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

Although the existence of legal obligations to notify different types of bio-incidents, namely: (1) incidents with human/animal/plant pathogens, (2) accidents during contained use activities or (3) some particular infectious diseases; there is no systematic reporting to the competent authorities. This implies that the opportunity to evaluate the incident and possibly improve the actual situation to avoid similar incidents in the future (re-occurrence) is not fully used. It is both in the interest of the involved laboratories and policy-makers to dispose of clear and solid (anonymised) data on the occurrence of LAIs to make a complete evaluation possible and to make it available to the biosafety community. Moreover, no systematic reporting of bio-incidents makes evidence based biosafety and its added value rather impossible.

To generate data that will identify possible gaps (in risk management, knowledge, …) and prevent re-occurrence, it is recommended to establish an internal system to register all bio-incidents happened in the institutions and to notify to the competent authorities when employee(s) exposure or spreading in the environment is confirmed. In that way LAIs and near-LAIs become registered and evaluated into detail. Moreover, we can learn from each notification to prevent similar bio-incidents in the future. ***For details about legal obligations to notify bio-incidents, please consult our website www.biosafety.be.***

Although it is recommended to follow up the lab worker by an occupational health practitioner when there is a possible risk of infection after an bio-incident, there is still a part of LAIs that remain unnoticed, especially when the LAIs occur asymptomatically, or with relatively mild symptoms or symptoms similar to endemic diseases and a low degree of awareness of the involved personnel. Moreover, 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 laboratory and could lead to a wrong diagnosis by the general practitioner (for example, a lab acquired infection with *Francisella tularensis* could be misdiagnosed as a case of influenza (25)). This could lead to inappropriate conclusions or treatment, and may even result in secondary infection (colleagues and family members) and death.

Within this context, personnel exposed to (a) pathogenic organism(s) should receive adequate information about the possible range of symptoms that can occur after a LAI with the pathogen(s) they manipulate. It is thus recommended to provide 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(s). Also, the occupational health practitioner should receive adequate information on the risks associated with potential occupational exposure to pathogenic organisms on the workplace and the expected symptoms.

7. ANNEXES

Annex 1: Summary of recently reported Laboratory-Acquired Infections worldwide (2000-2012)

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

Number	Organism		# cases	Risk class[25]	Route of exposure	Bio-incident	YEAR of publication	Country	Ref
2	***Bacillus spp.***	***B. anthracis***	1	3	Parenteral inoculation	Human error: No compliance with biosafety measures	2002	USA	LINK
		B. cereus	1	2	Unknown	Unknown	2011	USA	ProMEDmail
124	***Brucella spp.***	***B. abortus***	1	3	Inhalation or ingestion	Technical failure : breaking of centrifuge tube	2000	Italy (EU)	(26)
		B. bacteria	2	3	Unknown (2)	Human error: Ignorance (2)	2004	USA	(27)
		B. bacteria	2	3	Unknown (2)	Human error: No compliance with biosafety measures (2)	2008	USA	LINK
		B. bacteria	3	3	Inhalation? (3)	Unknown (3) (sniffing?)	2008	Turkey	(28)
		B. bacteria	1	3	undefined	undefined	2010	Australia	(29)
		B. bacteria	1	3	Inhalation	Human error: No compliance with biosafety measures (no use of BSC)	2008	United Arab Emirates	(30)
		B. bacteria.	75	3	Inhalation or ingestion???	Human error: No compliance with biosafety measures (60) Unknown (6)	2005	Spain (EU)	(31)
		B. melitensis	38	3	Unknown (50%) Inhalation (39%)	Human error No compliance with biosafety measures	2012	Turkey	(32)

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

2	***Burkholderia spp.***	***B. 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
1	***Chlamydophila psittaci***	***Chlamydophila psittaci (avian strain)***	1	3	Unknown (inhalation?)	Human error: Ignorance	2009	Belgium (EU)	(16)
2	***Clostridium difficile***	***Clostridium difficile***	2	2	Unknown	unknown	2008	Spain (EU) and The Netherlands (EU)	(33)
1	**Cowpox virus**	**Cowpox virus**	1	2	Contact?	Unknown	2011, 2012	USA	ProMEDmail; (34)
1	**Dengue virus**	**Dengue virus**	1	3	Contact	Human error : No compliance with biosafety measures	2011	Australia	(35)
5	**Filovirus**	**Ebola virus**	2	4	Parenteral inoculation (1) Undefined (1)	Human error: Needle stick or cut incidents	2004	Russia	LINK
		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)	(36)
		Marburg virus	2	4	Undefined (2)	Undefined (2)	2004	Russia	LINK
4	***Escherichia coli***	***E.coli O157:H7***	4	3(*)T	Inhalation or ingestion	Human error: No compliance with biosafety measures (2) Unknown (2)	2005	USA	(37)
6	***Francisella tularensis***	***F.tularensis***	1	3	Inhalation or ingestion	Human error: Ignorance	2002	USA	(38)
		F.tularensis	3	3	Unknown	Unknown	2005	USA	LINK
		F.tularensis	1	3	Undefined	Undefined	2009	USA	LINK
		F.tularensis	1	3	Unknown	Unknown	2012	USA	LINK
1	***Leptospirosis Bacteria***	***Leptospirosis bacteria***	1	2	Parenteral inoculation	Human error: Needle stick or cut incidents after breaking tube	2004	India	(39, 40)
4	***Mycobacterium spp.***	***M.kansasii***	1	2	Parenteral inoculation	Human error: Needle stick or cut incidents	2005	Belgium (EU)	(14)

		M. tuberculosis	3	3	unknown	Technical failure : leaky aerosol chamber	2005	US States, India, New Zealand, and Northern Ireland (EU)	LINK
11	***Neisseria meningitidis***	***N. meningitidis***	5	2	Inhalation or ingestion	Human error : No compliance with biosafety measures	2001	UK (EU)	(41)
		N. meningitidis	2	2	Inhalation or ingestion	Human error: No compliance with biosafety measures	2002	USA	LINK
		N. meningitidis	2	2	Undefined (2)	Undefined (2)	2005	USA	(42)
		N. meningitidis	1	2	Unknown	Human error : No compliance with biosafety measures?	2007	Sweden (EU)	(43)
		N. meningitidis (serogroup A)	1	2	Unknown	Human error : No compliance with biosafety measures?	2007	USA	(44)
130	***Salmonella bacteria***	***S. serotype enteritidis***	21	2	Unknown	Human error: Spill?	2007	USA	LINK
		S. typhimurium	109	2			2012	USA	LINK
1	**SARS**	***SARS***	1	3	Unknown (inhalation)	Human error: Ignorance / cross contamination	2004	Singapore	(45)
1	***Shigella sonnei***	***Shigella sonnei***	1	2	Contact	Human error: No compliance with biosafety measures	2006	Belgium (EU)	LINK
3	***Staphylococcus aureus***	***S. aureus (MRSA)***	2	2	Undefined (2)	Undefined (2)	2006	The Netherlands (EU)	(46)
		S. aureus (EMRSA-15)	1	2	Parenteral inoculation	Human error: No compliance with biosafety measures	2003	Australia	(47)
11	**Vaccinia virus**	**Vaccinia virus**	1	2	Unknown	Human error: No compliance with biosafety measures	2006	USA	LINK
		Vaccinia virus	1	2	Parenteral inoculation	Human error: Needle stick	2003	Brazil	(48)
		Vaccinia virus	1	2	Parenteral inoculation	Human error: No compliance with biosafety measures	2003	Canada	LINK
		Vaccinia virus	1	2	Parenteral inoculation	Human error: Needle stick or cut incidents	2004	USA	(49)
		Vaccinia virus	5	2	Parenteral inoculation (5)	Human error: Needle stick or cut incidents (5)	2008	USA	LINK

		Vaccinia virus	1	2	Unknown	Human error: No compliance with biosafety measures (cross contamination)	2009	USA	LINK
		Vaccinia virus (Recombinant)	1	2?	Contact?	Human error: No compliance with biosafety measures	2003	Germany (EU)	(50)
1	***Vibrio cholerae O1***	***Vibrio cholerae O1***	1	2T	Unknown	Technical failure : overtipping during culturing (spill)	2009	Austria (EU)	(51)
4	**West Nile Virus**	**West Nile Virus**	2	3	Parenteral inoculation	Human error: Needle stick or cut incidents	2002	USA	LINK
		West Nile virus	1	3	Parenteral inoculation	Human error: Needle stick or cut incidents	2009	South Africa	(52)
		West Nile virus	1	3	Contact	Ignorance / Human error: No compliance with biosafety measures?	2010	South Africa	LINK
1	***Yersinia pestis (attenuated)***	***Y.pestis (attenuated)***	1	2?	Unknown	Unknown	2011	USA	LINK

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