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Risk assessment of biological hazards in University laboratories: checklist and critical control points

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Abstract

Background and aim. *Biomedical research in academic settings is an important issue for Public Health and Environment protection. As workplaces, the facilities for research expose their personnel to different hazards and health risks. The University of L'Aquila (Italy) carried out a field study aimed at creating and applying a checklist intended for laboratory staff.*
Methods. *The proposed checklist was derived from the procedure illustrated in the Appendix (procedure followed for the identification of a numerical index of biological risk for university facilities) and consists of 9 items. The study was conducted in 42 laboratories.*
Results. *The results highlighted that 40 laboratories fall into the "low risk" and the remaining 2 into the "moderate risk" category.*
Conclusions. *Labs with risk factors are a minority. These were properly identified using the proposed methodology.*

Introduction

The Italian National Institute of Health (*Istituto Superiore di Sanità – ISS)* has issued regulatory requirements (1) which set out the operating procedures for assessing and managing risks associated with the handling of biological agents in the laboratory. The facility where scientific research is conducted is a working environment that involves numerous potential hazards and risks to the health and safety of the lab workers. Laboratory employees often have little awareness of the risk which they may be exposed to, presumably due to a limited knowledge of the potential severity of the hazard and of the long-term risks to human health (1).

Numerous safety rules and regulations are established in order to minimize the hazards and the levels of risk. The main regulations are part of the Italian legislation (Legislative Decree 81/08, as amended), and failure to comply may result in a sanctions or penalties (1).

The main risk factors associated with a

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laboratory can be summarized as follows: chemical, physical and biological agents; high voltage equipment, centrifuges, high and low temperatures; crowding, space limitation; organizational-management aspects, difficulties in communication between workers, lack of internal procedures, simultaneous presence of different workers in the laboratory, such as internal staff (researchers, technical-scientific workers), external personnel (adjunct researchers, PhD students, research fellows or associates, undergraduates), or visitors; lack of information, education and training of the staff (particularly the internal personnel) (1).

According to the Italian Legislative Decree 81/08, a biological agent is defined as "any micro-organisms, including those which have been genetically modified, cell cultures or human endoparasites, which may cause infection, allergy or toxicity." (2).

Thus, any organism, cellular or not-cellular, which is capable of replication or of transferring genetic material, falls into this definition; they are bacteria, virus, fungi, and toxins; biological entities which have an ubiquitous presence in any working environment and setting. However, different levels of virulence and severity exist, associated also with different exposures and routes of transmission. These have been classified into four hazard groups, based on the degree of infectious risk involved (2, 3).

The biohazard classification takes into account factors such as transmissibility, pathogenicity, no effective treatment or prophylaxis availability, with the most hazardous micro-organisms being classified into hazard class four.

Thus, an adequate biological risk assessment should identify both the inherent hazard of the micro-organism and the risk of transmission to the lab workers.

The phrase "biological risk in the workplace" defines all those situations where a risk to human health may exist from possible exposure to any biological agent, cell culture or human endoparasite, whether or not genetically modified, which may cause infection, allergy or toxicity (2).

The Annex to the legislative decree (list of the classified biological agents) summarizes the classification of the biological agents based on their impact on healthy workers (2).

Research laboratories involve exposure to biological agents which, in most cases, have a low level of pathogenicity (Class 1 and 2), their use is often "intentional", and a large number of micro-organisms are handled according to the aim of the research. In addition, laboratories have a high staff turnover, particularly of external personnel, namely, undergraduates, students working on their thesis and fellows. Thus, these categories are often not provided with biosafety training and education courses.

A thorough review of the data from national and international literature highlighted the lack of studies investigating biorisk in university laboratories; among these (4, 5) is a study conducted at the Faculty of Medicine and of Dentistry of the University of Indonesia, in Jakarta, where a checklist tool was applied for laboratory assessment of biosafety measures. The checklist was developed in agreement with the directives of the WHO and of the National University of Singapore (NUS) laboratories, regarding the management and handling of different biologically hazardous agents. The study has highlighted the failure of these laboratories to meet the relevant specifications of all the checklist items, and only two laboratories showed compliance with 50 percent of the checklist items. This may be due to the fact that the most virtuous laboratories are the BSL-2 and BSL-3 facilities, where the laboratory personnel have a higher awareness and receive a specific training regarding biosecurity procedures in advance of the establishment of the laboratories (5).

Our study aims to characterize the biorisk in university laboratories, to define the checklist items for biorisk management and the hierarchy of the specific domains as a preliminary phase in defining the assessment methods. The scores will be used only to identify the more frequent hazard domains in a biological laboratory (without algorithm), in order to plan a second research phase aimed at validating a score-weighing to assess the quantitative risks for the personnel.

Methods

Instruments and assessment process

The checklist is composed of 9 items evaluating different areas of the laboratory work covering the biological risk determination. For each item (excluding no.1) a sub-score was calculated by means of a structured self-report questionnaire with 30 questions and a scoring system based on an interval scale assessment as described in the Appendix, and summarized in Table 1.

In particular, biorisk components are: Item 1 – Personnel, simply computing the number of people working permanently in the laboratories; Item 2 - Activities, with 2 questions (sub-score from 2 to 34); Item 3 – Exposure, with 10 questions (sub-score from 6 to 30); Item 4 – Facility, with 3 questions (sub-score from 2 to 8); Item 5 – Hoods, with 4 questions (sub-score from 0 to 10); Item 6 – Decontamination, with 4 questions (sub-score from 1 to 15); Item 7 – Containment, with 3 questions (sub-score from 3 to10); Item 8 – Waste, with 2 questions (sub-score from 2 to 4); Item 9 – Personal Protective Equipment (PPE), with 2 questions (sub-score from 1 to 7).

Table 1 - Biorisk checklist (see Appendix)

Checklist items			Corresponding questions		Risk Score
#	Area	Description	No.	#	Min – Max
1	Personnel	Number of people working permanently in the laboratories	1		0 – 0
2	Activities	Assessment of the type of activities carried out in the laboratory	2	#1, #2	2 - 34
3	Exposure	Evaluation of the level and type of exposure to biological agents	10	#3, #4, #5, #6, #7, #8, #9, #19, #22, #28	6 - 30
4	Facility	Analysis of the structural characteristics of the laboratories, the collective protective equipment (CPE) and the safety and/or health signs	3	#10, #11, #12	2 - 8
5	Hoods	Type of hoods in the lab and maintenance	4	#13, #14, #15, #16	0 - 10
6	Decontamination	Assessment of the cleaning performance[(a)] of the equipment used	4	#17, #18, #20, #21	1 - 15
7	Containment	Assessment of the procedures for the containment of the exposure	3	#23, #24, #25	3 - 10
8	Waste	Management of medical waste	2	#26, #27	2 - 4
9	Personal Protective Equipment (PPE)	Assessment of the type and of the characteristics of the PPE used	2	#29, #30	1 - 7

[(a)] the check list and evaluation are based on the non-simultaneous use of products for cleansing and/or sanitization

The total sum of sub-scores was calculated as a measure of the overall biological hazard assessed for each laboratory. Taking into account the possible minimum and maximum values and dividing the interval between the two numerical values into four intervals, we could identify four categories of risks based on the interval within which the index of biological risk falls.

They are listed below according to the possibility or requirement of implementing risk protection measures and procedures: negligible risk (score from 0 to 30), low risk (31 - 60), medium risk (61 - 90), high risk (91 - 120). This scoring system, which is based on the index values associated with a laboratory, assigns the assessed situation to one of four bands, as follows: I negligible risk white band; II low risk green band; III medium risk yellow band; IV high risk red band (See Appendix).

The characteristics of each category are summarised below.

Negligible	Level of biological risk is equal to that of the general population
Low	The implementation of prevention and protection measures is not required; however, general hygiene rules and regulations, technical, organisational and procedural safety measures, as set forth by Italian Legislative Decree 81/08, should be implemented
Medium	Implementation of specific prevention and protection measures required
High	Implementation of specific and urgent prevention and protection measures required

Sample

Overall, 42 different laboratories, involving direct or indirect exposure to biological agents, were enrolled (39 at the University of L'Aquila and 3 in the Local Health Authority of L'Aquila, and 124 (37 males, 87 females) out of the 179 lab workers filled in the questionnaire.

Statistical analysis

Only a descriptive statistical analysis was carried out, comparing, for each laboratory, the overall score with limits of risk categories and calculating the average scores in the entire sample for the individual nine check-list items.

Results

Given the above analysis, it was highlighted that 40 out of a total of 42 assessed laboratories fall into the "low risk" category (with score ranging from 37 to 60), while the remaining 2 laboratories fall into the "moderate risk" class (with the score equal to 62 and 64) (Table 2).

The overall mean score is 55. For a full view of the scores obtained see Table 2.

The total number of lab workers is 179, while the average number is four per laboratory. The mean score of the various activities is 10 (range 2-34). The mean score related to the evaluation of the level and type of exposure to biological agents is 16 (range 6-30).

In the first "moderate risk" laboratory, several activities are conducted, including the preparation of samples/medicinal products, of culture media, and animal testing. The activity involves the manipulation of biological agents and pf genetically modified microorganisms (GMOs), and the use of Class I and II biological safety cabinets. No biological containment level sign is provided at the entrance to the laboratory. The routine laboratory tasks are carried out by approximately 5 workers.

The activities performed in the second "moderate risk" laboratory include preparation of microbiological culture media and animal testing. Research is conducted daily, neither a containment level nor a biohazard sign is posted at the entrance to the laboratory. Treatment of the medical waste is not performed prior to its delivery

Table 2 - Individual laboratory scores

# Laboratory	Personnel	Activities	Exposure	Facility	Hoods	Decontamination	Containment	Waste	PPE	Total
1.	1	6	12	4	4	4	3	3	1	37
2.	2	8	10	6	3	2	3	4	1	37
3.	1	5	9	8	3	1	4	3	5	38
4.	5	4	7	6	3	3	4	4	7	38
5.	3	6	6	6	3	4	8	4	3	40
6.	1	5	11	8	3	1	4	3	5	40
7.	4	7	14	3	6	4	3	3	3	43
8.	1	8	13	4	6	4	3	3	5	46
9.	4	8	14	3	6	7	5	2	1	46
10.	10	9	13	4	6	5	5	3	1	46
11.	10	10	14	5	6	4	4	3	1	47
12.	4	8	15	5	6	4	5	2	3	48
13.	2	7	15	5	6	7	3	2	3	48
14.	1	8	18	6	11	5	5	4	5	48
15.	3	10	14	6	7	4	3	4	1	49
16.	3	10	14	6	7	4	3	4	1	49
17.	3	14	17	2	6	4	3	2	1	49
18.	3	5	17	6	9	4	5	2	1	49
19.	10	11	15	5	6	4	5	3	1	50
20.	2	9	18	6	3	1	5	3	5	50
21.	1	8	18	6	6	6	3	3	1	51
22.	5	10	11	6	6	8	3	4	3	51
23.	5	11	15	4	8	5	4	1	3	51
24.	5	13	16	3	6	5	5	3	1	52
25.	4	7	16	5	6	9	5	3	1	52
26.	2	5	19	4	8	4	8	2	3	53
27.	3	5	19	4	8	4	8	2	3	53
28.	2	13	17	4	4	8	5	2	1	54
29.	10	8	18	6	5	7	7	2	1	54
30.	3	7	21	5	6	8	5	2	1	55
31.	6	12	26	5	3	1	4	3	1	55
32.	4	13	21	3	6	5	5	2	1	56
33.	20	9	27	5	4	6	3	2	1	57
34.	3	14	19	2	9	4	5	3	1	57
35.	1	12	21	4	7	4	3	3	3	57
36.	3	12	20	5	6	8	4	2	1	58
37.	2	13	17	7	6	7	5	2	1	58
38.	4	16	16	4	9	4	5	3	1	58
39.	4	11	20	6	6	8	5	3	1	60
40.	10	13	23	3	6	6	5	3	1	60
41.	5	19	19	4	7	4	5	2	2	62
42.	4	18	18	6	9	5	3	3	2	64
Mean value	**4**	**10**	**16**	**5**	**6**	5	4	3	2	55

1 Optical and electron microscopy biotechnologies
2 Radiobiology
3. Lab medicine
4 Waste recovery and treatment
5 Ultrastructural anatomy electron microscopy
6 Clinical pathology and Biomedical Laboratory Techniques
7 Medical genetics hospital services
8 Pharmacology and toxicology
9 Legionella testing and research
10 Biochemistry and molecular biology
11 Molecular biology
12 Applied biology and metabolism
13 Bioactive peptides
14 Signal transduction normal and pathological tissues
15 Anatomy and anatomic imaging
16 Reproductive biotechnologies
17 Genetics and mutagenesis
18 Molecular pathology
19 Biochemistry
20 Clinical Pharmacology
21 Endocrinology
22 Immunology
23 Applied biology and reproductive biotechnologies
24 Developmental biology
25 Clinical pathology
26 Confocal microscopy and neuroimaging
27 Visual neurophysiology
28 Applied biology and reproduction
29 Molecular biology and clinical biochemistry
30 Microbial biochemistry
31 Infectious diseases services
32 Neurobiology
33 Anatomical pathology
34 Molecular medicine
35 Experimental pathology
36 Biochemistry and cellular pharmacology
37 Microbiology
38 Skeletal system diseases lab
39 Cell biology
40 Hospital's clinical laboratory
41 Pharmacology
42 Bone biopathology lab

to the authorized disposal company. The routine laboratory tasks are carried out by approximately 4 workers.

In addition, 8 laboratories are at the higher limit of the "low risk" parameter, with a score between 57 and 60.

Discussion

The interpretation of the results is based on the number and type of activities conducted within the university laboratories and on the exposures to the biological agents.

Laboratories that reported a score rated as "low risk" (from lab no. 1 to no. 40) (Table 2) have low exposure levels to biological agents and often these agents are not intentionally handled. In addition, no critical factors emerged in relation to biological containment and the waste management.

With regard to the laboratories rated as "medium risk" (No 41 and 42), laboratory No 41 handles genetically modified biological agents, which require a more detailed analysis in order to define the containment level (Table 2). Additionally, laboratory No 42 does not perform the treatment of the medical waste before its delivery to the authorized disposal company. These parameters affect the score negatively and account for their inclusion into a higher risk group.

Our findings are quite positive and consistent with the results of the study conducted by the University of Jakarta (5). However, their findings are better because they have BSL 2 and BSL3 laboratories, which entail a higher awareness among the workers who receive suitable information, instruction and training in working safely with agents.

The current biotechnologies applied to the development of diagnostic devices, therapeutic agents and the manipulation of microorganisms, which employ genetic engineering techniques, should also raise new questions and introduce new perspectives concerning biorisks, as suggested by the literature (4). Laboratories require safety measures designed to protect the personnel, the community, and the environment that may be exposed to hazardous materials and organisms (4). Some infected individuals from the outbreaks of *Salmonella* tracked by the US Centers for Disease Control, which originated from didactic microbiology laboratories, reported that they do not remember the following: being trained at the biosafety level 2 (BSL2), wearing lab coats, having to leave their writing utensils in the laboratory, having to wash their hands before leaving the lab, and having a designated lab notebook that was not used for other classes. In addition, some people stated that they used BSL2 organisms in introductory biology labs. All of these issues go against the best practices put forward by the ASM (American Society of Microbiology) biosafety guidelines (6).

A critical factor in the preparation of this study was the lack of available data in literature, which limited the possibility of a debate or further discussion. The existing biological risk assessment methods are based on the probability of occurrence and on the severity of a potential adverse effect. Given the lack of suitable data and of exposure monitoring, it is difficult to define an acceptable risk level. With regard to the aspects that may be improved in order to minimize risks, we suggest: the integration of the item-based questionnaire to investigate the implications related to the genetically modified microorganisms (handled in some of the assessed laboratories), the evaluation of the level and type of exposure to biological agents, training and instruction as reported in literature, also concerning non-university laboratories (7-19). As to the latter aspects, some variables are accounted for by the extreme heterogeneity of the individuals attending the research laboratories, including

the lab personnel and the visitors, such as undergraduates, fellows, PhD students, and junior doctors.

One limit of the study is the absence of a weighing score calculation system. At the moment, the quantitative assessment of biological risk hasn't sufficient literature-based data for establishing evidence-based criteria for weighing single items in a check-list with a numeric variable treatment system, while semi-quantitative or qualitative-quantitative methods are in considerable development, in particular, chemical risk assessment, that is more advanced (20). Nevertheless, the present results could be improved in a second research phase, aimed at considering and validating a quantitative assessment system of risk for the laboratory personnel. The methodology could be realized by means of a structured analysis by an expert panel of scientists, i.e. a Delphi study, in order to establish quantitative weighted-scores.

In conclusion, this checklist has also proven to be a simple tool for evaluating laboratories that manage and store materials with biological hazards. The evolution of laboratory software applications could increase their effectiveness and reliability. This checklist can be further developed as a software application so that faster and more accurate assessment of biorisk management in the lab could be available. Its use is preliminary and could provide a plausible and future implementation by our university.

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Riassunto

Valutazione del rischio biologico in laboratori di ricerca universitari: checklist e punti critici di controllo

Background e scopo dello studio. La ricerca biomedica svolta in ambito universitario è una questione importante anche dal punto di vista della tutela della salute pubblica e dell'ambiente. Le strutture per la ricerca, in quanto luoghi di lavoro, espongono il personale a diversi pericoli e rischi per la salute. L'Università degli Studi dell'Aquila (Italia) ha realizzato uno studio sul campo finalizzato all'ideazione e applicazione di una checklist rivolta al personale di laboratorio.

Metodi. La checklist proposta deriva dalla procedura in appendice (procedura seguita per l'identificazione di un indice numerico di rischio biologico) e si compone di 9 item. Sono stati arruolati in 42 laboratori universitari.

Risultati. I risultati evidenziati che 40 laboratori rientrano nella categoria "rischio basso" e i restanti 2 nella categoria "rischio moderato"

Conclusioni. I laboratori 'a rischio' sono una minoranza e sono stati adeguatamente identificati utilizzando la metodologia proposta.

References

1. Morace G, Fazzi P, Ilari M, Salvatore M, Caiola S. Guida operativa per la manipolazione di materiali a rischio biologico (Guidance on the correct handling of biological materials). Roma: Istituto Superiore di Sanità, 2011 (Rapporti ISTISAN 11/31). Available on: www.iss.it [Last accessed: 2021 Feb 12].
2. D.Lgs. (Italian Republic. Legislative Decree) April 9, 2008, n. 81. s.m.i. Titolo X. Esposizione ad Agenti biologici. Art. 267. Def.
3. Istituto Superiore per la Prevenzione e la Sicurezza sul Lavoro (ISPESL). Linee guida sull'attività di sterilizzazione quale protezione collettiva da agenti biologici per l'operatore nelle strutture sanitarie (D.Lgs. 81/2008 e s.m.i.). Roma: ISPESL, maggio 2010. Available on: rev_1f_L.G._ISPESL_Sterilizzazione_versione_20_maggio_2010 (lisaservizi.it) [Last accessed: 2021 Feb 12].
4. Coelho AC, García Díez J. Biological risks and laboratory-biotechnology. Front Bioeng Biotechnol 2015; **3**: 56. doi: 10.3389/fbioe.2015.00056.
5. Naroeni A, Bachtiar EW, Ibrahim F, et al. Challenges in implementing a biorisk management

program at universitas Indonesia: A Checklist Tool for biorisk management. Health Secur 2016; **14**(6): 375-81. doi: 10.1089/hs.2016.0013. Epub 2016 Nov 10.
6. Byrd JJ, Emmert E, Maxwell R, Townsend H. Guidelines for biosafety in teaching laboratories Version 2.0: a revised and updated manual for 2019. J Microbiol Educ 2019; **20**(3): 20.3.57. doi: 10.1128/jmbe.v20i3.1975.
7. Aronson BD, Silveira LA. From genes to proteins to behavior. A laboratory project that enhances student understanding in cell and molecular biology. CBE Life Sci Educ 2009; **8**(4): 291-308. doi: 10.1187/cbe.09-07-0048.
8. Bayot ML, Limaiem F. Biosafety Guidelines. [Update 2020 Mar 25]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing, 2020 Jan-. Available on: https://www.ncbi.nlm.nih.gov/books/NBK537210/ [Last accessed: 2021 Feb 12].
9. Emery RJ, Rios J, Patlovich SJ. Thinking outside the box: biosafety's role in protecting non-laboratory workers from exposure to infections disease. Appl Biosaf 2015; **20**(3): 128-9. doi: 10.1177/153567601502000301.
10. Jonsson CB, Cole KS, Roy CJ, Perlin DS, Byrne G; members of the RBL-NBL Directors Network. Challenges and practices in building and implementing biosafety and biosecurity programs to enable basic and translational research with select agents. J Bioterror Biodef 2013; Suppl **3**(15): 12634. doi: 10.4172/2157-2526.S3-015.
11. Kipnis M, Hofstein A. The inquiry laboratory as a source for development of metacognitive skills. Int J Sci Math Educ 2008; **6**: 601-27. https://doi.org/10.1007/s10763-007-9066-y.
12. Kojima K, Makison Booth C, Summermatter K, et al. Risk-based reboot for global lab. New WHO guidance could expand access to lab facilities. Science 2018; **360**(6386): 260-2. doi: 10.1126/science.aar2231.
13. Lipsitch M, Bloom BR. Rethinking biosafety in research on potential pandemic pathogens. mBio. 2012; **3**(5): e00360-12. doi: 10.1128/mBio.00360-12.
14. Matza-Porges D, Nathan S. A biosafety level 2 virology lab for biotechnology undergraduates. Bochem Mol Biol Educ 2017 Nov; **45**(6): 537-43. doi: 10.1002/bmb.21080. Epub 2017 Jul 30.
15. Mourya DT, Yadav PD, Majumdar TD, Chauan DS, Katoch VM. Establishment of biosafety level-3 (BSL-3) laboratory: important criteria to consider while designing, constructing, commissioning and operating the facility in Indian setting. Indian J Med Res 2014; **140**(2): 171-83.
16. Peng H, Bilal M, Iqbal HMN. Improved biosafety and biosecurity measures and/or strategies to tackle laboratoty-acquired infections and related risks. Int J Environ Res Public Health 2018 Dec; **15**(12): 2697. doi: 10.3390/ijerph15122697. Epub 2018 Nov 29.
17. Rusnak JM, Kortepeter MG, Hawley RJ, Anderson AO, Boudreau E, Eitzen E. Risk of occupationally acquired illness from biological threat agents in unvaccinated laboratory workers. Biosecur Bioterror 2004; **2**(4): 281-93. doi: 10.1089/bsp.2004.2.281.
18. Trevan T. Biological research: rethink biosafety. Nature 2015; **527**(7577): 155-8. doi: 10.1038/527155a.
19. Wurtz N, Papa A, Hukic M, et al. Survey of laboratory-acquired infections around the world in biosafety level 3 and 4 laboratories. Eur J Clin Microbiol Infect Dis 2016 Aug; **35**(8): 1247-58. doi: 10.1007/s10096-016-2657-1. Epub 2016 May 27.
20. Mastrantonio R, Scatigna M, D'Abramo M, Martinez V, Paoletti A, Fabiani L. Experimental Application of Semi-Quantitative Methods for the Assessment of Occupational Exposure to Hazardous Chemicals in Research Laboratories. Risk Manag Healthc Policy 2020 Oct 6; **13**: 1929-37. doi: 10.2147/RMHP.S248469.

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APPENDIX

Procedure applied for the definition of a numerical index of biological risk for academic settings

In 2015, a questionnaire was administered to the Local Safety & Health Officers of the university facilities who are exposed to biological materials. One copy of the questionnaire was distributed to each facility where a biological hazard may exist.
The questionnaire was compiled by the user of the facility (in case of more users, it was compiled by the one, or by one of those, who use it for a longer time) and signed by the respondent and by the manager of the facility.
Each item included close-ended questions, each of which was assigned a numerical value, proportional to the risk associated with the surveyed activity.
As the questionnaires were collected, the numerical values were added together to obtain a numerical index of a site-specific biological risk.
By taking into account the minimum and maximum indices and dividing the interval between the two numerical values into four intervals, we could identify four categories of risk based on the interval within which the index of biological risk falls.
The categories are listed below according to the possibility or need to adopt protection measures and procedures against biological hazards.
NEGLIGIBLE (0 → 30)
LOW (31 → 60)
MEDIUM (61 → 90)
HIGH (91 → 120)

The characteristics of each category are summarised below.

Negligible	The level of biological risk is equal to that of the general population
Low	The implementation of prevention and protection measures is not required; however, general hygiene rules and regulations, and technical, organisational and procedural safety measures should be implemented under the Italian Legislative Decree 81/08
Medium	The implementation of specific prevention and protection measures is required
High	The implementation of specific and urgent prevention and protection measures are required

The following table reports the questions and the corresponding indices assigned to each answer:

Question		Answer and corresponding index	Notes
#	Content		
1	*Macro activity carried out in the facility*:		in case of different activities, add the indices
	Research	3	
	Diagnosis	3	
	Sample / pharmaceutical preparation	3	
	Culture media preparation	1	
	Animal testing	2	
	Food analysis	3	
	Other	Variable from 1 to 3	
2	*Activity carried out in the facility*:		in case of different activities, add the indices
	Molecular biology	2	
	Cytology	2	

	Microbiology / mycology	3	
	Animal cell culture	3	If they are human cells +1; If they are pathological cells +1
	Plant cell culture	1	
	Clinical laboratory analysis	3	
	Anatomical pathology	2	
	Other	Variable from 1 to 3	
3	*Intentional use ** of biological agents (BA) / Genetically Modified Organisms (GMOs)*	1	BA = any micro-organism, cell culture, or human endoparasite, which may cause any infection, allergy or toxicity
4	*Possible presence of BA /GMOs in the activity*	2	GMO = any micro-organism whose genetic material has been altered in a way that does not occur naturally
5	*Volume of each single sample*		
	< 10	1	
	> 10 e <100	2	
	> 100 e < 1000	3	
	>1000	4	
6	*Frequency of use (of the samples)*		
	Daily	3	
	Weekly	2	
	Monthly	1	
	Yearly	1	

7	*Classes of BA or GMO used*		
	Hazard **group 1** BAs *	1	Group 1 BAs = unlikely to cause human disease
	Hazard **group 2** BAs *	2	Group 2 BAs = can cause human disease and may be a hazard to employees; it is unlikely to spread to the community; there is usually effective prophylaxis or treatment available.
	Hazard **Group 3** BAs *	3	Group 3 BAs = can cause severe human disease and may be a serious hazard to employees; it may spread to the community; but there is usually effective prophylaxis or treatment available.
	Risk class 1 GMOs *	1	class 1: Contained uses of no or negligible risk, for which containment level 1 is appropriate to protect human health and the environment.
	Risk class 2 GMOs *	2	class 2: contained use of low risk for which containment level 2 is appropriate to protect human health and the environment.
	Risk class 3 GMOs *	3	class 3: contained use of moderate risk for which containment level 3 is appropriate to protect human health and the environment.

8	*Sample origin*		
	Internal origin	2	
	External origin, certified	1	
	External, not certified	3	
9	*Is an updated list of BAs and/or GMOs in place?*		
	YES	2	
	NO	3	
	not used	1	
10	*Does the work area have the basic requirements recommended for Biological Agents?*		
	YES	1	
	NO	3	
11	*Is the biosafety level posted on all access doors to the work area?*		A biosafety level is a set of biocontainment precautions required to isolate dangerous biological agents in an enclosed laboratory facility.
	NO	3	
	YES, level 1	1	
	YES, level 2	1	
	YES, level 3	2	

12	*Is the biohazard symbol posted on the access doors to the work area?*		It is recommended to post warning signs or symbols for BAs (or fluids / materials that may contain them) starting from hazard group 2.
	NO, not necessary	0	
	YES	1	
	NO	2	
13	A*re horizontal laminar fume hoods in place?*		If no flow system is in place but its need is acknowledged: + 4
	YES		
	NO		
14	*Are vertical laminar fume hoods in place?*		
	YES		
	NO		
15	*Are vertical laminar flow cabinets in place?*		Biosafety cabinets are divided into three classes: I, II and III based upon their containment capabilities when working with BA.
	YES, class I		
	YES, class II		
	YES, class III		
	NO		

16	*Are operation and maintenance manuals available and used for the fume hoods and the equipment?*		
	YES	1	
	NO	3	
17	*Are the fume hoods cleaned regularly?*		Only where fume hoods are in place
	At the end of each operation	1	
	Daily	2	
	Weekly	3	
	Each month	4	
18	*Which are the detergents used for the cleaning of the hoods?*		Only where fume hoods are in place. If different detergents are used, the lowest numerical index is applied.
	Sodium hypochlorite	1	
	Ethyl alcohol 70%	1	
	Distilled water	4	
	Saline solution	4	
	Formalin solution	1	
	Other	Variable from 1 to 4	
20	*Is the cabinet decontamination performed using formaldehyde gas?*		Only where fume hoods are in place
	NO, not necessary	1	
	NO	3	
	YES	2	

21	*Are the tools cleaned regularly?*		
	At the end of each operation	1	
	Daily	2	
	Weekly	3	
	Monthly	4	
22	*Are needles, blades, sharp objects used?*		
	YES	3	
	NO	1	
	If YES, which?		
19	*Are aerosol-generating activities performed?*		E.g.: vortexing, centrifuging, shaking bacterial solutions, use of flaming loops, use of pipettes.
	YES	3	
	NO	1	
	If YES; which?		
23	*Are procedures or technical system in place to limit the generation of aerosol?*		
	YES	3	
	NO	1	
	If YES, which?		
24	*Is the germicidal UV lamp in place?*		
	YES, in the lab	2	
	YES, under the fume hood	1	
	NO	3	

25	*Is the germicidal lamp used regularly?*		only where the germicidal lamp is in place
	at the end of the day	1	
	every week	3	
	every month	4	
26	*Is medical/animal waste management carried out?*		
	YES	1	
	NO	2	
27	*Is medical waste treatment carried out prior to its delivery to the authorized waste handlers?*		
	YES	1	
	NO	2	
28	*Have recurrent episodes of allergy been reported by the workers?*		
	YES	2	
	NO	1	
29	*Which are the PPEs in place and used?***		PPEs = Personal Protective Equipment
	Coat		
	Gloves		
	Goggles		
	Mask		
	Other		
30	*Are the PPEs certified?*		
	YES	1	
	NO	3	

The numbers assigned to each question are in bold. They are reported in the checklist grouped by each item. No question is included under item number 1 as it only reports the number of employees in each laboratory.
* If they are airborne, multiply by 2.
** "Intentional use of BAs" means the deliberate use, manipulation, culture and introduction in the work cycle.
*** The value assigned to the type of PPEs used varies according to the activity being carried out. For each PPEs that should be used but which is not, add +2. If no DPI is used, add +4.