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Biosafety principles in parasitology laboratories

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Abstract

Laboratories are designed for three main objectives; education, diagnosis and research. The concept of biosafety has begun to be prioritized due to the need to protect the personnel working in laboratories for education, routine diagnosis and research purposes, as well as people outside the laboratory and laboratory materials.

Since 1990s the concept of biosafety has started to be developed for laboratories and biosafety guidelines have began to be published by various universities and institutions. Precautions that are related to biosafety and standards are determined based on the risk assessment of microorganisms to be studied in the laboratory. According to these risk assessments, microorganisms have been classified into four risk groups and, four kinds of laboratory biosafety levels have been determined. In these laboratory biosafety levels, as well as standard microbiological applications, specific applications and precautions are taken that are mentioned as primary and secondary property barriers. Animal care units, arthropods production and care centers are also classified as laboratories.

The term of biosafety includes topics such as proper collection of waste generated in the laboratory, transportation and disposal of chemical and radioactive substances. The topics mentioned in this review are dealt based on parasitology laboratories. be useful in identifying patients who will require advanced support during admission for ARDS.

Key words: *Biosafety, Microbiology, Parasitology*

Introduction

After the development of science and technology, human and human values were taken into the forefront, the concept of 'Security First' was started to be used and this concept found its place in every unit of life. Laboratories and their associated centers which are among the most important institutions serving humanity have also been influenced by this concept.

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Laboratories are designed for three main purposes: education, diagnosis and research. There are laboratories for education in many disciplines. In these laboratories, practical applications related to theoretical education are carried out at the level of primary and secondary school education as well as undergraduate and graduate education. One of the most common and well-known laboratory classes is diagnostic laboratories. Samples are collected in routine diagnostic laboratories such as biochemistry, immunology, pathology, microbiology and parasitology. Samples collected into the parasitology laboratory have the possibility of containing parasites as well as bacteria, fungi, and dangerous viruses. For this reason, it is important that the personnel working in the routine diagnostic laboratories have adequate training in the areas ranging from sample acceptance to processing of samples and they should work in laboratories where necessary precautions are taken in terms of safety.

Microorganisms studied in research laboratories are usually pre-determined. The genus and species of bacteria, fungi, virus or parasites studied in these laboratories are known. Factors such as transmission route and transmission dose should be known about these microorganisms. In addition to the study with a specific microorganism, due to the potential contents of samples such as water and soil, which are accepted to the laboratory for research purposes, it is required to increase the security measures to be taken in this regard.

The concept of biosafety has begun to be prioritized due to the need to protect the personnel working in laboratories for education, routine diagnosis and research purposes, as well as people outside the laboratory and laboratory materials.

The History of Biosafety

Since the 1990s, the concept of Biosafety for Laboratories has started to develop and biosafety guidelines have been published by various universities and institutions.

Due to laboratory related infections (LRI) and accidents, and the damage to laboratory workers and environment as a result of these, concepts related to biosafety have been established and guidelines have been published. First notifications about LRI were made in 20th century. In 4 studies by Pike and Sulkin, there were 168 reported deaths due to 4079 LRI cases between 1930 and 1978. With these studies, the 10 most common pathogens among those who caused the death of laboratory workers were Brucella spp., Coxiella burnetii, Hepatitis B virus (HBV), Salmonella typhi, Francisella tularensis, Mycobacterium tuberculosis, Blastomyces dermatitidis, Venezuellaat encephalitis virus, Chlamydia psittaci and Coccidioides immitis (1-4).

A literature review by Harding and Byers 20 years after 1978 found 22 deaths in 1267 infections. In 5 of these, laboratory induced infectious agent caused fetal death and Mycobacterium tuberculosis, Coxiella burnetii, Hantavirus, Arbovirus, HBV, Brucella spp., Salmonella spp., Shigella spp., Hepatitis C virus and Cryptosporidium spp. were detected in 1074. Harding and Byers (5) reported that 45% of the LRIs occurred in clinical diagnostic laboratories and 51% in research laboratories, and revealed that LRIs were an important problem for both diagnosis and research laboratories.

The biosafety concept has come to the forefront in recent years due to the concerns about new pathogens and studies on important viruses such as HIV and Zika viruses in addition to combating bioterrorism activities including the use of microorganisms such as anthrax as a biological weapon.

Biosafety

Biosafety is called the optimal combination of laboratory infrastructure, design, equipment, techniques and applications in order to ensure that the studies with the potential pathogenic hazard-containing material, microorganisms or their genetic and toxic variations are safe for human and environment.

In biosafety, there are concepts that are often confused and used interchangeably. These concepts form the basis of biosafety.

Sterilization: The elimination of live microorganisms and viruses in any substance, device and solution. Sterilization is performed by physical methods such as heat (dry heat, steam heat) and irradiation (alpha, gamma rays), and by chemical methods such as alkylating agents (ethylene oxide, gluteraldehyde, formaldehyde), chlorine dioxide, hydrogen peroxide gas, gas, gas plasma, peracetic acid, ozone and ozone- gas. The sterilization process is practically measurable and controllable by means of a sterility assurance level (SAL = Sterility Assurance Level) (6-8).

Disinfection: It is a less lethal process than sterilization. While the pathogenic microorganisms on inanimate substances are removed, not all bacterial products such as bacteria spores are eliminated. This process is usually done by using chemicals and heat (pasteurization).

A classification has been proposed by Earl Spaulding in 1972 for the use of of liquid chemical germicides for the purpose of disinfection. This classification was gradually used in the USA by the Centers for Disease Control (CDC) and the Food and Drug Administration (FDA). In 1991, an additional classification was made considering the floors, walls and surfaces in the laboratories. Spaulding also categorized chemical disinfectants into high, medium and low level disinfectants (Hospital disinfectants) according to their effect levels (7-9) (Table 1).

Table 1. Categorization of Disinfectants (9).

High Level Disinfectants	**Medium Level Disinfectants**	**Low Level Disinfectants**
• Glutaraldehyde • Formaldehyde • Sodium hypochlorite • Paracetic acid • Hydrogen peroxide	• Ethyl and isopropyl alcohol • Phenol and phenol compounds • Iodophors • Glucoprotamine	• Ethyl and isopropyl alcohol • Phenol and phenol compounds • Iodophores • Sodium hypochlorite • Quaternary ammonium compounds

Asepsis: Processes to prevent contamination of an environment with microorganisms (6-8).

Antisepsis: Clearing of a living tissue from microorganisms using chemicals (6-8).

Decontamination: Decontamination is the process of removal of pathogenic microorganisms from the materials and living tissues used by laboratory workers through disinfection, sterilization, cleaning and/or antisepsis (6-8).

Microbiological Risk Assessment

World Health Organization (WHO) divided infectious microorganisms into four risk groups (Table 2) (10). Countries, regions, or institutions consider certain aspects of microorganisms when creating their own Biosafety Laboratory guidelines. These features are:

1- Pathogenicity and infectious dose of microorganism
2- Natural transmission pathways of infection
3- Result of exposure to microorganism
4- Other infection routes caused by laboratory applications (vascular, airway or oral routes)
5- Sensitivity or resistance of microorganism to environmental conditions
6- The volume and concentration of materials to be used in laboratory studies
7- The presence of a suitable host (human or animal)
8- Information obtained from animal studies, laboratory accidents and clinical reports
9- Laboratory activity plans (sonication, pipetting, centrifugation, etc.)
10- Any genetic manipulation of microorganism
11- The presence and availability of effective treatment facilities in the presence of resistance
12- Effective protection measures: Prophylaxis (application of vaccination or antiserum), control of reservoir animals and arthropods, hygiene of water and food.

Table 2. Risk groups and features of infectious microorganisms.

Risk Group	Personal Risk	Social Risk	Explanation
1	Low or no	Low or no	In this group, there are microorganisms which are not likely to cause disease in humans and animals.
2	Medium	Low	There are microorganisms in this group that can cause disease in humans and animals, but do not pose a serious threat to laboratory workers, society, pets and the environment. Laboratory workers may be exposed to serious infections, but there are effective treatment and prevention methods and the spread of the disease is limited.
3	High	Low	Although pathogens cause serious infections in humans and animals, they are rarely transmitted from an infected person to another. There are effective treatment and protection measures against these pathogens.
4	High	High	Pathogens in this group cause serious diseases in humans and animals. It is transmitted directly and indirectly from one person to another. There are usually no effective treatment and protection measures against these pathogens.

Microorganisms in Risk Group 1 have little or no personal or social risk. In this group, there are microorganisms which are not likely to cause disease in humans and animals. Microorganisms in Risk Group 2 have moderate personal risk and little social risk. There are microorganisms in this group that can cause disease in humans and animals, but do not pose a serious threat to laboratory workers, society, pets and the environment.

Microorganisms in Risk Group 3 have a high level of personal risk and a low social risk. Although pathogens cause severe infections in humans and animals, they are rarely transmitted from one infected person to another. Microorganisms in Risk Group 4 have a high level of personal risk and a high social risk. Pathogens in this group cause serious diseases in humans and animals (10).

Classification of infectious agents by risk group

Bacterial, fungal, viral and parasitic agents are grouped according to the microbiological risk group classification. Bacterial agents (E.coli, Bacillus subtilis, Bacillus licheniformis) that do not cause disease in healthy people are in Risk Group 1. The majority of bacterial agents are in Risk Group 2. Risk Group 4 does not contain any bacterial agents, while the bacteria in Risk Group 3 are shown in Table 3 (11).

Most of the fungal agents are in Risk Group 2. Blastomyces dermatitidis, Cladophialophora bantiana, Coccidioides immitis, Histoplasma capsulatum duboisii and Paracoccidioides brasiliensis are pathogens in this group. While fungal agents such as bacterial agents are not included in Risk Group 4, some fungal infectious agents are in Group 3 (Table 3) (11).

Viruses are the most risky microorganisms in terms of transmission. There are viruses in 4 risk groups. The Crimean-Congo hemorrhagic fever virus and Ebola virus are in the Risk Group 4 (Table 4) (11).

Most of the parasitic infections are in Risk Group 2. Risk Groups 3 and 4 do not contain any parasitic agents. According to CDC and National Institutes of Health (NIH), grouping of parasites by microbiological risk classification is presented in Table 5 (11).

Table 3. Risk Group 3 Agents.

Bacteria
• *Bartonella* • *Brucella* (*B. abortus, B. canis, B. Suis)* • *Burkholderia (Pseudomonas) mallei, B. pseudomallei* • *Coxiella burnetii* • *Francisella tularensis* • *Mycobacterium bovis* • *Pasteurella multocida* type B -"buffalo" ve other virulent strains • Rickettsia akari, R. australis, R. canada, R. conorii, R. prowazekii, R. rickettsii, R, siberica, R. tsutsugamushi, R. typhi (R. mooseri) • *Yersinia pestis*
Fungi
• *Coccidioides immitis* • *Histoplasma capsulatum, H. capsulatum var. duboisii*
Parasites
-
Virus
Alfaviruses (Togaviruses) - Group A Arboviruses Chikungunya virus Semliki forest virus St. Louis encephalitis virus Venezuelan horse encephalitis virus Other viruses Arenaviruses Flexall virus Lymphocytic choriomeningitis virus (LCM) (neurotropic strains) Bunyaviruses Hantaviruses Rift valley fever virus Coronaviruses Severe Acute Respiratory Syndrome associated coronavirus (SARS-CoV) Middle East Respiratory Failure Syndrome (MERS-CoV) Flaviviruses- Group B Arboviruses Japanese encephalitis virus West Nile virus (WNV) Yellow fever virus **Other viruses** Orthomyxoviruses Influenza viruses 1918-1919 H1N1 (1918 H1N1), human H2N2 (1957-1968), and high pathogen avian influenza virus H5N1 Poxviruses Monkey flower virus Prions Transmitted spongy encephalopathy (TSE) agents Retroviruses Human immunodeficiency virus (HIV) type 1 and 2

While the bacterial, fungal and parasitic factors were not found in Risk Group 4, the following viruses were classified in Risk Group 4 (Table 4).

Table 4. Viruses in Risk Group 4.

	Virus
	Arenaviruses
	Guanarito virus
	Lassa virus
	Junin virus
	Machupo virus
	Sabia virus
	Bunyaviruses (Nairovirus)
	Crimean-Congo hemorrhagic fever virus
	Filoviruses
	Ebola virus
	Marburg virus
	Flaviruses - Group B Arboviruses
	Tick-borne encephalitis virus complex, Central European encephalitis, Hanzalova, Hypr, Kumlinge, Kyasanur Forest disease, Omsk hemorrhagic fever, and Russian spring-summer encephalitis viruses
	Herpesviruses (alpha)
	Herpesvirus simiae (*Herpes B* or *Monkey B virus*)
	Paramyxoviruses
	Equine morbillivirus (*Hendra virus*)
	Hemorrhagic fever agents and viruses not yet defined

Table 5. Risk groups of parasites according to the microbiological risk classification.

	Parasite	**CDC Biosafety Level**	**NIH Risk Group**
1	*Acanthamoeba castellani*	2	-
2	*Ancylostoma duodenale*	2*	2
3	Ancylostoma ceylanicum	2*	2
4	Angiostrongylus cantonensis	-	-
5	Angiostrongylus costaricensis	-	-
6	Ascaris lumbricoides	2*	2
7	Ascaris suum	2*	2
8	Babesia divergens	2*	2
9	Babesia microti	2*	2
10	Brugia malayi	2*	2
11	Brugia timori	-	2
12	Capillaria philippinensis	-	-
13	Clonorchis sinensis	-	-
14	*Cyclospora cayetanensis*	-	-
15	*Cryptosporidium parvum*	2*	2
16	*Cysticercus cellulosae*	2	2
17	*Diphyllobothrium latum*	-	-
18	*Dipylidium spp*	-	-
19	*Dracunculus medinensis*	-	-
20	*Echinococcus granulosis*	2*	2
21	*Echinococcus multilocularis*	2*	2
22	*Echinococcus vogeli*	2*	2
23	*Entamoeba histolytica*	2	2
24	*Fasciola gigantica*	2*	2
25	*Fasciola hepatica*	2*	2
26	*Fasciolopsis buski*	-	-
27	*Giardia lamblia*	2*	2
28	*Hymenolepis diminuta*	-	2
29	*Hymenolepis nana*	2	2
30	*Leishmania braziliensis*	2*	2
31	*Leishmania donovani*	2*	2
32	*Leishmania ethiopica*	2*	2
33	*Leishmania major*	2*	2

34	*Leishmania tropica*	2*	2
35	*Loa loa*	2*	2
36	*Mansonella ozzardi*	-	-
37	*Mansonella perstans*	-	-
38	*Microsporidia* spp.	2*	2
39	*Naegleria fowleri*	2	2
40	*Necator americanus*	2	2
41	*Onchocerca volvulus*	2*	2
42	*Opisthorchis felineus*	-	-
43	*Paragonimus westermanii*	-	-
44	*Plasmodium falciparum*	2*	2
45	*Plasmodium malariae*	2*	2
46	*Plasmodium ovale*	2*	2
47	*Plasmodium simian parasites*	2*	2
48	*Plasmodium vivax*	2*	2
49	*Pneumocystis carinii*	-	-
50	*Sarcocystis* spp.	2	2
51	*Sarcocystis sui hominis*	2*	-
52	*Schistosoma haematobium*	2*	2
53	*Schistosoma intercalatum*	2*	2
54	*Schistosoma japonicum*	2*	2
55	*Schistosoma mansoni*	2*	2
56	*Schistosoma mekongi*	2*	2
57	*Strongyloides stercoralis*	2*	2
58	*Taenia saginata*	-	-
59	*Taenia solium*	2	2
60	*Toxocara canis*	-	2
61	*Toxoplasma gondii*	2*	2
62	*Trichinella spiralis*	-	2
63	*Trichomonas vaginalis*	-	-
64	*Trichostrongylus* spp.	2*	2
65	*Trichuris trichiura*	2*	2
66	*Trypanosoma brucei brucei*	2*	2
67	*Trypanosoma brucei gambiense*	2*	2

68	*Trypanosoma brucei rhodensiense*	2*	2
69	*Trypanosoma cruzi*	2*	2
70	*Trypanosoma* spp.	2	2
71	*Wuchereria bancrofti*	2*	2

(CDC; US Centers for Disease Control, NIH; US National Institutes of Health
2*; According to microbiology risk classification, risk group 2 is implied.)

Biosafety levels of laboratories are determined according to microbiological risk grouping. According to WHO microbiological risk assessment, four Biosafety levels were determined in the laboratory. The applications and necessary safety equipment in four biosafety level laboratories are shown in Table 6 (10). Table 7 shows the main characteristics of four biosafety level laboratories (10).

Table 6. Laboratory biosafety levels according to microbiological risk assessment.

Risk Group	**Biosafety Level**	**Laboratory Type**	**Laboratory Applications**	**Safety Equipments**
1	Basic laboratory Biosafety Level 1 (BSL-1)	Basic Education Laboratories Research Laboratories	Good laboratory practices (GLP)	Not necessary Open bench study
2	Basic laboratory Biosafety Level 2 (BSL-2)	Public Health Laboratories Diagnostic Laboratories Research Laboratories	In addition to GLP Protective clothing Biosafety mark	Biosafety cabinets (BSC) for potential aerosol near open benchs
3	Isolation Laboratory Biosafety Level 3 (BSL-3)	Special Diagnostic Laboratories Research laboratories	In addition to BSL-2 Special Protective clothing Controlled entry-exit One way airflow	BSC and/or other primary protection equipments for all activities
4	Maximum Isolation Laboratory Biosafety Level 4 (BSL-4)	Laboratories working with dangerous pathogens	In addition to BSL-3 Air locked entry Shower outlet Special waste system	Class III BSC or Class II BSC with positive pressure, special clothing, double door autoclave

(BSL, Biosafety Level; BSC, Biosafety cabinets; GLP, Good laboratory practices.)

Table 7. Biosafety level requirements.

Property	Biosafety Level			
	1	2	3	4
Laboratory isolation		No		
Switchable room for decontamination		No	Y	s
Ventilation				
- Airflow inside	No	Optional	Yes	Yes
- Controlled airflow	No	Optional	Yes	Yes
- HEPA filtration of discharged air	No	No	Yes/No	Yes
Double door entry-exit	No	No	Yes	Yes
Air lock	No	No	Yes	Yes
Air lock with shower	No	No	Yes	Yes
Entry area	No	No	Yes	-
Entry area with shower	No	No	Yes/No	Yes
Liquid waste treatment / special waste treatment system	No	No	Yes/No	Yes
Autoclave				
-In place of work	No	Optional	Yes	Yes
- In laboratory	No	No	Optional	Yes
- Double way	No	No	Optional	Yes
Biosafety cabinet	No	Optional	Yes	Yes
Staff monitoring	No	No	Optional	Yes

Biosafety Principles

The main purpose of biosafety programs is to eliminate or reduce the potentially harmful biological agents for laboratory workers, other people and those in the external environment. For this reason, the laboratory worker must combine three important factors to reduce the risks associated with agents:

1- Laboratory applications and techniques

2-Biosafety equipment (Primary barriers and personnel protective equipment)

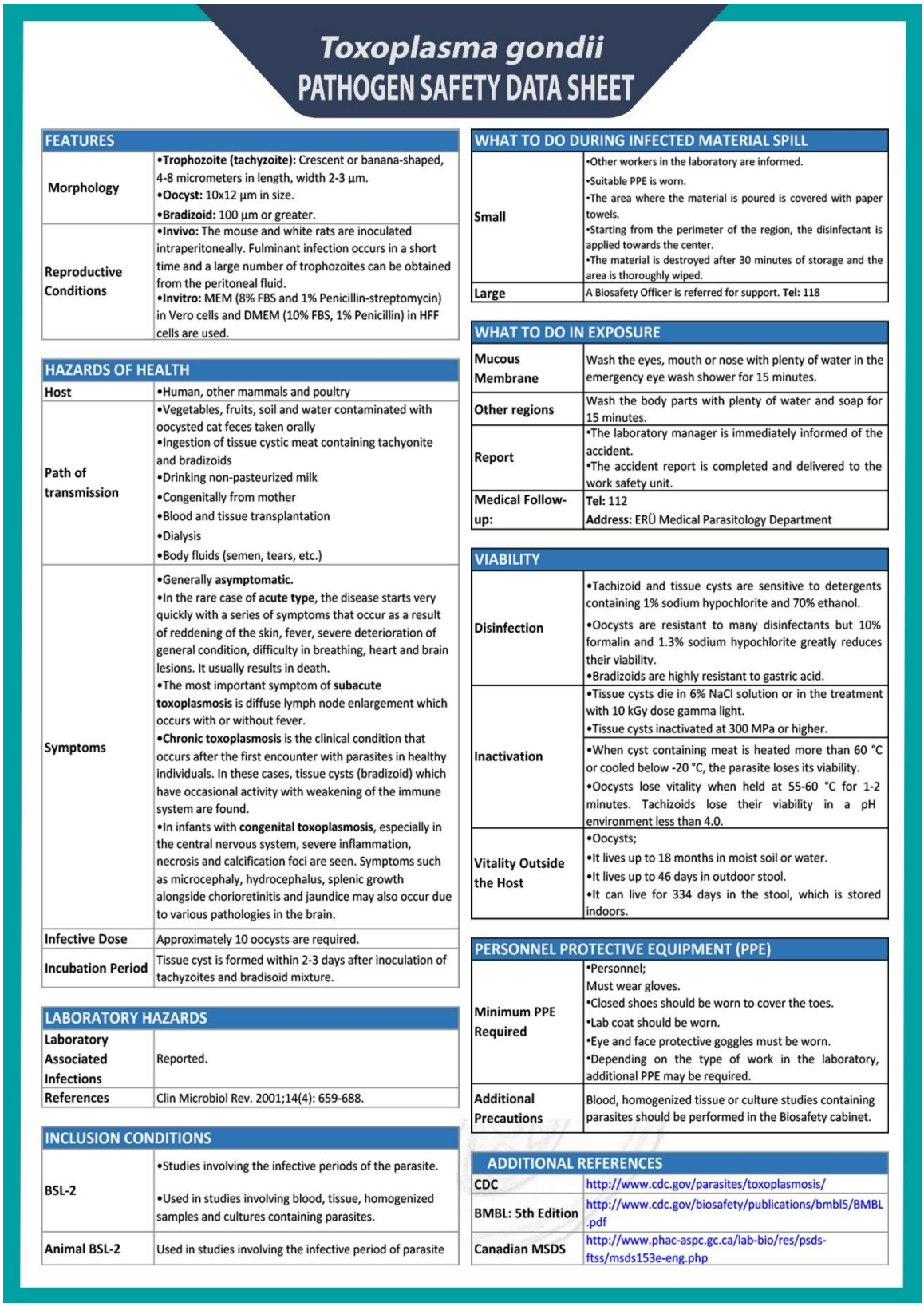
3-Laboratory design and models (secondary barriers)

The most important factor in biosafety protection is the strict adherence to standard microbiological applications and techniques. Additional measures may be required when standard applications and techniques are not sufficient to control pests. Laboratory managers are responsible for identifying and appliying these additional security measures. Biosafety equipment includes biosafety cabinets, closed containers, and design and modifications to reduce the exposure to potential pathogens, as well as to design and modifications related to operational flow. Biosafety cabinets (BSC) are the basic equipments used in the field of biosafety. There are 3 types of BSC (Class I BSC, Class II BSC and Class III BSC) used in laboratories designed for both diagnostic and research purposes. Class I and Class II BSC are the most important primary barriers, and when good microbiological techniques are used, they protect employees, other laboratory workers and the environment, significantly. Class II BSC also protect the material in the cabin from external contamination. Class III BSC provide a high degree of protection for laboratory

workers and the environment. One of the important equipments used as a primary barrier is the centrifugal safety containers. Using these containers can prevent aerosal contamination during centrifugation. Other than these, biosafety equipment used are personnel protective equipments (masks, glasses, face protection, gloves, lab coats, jackets, overshoes, boots).

Secondary barriers are measures that protect people and animals outside the laboratory from being accidentally infected. The secondary barriers to be kept in the laboratory depend on the risk of transmission of the factors studied. In BSL-1 and BSL-2 laboratories, it is required that the working areas are far away from the community, devices such as autoclaves for decontamination and hand washing basins should be found (12).

It is recommended that each institution and university should create "laboratory study guides" related to the pathogens by considering the microorganisms studied in the laboratories. "The pathogen safety data sheet" prepared for *Toxoplasma gondii* in Medical Parasitology Department of Erciyes University is shown in Figure 1. By observing the compliance of the laboratory studies with the mentioned guidelines, it can be ensured that the possible laboratory accidents and transmissions to the personnel and the environment can be prevented.



Toxoplasma gondii
PATHOGEN SAFETY DATA SHEET

FEATURES	
Morphology	•**Trophozoite (tachyzoite):** Crescent or banana-shaped, 4-8 micrometers in length, width 2-3 µm. •**Oocyst:** 10x12 µm in size. •**Bradizoid:** 100 µm or greater.
Reproductive Conditions	•**Invivo:** The mouse and white rats are inoculated intraperitoneally. Fulminant infection occurs in a short time and a large number of trophozoites can be obtained from the peritoneal fluid. •**Invitro:** MEM (8% FBS and 1% Penicillin-streptomycin) in Vero cells and DMEM (10% FBS, 1% Penicillin) in HFF cells are used.

HAZARDS OF HEALTH	
Host	•Human, other mammals and poultry
Path of transmission	•Vegetables, fruits, soil and water contaminated with oocysted cat feces taken orally •Ingestion of tissue cystic meat containing tachyonite and bradizoids •Drinking non-pasteurized milk •Congenitally from mother •Blood and tissue transplantation •Dialysis •Body fluids (semen, tears, etc.)
Symptoms	•Generally **asymptomatic.** •In the rare case of **acute type**, the disease starts very quickly with a series of symptoms that occur as a result of reddening of the skin, fever, severe deterioration of general condition, difficulty in breathing, heart and brain lesions. It usually results in death. •The most important symptom of **subacute toxoplasmosis** is diffuse lymph node enlargement which occurs with or without fever. •**Chronic toxoplasmosis** is the clinical condition that occurs after the first encounter with parasites in healthy individuals. In these cases, tissue cysts (bradizoid) which have occasional activity with weakening of the immune system are found. •In infants with **congenital toxoplasmosis**, especially in the central nervous system, severe inflammation, necrosis and calcification foci are seen. Symptoms such as microcephaly, hydrocephalus, splenic growth alongside chorioretinitis and jaundice may also occur due to various pathologies in the brain.
Infective Dose	Approximately 10 oocysts are required.
Incubation Period	Tissue cyst is formed within 2-3 days after inoculation of tachyzoites and bradisoid mixture.

LABORATORY HAZARDS	
Laboratory Associated Infections	Reported.
References	Clin Microbiol Rev. 2001;14(4): 659-688.

INCLUSION CONDITIONS	
BSL-2	•Studies involving the infective periods of the parasite. •Used in studies involving blood, tissue, homogenized samples and cultures containing parasites.
Animal BSL-2	Used in studies involving the infective period of parasite

WHAT TO DO DURING INFECTED MATERIAL SPILL	
Small	•Other workers in the laboratory are informed. •Suitable PPE is worn. •The area where the material is poured is covered with paper towels. •Starting from the perimeter of the region, the disinfectant is applied towards the center. •The material is destroyed after 30 minutes of storage and the area is thoroughly wiped.
Large	A Biosafety Officer is referred for support. **Tel:** 118

WHAT TO DO IN EXPOSURE	
Mucous Membrane	Wash the eyes, mouth or nose with plenty of water in the emergency eye wash shower for 15 minutes.
Other regions	Wash the body parts with plenty of water and soap for 15 minutes.
Report	•The laboratory manager is immediately informed of the accident. •The accident report is completed and delivered to the work safety unit.
Medical Follow-up:	**Tel:** 112 **Address:** ERÜ Medical Parasitology Department

VIABILITY	
Disinfection	•Tachizoid and tissue cysts are sensitive to detergents containing 1% sodium hypochlorite and 70% ethanol. •Oocysts are resistant to many disinfectants but 10% formalin and 1.3% sodium hypochlorite greatly reduces their viability. •Bradizoids are highly resistant to gastric acid.
Inactivation	•Tissue cysts die in 6% NaCl solution or in the treatment with 10 kGy dose gamma light. •Tissue cysts inactivated at 300 MPa or higher. •When cyst containing meat is heated more than 60 °C or cooled below -20 °C, the parasite loses its viability. •Oocysts lose vitality when held at 55-60 °C for 1-2 minutes. Tachizoids lose their viability in a pH environment less than 4.0.
Vitality Outside the Host	•Oocysts; •It lives up to 18 months in moist soil or water. •It lives up to 46 days in outdoor stool. •It can live for 334 days in the stool, which is stored indoors.

PERSONNEL PROTECTIVE EQUIPMENT (PPE)	
Minimum PPE Required	•Personnel; Must wear gloves. •Closed shoes should be worn to cover the toes. •Lab coat should be worn. •Eye and face protective goggles must be worn. •Depending on the type of work in the laboratory, additional PPE may be required.
Additional Precautions	Blood, homogenized tissue or culture studies containing parasites should be performed in the Biosafety cabinet.

ADDITIONAL REFERENCES	
CDC	http://www.cdc.gov/parasites/toxoplasmosis/
BMBL: 5th Edition	http://www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
Canadian MSDS	http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/msds153e-eng.php

Figure 1. The pathogen safety data sheet for *Toxoplasma gondii*.

Biosafety Level Laboratories-1 (BSL-1)

BSL-1 laboratory is a suitable laboratory type for agents that may potentially have minimal harm to laboratory workers and the environment. In BSL-1 laboratories, studies are generally carried out in open work areas using good microbiological techniques. Special equipment and areas are not required for the elimination of pests in such laboratories.

This type of laboratory staff is specially trained in the procedures carried out in the laboratory. In BSL-1 laboratory, standard microbiological techniques should be applied and primary and secondary barrier equipments should be provided.

A-Standard Microbiological Applications:

1-The laboratory manager should apply the institutional policies on controlled access to the laboratory

2-People who come into contact with the live material or come into contact with the animal should wash their hands after removing their gloves and before leaving the laboratory

3-Because of the risk of exposure to potential infectious material, eating, drinking, smoking, contact lenses and cosmetic use should be prohibited. Persons using contact lenses in the laboratory should wear glasses or face protection. Food should be stored in the cabinet and refrigerator outside the laboratory.

4-The use of pipettes with mouth should be prohibited. Mechanical / automatic pipette tools should be used.

5-Policies on the use of cutting tools (needles, surgical knives, glass fractures) should be developed and implemented. The following precautions should always be taken regarding sharp objects:

a) It is of utmost importance that a careful control of the needle and other cutting tools is carried out. The syringe needles must not be twisted, uncut or broken. After use, the syringe cap should not be closed again or moved by hand before being discarded.

b) Used disposable needles should be placed in puncture-resistant containers for disposal.

c) Non-disposable cutting tools should be placed in a hard-walled container.

d) Broken glass should not be handled directly. Instead, it must be removed using a brush, dustpan, tongs or forceps. If possible, use plastic containers instead of glass containers.

6-All applications should be done carefully so as to minimize splashing or aerosol formation.

7-Working areas should be decontaminated immediately after the study is completed and also with appropriate disinfectants when any infectious material is poured or spilled.

8. All culture, stock and wastes must be decontaminated in a suitable manner, such as an autoclave, before disposal.

a) Decontaminated materials should be transported with sealed and closed containers to the non-laboratory area where they will be kept for a long time.

b) Materials that are decontaminated in the laboratory should be packaged according to the rules to which the laboratory is attached.

9-Universal "Biosafety sign poster" should be hung at the entrance of laboratories with infectious agent. In this poster, the identity of the agent, laboratory manager and other responsible personnel working in the laboratory and the phone should be found. Information about the agents studied in the laboratory should also be posted. This information should be determined in line with institution policies.

10-Control programs for rodents such as insects and mice should be applied.

11-The laboratory manager should take the necessary measures to ensure that the laboratory personnel are not exposed to the infection, taking into account their duties, and ensure that the staff receives appropriate training on the subject. The staff should renew their information once a year and receive additional training when there are changes in the working principles. The health status of the staff may affect the susceptibility to infection, vaccination and prophylactic application. For this reason, all laboratory personnel, especially women in childbearing age, should be monitored for their immune status. Appropriate advice and guidance should be provided by the institution's health care provider for the people in these conditions

B-Special Applications

BSL-1 laboratories do not require special applications to be added to standard microbiological techniques.

C-Primary Barriers (Safety Equipments)

1- Special devices and equipment such as biological safety cabins are not required when working with BSL-1 agents.

2-It is recommended to wear laboratory clothes, lab coats or uniforms to prevent contamination of clothing used outside.

3-Protective goggles should be worn for protection against contact of microorganism splashes or various harmful materials. Personnel using contact lenses among laboratory workers should wear glasses during work.

4-Gloves must be worn to protect hands from potential pests. Glove selection should be made according to risk assessment. Hands should be washed before leaving the laboratory. In addition to the above, BSL-1 employees;

a) gloves; they must be replaced when it is contaminated, its integrity deteriorated, or whenever it is needed.

b) When the work with dangerous material is finished, gloves must be removed and hands washed before leaving the laboratory.

c) Disposable gloves must not be reused or washed. Gloves that have been used with another contaminated laboratory waste should be replaced by disposing of. Hand wash protocols must be applied with care.

D-Secondary barriers (laboratory infrastructure and design)

1- Laboratories must have controlled entry doors.

2- Each laboratory must have a wash basin for hand washing.

3- Laboratories should be designed as easy to clean, carpets and rugs should not be used.

4- Furniture in the laboratory must be sound; no fabric upholstery; Areas and gaps between benches, cabins and equipment should be easy to clean. For this purpose:

a) Bench surfaces; It must be waterproof, resistant to acids, alkalis and organic solvents and should be made of heat resistant material.

b) The seats used in laboratory work must be coated with a non-porous material, should be easily cleaned and decontaminated with suitable disinfectants.

5-If there are windows that can be opened in laboratories, they should be covered with mosquito nets (13).

Biosafety Level Laboratories-2 (BSL-2)

BSL-2 is similar to BSL-1 in many aspects. Such laboratories are places where agents that are moderately harmful to laboratory workers and environment are involved. However, there are 4 important differences from BSL-1.

1-Laboratory personnel should receive a special training on the microorganism to be studied.

2- Access to the laboratory should be limited during the study.

3-Extreme caution should be exercised when working with contaminated cutter instruments.

4-Biosafety cabinets or other necessary safety equipment should be used when performing applications that cause contamination by aeorosol or splash.

A-Standard Microbiological Applications

Standard microbiological applications are as in BSL-1.

B-Special Applications

1-All personnel entering the laboratory should be informed of potential hazards and meet the requirements for entry / exit to the laboratory.

2. In addition to providing appropriate medical assistance to laboratory personnel, vaccines that are related to agents studied or having potential to be encountered in the laboratory should be provided.

3-Each institution should collect the serum samples of the staff at risk and take appropriate conditions, taking into account the future needs.

4. Biosafety guidelines specific to each laboratory should be prepared and this guideline should be adopted as the policy. This prepared biosafety manual should be accessible to the staff.

5-The laboratory manager and laboratory personnel should ensure the adequacy of the personnel in standard and special microbiological applications before working with BSL-2 agents.

6-Potential infectious materials should be placed in durable, sealed containers during collection, transportation, processing, storage or transportation.

7-Laboratory equipment should be routinely decontaminated in addition to decontamination after any spill.

a) Spills on infectious materials should be decontaminated and cleaned by trained and equipment-trained personnel to work with infectious materials.

b) Equipment must be decontaminated before taken out of laboratory for repair, maintenance or any other reasons.

8-Conditions for exposure to infectious agents should be evaluated and treated immediately according to the procedures described in the laboratory biosafety manual. The laboratory manager should be informed immediately when all these events occur. Medical evaluation, supervision and treatment should be provided and events recorded.

9-Any animals and plants not related to the work in the laboratory should not be kept.

10- All infectious material processing involving aerosol should be performed in a BSC or appropriate physical device.

C-Primary barriers (Personal protective equipment)

1-Maintained BSCs, other appropriate personal protective equipment, or other physical transmission preventive devices should be used as a primary barrier in the following cases.

a) Procedures are carried out according to the probability of creating an aerosol or splash. These procedures are: pipetting, centrifugation, rinsing, mixing, grinding, sonication, opening of the packaging covers of infectious materials, intranasal processes in animals, obtaining a new generation from animals or eggs.
b) Conditions in which large volumes or intense concentrations of infectious agents are used. Such materials can be centrifuged using capped rotor or centrifugal safety containers.
2-For laboratory use when working with dangerous substances; lab coat, shirt, uniform or coat should be worn. When leaving the laboratory (to cafeteria, library, etc.), protective clothing should be removed. Laboratory clothes should not be disposed of and should be washed by the institution. It is not recommended to take the clothes used in the laboratory to home.
3-When microorganisms are taken out of the BSC or protective device; Eye and face protection equipment (glasses, mask, face protection) should be used to prevent splashing and spilling of infectious or other harmful substances. Eye and face protectors should be disposable as other contaminated laboratory waste. Laboratory personnel using contact lenses should wear eye protection.
4-Gloves should be worn to protect hands from harmful materials. The choice of gloves should be made according to the risk assessment. Personnel with latex allergy should use alternative gloves. Gloves should not be used outside the laboratory. In addition, for BSL-2 employees:
a) gloves; it must be changed when it is contaminated, its integrity deteriorated, or any requirements.
b) Remove the gloves and wash hands after the work is finished and before leaving the laboratory.
c) Disposable gloves should not be reused or washed. Gloves used with other contaminated laboratory waste should be disposed of. Hand wash protocols must be followed carefully.
5-Eye, face and respiratory protection equipment should be used in the rooms with infected animals according to the risk assessment.

D-Secondary barriers (laboratory infrastructure and design)

1-Laboratory doors must be self-closing and locked in accordance with institutional policies.
2-The laboratory must have a wash basin for hand washing. Wash basins can be manual or automatic, but should be positioned close to the exit door.
3-Laboratories should be easily cleaned and decontaminable, carpet and rugs should not be allowed to use.
4-Laboratory furniture should be intact, there should be no fabric upholstery. Areas and spaces between benches, cabins and equipment must be easy to clean.
a) Bench surfaces should be waterproof, resistant to acid, alkalis and organic solvents and made of heat resistant material.
b) The seats used in laboratory work must be covered with a non-porous material, easily cleaned and decontaminated with suitable disinfectants.
5-It is not recommended that the lab windows are openable and should be covered with mosquito nets if there is a window.
6-BSC should be used. BSC should be away from doors and openable windows, should not be where the traffic of the laboratory is dense and the air flow cannot be provided.

7- Vacuum lines should be protected with liquid disinfectant traps.
8-It should be an emergency eye wash unit.
9-There is no specific requirement for the ventilation system. However, mechanical ventilation systems that do not require air circulation outside the laboratory area should be considered in the planning of new facilities.
10- If the BSC are tested and verified at least once a year according to the manufacturer's recommendation, HEPA filtered Class II BSC-treated waste gases can be recirculated safely to the laboratory environment. The waste gases from the BSC can be exported directly or they can be disposed of in the laboratory waste system.
11- It must be a suitable method for decontamination of all laboratory waste in the laboratory (autoclave, chemical disinfection, incineration and other valid decontamination methods) (13).

Biosafety Level Laboratories-3 (BSL-3)

Biosafety Level Laboratory-3; It is suitable for studies with agents that can cause serious or potentially fatal diseases through clinical diagnosis, training, research or inhalation. Laboratory staff should receive specific training on pathogenic and potentially lethal substances, and their mastery of the application of infectious agents and related procedures should be supervised by authorized scientists.
All procedures involving applications to infectious agents must be performed in BSC or other physical devices.
BSL-3 has special engineering and design features.
The following standard and special safety applications, equipment and plant requirements is also valid for BSL-3.

A-Standard Microbiological Applications

Standard microbiological applications should be performed as in BSL-1.

B-Special Applications

Special applications in BSL-2 must be carried out. In addition, all procedures involving manipulation of infectious substances should be performed within BSC or other physical cabin devices. No work should be done on open benchs. When aplications are not made in BSC, other protective equipment, such as personnel protective equipment and capped rotor, centrifuge covers, is mandatory.

C-Primary Barriers (Safety Equipments)

1-All procedures related to infectious material should be performed in BSC (preferably Class II or Class III BSC).
2-Lab workers should be wearing knitted, surgical clothes or overalls with protective covers in the back and should not wear these clothes outside the laboratory. Reusable clothing must be decontaminated before washing. It should be replaced when it is contaminated again.
3-When microorganisms are removed from the BSC or protective device, eye and face protection equipments (glasses, mask, face protection) should be used to prevent splashing and spilling of infectious or other harmful substances. Eye and face protectors should be disposable as other contaminated laboratory waste. Laboratory personnel who use contact lenses should wear eye protection.

4-Gloves should be worn to protect hands from harmful materials. Glove selection is made according to risk assessment. There are alternative gloves for latex for people with latex allergy. Gloves should not be used outside the laboratory. In addition, BSL-3 employees must comply with the following rules:

a) Gloves; it must be changed when it is contaminated, its integrity deteriorated, or any requirements. Two layers of gloves can be worn if appropriate.

b) When the work is finished with harmful material and before leaving the laboratory, gloves should be removed and hands washed.

c) Disposable gloves should not be reused or washed. Gloves used with other contaminated laboratory wastes should be disposed of and hand washing protocols should be followed carefully.

5-Eye, face and respiratory protection equipment should be used in the rooms with infected animals as a result of the risk assessment.

D-Secondary barriers (laboratory infrastructure and design)

1-Laboratory doors must be self-closing and locked in accordance with institutional policies. The laboratory should be located in a place away from the traffic density in the building. The laboratory entry should be closed with two automatical doors. The staff dressing room can be located in the section between these two doors.

2-The laboratory must have a wash basin for hand washing. Sinks can be manual or automatic. Sinks should be positioned close to the exit door. If you have two separate laboratories intertwined, you must have the sink to wash the hand in both laboratories. Additional sinks may also be required according to the risk assessment.

3-Laboratories should be easily cleanable and decontaminable. Carpet and rugs should not be allowed in the laboratory. The floor, wall, ceiling and connection areas must be closed. The space around the door and ventilation openings must be closable to facilitate decontamination.

a-Floors should be made of liquid-proof material that is resistant to sliding and chemicals.

b-Walls should be constructed so that their smooth surface can be easily cleaned and disinfected.

c-Ceilings should also be like walls in general.

When the laboratory is closed for major renovations or maintenance, or in case of large contamination and significant changes in laboratory use, decontamination of the entire laboratory should be ensured. The selection of appropriate materials and methods for laboratory decontamination should be based on the risk assessment used.

4-Laboratory furniture should be intact, there should be no fabric upholstery. Areas and spaces between benches, cabins and equipment should be easy to clean.

a) Bench surfaces should be waterproof, resistant to acid, alkalis and organic solvents and made of heat resistant material.

b) The seats used in laboratory work must be covered with a non-porous material, easily cleaned and decontaminated with suitable disinfectants.

5-All windows in the laboratory must be closed system.

6-BSC should be used. BSC should be away from doors and openable windows, should not be where the traffic of the laboratory is dense and the air flow cannot be provided.

7-Vacuum lines should be protected with HEPA filter or an equivalent filtration system. If necessary, filters must be replaced. There may also be disinfectant traps in the vacuum lines.
8-It should be an emergency eye wash unit.
9- Channel ventilation system is required. This system must provide a continuous flow of air from "clean" areas to "potentially contaminated" areas by pulling air into the laboratory. The laboratory should be designed so that the air flow is not reversed in case of malfunction.
a-Laboratory staff should ensure the directional air flow with the visual observation of a device confirming the directional air flow at the laboratory entrance. There should be an audible warning system that warns of disturbances in the air flow.
b-Laboratory waste air should not be circulated to other areas of the building.
c-Laboratory waste air must be passed through HEPA filter. The outlet area of the laboratory waste air should be away from the air intake areas and the places people use.
HEPA filter housings must have gas-tight insulation dampers and decontamination ports. Each HEPA filter and assembly must be tested for leakage. Filters should be subjected to at least yearly certification.
10- Class II BSC with HEPA filter that filters dirty air should be tested and inspected at least once a year, and it can be recycled more safely if the user is working according to the recommendations of the manufacturer. The output of the BSC can be connected to the output system of the laboratory, or it can be directly connected outside with a robust system. Appropriate safety cabinet performance and air system must be verified. The BSC needs to be inspected at least once a year for the correct performance. Class III BSC must be connected directly to the second outlet HEPA filter of the cabinet. It must be designed to protect air from the positive pressure of the cabinet.
11- There must be a suitable method for decontamination of all wastes in the laboratory (autoclave, chemical disinfection, incineration and other valid decontamination methods).
12-For the decontamination of all laboratory wastes, there should be a suitable method in the laboratory or in the facility (such as an autoclave, chemical disinfection or other approved decontamination method).
13-When designing the laboratory, it should be considered that large parts of the equipment will be decontaminated before being removed from the laboratory.
14- As a result of enhanced environmental and personal protection, certification reports or risk assessment analysis; applicable formal, local or national regulations may be required. These advanced laboratories may include at least one of the examples: a waiting room for clean storage and safety shower; gas-tight shock absorbers for laboratory insulation; final HEPA filtration for laboratory waste air; waste disposal and advanced control devices such as biometric devices.
15-BSL-3 laboratory design must be validated in terms of operating parameters and procedures and this must be documented before starting laboratory work. BSL-3 laboratory standards must be approved once a year (13).

Biosafety Level Laboratories-4 (BSL-4)

BSL-4 is necessary to work with dangerous pathogens. It is usually not known how these pathogens are transmitted, these pathogens can infect people through aerosol, can be life-threatening and often fatal. There is no vaccination and treatment against these pathogens. The laboratory staff should receive special and comprehensive training because they work

with highly dangerous infectious agents. Laboratory staff should be familiar with standard and specific applications, anti-infection equipment and laboratory design issues. All laboratory personnel and supervisors must be competent in materials and procedures that require work in the BSL-4 laboratory. The laboratory supervisor controls access to the laboratory in accordance with the institutional policies.

BSL-4 laboratories have 2 models:

1-Cabin Laboratory: Pathogen in this laboratory type should be studied in Class III BSC.

2-Suit Laboratory: In this laboratory, personnel should wear an air-protective suit that gives positive pressure.

A-Standard Microbiological Applications:

Standard microbiological applications should be performed as in BSL-1.

B-Special Applications:

1-All personnel entering the laboratory should be informed about the microorganisms to be studied and the precautions to be taken regarding the access to the laboratory.

Only persons who will be working in the laboratory and assisting them should be allowed to enter.

Access to the laboratory should be through safe and locked doors.

The date and time of all people entering and exiting the laboratory should be recorded in a notebook.

Apart from emergency situations, personnel should change clothes and use shower at the entrance and exit of the laboratory. All personnel should change their clothes in the changing room. All persons entering the laboratory should use laboratory clothes, such as underwear, pants, shirts, overalls, shoes and gloves. All staff should take a shower while leaving the laboratory. In case of doubt, some materials should be considered as contaminated material and decontaminated before washing.

After the laboratory has been completely decontaminated and all infectious agents have been secured, personnel can make the entry and exit without the clothing and the shower described above.

2-Laboratory personnel and support personnel should be provided with medical services such as vaccination and medical assistance. For the medical follow-up of laboratory-related accidents, a system should be established for reporting and documenting laboratory accidents, exposure to agents and the absences of employees. There should be a facility and system for medical assistance and isolation in the case of a laboratory-related infection.

3-Each institution should collect and store serum samples of staff at risk.

4-Laboratory specific biosafety guidelines should be prepared. This manual should be accessible at any time.

5-The laboratory manager is responsible for ensuring for staff that:

a) To provide competence in standard and special microbiological practices and techniques in applications with agents requiring work under BSL-4 conditions.

b) To receive appropriate training for laboratory-specific applications and operations.

c) To receive annual updates and additional training when procedural or policy changes related to laboratory studies occur.

6-The materials which are taken either alive or intact out of the laboratory are transferred to a non-corrosive, leak-proof container and then taken into a second non-corrosive, leak-

proof container. These materials should be transferred through a disinfectant tank, fumigation chamber or decontamination shower.

7-Laboratory equipment should be routinely decontaminated as is done after spills, splashes or other potential contamination.

a) Spills containing infectious materials should be handled, decontaminated and cleaned by appropriate professional personnel who are trained and capable of working with infectious material. The procedure to be applied after the spill must be determined and put into a visible place.

b) The equipment must be decontaminated using an effective and valid method before taken out of the laboratory for repair or maintenance purposes.

c) Equipment and material that can be damaged by high temperature or steam should be cleaned in an air chamber or in a room designed for this purpose using an effective and valid procedure, such as a gas or steam method.

8-Events that may cause exposure to infectious agents should be evaluated and treated according to the procedures described in the laboratory biosafety manual. All events occurring in the laboratory should be reported to the laboratory manager and the responsible laboratory staff as defined in the laboratory biosafety manual. Events related to the subject should be recorded, necessary medical evaluation, follow-up and treatment should be done.

9- Animals and plants not associated with laboratory studies should not be allowed to enter the laboratory.

10- If the materials to be entered into the BSL-4 laboratory are not brought from the exchange chamber, they should be firstly entered after decontaminating with a double-door autoclave or fumigation chamber or airtight cabinet. The doors of the autoclave or fumigation rooms must be interlocked to prevent contamination by opening the outside door.

Only necessary consumables and equipment should be included in the BSL-4 laboratory. In addition, all equipment and consumables must be decontaminated before removal from the laboratory.

11- Before starting the laboratory studies, it should be ensured that the laboratory works according to the system and the daily supervision of life support systems should be completed and documented.

12- Effective and practical protocols should be established for emergency cases. These protocols should include plant failures, fires, animal escape in the laboratory and other possible emergencies. Training on emergency response procedures should be provided by emergency response personnel and other responsible personnel according to institutional policies.

C- Primary Barriers (Safety Equipment)

Cabin Laboratories

1-All procedures related to infectious substances should be performed in the laboratory in Class III BSC.

Class III BSC gloves should be checked for damage before use and replaced if necessary. Cabin gloves should be renewed during the annual certification.

Work with high concentrations or large volumes of infectious material in the Class III cabin must be performed in a device to prevent contamination within the cabin. Such materials should be centrifuged using closed-rotor or mouth-capped centrifuge tubes in the cabinet.
The certification of the Class III biological safety cabinet must be carried out at least once a year.
2-Laboratory workers should use tight protective laboratory clothes such as protective apron or overalls with their front sides closed. No personal items, such as clothes, jewelry, other than glasses, should be taken into the personal shower area. All protective clothing must be removed in the dirty replacement room before showering. The clothes to be re-used must be autoclaved before they are removed from the laboratory to wash.
3-Eye, face and respiratory protection materials should be used according to the risk assessment in the rooms with infected animals. Glasses used by personnel in daily life should be decontaminated before leaving the body shower.
4-Disposable gloves should be worn inside the cabin gloves and caution should be taken against tearing of cabin gloves. Gloves should not be worn outside the laboratory. Gloves should not be washed and reused. Gloves used with other contaminated products must be disposed immediately.
Suit laboratories
1-All procedures should be done by wearing one-piece positive pressure protective clothing.
All procedures related to infectious agents should be performed within BSC or other primary barrier system.
Aerosol-producing equipment must be in the primary barrier devices that pump out the air after passing it through the HEPA filter. HEPA filters should be tested annually and replaced if necessary.
Class II booths must be inspected and certified at least once a year. The air discharged through HEPA filters can be safely recirculated in the laboratory.
2-Employees should wear a special laboratory suit covering the entire body before entering the laboratory. All clothes used must be changed in the dirty room before entering the shower.
3- In addition to suit laboratory gloves, the inner disposable gloves should also be worn. Disposable gloves should not be worn outside the dress change area. Disposable gloves should not be washed and reused. Inner gloves should be removed and disposed of in the interior of the dress change chamber before entering the shower. Used gloves should be discarded with other contaminated waste.
4-During laboratory studies; In order to eliminate contamination and to minimize contamination of the laboratory, decontamination of outer gloves is performed.

D-Secondary barriers (laboratory infrastructure and design)
In BSL-4 laboratories, in addition to BSL-3, the following secondary barriers should be provided.
1-Completely separated building or isolated areas
2-The presence of ventilation and disinfection systems for the removal of infected air (13).
Personnel protective biosafety equipment
Biosafety personnel protective equipment include biological safety cabins, face protectors, emergency head and eye showers, masks, goggles, clothing, gloves and others.

Biosafety Cabinets

Biosafety cabinets are designed to protect the laboratory environment, staff and working material from aerosols and splashes that may occur when working with infectious agent-containing materials such as primary cultures, stocks and diagnostic samples. BSC have undergone some changes in parallel with major developments over time. The most important change is the addition of HEPA (high-efficiency particulate air) filter to BSC. The HEPA filters have a 99.97% potential to hold particles of 0.3 micrometers. Together with the developments in biotechnology, BSC are classified in three main groups as Class I, Class II and Class III (14-16). According to this:

Class I Biological Safety Cabinets

Class I BSC are first designed and widely used cabinets in the world. The most important advantage is that it is used frequently in radioactive and volatile chemical toxicity studies due to its personnel protection feature.

The room air is drawn at a speed of at least 0.38 m/s from the front opening of the BSC and is discharged through the work surface and through the discharge channel. These class cabins are not used in the studies where the air in the cabinet, like cell culture, must be protected because there is a direct penetration of the room air into the working surface in these cabinets (17, 18).

Class II Biological Safety Cabinets

Class II BSC have the ability to protect both the personnel and the work surface from the contaminated air. It is more widely used than Class I BSC because of its protection of the studied product. In this class of cabinets, HEPA filtered air is supplied inside the cabinet. Class II BSC are used for studies with microorganisms in risk groups 2 and 3. The cabinets in this class can also be used for studies with risk group 4 microorganisms when a positive pressure suit is used. Class II BSC have four types, A1, A2, B1 and B2:

The flow velocity of air in Class II A1 BSC should be at least 0.38 m/s across the front of the cabin. 70% of the hood air is recirculated in the cabinet after passing through the HEPA filter. The remaining 30% of the air is discharged into the laboratory or expelled. The air coming out of the Class II A1 BSC waste system can either be delivered to the room or thrown out of the building or into the building's air waste system by means of a hood.

Class II A2 BSC are connected outside to through a hood. Class II B1 and B2 cabinets are like Class I cabinets.

Biological safety cabinets are classified according to factors such as the speed of air entering from the cabin front opening, the ratio of air being recirculated in the cabin and the amount of exhausted air after being passed through the HEPA filter and the waste system (Table 8) (17, 19).

Table 8. Differences between biological safety cabinets.

Cabinet Type	Air Flow Speed inside the Cabinet (m/s)	Air Flow (%)		Exhaust System
		Recirculation inside the Cabinet	Exhausted Air	
Class I	0.36	0	100	Direct
Class II A1	0.38-0.51	70	30	In-room or hood connected

Class II A2	0.51	70	30	In-room or hood connected
Class II B1	0.51	30	70	Direct
Class II B2	0.51	0	100	Direct
Class III	*	0	100	Direct

*; This type of cabinet has no front opening and has a cabinet glove system.

Class III Biological Safety Cabinets

This type of cabinets are the cabinets with a high degree of protection in the work with microorganisms in the risk group 4.

Class III cabinets are a completely closed system and cabin work is carried out with the help of long-sleeved gloves mounted on the cabin. Cabinet-mounted double-door autoclave is used to provide input and output of the cabinet.

Class III cabinets are required for studies in BSL-3 and BSL-4 laboratories (10).

Biosafety in animal facilities (maintenance units)

Experimental animals are used for diagnostic purposes as well as for research purposes. The experimental animals used for this purpose should be housed in a comfortable, hygienic environment and should be fed with adequate and healthy food and water. Ethical and institutional permits should be obtained in all studies with animals. At the end of the experiments, animals should be evaluated according to ethical rules.

During the use of experimental animals; the drug to be tested in animals and any material to be injected are important. On the other hand, there are very important procedures in terms of maintenance and care of these animals. For security reasons, animal shelters should be independent or self-contained. If a separate building cannot be allocated and is positioned adjacent to other buildings, necessary precautions should be taken for decontamination and disinfection.

Just like laboratories, animal care units are also classified according to the microorganism risk assessment and classified as Animal Biosafety Level-1 (ABSL-1, ABSL-2, ABSL-3 and ABSL-4). The differences of these units according to microorganism risk level of these units, biosafety equipments and the required applications are summarized in Table 9 (20-22).

Table 9. Biosafety levels of animal care units and equipments necessary to be kept in these units and Biosafety applications.

Risk Group	Laboratory level	Laboratory applications and biosafety equipment
1	ABSL-1	Limited entry, protective clothing and goggles
2	ABSL-2	In addition to ABSL-1; Biosafety hazard sign, Class I and II BSC for aerosol producing applications, Decontamination of waste and cages before washing
3	ABSL-3	In addition to ABSL-2; controlled access, BSC and special protective clothing for all applications
4	ABSL-4	In addition to ABSL-3; very limited entrance, change of dress before entrance, Class III BSC or positive pressure dress, shower at the exit, decontamination before disposal of all waste from the unit

Animal Biosafety Level-1

In Animal Biosafety Level-1; risk group 1 animals in which the microorganisms are inoculated are examined and associated applications are performed. In these laboratories, good microbiological application conditions called GMP should be considered. The animal plant manager should set procedures, protocols and policies for access to the facility and for all applications. An appropriate medical follow-up method should be established for employees. A guideline approved by relevant institutions should be prepared and put into effect (20-22).

Animal Biosafety Level-2

These laboratories are suitable for studies with animals in which the microorganisms in risk group 2 are inoculated. In addition, the following security measures apply:

1-All the requirements for the animal plant should meet ABSL-1 criteria.
2-Biosafety warning sign should be hung on the door and appropriate places.
3-The plant must be designed to be easy to clean and maintain.
4-Doors must be opened inwards and self-closing.
5-Heating, ventilation and lighting should be sufficient.
6-If the mechanical ventilation is provided, the air flow must be inward. The exhaust air must not be recirculated in any part of the building.
7-Entries should be limited to authorized persons only.
8-Animals should not be allowed to be used other than animals used in the experiments.
9-Arthropod and rodent control program should be applied.
10- If there is a window, it should be safe and resistant to breakage. If the windows can be opened, it should be covered with arthropod proof mosquito-nets.
11-Working surfaces should be disinfected with effective disinfectants after the work.
12- For aerosol forming studies, BSC Class I and II or isolated cages with appropriate air intake and HEPA filtered waste system should be provided.
13-Autoclave should be in or near the facility.
14-Animal bedding materials should be removed to minimize aerosol and dust formation.
15- All waste materials and animal bedding materials must be decontaminated before disposal.
16-The use of cutting tools should be limited as much as possible. After use, the cutting tools must be kept in a puncture-proof, sealed container and considered to be infectious.
17- The materials to be autoclaved and incinerated shall be carried safely in closed containers.
18-Animal cages should be decontaminated after use.
19-Animal carcasses must be destroyed.
20-Protective clothing and equipment should be worn at the facility and should be removed when exiting.
21-Hand washing unit must be available. Staff should wash their hands after leaving the facility and entering.
22- Small injuries should be treated, all injuries should be reported and recorded.
23-Eating, drinking, smoking and cosmetic application should be prohibited in the facility.
24-All staff should take the necessary training for this laboratory (20-22).

Animal Biosafety Level-3

These laboratories are suitable for studies with animals in which the microorganisms in group 3 are inoculated. All systems, applications and procedures in such places should be reviewed and annual certification should be completed. The following safety measures should also be taken:

1- For these animal facilities - Biosafety Level 1 and 2 requirements must be met.

2-Strictly controlled entry rules should be applied.

3-The facility should be separated from the other laboratory and animal housing areas by two doors with an entree area.

4- There should be a hand washing unit and shower in this entree area.

5-All rooms should have mechanical ventilation to ensure continuous airflow. The exhaust air must be filtered through the HEPA before being discharged into the atmosphere. The system should be designed so as to avoid countercurrent and positive pressurization anywhere on the plant.

6-Autoclave should be at the place where biological harmful agents and substances are present in the animal plant.

7-The incineration furnace shall be present in the facility or the measures to be taken to perform this function shall be taken by the relevant institution.

8- Risk group 3 animals infected with microorganism should be fed in rooms with ventilation waste system located behind their cages.

9-Animal beds should be as dust-free as possible.

10- All protective clothing should be decontaminated before washing.

11-Windows must be closed, locked and resistant to breakage.

12-As far as possible, staff should be vaccinated (20-23).

Animal Biosafety Level-4

In this facility, the work should be regulated according to the level of biosafety level 4 in maximum isolation conditions and according to local and national rules. The following safety measures should also be taken:

1- For these animal facilities - Biosafety Level 1, 2 and 3 requirements must be met.

2-Strictly controlled entry rules must be applied: only those designated by the staff manager must have access authorization.

3-Persons should not work alone: at least two people should work together.

4-Personnel should be trained as much as possible in the field, should be aware of the hazards in their work and take the necessary measures.

5-Risk group 4 microorganism infected animals should be maintained in accordance with the laboratory conditions of the maximum isolation level of biosafety level 4.

6- Personnel should enter the facility through an air locked hallway. The clean area should be separated from the areas where the clothes are changed and the shower is located.

7. When entering the facility, the personnel must change their outer clothing and wear special protective clothing. After the work is finished, protective clothing should be removed and sent to the autoclave before taking a shower.

8-The system must be ventilated with HEPA filter waste air system designed to provide negative pressure.

9-The ventilation system should be designed in a way to prevent the reverse flow and positive pressurization at any place.

10 - The transfer of the material to the clean area should be done by a double door autoclave.
11-Materials that are not autoclavable should be stored in a part of the clean room except where contaminated materials are located.
12- All the applications related to animals infected with risk group 4 microorganism should be performed under Biosafety level 4 conditions, including maximum isolation conditions.
13-All animals must be isolated.
14- All animal wastes and deposits should be autoclaved before disposal.
15- The personnel working in this facility must be subjected to medical supervision (20-22).

Arthropod biosafety level laboratories (Arthropod BSL)

Risk assessment for arthropods important for public health is based on the arthropod biosafety level laboratory guidelines. Arthropods carry many parasites such as *Plasmodium, Leishmania,* in addition, they carry many viral and bacterial agents or become biological vectors for these agents. In advanced research with parasites such as *Plasmodium* and *Leishmania*, the life cycle of these parasites must be ensured. In addition, it is necessary to meet certain criteria for laboratory conditions to keep the continuity of arthropods due to the reasons such as the cultivation of arthropods such as *Lucilia sericata* for the treatment of maggot debridement or similar studies with this kind of arthropods.
In these laboratories, increased safety measures such as those in biosafety level laboratories should be taken. In parallel, four Arthropod BSLs were identified. The most flexible in terms of arthropod biosafety level laboratories is Arthropod BSL-2. This level covers the most exotic and transgenic arthropods and is necessary for studies to be performed with microorganisms requiring BSL-2. For each Arthropod BSL standard design, special applications, primary barrier equipment and the design of laboratory infrastructure and design with secondary barrier are required, same as in BSL laboratories (24).

Chemical, Electrical and Fire Safety

Only pathogenic microorganisms are not dangerous for staff working in both routine diagnosis and research laboratories such as parasitology. At the same time, various chemicals are dangerous for the personnel. For this reason, it is important to have accurate information about the toxic effects of chemicals, exposure routes, hazards that may be associated with transport and storage. Chemicals that are not compatible with one another should not be stored together. Material safety data sheets (MSDS) or information on other chemicals must be obtained from the manufacturer or supplier.
With the use of chemical materials, the respiratory system, blood, lungs, liver, kidneys, the gastrointestinal system and other organs and tissues, can be seriously damaged. Some chemicals used may be carcinogenic or teratogenic. Some inhaled solvent vapors are also toxic. In addition, prolonged and repeated exposures to the liquid phase of some organic solvents can cause serious skin damage.
Storage should be demonstrated with caution in explosive chemicals such as ether, picric acid, perchloric acid and azides. Necessary measures should be taken and equipment should be made available taking into consideration the possible situations related to the spillage and leakage of chemicals. Measures should be taken to store compressed and liquefied gases in suitable places and environments.

Laboratory personnel should be taken into consideration for the possible hazards of different forms of energy, including fire, electricity, radiation and noise, and the necessary precautions should be taken into account (25, 26).

Transport of Infectious Agents

An infectious agent is a substance that is pathogenic or suspected to be pathogenic. Pathogens are infectious microorganisms, such as bacteria, viruses, fungi, and parasites, or infectious particles such as prions that cause disease in humans and animals. Infectious substances can be found in cultures, body fluids and tissues.

Transport of infectious substances and materials is carried out in accordance with the rules, set by the Ministries of Transport and Civil Aviation Organizations of each country, governing the transport of risky materials.

Rules have been set up to protect the carriers, the public and the environment from the harmful effects caused by the infectious material carried. A healthy transport of the infectious material is possible with a rigorous packaging and sufficient information about transport.

The packaging and transport of infectious substances is carried out in the world by the International Air Transport Association (IATA) under the code of "Regulation on Hazardous Substances". In Turkey, it is carried out under the regulatory framework entitled "*Infectious Diagnosis with infectious substances and Transport Regulations of Clinical Samples*" which is published in the Official Gazette No. 27710 on September 25, 2010. The infectious agents within the scope of these regulations are classified into two categories as "Biological substance, Category A" and "Biological substance, Category B".

Category A is used to describe infectious substances that can cause permanent disability, life-threatening or lethal disease in healthy people or animals when exposed; Category B is the term used to describe infectious substances that do not meet Category A criteria.

The packaging uses the PT602 code for category A and the PT650 code for category B. The PT904 code is also used if it is necessary to transport, for example, on dry ice (carbon dioxide ice) during transport. PT650 rules are generally applicable with some exceptions in the packaging of patient blood and urine samples to be sent for the tests for organ function tests, biochemical tests, hormone tests and cancer markers for non-infectious diseases.

Packages should be packed with a triple packaging system consisting of nested internally, first, second and third containers, respectively. The first container must be of a sealed, preferably screw-capped and/or sealed type, and controlled by the sender and/or the packager. The second container must be durable, waterproof and impermeable. The second containers must be certified/approved by the United Nations (UN), including free fall and pressure testing. The third container or outer container shall be made of material resistant to trauma and pressure effects, such as corrugated board, corrugated plastic, foam, thick cardboard or board. It is compulsory to have a pocket or compartment, which is designed to carry related documents together with the package and to prevent these documents from falling, wetting, mixing and disappearing, on a surface other than the bottom surface of the container. On the third container, the symbol of the United Nations, the type code of the package, the class, the package production date, the manufacturer's code, the recipient and the sender contact information should be included.

Infectious substances must be transported in accordance with the official newspaper directive, the European Agreement on the Carriage of Dangerous Goods by Road (ADR) or the IATA (International Air Transport Association), in a controlled, safe, fast and environmentally sound manner.

For the transport of substances in Category A, it is mandatory to obtain permission from the Ministry of Health or Provincial Health Directorates for the transport of substances with UN code 2814 (Infectious substances affecting only people). For the transportation of substances with UN code 2900 (infectious substances affecting only animals), it is mandatory to obtain permission from the Ministry of Food, Agriculture and Livestock or Provincial Directorates of Agriculture.

"Biological Substance, Category B" and UN3373 code number are used for the transport of substances in Category B. If the infectious substance contained in the infectious diagnostic specimen is known for certain or is highly predictable, then the appropriate name for that category and the UN2814 or UN2900 code numbers are used. The UN3373 code number should be used if the infectious substance it contains is unknown (21, 27-29).

Biological Waste and Disposal

Wastes should be separated according to the following criteria and necessary actions should be taken.

1. Infectious, potentially infectious, recombinant or synthetic nucleic acid biological wastes
2. Non-infectious biological wastes
3. Cutter waste
4. Radioactive / biological wastes
5. Chemical / biological wastes

1-Infectious, Potential Infectious, Recombinant or Synthetic Nucleic Acid biological wastes: Such wastes are substances that contain or are contaminated with:

- Human, animal and plant pathogens
- Recombinant or synthetic nucleic acids and recombinant organisms
- Laboratory and clinical wastes containing human and animal blood, blood products, tissues, cell cultures and other potentially infectious materials
- Cultures

Infectious, potentially infectious, recombinant or synthetic nucleic acid biological laboratory wastes must be inactivated before removal from the facility. Although combustion and chemical inactivation are appropriate in some cases, the most preferred method is steam sterilization (autoclave). The storage of all wastes in this category which are not inactivated should be in the laboratory. Infectious or pathogenic waste should be kept in closed/enclosed waste cans. Such wastes should not be stored for more than 24 hours until inactivation. Biological waste bins or bags should be marked with a biohazard mark. Full or partially filled waste bins should not be kept for more than 30 days.

2-Non-infectious biological wastes

Such wastes are used in clinical and research laboratories and are not defined as infectious biological wastes:

- Laboratory materials (cell culture tubes and flasks, vials, centrifuges and test tubes)

• Unused medical devices
• Gloves and other protective equipment the personnel
• Blood, blood products or tissues that are not contaminated with or are not known to be contaminated with pathogens.
Inactivation of such wastes is not necessary before being removed from the plant. Such products should be placed in red biological waste containers or sachets.

3- Cutter wastes
Tools that cut and injure the skin (lancet, scalpel blades, needles or syringe / needle combinations) should be placed in red hard plastic boxes. The cutter waste should be closed when the waste container is full and must be discarded within 30 days of being closed. Cutter waste bins are placed in red biological waste containers or sachets. If there is contamination with infectious, potentially infectious, recombinant and synthetic nucleic acids, the cutter waste bins must be autoclaved before disposal. Tools that can cut the skin (brittle glass, glass slides and cover slips, razor blades, pipettes and pipette tips) should be removed in such a way that they do not damage anyone or anything.

4-Radioactive / biological waste mixtures
Radioactive wastes contaminated with infectious, potentially infectious, recombinant, and synthetic nucleic acid components, if possible, should be inactivated prior to collection by the Radiation Safety Service.

5-Chemical/biological waste mixtures
Chemical wastes contaminated with infectious, potentially infectious, recombinant, and synthetic nucleic acid components, if possible, should be inactivated prior to collection for disposal by the service responsible for the chemical/biological waste. Necessary precautions should be taken against the formation and release of toxic chemicals during inactivation. For this process autoclave is generally not recommended.
Boxes of biohazardous waste, bags and cutting tool boxes must be closed in laboratories in order to be disposed without waiting for full filling. The waste bag or the box should be labeled with the name of the place where the waste was obtained, the name of the laboratory and the phone and the name of the person who prepared the bag, and left to the appropriate place where the wastes will be taken within a month (11).

Biosafety and Recombinant DNA technology
With the human-centered approach in every field of work, the concept of Biosafety has been used in laboratories in parallel with the prominence of the security first slogan. In particular, the development of recombinant DNA technology, the emergence of new products and the concept of biosafety have necessitated the determination of certain criteria in terms of biosafety.
PUC-based vectors and E.coli cells are extensively used in recombinant DNA technology. The studies to be performed using these systems are performed in the BSL-1 laboratory, usually there is no need for higher level Biosafety laboratory conditions. Biosafety level higher than BSL-1 in recombinant DNA technology studies is required in the following cases:

1. Where the expression of DNA sequences produced from pathogenic organisms increases the virulence of genetically modified organisms,
2. Studies on DNA sequences with unknown character,
3. Studies on gene products with potential pharmacological activity,
4. Studies on gene products of toxins.

Viral vectors such as adenovirus vector are commonly used for transfer to other cells. These vectors should be considered at the same level of biosafety as the parent Adenovirus from which they were derived.
Transgenic plants, transgenic or 'knock-out' animals have been used extensively in many studies in recent years. For the production of such plants and animals, risk assessment should be carried out to determine the appropriate level of biosafety.

Risk assessment of genetically modified organisms: During the risk assessment of genetically modified organisms, the biological safety level of the medium to be studied should be determined by separate evaluation of the donor or recipient/host organisms. Genetic hazards introduced by recombinant DNA technology: It should be noted that when working with the following genes known for their active biological and pharmacological properties there may be increased risk:
• Toxins
• Cytokines
• Hormones
• Gene expression regulators
• Virulence factors and enhancers
• Oncogenic gene sequences
• Antibiotic resistance
• Allergens
The conditions to be considered for the recipient or the host are: host sensitivity, virulence, infectivity, immune status and exposure of the recipient (30-32).

Biosafety Checklist
After the laboratory and laboratory-related safety and biosecurity measures have been identified and implemented, a checklist that does aim to assist in the assessment of the safety status of the laboratory should be established. This evaluation should include services such as: laboratory buildings, storage facilities, sanitation and personnel facilities, heating and ventilation systems, lighting, laboratory biosecurity, fire prevention and fire protection measures, evaluation of flammable liquid tanks, compressed and liquefied gases, electrical installation and related measures, personnel measures for protection and monitoring of personnel health and safety, laboratory equipment and calibration, water, gas, malfunctions and repair. Evaluations related to these operations should be identified and corrected (10).

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