Working in a Microbiological Laboratory: face-to-face with infectious risk

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Introduction

A large number of workers are employed in microbiological laboratories that range in size and complexity from large research and clinical laboratories to the physician's office laboratory. These workers are exposed to a variety of potential occupational health risks that include infectious materials and cultures, toxic-carcinogenic and flammable chemicals, radiation, mechanical and electrical hazards. Although all occupational hazards are important, the most serious one in the laboratory is the biological agents (microorganisms), which may be found in infectious materials and cultures and are frequently associated with laboratory infections. Published reports describe a big number of laboratory-associated infection cases such as brucellosis, Q-fever, typhoid fever, hepatitis, tularemia, tuberculosis, AIDS, etc. The advent of the AIDS epidemic in the early 1980s and the associated rise in tuberculosis and hepatitis B and C infections have renewed interest in laboratory safety programs.

Guidelines and Legislation

Agencies and associations such as the World Health Organization (WHO), the Occupational Safety and Health Administration (OSHA), the National Institute for Occupational Safety and Health (NIOSH), the Advisory Committee on Dangerous Pathogens (ACDP), the Centers for Disease Control (CDC), have set safety standards, which have been adopted through legislation by the Governments of various countries. Thus, licensing clinical laboratories to work with various infectious agents, packaging and shipment of infectious material, safe operation of laboratories, prevention of employee exposure to infectious agents, as well as disposal of biohazardous waste, are the main topics regulated by law.

The European Parliament and the Council of the European Union originally adopted the Directive 90/679/EEC "On the protection of workers from risks related to exposure to biological agents at work", which has been substantially amended by the Directives 93/88/EEC, 95/30/EC, 97/59/EC, 97/65/EC. Recently, the Directive 2000/54/EC including all of the above-mentioned has been adopted. Until the Greek law complies with this Directive, the provisions of Directive 90/679/EEC and its amendments are enforced, as defined in the Presidential Decrees 186/95, 174/97 and 15/99. According to Council Directives, special measures have been taken for laboratories carrying out

work which involves handling biological agents for research, development, teaching or diagnostic purposes, in order to minimize the risk of infection.

Developing a safety policy

The strategy for minimizing the exposure of laboratory workers to infectious agents is based on the concept of biological agent "containment". The term "containment" describes the way in which biological agents are managed in the laboratory so as to prevent the exposure of laboratory workers, other people and the outside environment to the agent(s) in question.

Primary containment provides protection of the worker and the immediate laboratory environment from exposure to infectious agents. This can be achieved by a combination of strict adherence to proper microbiological practices or techniques and the use of appropriate containment devices or safety equipment such as microbiological safety cabinets (MSCs) and personal protective equipment (e.g., laboratory coats and gowns, gloves, masks, face shields and glasses).

Secondary containment provides protection of all workers within the facility and the environment outside the laboratory. This can be achieved by a combination of laboratory design and operating procedures (e.g. restriction of access, air handling and safe disposal of waste).

In developing a satisfactory safety system, the main areas for consideration are:

1. Assessment of the risks of infection transmission
2. Determination of containment level – Application of containment measures
3. Monitoring of safety – Staff training and occupational health

Assessment of the risks

In the case of any activity within the laboratory involved a risk of exposure to biological agents, the nature, degree and duration of workers' exposure must be determined in order to make it possible to assess any risk and to lay down the measures to be taken.

Risk assessment criteria are influenced by:

- The type of laboratory as related to the quantities of infectious materials (e.g., clinical, research laboratory) and specific procedures performed (e.g., aerosol-generating activities).
- The susceptibility of laboratory worker to infections (e.g., pre-existing disease, medication, compromised immunity, pregnancy or breast feeding); additional risk to such workers should be considered as part of the risk assessment required.
- The infectious agent; the most important determinant in risk assessment is the pathogenicity of the microorganism.

Biological agents are classified into four risk groups, according to the degree of infection risk (Table 1).

Group 1	- unlikely to cause human disease
Group 2	- possibility to cause of human disease and might be a hazard to workers - unlikely to spread to the community - effective prophylaxis or treatment usually available
Group 3	- possibility to cause severe human disease and present a serious hazard to workers - risk of spreading to the community - effective prophylaxis or treatment usually available
Group 4	- cause of severe human disease with serious hazard to workers - high risk of spreading to the community - no effective prophylaxis or treatment usually available

Table 1. Risk groups of Biological agents

In line with the scope of the Council Directives, only agents that are known to infect humans are to be included in the classification list (groups 2, 3 and 4) (Table 2).

Table 2. Examples of bacteria, viruses, parasites and fungi defined by risk group (according to Directive 2000/54/EC)

Risk group	**Bacteria**	**Viruses / prions**	**Parasites**	**Fungi**
2	*Staphylococcus aureus* *Streptococcus pneumoniae* *Escherichia coli* *Neisseria meningitidis* *Chlamydia trachomatis*	Adenovirus Hepatitis A virus Influenza viruses types A, B, C Herpes virus varicella-zoster	*Plasmodium* spp. *Entamoeba histolytica*	*Candida albicans* *Aspergillus fumigatus*
3	*Bacillus anthracis* *Brucella melitensis* *Mycobacterium tuberculosis* *Chlamydia psittaci* *Coxiella burnetii* *Salmonella typhi**	Rabies virus* Hepatitis B,C,D,E,G viruses*[D] Human immunodeficiency viruses (AIDS)*[D] Hantaan (Korean haemorrhagic fever) Prions *[D]	*Plasmodium falciparum** *Leishmania donovani** *Trypanosoma cruzi* *Naegleria fowleri* *Echinococcus granulosus**	*Coccidioides immitis* *Paracoccidioides brasiliensis* *Histoplasma capsulatum*
4	–	Ebola Virus Lassa Virus Crimean-Congo haemorrhagic fever	–	–

* Certain biological agents classified in group 3 may present a limited risk of infection for workers because they are not normally infectious by the airborne route

[D] List of workers exposed to this biological agent to be kept for more than 10 years after the end of last known exposure

In the case of activities involving exposure to several groups of biological agents, the risk is assessed on the basis of the danger presented by all hazardous biological agents present.

Containment levels - Containment measures

Measures are taken following the assessment, as well as deciding upon the containment level required for the biological agents according to the degree of risk. Activities involving the handling of a biological agent of groups 2, 3 or 4 must be carried out only in working areas corresponding to at least containment level 2, 3 or 4, respectively (CL-2, CL-3, CL-4).

In particular, the following measures must be applied on the basis of the results of the assessment (Table 3):

Table 3. Containment measures for CL-2, CL-3 and CL-4 laboratories (according to Directive 2000/54/EC)

Containment Level	Security and access	Air handling system	Disinfection and disposal procedures	Protective equipment and procedures
2	• Biohazard sign • Access restricted to nominated workers only* • Observation window present, so that occupants can be seen* • Efficient vector control, for example rodents and insect* • Safe storage of biological agents		• Specified disinfection procedures • Surfaces of benches impervious to water and easy to clean • Surfaces resistant to acids, alkalis, solvents, disinfectants* • Incinerator for disposal of animal carcasses* • Autoclave available - Decontamination of identified wastes	• **Safety cabinets Class I or II** or other suitable containment (where appropriate) • Personal protective equipment+ • Standard microbiological practices++
3	• Biohazard sign • **Access restricted to authorized personnel only** • Observation window present, so that occupants can be seen* • Efficient vector control, for example rodents and insect • Safe storage of biological agents • **Laboratory contain own equipment*** • **Workplace separated from any other activities in the same building***	• **Workplace maintained at an air pressure negative to atmosphere*** • **Extract air to the workplace filtered using high efficiency particulate absorption (HEPA) or likewise**	• Specified disinfection procedures • Surfaces of benches and floor impervious to water and easy to clean • Surfaces resistant to acids, alkalis, solvents, disinfectants • Available incinerator for disposal of animal carcasses • Autoclave available - Decontamination of all waste • **Workplace sealable to permit disinfection***	• **Safety cabinets Class I or II** or other suitable containment (where infection is by airborne route) • All necessary personal protective equipment+ (respiratory protection) • Standard microbiological practices++
4	• Biohazard sign • **Access restricted to authorized personnel only – via air-lock key procedure (change to protective clothing before entering-shower on exit)** • Observation window present, so that occupants can be seen • Efficient vector control, for example rodents and insect • Secure storage of biological agents • **Laboratory contain own equipment** • **Workplace separated from any other activities in the same building or in a separated building**	• **Workplace maintained at an air pressure negative to atmosphere** • **Input and extract air to the workplace filtered using high efficiency particulate absorption (HEPA) or likewise**	• Specified disinfection procedures • All of surfaces impervious to water and easy to clean • Surfaces resistant to acids, alkalis, solvents, disinfectants • Incinerator for disposal of animal carcasses, available on site • Autoclave available on site **- Decontamination of all liquid effluent** and solid wastes • **Workplace sealable to permit disinfection**	• **Safety cabinets Class III** or other suitable containment • **Full body, air-supplied, positive-pressure personnel suit for all procedures** • Standard microbiological practices++

* Recommended measures for CL-2 and/or CL-3

\+ Laboratory coats and gowns, gloves, masks, face shields and glasses

++ Use mechanical pipetting devices, minimization splashes and aerosols, needles and sharps precautions, use leak-proof transport containers, wash hands, prohibition of eating, drinking and smoking in laboratory

- determination of laboratory's facilities and appropriate equipment
- development of laboratory protocols by safety procedures
- collective and individual protection measures
- hygiene measures compatible with the aim of the prevention or reduction of the accidental release of a biological agent from the workplace
- arrangements for the safe handling and transport of biological agents within the workplace
- means for safe collection, storage and disposal of waste, including the use of secure and identifiable containers.

All microbiology laboratories should be equipped with a microbiological safety cabinet (MSC). A MSC is a ventilation enclosure intended to offer protection to the user and the environment from aerosols generated when handling biological agents or infectious material that may contain such agents. There are three types of MSCs (Class I, II, III) (Table 4).

Table 4. Summary of characteristics of microbiological safety cabinets (MSCs, Class I, II, III)

Safety cabinets (MSCs)	**Containment Level**	**Inflow**	**Outflow**	**Protection Provided**		
				Work (Product)	**Worker**	**Environment**
Class I	2, 3	Room air	HEPA* filtered air	No	Yes	Yes
Class II	2, 3	Room air HEPA* filtered	HEPA* filtered air	Yes	Yes	Yes
Class III	4	HEPA* filtered air		Yes	Yes	Yes

* HEPA: High efficiency particulate absorption

Monitoring of safety

Each laboratory should have a safety manual consisting of written policies for the operating procedures, the cultivation, storage and disposal of biohazardous materials, and the details for disinfecting laboratory equipment. The head of department should appoint a safety officer who is accountable for facilitating translation of the main aims of the safety protocol into working practice, identifying potential hazards, monitoring safety standards, and educating members of laboratory staff. Any accident or incident, which may have resulted in the release of a biological agent, should be directly referred to the safety officer.

Training of workers

All laboratory workers should receive sufficient and appropriate training, in particular in the form of information and instructions, concerning potential health risks, precautions to be taken to prevent exposure, hygiene requirements, use of protective equipment and clothing, steps to be taken in the case of incidents and incident prevention. The training shall be given at the beginning of operation, be repeated periodically and adapted to take account of new or changed risks.

Health surveillance of workers

Each worker should undergo a pre-employment medical examination that identifies prior exposure of the individual to infectious agents and underlying conditions (e.g., pre-existing disease, medication, compromised immunity, pregnancy etc.) that require special employment placement. Thereafter, each worker shall be able to undergo relevant health surveillance at regular intervals. Usually, the facility provides these services through an employee health scheme or contract with an outside organization.

Effective vaccines should be made available for those workers who are not already immune to the biological agents to which they are exposed or are likely to be exposed, e.g. to blood-borne pathogens.

A list of workers exposed to group 3 and/or group 4 biological agents, should be kept for at least 10 years or an appropriately longer time. Each case of disease or death identified as resulting from occupational exposure to biological agents shall be notified to the competent authority. A systematic reporting system must be established and the number of laboratory workers and infections associated with their workplace has to be published each year.

Remarks – Suggestions

The Member States shall establish, in accordance with national laws and practice, arrangements for the application of the Council Directives provisions "On the protection of workers from risks related to exposure to biological agents at work".

Greek legislation has been adjusted in accordance with the provisions of the above -mentioned Directives. However, the specific measures legislated to minimize infection hazards in laboratories are not always applied. Most of the microbiological laboratories in Greece, despite conducting procedures involving Group 2 and 3 biological agents either for diagnostic purposes (Hospital Laboratories) or for research or educational purposes (University Laboratories), are classified on Containment Level 2 or lower. Some laboratories comply with the provisions of the Directives concerning the assessment of risks and the levels and measures of containment, but do not do so when it comes to the surveillance and safety control. The question of the laboratory staff safety might have been covered in terms of legislation, but the application of Directives has been delayed due to financial and bureaucratic reasons. The keeping of lists of exposed personnel could lead to better knowledge of the hazards involved in the exposure to biological agents at work. How many laboratories keep such lists, though? Part of this responsibility belongs to the employees themselves. They should demand abiding to all Directives as it primarily concerns their safety and health.

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