LABORATORY BIOSAFETY MANUAL

WORLD HEALTH ORGANIZATION GENEVA

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INTRODUCTION

Manuals, codes of practice and other publications on biosafety have proliferated in recent years to such an extent that to produce another might seem superfluous. Yet reading the existing publications makes it evident that most reflect particular features of the organization and administration of laboratories, the health and safety legislation, and the categorization of pathogenic micro-organisms obtaining in their countries of origin. The character of these publications varies from one country to another, as does the emphasis of the advice proffered in the texts. Some manuals deal only with a defined aspect of the subject, such as the handling of highly dangerous pathogens or the putative hazards of genetic manipulation; others are written solely for use in a particular institution or type of laboratory. None of the existing publications is, for these reasons, entirely suitable for international application. This consideration has led the World Health Organization, through its Special Programme on Safety Measures in Microbiology, to publish this manual as a basis for general guidance on biosafety.

The manual represents a synthesis of the advice formulated by a number of international working groups of experts established under the Programme from 1976 onwards, and is framed in the light of three main factors that affect international application. The first is that the risks ascribable to certain biological agents vary in different countries, for what may be an important pathogen in one part of the world may be a lesser one in another. Secondly, the varying levels of development of laboratory facilities throughout the world are such that it is essential that any safety measures proposed should fit the available resources. Thirdly, the needs of laboratory personnel in different countries vary according to their training and the work they are required to do. Thus, recommended operational procedures must be adaptable to widely varying patterns of educational attainment and laboratory practice.

The manual is designed for microbiological laboratories and its emphasis is therefore on biosafety. It must be borne in mind, however, that chemical, physical, and radiation safety measures also apply.

It is in three main parts that deal, respectively, with:

— guidelines on basic standards of laboratory operation, design and equipment;

— procedures for safe laboratory practice; and

— the selection and use of essential biosafety equipment.

The information is intended primarily for the guidance and use of laboratory

supervisors, biosafety officers, and others responsible for laboratory safety programmes.

The guidelines in Part I may be used as a source document from which individual laboratory manuals applicable to local circumstances can be derived. Those responsible for compiling biosafety manuals should be aware of the "overkill" that can result from devising precautions that are unduly stringent or from excessive reliance on physical safeguards. They should recognize that the principal element in biosafety is the inculcation of sound microbiological practices in both microbiologists and non-microbiologists.

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GENERAL PRINCIPLES

Throughout the manual, reference is made to the relative hazard of infective microorganisms by risk group (Risk Group I, Risk Group II, Risk Group III, and Risk Group IV). Laboratories are referred to by design features, construction, and containment equipment (basic laboratory, containment laboratory, and maximum containment laboratory).

Laboratory containment requirements are found in Part I. Risk group descriptions and classification follow. Table 1, overleaf, relates risk groups to various categories of laboratory.

Classification of Infective Microorganisms by Risk Group

Risk Group I (low individual and community risk).
A microorganism that is unlikely to cause human disease or animal disease of veterinary importance.

Risk Group II (moderate individual risk, limited community risk).
A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock, or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread is limited.

Risk Group III (high individual risk, low community risk).
A pathogen that usually produces serious human disease but does not ordinarily spread from one infected individual to another.

Risk Group IV (high individual and community risk).
A pathogen that usually produces serious human or animal disease and may be readily transmitted from one individual to another, directly or indirectly.

Each country should draw up a classification by risk group of the agents encountered in that country, based on the following factors:

— Pathogenicity of the agent.

— Modes of transmission and host range of the agent. These may be influenced by existing levels of immunity, density and movement of the host population, presence of appropriate vectors and standards of environmental hygiene.

— Availability of effective preventive measures. Such measures may include: prophylaxis by vaccination or antisera; sanitary measures, e.g., food and water hygiene; the control of animal reservoirs or arthropod vectors; the movement of people or animals; and the importation of infected animals or animal products.

— Availability of effective treatment. This includes passive immunization and postexposure vaccination, antibiotics, and chemotherapeutic agents, taking into consideration the possibility of the emergence of resistant strains.

In assessing the various criteria for classification, it is also important to take into account conditions prevailing in the geographical area in which the microorganisms are handled. Individual governments may decide to prohibit certain pathogens from being imported or handled other than for urgent diagnostic purposes.

Table 1. Risk groups in relation to category of laboratory

Risk group	Laboratory classification	Examples of laboratories	Examples of organisms
I Low individual risk and low community risk	Basic	Basic teaching	*Bacillus subtilis* *Escherichia coli* K12
II Moderate individual risk and limited community risk	Basic (with biosafety cabinets or other appropriate personal protective or physical containment devices when required)	Primary health services; primary level hospital; doctors' offices; diagnostic laboratories; university teaching; public health laboratories	*Salmonella typhi* Hepatitis virus B *Mycobacterium tuberculosis*[a] LCM virus[b]
III High individual risk and low community risk	Containment	Special diagnostic laboratories	*Brucella* spp. Lassa fever virus *Histoplasma capsulatum*
IV High individual risk and high community risk	Maximum containment	Dangerous pathogens units	Ebola-Marburg virus Foot-and-mouth-disease virus

[a] When larger volumes or high concentrations are used, or when techniques may involve aerosol production, these and other agents should be promoted to Risk Group III.

[b] Includes research laboratories at appropriate risk group level.

PART I

GUIDELINES

A. THE BASIC LABORATORY

The basic laboratory encompasses all laboratories working with Risk Group I and Risk Group II agents—those that present low or moderate risk to the laboratory worker and low or limited risk to the community. In some instances, particularly in clinical laboratories of hospitals, exposure to agents of high individual risk may occasionally or unexpectedly occur in the course of routine work. These possibilities must be recognized in developing safety plans and policies.

The basic laboratory guidelines presented here are comprehensive and detailed as they are fundamental to all classes of laboratory. The guidelines for containment laboratories that follow later are modifications of the basic guidelines designed for work with the more dangerous pathogens.

Code of practice

This code is a listing of the most essential laboratory procedures that are basic to safe laboratory practice. In many laboratories and national laboratory programmes, such a code may be given the status of "rules" for laboratory operation. In these guidelines various parts of the "code of practice" will be elaborated and explained.

It is emphasized that good laboratory practice is fundamental to laboratory safety and cannot be replaced by specialized equipment, which can only supplement it.

The most important rules are listed below, not necessarily in order of importance:

1. Mouth pipetting should be prohibited.

2. Eating, drinking, smoking, storing food, and applying cosmetics should not be permitted in the laboratory work area.

3. The laboratory should be kept neat, clean, and free of materials not pertinent to the work.

4. Work surfaces should be decontaminated at least once a day and after any spill of potentially dangerous material.

5. Members of the staff should wash their hands after handling infectious materials and animals and when leaving the laboratory.

6. All technical procedures should be performed in a way that minimizes the creation of aerosols.

7. All contaminated liquid or solid materials should be decontaminated before disposal or re-use; contaminated materials that are to be autoclaved or incinerated at a site away from the laboratory should be placed in durable leakproof containers, which are closed before being removed from the laboratory.

8. Laboratory coats, gowns, or uniforms should be worn in the laboratory; laboratory clothing should not be worn in nonlaboratory areas; contaminated clothing should be disinfected by appropriate means.

9. Safety glasses, face shields, or other protective devices should be worn when necessary to protect the eyes and face from splashes and impacting objects.

10. Only persons who have been advised of the potential hazards and meet any specific entry requirements (e.g., immunization) should be allowed to enter the laboratory working area; laboratory doors should be kept closed when work is in progress; access to animal houses should be restricted to authorized persons; children are not permitted in laboratory working areas.

11. There should be an insect and rodent control programme.

12. Animals not involved in the work being performed should not be permitted in the laboratory.

13. The use of hypodermic needles and syringes should be restricted to parenteral injection and aspiration of fluids from laboratory animals and diaphragm vaccine bottles. Hypodermic needles and syringes should not be used as a substitute for automatic pipetting devices in the manipulation of infectious fluids. Cannulas should be used instead of sharp needles wherever possible.

14. Gloves should be worn for all procedures that may involve accidental direct contact with blood, infectious materials, or infected animals. Gloves should be removed aseptically and autoclaved with other laboratory wastes before disposal. When disposable gloves are not available, re-usable gloves should be used. Upon removal they should be cleaned and disinfected before re-use (see Part II: G. "Disinfection and sterilization").

15. All spills, accidents and overt or potential exposures to infectious materials should be reported immediately to the laboratory supervisor. A written record should be prepared and maintained. Appropriate medical evaluation, surveillance, and treatment should be provided.

16. Baseline serum samples may be collected from and stored for all laboratory and other at-risk personnel. Additional serum specimens may be collected periodically depending on the agents handled or the function of the facility.

17. The laboratory supervisor should ensure that training in laboratory safety is provided. A safety or operations manual that identifies known and potential hazards and that specifies practices and procedures to minimize or eliminate such risks should be adopted. Personnel should be advised of special hazards and required to read and follow standard practices and procedures.

Laboratory design and facilities

In designing a laboratory and assigning certain types of work to a laboratory, special attention should be paid to conditions that are known to pose problems. These include:

— creation of aerosols;

— work with large volumes and/or high concentrations of microorganisms;

— overcrowded, overequipped laboratories;

— infestation with rodents or insects;

— unauthorized entrance.

Design features for basic laboratories

1. Ample space must be provided for the safe conduct of laboratory procedures.

2. Walls, ceiling, and floors should be smooth, easily cleanable, impermeable to liquids, and resistant to the chemicals and disinfectants normally used in the laboratory. Floors should be slip-resistant. Exposed pipes and ducting should stand clear of walls. (Horizontal runs should be avoided to prevent dust collection.)

3. Adequate illumination should be ensured for carrying out all activities. Undesirable reflection is to be avoided.

4. Bench tops should be impervious to water and resistant to disinfectants, acids, alkalis, organic solvents, and moderate heat.

5. Laboratory furniture should be sturdy, and open spaces between and under benches, cabinets, and equipment should be accessible for cleaning.

6. Storage space must be adequate to hold supplies for immediate use and thus prevent clutter on benchtops and in the aisles. Additional long-term storage space, conveniently located outside the working areas, should also be provided.

7. Wash-basins, with running water if possible, should be provided in each laboratory room, preferably near the exit.

8. Doors should have appropriate fire ratings, be self-closing, and have vision panels.

9. An autoclave (or a suitable substitute) for decontamination of infectious laboratory wastes should be available in the same building as the laboratory.

10. Facilities for storing outer garments and personal items and for eating, drinking, and smoking should be provided outside the working areas.

11. There are no specific ventilation requirements. In planning new facilities, consideration should be given to providing a mechanical ventilation system that provides an inward air flow and exhaust without recirculation. If there is no mechanical ventilation, windows should be openable, preferably having fly-proof screens. Skylights should be avoided.

12. Space and facilities should be provided for the safe handling and storage of solvents, radioactive materials, and compressed gases.

13. Safety systems should cover fire, electrical emergencies, emergency shower, and eyewash facilities.

14. First-aid areas or rooms suitably equipped and readily accessible should be available.

15. A good-quality and dependable water supply is essential. There should be no cross-connexions between sources for laboratory purposes and the drinking-water supply. The public water system must be protected by a back-flow preventer.

16. A reliable electricity supply with adequate capacity should be available. There should be emergency lighting to permit safe exit. A standby generator is desirable for the support of essential equipment – incubators, freezers, etc. In particular, it is indispensable for the ventilation of animal cages.

17. A reliable supply of town, natural, or bottled gas to each working area is essential. Good maintenance of the installation is mandatory.

18. Three aspects of waste disposal need special attention to meet performance and/or pollution control requirements:

— autoclaves and sterilizers for treatment of solid wastes need specially designed accommodation and services;

— wastewater and sewage discharged from laboratories may have to be pretreated;

— incinerators may need to be of special design and equipped with afterburners and smoke-consuming devices.

19. Laboratories and their animal houses are occasionally the targets of vandals. Security may be augmented by strong doors, screened windows, and restricted issue of keys.