Fifth Edition

Microbiological Methods

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Butterworths

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Preface to the fifth edition

During the preparation of the fifth edition of this book we have frequently referred to earlier editions and revisions and it has been interesting to note how much of the basic technique and information of 20 years ago still holds good and how much has changed, not necessarily for the good of the art.

Once again, small changes in the method of presentation have been necessary, but we have held fast to the general format which, to judge from sales, has been successful in the past. We are, however, indebted to our publishers for giving this format a new and more attractive style.

As in previous editions, the names of some organisms have been changed to accommodate the whims of 'taxonomic tour operators', but for some microbes we have firmly retained the nomenclature in common use.

While we have incorporated much new material we are conscious that the book reflects our own interests, but we hope that these are also those of our colleagues and their students who work at the 'sharp' end in routine medical, public health and food control laboratories where unlimited money is not available for new and exciting equipment. We believe that 'manual' microbiology still has a long way to go before automation takes over completely.

We have also tried to increase the numbers of 'nuggets' and 'titbits' of useful information, acquired from other workers too numerous to name individually, and either unpublished or printed in journals which may not be available in many laboratories.

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Contents

Chapter 1

Safety in microbiology

Over 4000 laboratory-associated infections have been reported so far this century.[1,2] Many more have probably gone unreported. Most of the victims worked in research laboratories with organisms whose potential pathogenicity was unknown at the time, and/or used techniques now known to be hazardous. As a result of investigations over the last 25 years the causes of many of these infections have been discovered and methods for preventing them have been developed. There is now no need for microbiological laboratory workers (with certain notable exceptions which are outside the scope of this book) to feel at risk from the organisms they handle providing that they are aware of:

(1) the potential hazards of the organisms they handle,
(2) the routes by which these organisms may enter the body and cause infections,
(3) the correct methods of 'containing' these organisms so that they do not have access to those routes.

Classification of micro-organisms on the basis of risk

Micro-organisms vary in their ability to cause infections. Some are harmless; some may be responsible for diseases with mild symptoms; others can cause serious illnesses; a few have the potential for spreading in the community and causing serious epidemic disease. Experience and research into laboratory-acquired infections have enabled investigators to classify organisms and viruses into three or four groups. Three separate systems[3,4,5] have been published and are shown in *Tables 1.1* and *1.2*. The most recent, that of the Safety Measures in Microbiology Programme of the World Health Organization (*Table 1.2*)[5,6] is the best and is adopted in this book. This uses four Risk Groups (I–IV) in increasing order of hazard to the laboratory worker and the community. The Risk Group numbers also indicate the levels of 'containment', i.e. precautions and techniques necessary to prevent infections.

The World Health Organization published no lists of micro-organisms within each Risk Group. Member states are encouraged to prepare their own

TABLE 1.1. Summary of systems for classifying micro-organisms on the basis of hazards to laboratory workers and the community

	Hazard			
	Low			*High*
USPHS (1974)	Class 1 none or minimal	Class 2 ordinary potential	Class 3 special, to individual	Class 4 high, to individual and community
UK (1978)[a]		Category C no special potential	Category B special, to individual	Category A high, to individual and community
WHO (1979)	Risk Group 1 low individual low community	Risk Group II moderate individual limited community	Risk Group III high individual low community	Risk Group IV high, to individual and community
Appropriate laboratory	Basic	Basic	Containment[a]	Maximum containment[b]

[a] DHSS, 1978.
[b] Refers to precautions, equipment and design of laboratory.

TABLE 1.2 Classifications of micro-organisms on the basis of hazard. WHO Risk Group system[5, 6]

Risk Group I (low individual and community risk).
A micro-organism 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.

lists because organisms which may present a high risk in one country may offer few or none in another; there is also no point in taking elaborate and expensive precautions against an organism in the laboratory if the workers are exposed to it outside.

A few lists have been proposed, e.g. in the UK[4], and in the USA[3, 7] but there is still no general agreement within and between those countries. In *Tables 1.3* and *1.4* the organisms mentioned in this book have been classified into Risk Groups I, II and III, with reference to existing lists[4, 7], after seeking the advice of British, American and European microbiologists who actually work on the organisms and after considering the histories of infections which they have caused[1, 2]. As Risk Group IV contains only viruses no reference is made to it in this book.

TABLE 1.3 Risk Groups of bacteria mentioned in this book

Organism	*Risk Group*	*Organism*	*Risk Group*
Acetobacter	I	*Brevibacterium*	I
Achromobacter	I	*Brochothrix*	I
Acinetobacter	I	*Brucella*	III
Actinobacillus	I	*Campylobacter*	I
Actinomyces bovis	II	*Chromobacterium*	I
eriksonii	II	*Citrobacterium*	I
israelii	II	*Clostridium botulinum*	III
naeslundii		*difficile*	II
other spp.	I	*fallax*	II
Aerococcus	I	*novyi*	II
Aeromonas	I	*perfringens*	II
Alcalescens	I	*septicum*	II
Alkaligenes	I	*sordelli*	II
Arizona	I	*tetani*	II
Bacillus anthracis	II	other spp.	I
other spp.	I	*Corynebacterium diphtheriae*	II
Bacteroides	I	*equi*	I
Bifidobacterium	I	*pyogenes*	I
Bordetella	II	*renale*	I
Borrelia	II	*ulcerans*	II
Branhamella	I	other spp.	I

TABLE 1.3 (*continued*)

Organism	*Risk Group*	*Organism*	*Risk Group*
Edwardsiella	I	*Mycobacterium* (*continued*)	
Eikenella	I	*xenopi*	III
Enterobacter	I	other spp.	I
Erwinia	I	*Neisseria gonorrhoeae*	II
Erysipelothrix	II	*meningitidis*	II
Escherichia	I	other spp.	I
Flavobacterium meningo-		*Nocardia*	II
septicum	I	*Pasteurella*	II
other spp.	I	*Pediococcus*	I
Francisella	III	*Photobacterium*	I
Fusobacterium	I	*Plesiomonas*	I
Gardnerella	I	*Propionibacterium*	I
Gemella	I	*Proteus*	I
Gluconobacter	I	*Providence*	I
Klebsiella	I	*Pseudomonas mallei*	III
Lactobacillus	I	*pseudomallei*	III
Legionella	II	other spp.	I
Leptospira	II	*Salmonella paratyphi A*	III[a]
Leuconostoc	I	*typhi*	III[a]
Listeria	II	other serotypes	II
Microbacterium	I	*Serratia*	I[b]
Micrococcus	I	*Shigella*	II
Moraxella	I	*Staphylococcus aureus*	II
Mycobacterium		other spp.	I
africanum	III	*Streptobacillus*	II
avium	III	*Streptococcus* human and	
bovis	III	animal pathogens	II
chelonei	II	milk spp.	I
fortuitum	II	*Streptomyces madurae*	II
intracellulare	III	*pelleteri*	II
kansasii	III	*somaliensis*	II
leprae	II	*Treponema*	II
marinum	II	*Veillonella*	I
malmoense	III	*Vibrio cholerae*	II
scrofulaceum	III	*parahaemolyticus*	I
simiae	III	other spp.	I
szulgai	III	*Yersinia pestis*	III
tuberculosis	III	other spp.	I
ulcerans	II		

[a] Require a Containment laboratory but not a safety cabinet; airborne infection unlikely.
[b] Use a safety cabinet for experiments with aerosols.

TABLE 1.4 Risk Groups of fungi and yeasts mentioned in this book

Organism	*Risk Group*	*Organism*	*Risk Group*
Acremonium	I	*Epidermophyton*	III
Alternaria	I	*Fonseceae*	I
Aspergillus	I[a]	*Geotrichum*	I
Blastomyces	II	*Gliocladium*	I
Botrytis	I	*Hansenula*	I
Candida	I	*Helminthosporium*	I
Cladosporium	I	*Histoplasma capsulatum*	III
Coccidioides immitis	III	*Kloeckera*	I
Cryptococcus neoformans	II	*Madurella*	II
Debaromyces	I	*Microsporon*	II
Endomyces	I	*Neurospora*	I

TABLE 1.4 (*continued*)

Organism	*Risk group*	*Organism*	*Risk group*
Paecilomyces	I	*Saccharomyces*	I
Paracoccidioides braziliensis	II	*Scopulariopsis*	I
Penicillium	I	*Sporobolomyces*	I
Phialophora	I	*Sporothrix*	II
Pichia	I	*Torulopsis*	I
Pullularia	I	*Trichoderma*	II
Rhodotorula	I	*Trichophyton*	II

[a] Use a safety cabinet for experiments which generate spores. These may be allergenic.

Routes of infection

Micro-organisms can enter the body through the mouth (ingestion), through the lungs (inhalation), through the skin (injection) and through the eyes.

They may be ingested during mouth pipetting and may also enter the mouth from contaminated fingers and articles which have been contaminated from the laboratory benches, e.g. cigarettes, food, pencils. Such environmental contamination may be the result of spills and splashes, either unrecognized or inadequately disinfected.

Inhalation of infected airborne particles (aerosols) released during many common laboratory manipulations have probably caused the largest number of laboratory-associated diseases.[1, 2]

Injection may result from accidental stabbing with hypodermic needles, pasteur pipettes or broken and infected glassware. Organisms may also enter the body through cuts and abrasions in the skin, some of which may be too small to be obvious to the worker himself.

Splashing of infected liquids into the eyes has been the cause of a number of infections, some fatal.

The routes of infection in laboratory-acquired infections are not necessarily the same as those of 'naturally'-acquired infections. In addition, the infecting dose may be much greater and the symptoms may therefore be different.

Aerosols: infected airborne particles

These are likely to be released and may be inhaled or may contaminate the hands, bench, etc. during the following manipulations: work with loops and syringes; pipetting, centrifuging, blending and homogenizing, pouring; opening culture tubes and dishes. (For details *see* Collins[2].)

Prevention of laboratory-acquired infections: a code of practice

The principles of containment involve the provision of:

(1) primary barriers around the organisms to prevent their dispersal into the laboratory,
(2) secondary barriers around the worker to act as a safety net should the primary barriers be breached,

(3) tertiary barriers to prevent the escape into the community of any organisms which are not contained by the primary and secondary barriers.

This Code of Practice draws very largely on the requirements and recommendations published in other works.[2, 3, 4, 7, 8, 9]

Primary barriers

These are techniques and equipment designed to contain micro-organisms and prevent their direct access to the worker and their dispersal as aerosols.

(1) Mouth pipetting should be banned in all circumstances. Pipetting devices (p. 29) should be provided.
(2) No article should be placed in or at the mouth. This includes mouthpieces of rubber tubing attached to pipettes, pens, pencils, labels, smoking materials, fingers, food and drink.
(3) The use of hypodermic needles should be restricted. Cannulas are safer.
(4) Sharp glass pasteur pipettes should be replaced by soft plastic varieties (p. 28).
(5) Cracked and chipped glassware should be replaced.
(6) Centrifuge tubes should be filled only to within 2 cm of the lip. All Risk Group III materials should be centrifuged in sealed centrifuge buckets (p. 14).
(7) Bacteriological loops should be completely closed, with a diameter not more than 3 mm and a shank not more than 5 cm and be held in metal, not glass, handles.[2]
(8) Homogenizers should be inspected regularly for defects which might disperse aerosols (p. 15). Only the safest models should be used[2]. Glass Griffith's tubes and tissue homogenizers should be held in a pad of wadding in a gloved hand when operated.
(9) All Risk Group III materials should be processed in a Containment Laboratory (*see* Tertiary barriers *below*) and in a Microbiological Safety Cabinet (p. 16) unless exempted in *Table 1.3*.
(10) A supply of suitable disinfectant (p. 40) at use dilution should be available at every work station.
(11) Benches and work surfaces should be disinfected regularly and after spillage of potentially infected material.
(12) Discard jars for small objects and for re-usable pipettes should be provided at each work station. They should be emptied, decontaminated and refilled with freshly prepared disinfectant daily (p. 49).
(13) Discard bins or bags supported in bins should be placed near to each work station. They should be removed and autoclaved daily (p. 49).
(14) Broken culture vessels, e.g. after accidents, should be covered with a cloth. Suitable disinfectant should be poured over the cloth and the whole left for 30 min. The debris should then be cleared up into a suitable container (tray or dustpan) and autoclaved. The hands should be gloved and stiff cardboard used to sweep up.
(15) Infectious and pathological material to be sent through the post or by airmail should be packed in accordance with government, post office and

airline regulations, obtainable from those authorities. Full instructions are also given elsewhere.[2, 4, 6, 8]

(16) Specimen containers should be robust and leak proof.

Secondary barriers

These are intended to protect the worker should the primary barriers fail. They should, however, be observed as strictly as the latter.

(1) Proper overalls should be worn at all times and fastened. They should be kept apart from outdoor and other clothing.
(2) Laboratory protective clothing should be removed when the worker leaves the laboratory and not worn in any other area such as canteens and rest rooms.
(3) Hands should be washed after handling infectious material and always before leaving the laboratory.
(4) Any obvious cuts, scratches and abrasions on exposed parts of the worker's body should be covered with waterproof dressings.
(5) In laboratories where pathogens are handled there should be medical supervision.
(6) All illnesses should be reported to the medical or occupational health supervisors. They may be laboratory associated. Pregnancy should also be reported. It is inadvisable to work with certain organisms during pregnancy.
(7) Any member of the staff who is receiving steroids or immunosuppressive drugs should report this to the medical supervisor.
(8) Workers should be immunized where possible and practicable against likely infections.[2, 4]
(9) In laboratories where tuberculous materials are handled the staff should have received BCG or have evidence of a positive skin reaction before starting work. They should have annual chest X-rays thereafter[2, 4].

Tertiary barriers

These are intended to offer additional protection to the worker and to prevent the escape into the community of micro-organisms that are under investigation in the laboratory.

They concern architectural and engineering design and the reader is referred to other publications.[2, 6, 10] The WHO Risk group system requires three levels of laboratory design, summarized below.[6]

The Basic laboratory

This is intended for work with organisms in Risk Groups I and II. Ample space should be provided. Walls, ceilings and floors should be smooth, nonabsorbent, easy to clean and disinfect and resistant to the chemicals that are likely to be used. Floors should also be slip resistant. Lighting and heating should be adequate. Hand basins, other than laboratory sinks, are essential.

Bench tops should be wide, the correct height for work at a comfortable

sitting position, smooth, easy to clean and disinfect and resistant to chemicals likely to be used. Adequate storage facilities should be provided.

Access should be restricted to authorized persons.

The Containment laboratory

This is intended for work with organisms in Risk Group III. All the features of the Basic laboratory should be incorporated, plus the following.

The room should be physically separated from other rooms, with no communication (e.g. by pipe ducts, false ceilings) with other areas, apart from the door, which should be lockable, and transfer grilles for ventilation (*see below*).

Ventilation should be one way, achieved by having a lower pressure in the Containment laboratory than in other, adjacent rooms and areas. Air should be removed to atmosphere (total dump, not recirculated to other parts of the building) by an exhaust system coupled to a microbiological safety cabinet (*see* p. 19) so that during working hours air is continually extracted by one or the other and airborne particles cannot be moved around the building. Replacement air enters through transfer grilles.

Access should be strictly regulated. The international biohazard warning sign, with appropriate wording, should be fixed to the door (*Figure 1.1*).

Figure 1.1 International biohazard sign

The Maximum Containment laboratory

This is required for work with Risk Group IV materials and is outside the scope of this book. Construction and use generally requires government licence or supervision.

For further information about the safe design of Basic and Containment laboratories *see* Collins[2] and WHO[6].

Instruction in safety

This instruction should be part of the general training given to all microbiology laboratory workers and should be included in what is known as Good Laboratory Practice. In general, methods that protect cultures from

contamination also protect workers from infection but this should not be taken for granted. Personal protection can be achieved only by good training and careful work. Programmes for training in safety in microbiology have been published.[2, 6]

For a review of the history, incidence, causes and prevention of laboratory-acquired infection *see* Collins[2].

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Chapter 2

Laboratory equipment

It is important to choose the correct equipment for microbiological work. A wide range of laboratory apparatus, glassware and plastic goods is available from scientific equipment suppliers, but unfortunately not all of it is suitable for microbiological use. Before purchasing any new piece of equipment, it is best to obtain the personal advice of microbiologists who have had experience in its use. It is bad policy to rely entirely on advertisements, catalogues, extravagant claims of representatives and the opinions of purchasing officers who are mainly concerned with balancing budgets. The best is not always the most expensive, but it is rarely the cheapest. Few microbiologists require very expensive research-type microscopes, but centrifuges should be the very best available. Laboratory autoclaves should be designed for laboratory, not pharmacy or hospital, use. Thermostatic equipment designed for chemical work is usually suboptimal for microbiological use.

In this chapter, we have indicated the types of equipment that we and our associates and friends have found adequate for use in medical, veterinary and food microbiology (except virology) work and have given the names of some suppliers in the UK, and a few in the USA, with whose equipment we are familiar or which has been recommended to us by our colleagues. In the UK regular reviews of equipment and information about new apparatus are given in *Laboratory Practice*, *Medical Laboratory World* and *The Medical Technologist*, all of which appear monthly. Our experience of American equipment is limited but we are advised that two publications are likely to be very useful to American and Canadian microbiologists. These are *The Guide to Scientific Instruments*, published annually in *Science* (NY) by the American Association for the Advancement of Science, and the *Annual Directory of Medical Laboratory Product and Service Literature*, published in *Laboratory Management* by United Business Publications.

Failure to mention any other products or suppliers merely indicates that we have had no personal experience of them. We welcome information from other suppliers about their products.

The operation of some microbiological equipment may result in the release of infectious aerosols (*see* Chapter 1 and Collins[1]).