Microbiological Methods

Fourth Edition

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MICROBIOLOGICAL METHODS

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BUTTERWORTHS
LONDON - BOSTON
Sydney - Wellington - Durban - Toronto

THE BUTTERWORTH GROUP

ENGLAND
Butterworth & Co (Publishers) Ltd
London: 88 Kingsway, WC2B 6AB

AUSTRALIA
Butterworths Pty Ltd
Sydney: 586 Pacific Highway,
NSW 2067
Also at Melbourne, Brisbane,
Adelaide and Perth

SOUTH AFRICA
Butterworth & Co (South Africa)
(Pty) Ltd
Durban: 152–154 Gale Street

NEW ZEALAND
Butterworths of New Zealand Ltd
Wellington: 26–28 Waring Taylor
Street, 1

CANADA
Butterworth & Co (Canada) Ltd
Toronto: 2265 Midland Avenue,
Scarborough, Ontario, MIP 4SI

USA
Butterworth (Publishers) Inc
Boston: 161 Ash Street, Reading
Mass. 01867

First Edition 1964
Reprinted 1966
Second Edition 1967
Reprinted 1970
Third Edition 1970
Fourth Edition 1976

ISBN 0 408 70716 X

Library of Congress Cataloging in Publication Data

Collins, Christopher Herbert.
Microbiological methods.

(Laboratory techniques series)
Includes bibliographical references and index.
1. Microbiology – Technique. I. Lyne, Patricia M., joint author. II. Title.
III. Series:
[DNLM: 1. Bacteriological technics. 2. Microbiology – Methods. QW25
M626]

QR65.C6 1976 576'.028 75-35745

ISBN 0-408-70716-X

Set in Great Britain by Cold Composition Ltd, Tonbridge
and printed by Billing & Sons, Guildford.

PREFACE TO THE FOURTH EDITION

It is eleven years since the first edition of this book was published. In the second and third editions we retained the original format, but so much has happened in the last few years in the techniques and the teaching of microbiology that we felt the need to change the way in which the information is presented. We have omitted the Introduction to Microbiology (Part I of the previous editions). To bring this section up to date while keeping it concise would have been a monumental task and it has already been done well by other authors. We decided also to rearrange the text and to confine it to technical methods for the culture and identification of bacteria and fungi of importance in medicine, public health, veterinary practice and the food industry.

Hitherto we restricted the text to methods used in the United Kingdom. In this edition, we were asked by our publishers to include some of the techniques used in the United States.

We acknowledge with gratitude the help given by our contributors and the help, advice and criticism given freely by bacteriologists on both sides of the Atlantic (who are too numerous to name), and we are also grateful to a number of commercial organisations for placing much of their technical material at our disposal.

As usual, we have named many products and suppliers. This does not imply that these are necessarily superior to any that we have not mentioned and of which we have not as yet had experience or received recommendations from our associates.

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CONTENTS

CHAPTER 1

PREVENTION OF LABORATORY-ACQUIRED INFECTION *

The first account of a laboratory-acquired infection was probably given by Koch in 1886[1]. A man who had attended a course in bacteriology in Berlin became ill with cholera when there were no other cases of that disease in Germany. Since then there have been too many reports and cases to be listed; until recently, those who suffered or died were considered to be either unlucky or martyrs to medical science.

In the last decade, when hospital laboratories and laboratory workers have increased considerably in numbers, the reports of Sulkin[2], Sulkin *et al.*[3] and Phillips[4] have suggested to laboratory directors and health authorities that unnecessary hazards exist in microbiological laboratories and pathology departments.

Work in a hospital laboratory in the U.K. will, in future, be subject to the provisions of the Health and Safety at Work Act, 1974.

Laboratory work, like many other occupations, involves specific hazards; these may be considerably reduced by understanding what they are and planning and executing a comprehensive scheme of preventive measures. These comprise: (1) provision of a safe building and equipment and their proper maintenance; (2) education of all staff in safety precautions; and (3) continuous supervision to ensure that these precautions are observed.

HAZARDOUS PROCEDURES

A microbiological risk arises from any procedure which releases micro-organisms into the environment or which otherwise allows them access to the human body. Infection may be initiated by inhalation,

*Reproduced by permission of Her Majesty's Stationery Office and the Public Health Laboratory Service Board from the contribution by one of us to Public Health Laboratory Service Monograph No. 6, 'The Prevention of Laboratory-Acquired Infection' (C. H. Collins, E. G. Hartley and R. Pilsworth). London: HMSO (1974; revised 1975). This monograph also contains 'Primate Disease Hazards and their Prevention' (E. G. Hartley) and 'Protective Inoculations in the Control of Laboratory Infections' (R. Pilsworth). Material in the footnotes in this chapter is not present in the original text, and the references have been changed to the style of this book.

ingestion, through the broken or unbroken skin or through the conjunctiva.

Aerosols and Infected Air-Borne Particles – Many laboratory activities with fluids produce aerosols[5]. When a fluid surface is broken, large numbers of small droplets are produced. This may happen when a bubble bursts, when two solid surfaces separated by a film of liquid are parted, when one liquid is poured into another and when drops of liquid are allowed to fall on to a solid surface[6].

The larger droplets fall to the ground, but the smaller ones evaporate rapidly and, if the fluid contains bacteria, these remain suspended as infected air-borne particles or droplet nuclei. The small particles, between 1 and 5 μm in diameter, remain suspended for long periods and if inhaled penetrate directly into the lung. Larger particles are removed in the upper air passages.

Aerosol production is dangerous not only to the operator but to all persons in the vicinity.

Breakage and Spillage – Gross contamination of surfaces and of clothing by spilled cultures or broken culture tubes may result in infection by the hand-to-mouth route, to infection of existing skin lesions and to eye infections. The possibility of systemic infection by the conjunctival route must also be considered[7].

Direct Ingestion –This is usually associated with mouth pipetting, failure to wash the hands, consumption of food in the laboratory and smoking at the work bench.

Self-Inoculation and Wounding – Faulty techniques with hypodermic needles and accidents with broken glass may lead to direct inoculation into the blood stream. Contamination of the skin with blood may lead to infection directly or through minor abrasions (e.g. with Australia-antigen positive material, B virus and Marburg virus).

Levels of Hazard

Obviously the hazards of microbial infection are not the same in all laboratories. The small hospital laboratory, which handles urines, wound swabs and a few blood specimens; the larger unit, which examines many specimens for brucella or salmonella organisms; the tuberculosis laboratory and the haemodialysis laboratory present different levels of risk to laboratory staff. Different units of a large laboratory complex may have different risk levels. Precautions that are necessary at one end of the scale ('high risk') would be irksome and restrictive, as well as expensive, at the other ('low risk').

HAZARDOUS PROCEDURES

A working party set up to review the laboratory use of dangerous pathogens (Department of Health and Social Security, 1975[7]) identifies two categories of pathogenic organisms. Category A includes 'organisms so dangerous as to present great risks to the health, either of laboratory workers or of the human or animal communities. . .' and Category B 'organisms which present considerable dangers to laboratory workers and/or animals. . .' The report recommends that Category A pathogens should not knowingly be held in this country without authorisation and that Category B pathogens should only be held in a laboratory under the supervision of suitably qualified staff. A Code of Practice for handling Category A pathogens is included and recommendations are made for the use of these and other pathogens.

In the US micro-organisms are classified (Groups 1–5) according to the level of hazard they present. Standards of microbiological competence and precautions are prescribed for each group (U.S. Department of Education, Health and Welfare[9]).

High-Risk Specimens

At what level between 'low-risk' and 'high-risk' laboratory activities the more stringent precautions should be introduced must be decided by the laboratory director. He is familiar with the nature of the pathological material submitted for examination in all departments and with the variety of organisms it contains or is likely to contain. Unfortunately, no laboratory can choose what organisms are present in the material it receives. It is the unexpected organisms which present the risks.

It is known, however, that some organisms and viruses offer a greater risk of laboratory infections than others. Examples are tubercle bacilli, *Brucella, Francisella,* glanders bacilli, *Pasteurella, Coccidioides immitis,* rickettsiae, psittacosis-ornithosis agent, herpes B virus, Marburg virus and hepatitis B virus.

The policy of identifying certain specimens as 'high risk' material with coloured labels* seems to be desirable. The Department of Health and Social Security[8] suggests that because of the risk of infection from Australia-antigen positive material, blood specimens from the following should be so labelled: (1) patients in renal-failure clinics, e.g. patients undergoing repeated haemodialysis or kidney transplantation; (2) patients suffering from diseases of the liver; (3) patients with defective or altered immunological competence, e.g. leukaemia, Down's syndrome; and (4) patients in other 'at risk' groups, e.g. drug addicts. Patients whose serum is known to contain hepatitis B surface antigen (Hb_SAg)

*Supplied by Sessions

and from patients who receive repeated blood transfusions, e.g. haemophiliacs, should be added to this list.

In the most recent of these reports (Department of Health and Social Security, in print[8a]) the incidence of Hb_sAg positive blood donors tested at Regional Blood Transfusion Centres in England and Wales between January 1973 and June 1974 was between 1:1000 and 1:1200. There seems to be no reason, therefore, to label other blood samples as 'high risk'.

The danger of the labelling system is that it might imply that specimens not so labelled are 'safe'. This indicates the necessity for educating all pathology laboratory staff in microbiological safety procedures. Haematological, biochemical and histological laboratory staff often fail to regard their specimens as potentially infective.

Special Laboratories

In the last 20 years, there has been an increased specialisation in microbiology and more Reference and Regional laboratories have been set up to handle 'difficult' bacteria and viruses. Some of these 'difficult' organisms are the most infectious, and this tends to remove them from general laboratories to those which are more adequately staffed and equipped to handle them. This policy of containment of hazardous material is also emphasised by the recommendations that special laboratories within larger units should be equipped for dealing with 'high risk' material, e.g. for tuberculosis bacteriology and testing for hepatitis B surface antigen (Hb_sAg)[8,8a,10–12]

Special rooms, however, are of limited value unless restrictions are placed on the number of people entering them, and unless they are large enough for the safety of those who work in them. Ideally, there would be not less than 1 000 ft^3 of air space per person[10].

Design of Safe Laboratories

There should be close cooperation and consultation at all levels between the architects and engineers who design laboratories and those who will actually work in them. This policy will avoid sub-optimum working conditions, unsafe areas and expensive afterthoughts such as making holes in new roofs or walls to accommodate the trunking for exhaust protective cabinets. Safety must take precedence over cost and appearance.

Valuable information on the design of laboratories and specific requirements are given by Runkle and Phillips[13], Steere[14], the Depart-

ment of Health and Social Security[15] and British Standard 3202[16] (slightly outdated).

LABORATORY STAFF

Reception Staff

A laboratory has no control over the nature of the specimens it receives. Even if the precautions outlined below are taken, some specimens will arrive in an unsatisfactory, messy, or even dangerous condition, and staff must be protected from the consequences of handling them inexpertly.

The reception of specimens must therefore be the responsibility of someone with training and skill in microbiology. In too many laboratories specimens are unpacked and distributed by clerical or very junior and untrained laboratory staff.

It would be unreasonable to place a Senior, or even a State Registered Technician in sole charge of a reception area but a person of such rank and experience should be appointed to supervise these activities. He should be responsible for training suitable staff who could be recruited from the laboratory assistant (laboratory aide) grades and given this special responsibility and commensurate reward. This would be in accord with the job satisfaction principle in management theory. In laboratories which are attended by large numbers of patients, reception staff with nursing experience are desirable. Nurses usually have no difficulty in accepting laboratory disciplines.

Scientific and Technical Staff

Frequent changes in staff are undesirable. Where rotation through departments is practised this should be so organised that people have sufficient time to accustom themselves to local conditions. It is particularly confusing to junior staff if they are transferred rapidly from departments such as microbiology, where there is an awareness of the hazards of pathological material, to biochemistry and haematology departments where the same material may be treated casually.

Domestic and Cleaning Staff

These present problems. They are often not under the control and supervision of the laboratory staff and may be changed frequently because of the pressures and problems which beset a hospital domestic superintendent. Cleaning is often done in the early morning or in the evening when no laboratory staff is present. Infections which may

occur innocently or due to natural curiosity – sniffing at bottles or opening culture plates – are unlikely to be suspected and recorded as being of laboratory origin.

All domestic staff should receive instruction from a senior member of the laboratory staff. They should wear protective clothing and leave this behind in the laboratory when they have finished working there. They must be made aware of the importance of hand washing.

There is a strong argument for placing all cleaning and domestic staff as well as washing up staff under the permanent supervision of a senior member of the technical staff. The two jobs, washing laboratory glassware and cleaning floors and benches, might be combined. Being part of a laboratory team instead of a casual employee who might one day clean a laboratory and the next day an office would give greater job satisfaction and make safety education easier. Such staff would then be included in the laboratory health records.

If this arrangement cannot be made and it is not possible to supervise cleaning activities these might be restricted to floors, stairs, corridors and offices. The benches could be the responsibility of laboratory aides. In some laboratories each junior technician is responsible for cleaning a specified bench area. In laboratories where the domestic staff is not assimilated each individual must be carefully instructed to touch nothing on the benches and not to empty any discard bins. They may tend to regard these as the wastepaper baskets which they empty when they clean offices. It is advisable to have no waste paper baskets in the laboratory and to put all waste into the discard bins. These should have a distinctive colour.

Visitors and the General Public

Visitors, members of the public, patients and non-medical staff of other departments should not be allowed to enter laboratory areas unless accompanied by a member of the laboratory staff. Service engineers should similarly be escorted, and if they are to remain in the laboratory to work they should be given protective clothing and warned of the dangers of touching any material and equipment on the benches. Window cleaners, who walk on benches and who may tread on cultures, must also be supervised. All these people should be required to wash their hands, under supervision, before they leave the laboratory.

No person other than authorised laboratory staff and escorted service engineers should be allowed to enter 'high risk' rooms. Any cleaning should be done by laboratory staff; services and repairs should be carried out only when laboratory work is not being done and

protective clothing should be worn. These rooms should have the international 'Biohazard' motif displayed on the door.

Eating, Drinking and Smoking

Eating and drinking should not be permitted in any microbiological laboratory. Smoking should not be permitted in any working area. It might be pointed out that the risk of personal infection from handling cigarettes in the laboratory is of the same order as that incurred in eating food there.

Safety Codes and Safety Officers

Safety Codes – Most large institutions have their own printed safety codes. The Medical Research Council[17] issues a booklet to each member of its staff. The Department of Health and Social Security[11] has published a handbook and requires that this is issued to all laboratory workers in the National Health Service. It is also available to other authorities. Other instructions and precautionary advice may be added to these for particular or local circumstances and all members of the staff should be instructed to familiarise themselves with both the general and local safety codes and regulations.

It is desirable that personal instruction and explanations are given to all new members of the staff by the Safety Officer or other person appointed by the Director. At the end of the Medical Research Council booklet there is a slip which members of the staff are required to sign to certify that they have read, understood, and noted the contents. This slip is then filed with the personnel records. This is an excellent policy.

Safety Officers – Proposals to appoint Safety Officers have generated much heat. Opinions vary between the appointment of an Inspectorate like that operating under the Factories Act and the equally impracticable 'every man his own safety officer'.

In fact, every laboratory already has a safety officer in the person of the Director, who is ultimately responsible for all laboratory activities. He may delegate responsibility to another person, possibly to more than one in a laboratory which serves several disciplines. The Director and his safety officer(s) should have access to the necessary books and information and to advice from other institutions, but for proper management the laboratory should proceed like a ship, under 'master's orders, pilot's advice'.

The prime functions of the safety code and the safety officer should be education of staff, not merely by giving instructions but by involving

all laboratory workers in discussions and explanations. The more that each worker knows about the technical methods used by his associates and the hazards which these techniques may present the greater will be his interest and participation in the formulation and implementation of safety codes and safe procedures.

It is the duty of the senior staff, especially of the senior technical staff, to take all the responsibility for the observance of safety precautions by those who work under them. It is also the responsibility of all trained staff to encourage discussion and questioning about procedures.

Accident and Health Records

Two Accident books should be kept. One of these is for records of accidents involving injury to staff; and even minor cuts and abrasions should be recorded. The other book should record accidents where no injury follows, e.g. spillage, breakage, excessive aerosol production, and centrifuge accidents. Subsequent sickness may be traced to these events[2].

When a member of the staff returns from sick leave the nature of the illness should be recorded in a Sick Report book which is kept separate from the Accident books and in the care of a member of the medical staff. It should be regarded as confidential and not made accessible to staff other than those authorised by the Director. Lack of secrecy might lead to inaccurate reporting and therefore failure to connect sickness with a laboratory accident. Domestic and portering staff who are in any way associated with the laboratory should be included in these records.

Hand Washing Facilities

Hand basins with hot and cold running water must be provided in all laboratory rooms. There should be at least one basin in each room. In rooms where a large number of people work, e.g. more than ten, it is desirable that two basins be provided. The basins should be near to the doors.

No member of the staff should be expected to wash his hands in a sink which is used for other purposes.

The basins should have foot-operated or elbow-operated mixer taps which should be fitted with a spray nozzle. Hands should be washed under running water.

Automatic liquid soap dispensers should be avoided in favour of tablets of toilet soap regardless of the alleged economy of the former.

Many of these dispensers must be operated by dirty or contaminated hands before they yield any soap. It is often not obvious when they are empty.

Paper towels in dispensers are much more satisfactory than turkish or huckaback towelling. These cloth towels are usually changed on a rigid time basis and may remain wet and dirty for long periods. Roller towels are acceptable only if they are of the continuous flow type and are properly maintained and promptly replaced.

Used paper towels should be discarded into paper sacks in the usual metal frames with pedal-operated lids. The sacks should be removed daily and tied up for autoclaving and incineration. This may appear to be unnecessary but should be considered as part of the policy of containing within the laboratory any material which may conceivably be infected. Contaminated papers and wrappings may accidently find their way into these bins.

Staff should be encouraged to wash their hands frequently and always when leaving the laboratory. The provision of hand cream often encourages this practice.

Hands may be contaminated by the removal of soiled protective clothing and therefore should be washed again after removing it.

Protective Clothing

The traditional white coat with buttoned front is far from ideal. Even if properly fastened it does not protect the neck and upper chest from infected sprays and it gapes at the knees when the wearer sits down. The sleeves, unless rolled up, rapidly become dirty and probably infected from contact with the bench.

Laboratory workers, however, are strongly prejudiced in favour of these coats. They dislike those which wrap over the thorax and fasten at the neck ('dental' or 'Kildare' coats) and those which they regard as 'medical orderly' or 'theatrical' (*sic*) gowns. The latter, however, are probably the safest form of protective clothing for ordinary laboratory use. They protect the whole of the front of the body; their sleeves may be fastened tightly around the wrists to prevent the entry of aerosols; and they can be removed with the contaminated side inwards. Unfortunately they do not appear to be generally available with adequate pockets and they are difficult to fasten up. The single breast pocket which sheds its contents when the wearer bends down is useless and annoying and may lead to accidents. Tape fastenings break, are difficult to fasten up behind and become knotted.

A coat which goes a long way to meet these objections has been designed by Dowsett and Heggie[18] and is available commercially*.

Protective clothing is likely to be contaminated and may require autoclaving before laundering. Nylon and other synthetic fabrics cannot withstand this process. Most people prefer cotton or linen, anyway; nylon is non-absorbent and it has a high heat transfer and static build-up.

If the traditional coat is retained for normal or 'low risk' work it must be supplemented or replaced in 'high risk' areas. For work with tuberculous material, blood samples or other 'high risk' material a thin, impervious plastic disposable apron† should be worn over the white coat, or better, over a gown. In rooms set aside for work with exceptionally hazardous material, disposable gowns, plastic aprons and gloves should be worn. Staff should leave their white coats outside these rooms, don the protective clothing before entering, and discard it into suitable bins or paper sacks when leaving (*see* Treatment and Disposal of Infected Material, p. 37). Surgeon's masks are of doubtful value. Plastic aprons should be worn once only.

Protective clothing must be worn at all times in the laboratory but must be removed before visiting the rest room, canteen or library. If it is necessary to visit a hospital ward or department where it is customary to wear an overall the laboratory coat must be exchanged for a clean one.

Washable protective clothing is not changed frequently enough in most laboratories. The usual weekly change is not adequate for a reasonable level of safety and cleanliness except possibly for members of the staff who do not work at the bench or who do not handle specimens. A minimum of two changes per week should be maintained. Clean coats should always and instantly be available to any member of the staff who has contaminated or who suspects that he has contaminated his overall.

White coats or overalls should not be kept in the lockers where personal clothing is stored but placed on convenient hooks, preferably in the laboratory where they are worn.

Full Protective Clothing — This is rarely needed in hospital and public health laboratories. For an emergency, such as a major accident with a centrifuge or with a highly infectious culture a few sets of the clothing used in operating theatres or 'sterile areas' should be available. These should include vests, trousers, rubber boots, gowns, 'Porton'

*Supplied by Bassett
†Supplied by Jennings