Basic Medical Laboratory Technology

Second Edition

C J C Kirk
R N Peel
K R James
K S Lewis
A D Waft

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Editor
CLIVE J C KIRK, *TD, FIMLS*
Senior Chief Medical Laboratory Scientific Officer
Department of Surgery
Charing Cross Hospital Medical School, London

Authors
R NIGEL PEEL, *MB, BS, MRCPath*
Consultant Microbiologist
York District Hospital

KEITH R JAMES, *FIMLS*
Senior Chief Medical Laboratory Scientific Officer
Department of Histopathology
Charing Cross Hospital and Medical School, London

KEITH S LEWIS, *FIMLS, MIBiol, MSc*
Senior Chief Medical Laboratory Scientific Officer
Department of Chemical Pathology
Charing Cross Hospital and Medical School, London

A DOUGLAS WAFT, *FIMLS*
Senior Chief Medical Laboratory Scientific Officer
Department of Haematology
York District Hospital

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BASIC MEDICAL LABORATORY TECHNOLOGY

Preface to the Second Edition

Since the publication of the first edition changes have been made in the educational schemes for medical laboratory scientists in the United Kingdom. From the point of view of this book the most important of these changes has been the replacement of the Ordinary National Certificate by the Technician Education Council's Certificate in Science. However the body of technical knowledge required of medical laboratory scientists at this stage in their careers remains essentially the same, with due regard of course to the advances made since the publication of the first edition.

Another aspect of laboratory work that has seen considerable changes in recent years is safety. The introduction of the Health and Safety at Work Act, and the publication of the Howie Report, along with numerous other reports and items of legislation, have brought about a far greater awareness of factors affecting safety. This edition has attempted to highlight the more important safety precautions necessary in the somewhat hazardous environment of the medical laboratory. However it is not possible to cover all such precautions and the reader is reminded that no procedure should be attempted without due regard to its possible health and safety implications.

With these changes in mind, what was written in the Preface to the first edition concerning the aims of this book remains true for this edition.

In the first edition we thanked a number of people for their assistance. This assistance has proved to be of value either directly or indirectly in this edition and we would like to reiterate our gratefulness. In addition our gratitude is due to the following for their helpful advice and criticism of the sections indicated: Mr. D. Freer (Microbiology), Mrs. J. Gauntlet (Cytology), Mr. R. Hall (Haematology), Mr. N. Harling (Experimental Animals) and Mr. M. Pepper (Blood Group Serology); also to Mrs. C. Hiscoke, Miss A. Matcham and Miss E. Simpson for their able secretarial help.

C.J.C.K.

Preface to the First Edition

This book has been written primarily for the technician studying for the Ordinary National Certificate in Medical Laboratory Sciences but it is hoped that it will prove of value to all newcomers to Pathology Laboratories. It is intended to provide a working knowledge of the basic range of techniques in each of the major branches of Pathology.

The authors felt the need for a book that would provide instruction in those practical aspects of laboratory work that form part of the student's inservice training and that would augment the instruction in theory which is available at Technical Colleges. It is therefore essentially a practical book, although some theory has been included where required for the intelligent use of the methods described.

The methods recommended have all been proven in routine use as also have any commercial products mentioned. No claim is made that others may not be just as useful.

The authors wish to thank the following people: Miss M. Kenwright, Mr. L. C. Wilson and Mr. K. S. Lewis for their helpful criticisms of the Haematology, Microbiology and Clinical Chemistry sections respectively, Miss J. Willoughby for the effort and ability necessary to produce the line drawings, and Mrs. L. Thomas and Mrs. S. Paxton for their painstaking secretarial assistance. Professor A. J. Harding Rains has given us much help, encouragement, and the benefit of his considerable experience as an author and editor. Mr. Stephen Neal of Pitman Medical has brought us through the long gestation period to the birth of this book as painlessly as is possible. Our families also deserve our grateful thanks for tolerating the upheaval that seems inseparable from authorship. Naturally any mistakes remaining are our own.
C.J.C.K.; R.N.P.; K.R.J.; Y.K.

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Contents

PART ONE

General techniques

1. Professional conduct, health and safety

CODE OF BEHAVIOUR

Medical laboratory science is a profession supplementary to medicine and a high standard of ethics is required of those who practise this profession. In the United Kingdom the Professions Supplementary to Medicine Act of 1960 requires the Disciplinary Committee of the Medical Laboratory Technicians Board, in consultation with its Board and with the Council for Professions Supplementary to Medicine, to prepare a statement as to the kind of conduct which the Committee considers to be infamous conduct in a professional respect, and to send it to every registered practitioner of the profession. This statement is as follows:

No registered medical laboratory scientific officer should:

1. Hold himself out as a person who, by training and experience, is professionally qualified to diagnose or treat disease in man or animal.

2. Knowingly accept, obtain, assist in obtaining or report on any specimen for the purpose of the diagnosis and/or treatment of disease, or make any investigation for those purposes unless the diagnosis and/or treatment are to be performed by a registered medical, dental, or veterinary practitioner.

Unless there is specific evidence to the contrary, it is considered that a medical laboratory scientific officer who has carefully followed procedures which have been approved by local health managements for requesting laboratory tests will not be considered to be in breach of this requirement. Even in those cases where there is specific evidence, the Disciplinary Committee of the Board may take into account special circumstances, such as emergencies, which may enable them to consider that a breach of this rule should not make the registrant guilty of infamous conduct in a professional respect.

3. Knowingly disclose to any patient or to any other unauthorised person the result of any investigations or any other information of a personal or confidential nature gained in the course of practice of his profession. Unless there is specific evidence to the contrary it is considered that a medical laboratory scientific officer who has carefully followed procedures which exceptionally have been approved by local health managements for reporting results to patients (or in the case of children, their parents or guardians) will not be considered to be in breach of this requirement.

4. Advertise, whether directly or indirectly, or associate himself in any way with advertisement for the purpose of obtaining specimens for laboratory investigations.

5. Knowingly falsify or suppress a report of any laboratory investigation with which he may be concerned.

STAFF HEALTH AND IMMUNISATION

Before persons are employed in a pathology laboratory in the United Kingdom the employing authority will insist on a medical examination to see if they are fit. This will include a chest X-ray examination and this will be repeated every 3 years, except for staff who handle known or suspected tuberculous material, where it will be carried out annually. Prospective employees will also be given a skin test for tuberculosis, e.g. Mantoux or Heaf test, which if negative will be followed by inoculation with BCG (Bacille Calmette Guérin) since they must not handle tuberculous material until they have a positive reaction. All staff will also be tested for presence of hepatitis B surface antigen for their record, and females of child-bearing age for the presence of rubella (German measles) virus antibodies. If a high enough titre of rubella antibodies to suggest immunity is not found she will be offered the rubella vaccine. Pregnant women will not usually work with the rubella virus, cytomegalovirus or *Toxoplasma gondii* because of the risks of intrauterine infection.

It is clear that staff in pathology laboratories are at risk from a number of infective agents by the nature of their work. In addition to good working practices and proper conditions, protection from some infectious diseases can be achieved by artificial active immunisation. It is recommended that each member of the laboratory staff should be offered such protective inoculations (see Table 1.1) as are considered to be necessary by the medical staff of the laboratory or the local occupational health service unless there are indi-

vidual contraindications. This will necessitate liaison with the general medical practitioner of each person and a knowledge of previous immunisations and other medical history. Employing authorities should make some immunisation procedures a condition of employment, such as BCG in those who are tuberculin-negative. Until recently it was advised that smallpox vaccination should be repeated every 3 years but now that Africa has been declared free of smallpox by WHO the advice is likely to be changed for laboratories not known to keep or handle this dangerous virus. Sometimes passive immunisation with gamma-globulins may be used, such as following an accident where a member of staff has been accidentally inoculated with material containing a pathogen-like hepatitis virus. In this case, if it is virus B, a hyperimmune serum containing anti-virus B antibodies or gamma-globulin will be given.

Table 1.1. Examples of diseases for which protective immunisation is available

Anthrax	Plagus	Tetanus	Paratyphoid fevers
Cholera	Poliomyelitis	Tuberculosis	Typhus
Diphtheria	Rubella	Typhoid	

SAFETY

Medical laboratories are potentially hazardous working environments and this has been increasingly recognised in recent years. Consequently several relevant Acts of Parliament and Codes of Practice have been promulgated in the United Kingdom. Foremost among these are the 'Health and Safety at Work [etc.] Act 1974' and the 'Code of Practice for Prevention of Infection in Clinical Laboratories and Post Mortem Rooms 1978', commonly known as the Howie Report after the Chairman of the committee which produced the code. In addition several books, papers and circulars have been published and a short safety bibliography is appended to this chapter.

In general terms safety can be viewed in the light of common sense, specialist knowledge and statutory requirements. Most of the dangers encountered in clinical laboratories stem from infectious material, chemicals, ionising radiations, fire, faulty apparatus and careless behaviour. The safety information given in this chapter is by no means complete; that would be impossible. However this chapter includes some general safety considerations and elsewhere in this book mention is made of hazards associated with particular techniques. Two points should always be borne in mind. Firstly it is the duty of all members of the laboratory staff to co-operate in the prevention of accidents. Secondly some hazards seem more applicable to one laboratory discipline than another; this can lead to complacency.

All laboratory personnel automatically have a responsibility for their own safety as well as that of other workers. Ultimate responsibility for safety is shared between employer and employee.

Safety officers

In the United Kingdom the Health and Safety at Work Act requires the appointment of Safety Officers and the formation of Safety Committees. The Safety Officer will be a senior member of staff and will have specific responsibilities aimed at ensuring a safe environment and safe techniques. The advice of the Safety Officer should be sought before any new technique is introduced into the laboratory, after any accident or if any potential danger is identified.

The duties of the Safety Officer will also include keeping written records of all accidents, ensuring that new staff are adequately instructed on safety matters, periodic safety 'auditing' of methods and equipment and advice on the safe handling and disposal of hazardous materials and spillage.

Infection

The hazards of working with live germs in a microbiology laboratory are well recognised. There is an obvious danger from direct contact with cultures of pathogenic organisms, but the greatest danger is from breathing aerosols produced by careless handling of infected material. All materials sent to pathology laboratories are potentially infectious with the possible exception of tissues in histological fixative. Specimens sent for chemical or haematological examinations are just as likely to contain pathogens as specimens sent to the microbiology laboratory.

In the United Kingdom the 'Code of Practice for the Prevention of Infection in Clinical Laboratories and Post Mortem Rooms 1978' describes the way in which infective materials should be handled. The code divides micro-organisms, viruses and materials into four groups denoting their relative hazard levels and the minimum standard for handling them. A brief resumé of some aspects of the code of practice follows. However the code will be available in all UK laboratories and should be studied by all members of staff. For those readers outside the UK the code can still be used as a guide to

good laboratory practice in conjunction with any relevant local codes or legislation.

Classification of micro-organisms, viruses, and materials

The most hazardous organisms are viruses in Category A which includes rabies, smallpox and haemorrhagic fever viruses. Fortunately these viruses are not normally encountered in routine clinical laboratories and as only a limited number of specially approved centres are allowed to work on them further discussion of them would be out of place in this book.

Category B1 contains organisms, viruses and materials that present special hazards to laboratory workers and therefore require special containment precautions. Included in B1 are *Mycobacterium tuberculosis* and *Salmonella typhi*, which are not infrequently isolated in clinical microbiology laboratories in the UK. In addition materials and reagents containing hepatitis B viruses that are deliberately introduced into the laboratory as test materials and controls are included in this category.

Category B2 is mainly concerned with specimens known to be hepatitis B surface antigen (HB_sAg)-positive or those specimens coming from 'at-risk' patients who have not been screened and shown to be HB_sAg-negative. The at-risk groups include patients suffering from infective or suspected infective diseases of the liver, those in renal units and drug addicts.

Category C covers those organisms, viruses and materials not covered in the above groups which, provided that high standards of microbiological technique are maintained, do not present any special hazard to laboratory workers.

Laboratory accommodation for Category B1

Special accommodation, as described in the code, must be provided for work on Category B1 material. Naturally the organisms in this group may be isolated unexpectedly during normal laboratory work. When this occurs all the materials, cultures, etc., must be transferred to the special accommodation before any further work is carried out.

Work on materials that are tuberculous or might contain mycobacteria, the testing of sera for the presence of HB_sAg and on other organisms on the B1 list must be performed in a separate room which should not be used simultaneously for handling Category C items.

In addition to facilities normally associated with a microbiology laboratory the room must have at least a Class 1 exhaust protective cabinet and sufficient deep-freeze, and storage space so that all Category B1 specimens, materials and reagents are kept exclusively in the room.

Adequate security must be maintained by restricting admission to authorised personnel and locking the door when the room is not in use.

The door must have a glass panel so that the occupants of the room can be seen from the outside. The door, refrigerators, incubators and all equipment containing B1 items must be labelled 'Danger of Infection' with the international 'Biohazard' symbol (*see* Fig. 1.1).

Fig. 1.1. The International biohazard sign (red on yellow ground).

Aerosols

As is stated above infective droplets are probably the major source of laboratory infection and some droplets are formed whenever a fluid surface is broken. The smaller droplets evaporate at once to form nuclei of dried airborne material which can travel some distance in the area and which, if inhaled, may reach the alveoli of the lung. Aerosols may persist in the air for some time. Careful technique can reduce the droplet formation but they are formed in many procedures, some of which are detailed in Table 1.2 together with specific ways to reduce the aerosol risk. As many of these procedures as possible should be done in an exhaust protective cabinet and always if there is a likelihood of material being infected with particularly dangerous organisms such as tubercle bacillus. After accidents in which there has been an aerosol produced, it is recommended that the windows should be opened and that the room be evacuated for 10 minutes before clearing up and disinfection.

If the skin becomes contaminated it should be washed immediately and contaminated clothing should be removed and autoclaved or disinfected. If the accident involves the breakage of a glass vessel containing infective material the area must at once be covered by cloths soaked in a phenolic disinfectant. After at least 10 minutes the broken glass is picked up, using a pan and brush, and discarded into an infected waste container along with the cloths. The pan and brush should then be autoclaved or disinfected for 24 hours. All such accidents must be reported to the safety officer or a senior member of staff.

Personal hygiene

The individual laboratory worker can do a great deal to ensure his or her own safety and the safety of others by following a few simple rules of hygiene. These include:

1. Never eat, drink, smoke or apply cosmetics in a laboratory. Food and drink must not be taken into or stored in laboratories.
2. Always wear the appropriate protective clothing.
3. Hand-washing and the removal of protective clothing before leaving the laboratory will help to prevent the spread of laboratory hazards.
4. Remove immediately any chemical or biological contamination from clothes or skin.
5. Avoid such activities as nail-biting, pencil-chewing, sitting on benches and licking gummed labels.
6. Report all accidents however trivial they may seem.

Protective clothing

The simple white coat with buttons down the front has traditionally been the standard, and often only, form of protective clothing issued to laboratory workers. For many purposes it remains adequate so long as it fits properly and is buttoned up. However more advanced designs are now available and these are to be recommended especially in environments where infective material is handled. The new design of coat includes a wrap-over double-front, fastened at the side with press studs, a high neck, long sleeves and close-fitting waist bands. Coats should be changed at least twice a week, and immediately if contaminated or thought to be contaminated.

An impervious wrap-around plastic or rubber apron reaching from ankle to chest should be worn whenever HB_sAg-positive plasma is being handled. In addition plastic over-shoes or rubber boots should be worn under these conditions.

Disposable gloves must be used for handling Category B1 and B2 materials and are recommended at any time when the hands could become contaminated (e.g. with blood) or when there are cuts or abrasions on the hands. Heat-resistant gloves should be available in the laboratory for handling hot apparatus and standard household rubber gloves are useful for such tasks as washing up.

Goggles or face visors should always be used where there is a danger of splashing of chemicals or other potential eye hazards such as when cutting glass.

Table 1.2. Common causes of aerosol production and methods of prevention

Cause	Prevention
Pipetting (Pasteur)	Discharge the pipette slowly; forceful ejection of contents is usually inaccurate and causes aerosol formation
Centrifuging	Use sealed centrifuge buckets (*see* Chapter 4)
Decanting supernatants	Pipette whenever possible; pouring produces aerosols and usually contaminates the outside of containers
Opening screw-top bottles	Open carefully; avoid if possible filling or tipping to prevent the contents getting into the thread or cap
Opening snap-off caps or plug stoppers	Use screw-tops whenever possible
Bacteriological loops	Short loops cause less spraying than long ones. Use micro-incinerators for flaming rather than open burners. Use disposable swabs for viscous material (e.g. sputum)
Use of discard jars	Place items into disinfectant carefully, submerge completely, change disinfectant daily
Accidents	Keep benches tidy, do not hoard infective material unnecessarily, work carefully and methodically. CONCENTRATE

Suitable protective clothing properly fastened must be worn at all times in the laboratory and removed before leaving.

Biological safety cabinets, laminar flow cabinets and fume cupboards

All laboratories must be provided with cabinets and fume cupboards, of an approved design and standard, appropriate to the nature of the investigations carried out. These installations should be regularly inspected to ensure that they are functioning to the required specification of their type. If there is any doubt as to the suitability of the cabinet for a particular function the safety officer or a senior member of staff should be consulted.

There should be a minimum of equipment and material in the cabinet when in use and gas burners should not be used in biological safety or laminar flow cabinets. If it is necessary to flame loops a micro-incinerator should be used, but disposable plastic loops are often suitable.

To disinfect the cabinet 25 ml of formalin should be boiled away on an electric heater with the front closed down. The cabinet is then left overnight before opening the front and turning on the fan to vent the remainder of the formaldehyde.

It should be noted that exhaust protective cabinets are not the same as fume cupboards found in chemistry laboratories. Fume cupboards are designed to remove toxic or unpleasant vapours and to provide a measure of protection in certain chemical procedures, and should only be used for these purposes.

Chemical hazards

Chemicals may be hazardous in one or more of many different ways. The potentially hazardous properties of chemicals are dealt with under storage in Chapter 3. The same considerations must be borne in mind when using these chemicals.

Mouth pipetting is always potentially dangerous and some form of safety pipette *must* be used instead (*see* Chapter 2). Procedures involving boiling solvents, toxic gases and vapours must be carried out in an efficient fume cupboard such as that described by the British Standards specification.

Equipment including rubber gloves, goggles, rubber boots, a respirator, dust pan and brush, mop and sand should be available for dealing with spillage of noxious chemicals. The procedure to be adopted will depend upon the nature of the spilt chemicals and should be directed by a senior member of the staff. To help prevent spillage bottles and other containers should be carried with both hands, with one hand underneath giving support. Larger bottles, particularly those containing dangerous chemicals, should be transported in carriers.

Carcinogens are chemicals capable of provoking uncontrolled neoplastic change in tissues—in simple terms, cancer production. These chemicals are avoided as far as possible in the laboratory. Like infection, exposure can occur from inhalation, through the skin, and by ingestion. The risk involved depends mainly upon the length and frequency of exposure and the concentration of the carcinogen, but it is possible for even quite small exposures to be potentially hazardous. Junior laboratory staff should not, in general, be expected to use these substances.

Organic solvents are toxic and can be absorbed through the skin as well as being inhaled or ingested. Recent evidence suggests that they may cross the placental barrier and harm the developing fetus. Care should be taken to minimise the exposure to these solvents by adequate ventilation and protective clothing.

Fire

Fire is one of the most serious and most likely hazards to occur in a laboratory. All laboratory staff should know where the fire extinguishers are and how to use them, the rules concerning the fire-alarm system, and action to be taken in the event of a fire and the location of the fire exits. The most generally useful fire extinguisher in the laboratory is the carbon-dioxide cylinder which can be safely used with most chemicals and electrical equipment, and is clean. Dry powder extinguishers are also useful and may be more effective with some types of fire but are messy in use. Carbon tetrachloride, and water-based extinguishers such as the foam extinguisher, can be dangerous in laboratories and should be avoided. Glass-fibre blankets are useful for smothering small fires and clothing which is burning. These should be placed near at hand in the laboratory for instant use. Only the absolute minimum of flammable solvents should be kept in the laboratory. Serious laboratory fires have been started by ignition of flammable liquids by sparking contacts in electrical apparatus, and for this reason such liquids if necessary should be stored in spark-proof refrigerators. Laboratories, corridors and fire exits should also be kept

clean, tidy and free of rubbish. This will help prevent the outbreak or spread of fire. Rooms containing special hazards (e.g. gas cylinders, radioactive substances and large quantities of inflammable liquids) should be suitably labelled on the outside as a warning to the fire service.

Electrical hazards

It is possible to receive a fatal shock from as little as 60 V under adverse conditions. Mains-operated apparatus must be safely constructed with correctly connected earthing points and switches in the live wires. All electrical apparatus must be kept clean and dry, and must be regularly serviced by a competent electrician. Faulty apparatus must be labelled as such and withdrawn from service until properly repaired and checked.

RECEPTION OF SPECIMENS

There should be a separate room available for the reception of specimens to which access is restricted to authorised staff. Specimens should be delivered to the room through a hatch or across a fixed counter. Staff must be instructed in the way to deal with specimens and be aware of the risks involved such as contracting viral hepatitis from live viruses in blood on the outside of a leaking container.

Hypochlorite (e.g. 10% chloros) freshly prepared daily must be available to mop up any splashes or spillage, and pipettes must be completely submerged in hypochlorite (e.g. 2.5% chloros) overnight before being discarded.

Specimens known to be, or likely to come, within category B1 or B2 referred to above must be clearly labelled 'Danger of Infection' at all stages and special care must be taken. This will include the use of disposable gloves, visors and sealed centrifuge buckets.

Broken or leaking containers should be discarded in the normal way for infective materials and the sender should be informed of the loss and another specimen requested. The information on the soiled request form may be copied on to a fresh form (and the old one discarded) if the writing is still legible. If not, a copy from the sender is needed.

Specimens must be clearly labelled with the patient's name and the date as a minimum. Unlabelled and inadequately labelled specimens without proper request forms should not be accepted. Request forms must contain enough information for patient identification. The name alone is not enough since laboratory files contain records of thousands of patients, some of whom will have the same name. The patient's name; age or date of birth, if possible; hospital number and address, ward or department should be clearly written, as should the date, the name of the medical practitioner making the request, the nature of the specimen and the investigation required. In addition, it is always helpful to have some relevant clinical details to assist in deciding if further tests should be applied, and to help in assessing the result. The large variety of request forms in use suggests than an ideal form has not yet been devised, but they nearly all provide space for the above essential information.

POSTING OF SPECIMENS

Any of the smaller containers for pathological specimens selected for use from the large number on the market must be suitable for transmission through the post. The regulations which apply to the posting of specimens are to be found in the *Post Office Guide* under the heading 'Pathological specimens, Deleterious liquids or substances, Articles sent for medical examination or analysis'. The regulations are designed to protect the staff of the Post Office from infection. They may be summarised as follows. Specimens should be sent by first-class letter post and never by parcel post. The specimens must be enclosed in sealed containers which themselves must be placed in cases approved by the Post Office, and they must be padded with a quantity of some absorbent material such as cotton wool to prevent possible leakage from the package in the event of damage to the receptacle. The package must be marked 'Fragile with Care' and have the words 'Pathological Specimens' on it. Failure to comply with these regulations leaves the sender liable for prosecution. Receptacles supplied by laboratories should be submitted to the Postal Headquarters, Operations and Overseas Department, St. Martin's le Grand, London, EC1A 1HQ, in order to ascertain whether they are regarded as complying with the regulations. The Post Office totally prohibits Category A pathogens from carriage by the postal services.

Air mail

The Universal Postal Union has revised the regulations for the carriage of hazardous goods by air both on