7 Microbiological tests

7.1 Microbiology practice and quality assurance in district laboratories

In tropical and developing countries, there is an urgent need to strengthen clinical microbiology and public health laboratory services in response to:

- The high prevalence and increasing incidence of infectious diseases.

  HIV disease/AIDS, acute respiratory tract infections (particularly pneumonia), typhoid, cholera, dysentery, tuberculosis, meningitis, whooping cough, plague, sexually transmitted diseases (including gonorrhoea and syphilis), viral hepatitis, yellow fever, dengue, and viral haemorrhagic fevers are major infectious diseases that cause high mortality and serious ill health in tropical and developing countries. Climatic changes, particularly global warming and extreme rainfall, are increasing the distribution of some infectious diseases, especially those that are mosquito-borne and water-borne.

- The threat posed by the re-emergence and rapid spread of diseases previously under control or in decline such as tuberculosis, plague, diphtheria, dengue, cholera and meningococcal meningitis.

- The emergence of opportunistic pathogens associated with HIV, new strains of pathogens such as *Vibrio cholerae* serotype 0139 and viruses causing severe acute respiratory syndrome (SARS) and avian influenza.

- The rapid rate at which bacterial pathogens are becoming resistant to commonly available and affordable antimicrobials.

  Drug resistance is causing problems in the treatment and control of infections caused by pathogens such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Neisseria gonorrhoeae*, and enterococci. Some strains of *M. tuberculosis* have developed multi-drug resistance.

- The need for reliable microbiological data to develop and validate standard treatments and control interventions, and ensure antimicrobial drugs are purchased appropriately and used correctly.

  Infections are particularly prevalent where poverty, malnutrition, and starvation are greatest; sanitation is inadequate, personal hygiene poor, water supplies are unsafe or insufficient; health provision the least developed, and disease control measures are lacking or ineffective.

  War and famine in developing countries have greatly increased the number of people that have become refugees, suffer ill health and die prematurely from infectious diseases.

  In rural areas, distances to health centres and hospitals are often too great to be travelled by patients or mothers with young children requiring immunization.

  In many countries, increasing urbanization has resulted in an increase in the incidence of diseases associated with inadequate and unsafe water, poor sanitation, and overcrowded living conditions.

  In areas of high HIV prevalence, major pathogens such as *M. tuberculosis* and *Streptococcus pneumoniae* and a range of opportunistic pathogens associated with immunosuppression, are responsible for infections, often life-threatening, in those infected with HIV.

  This subunit includes information on:

  - Clinical microbiology and public health laboratory activities at district level.
  - Quality assurance and standard operating procedures (SOPs) in microbiology.
  - Collection of microbiological specimens.
  - Safe working practices.

**CLINICAL MICROBIOLOGY AND PUBLIC HEALTH LABORATORY ACTIVITIES AT DISTRICT LEVEL**

A network of district microbiology and regional public health laboratories is needed to provide to the community, accessible microbiological services.
Important: District laboratories require the support of the regional public health laboratory in the preparation and implementation of microbiological standard operating procedures (SOPs), safe working practices, on-site training, quality assurance, and provision of essential supplies (e.g. reagents, culture media, controls, antisera).

Operating microbiological laboratory services with minimal resources

The high cost of culture media and reagents, lack of a rational approach to the selection and use of microbiological investigations, and a shortage of trained technical staff and clinical microbiologists are important factors in preventing the establishment and extension of essential microbiological services in developing countries.

To ensure the optimal use of available resources, it is important for health authorities to identify those pathogens of greatest public health importance which require microbiological investigations based on a consideration of:
- local disease patterns,
- clinical relevance and frequency of isolation,
- severity of disease and outcome,
- possibility of effective intervention,
- need for surveillance to monitor drug resistance and epidemic potential,
- cost benefit ratio of isolation and, or, identification,
- laboratory capacity and resources available,
- availability of trained personnel to perform microbiological investigations and ensure the quality of work and reports.

Such an approach helps to target resources where they are most needed, enables a list of essential culture media and diagnostic reagents to be identified, sourced and costed, and training in microbiology techniques and their application to be more specific.

QUALITY ASSURANCE AND SOPs IN MICROBIOLOGY

The principles of quality assurance (QA) and general guidelines on how to prepare standard operating procedures (SOPs) are described in subunit 2.4 in Part 1 of the book.

Providing appropriate, reliable and affordable microbiological services

Laboratory personnel, clinicians, community health officers and sanitary officers must work closely together in deciding the microbiological services that are required and ensuring the services provided are appropriate, reliable, and affordable.

This involves identifying:
- The infectious diseases that require laboratory investigation (priority pathogens).
- Role of district laboratories in surveillance work and the investigation of epidemics.
- Techniques (SOPs) to be used to collect specimens, identify pathogens and perform antimicrobial susceptibility tests.
- Most appropriate systems for reporting and recording the results of microbiological investigations, collating and presenting data for surveillance purposes.
- Quality assurance.
- Training requirements, supervision, and ongoing professional support.
- Equipment and microbiological supplies needed and systems for distribution of supplies.
- Costs involved.

Need for quality assurance and SOPs in microbiology

Microbiological investigations are important in the diagnosis, treatment, and surveillance of infectious diseases and policies regarding the selection and use of antimicrobial drugs. It is therefore essential that test reports:
- are reliable,
- standardized,
- provide the information that is required at the time it is needed,
- in a form that can be understood.

Quality assurance is also required to minimize waste and ensure investigations are relevant and used appropriately.

WHO in its publication Basic laboratory procedures in clinical bacteriology states that quality assurance in microbiology must be:
- comprehensive: to cover every step in the cycle from collecting the specimen to sending the final report to the doctor as shown opposite;
rational: to concentrate on the most critical steps in the cycle;
regular: to provide continuous monitoring of test procedures;
frequent: to detect and correct errors as they occur.

The following apply to the QA of the pre-analytical, analytical, and post-analytical stages of microbiological procedures and should be incorporated in microbiological SOPs.

Pre-analytical stage
SOPs need to describe:
- Selection and appropriate use of microbiological investigations.
- Collection and transport of specimens.
- How to fill in a request form correctly.
- Checks to be made when the specimen and request form reach the laboratory.

Appropriate use of microbiological investigations
This aspect of QA requires collaboration between laboratory personnel, clinicians, and public health officers as discussed at the beginning of this subunit. The fewer the resources the more important it is to establish priorities based on clinical and public health needs. Clear guidelines should be provided on the use and value of specific microbiological investigations.

Collection and transport of microbiological specimens
Specimens for microbiological investigation must be collected correctly if pathogens are to be successfully isolated and identified, reports are not to be misleading, and resources are not to be wasted. Written instructions for the collection of specimens must be issued by the laboratory to all those responsible for collecting microbiological specimens. The collection of microbiological specimens is described at the end of this subunit.

Request form
Each specimen must be accompanied by a request form which details:
- the patient’s name, age (whether an infant, child, adult), gender, outpatient or inpatient number, ward or health centre, and home area/village.
- type and source of specimen, and the date and time of its collection.
- investigation(s) required.
- clinical note summarizing the patient’s illness, suspected diagnosis and information on any antimicrobial treatment that may have been started at home or in the hospital.

Note: The clinical note will help to report usefully the results of laboratory investigations.
- name of the medical officer requesting the investigation.

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More information
Checking a specimen and request form
SOPs should include the procedures to be followed when specimens reach the laboratory, particularly checks to ensure that the correct specimen has been sent and the name on the specimen is the same as that on the request form. Also included should be how to handle and store specimens that require immediate attention, e.g. c.s.f., blood cultures, unpreserved urine, swabs not in transport media, faecal specimens containing blood and mucus, and wet slide preparations.

Examples of specimens which should not be accepted for microbiological investigations include:
- dry faecal swabs,
- saliva instead of sputum,
- eye swabs that have not been freshly collected,
- any specimen not collected into a correct container,
- a leaking specimen (sample may be contaminated).

Analytical stage
The following should be included in microbiological SOPs, covering the analytical stage:

- Detailed procedures for examining different specimens (described in subsequent subunits).
- Staining techniques and quality control (QC) of stains (see following text).
- Aseptic techniques and safe handling of infectious material as described in subunit 7.4.
- Preparation and QC of culture media and preservation of stock strains used in performance testing (see subunit 7.4).
- Inoculation of broth and agar culture media and plating out techniques (see subunit 7.4).
- Reading and interpretation of cultures (see subunit 7.4).
- Techniques used to identify pathogens and the QC of diagnostic reagents, strips, and discs as described in subunit 7.5.
- Antimicrobial susceptibility testing and QC of procedure and discs as described in subunit 7.6.
- Cleaning and QC of equipment used in the microbiology laboratory, e.g. microscope, incubator, anaerobic jar, centrifuge, waterbath/heat block, autoclave, hot-air oven, and refrigerator (see following text).
- Immunological techniques and QC of antigen and antibody reagents.
- Safe working practices (see end of this subunit).

- Disposal of specimens and cultures (see subunit 3.4 in Part 1 of the book).
- Cleaning of glassware, plasticware, etc (described in subunits 3.4 and 3.6 in Part 1 of the book).
- Sterilization procedures and their control (see subunits 3.4 and 4.8 in Part 1 of the book).

Important: As part of QC, the performance of staff must be monitored, all techniques must be demonstrated to new members of staff, the results of QC tests must be recorded and signed, and the work of newly qualified staff supervised (see also subunit 2.4 in Part 1 of the book).

Control of stains and reagents
All stains and reagents must be clearly labelled, dated, and stored correctly. The preparation, fixation, staining and reporting of smears as detailed in the department’s SOPs must be followed exactly. Stains and reagents should not be used beyond their expiry date (where this applies) or when they show signs of deterioration such as abnormal turbidity or discoloration.

At regular intervals and whenever a new batch of stain is prepared, e.g. basic fuchsin in the Ziehl-Neelsen technique or crystal violet in the Gram technique, control smears of appropriate organisms should be stained to ensure correct staining reactions. Control smears used in the Ziehl-Neelsen technique should include smears with few to moderate numbers of AFB. Smears for controlling Gram staining can be prepared from a mixed broth culture of staphylococci and *Escherichia coli*. All control smears should be alcohol-fixed and stored in labelled, dated, airtight containers.

Use efficient (non-leaking), preferably light-proof stain dispensing containers to avoid stains being wasted. Ensure containers can be closed when not in use to avoid evaporation and contamination of stains.

A common cause of poor staining is attempting to stain a smear that is too thick, e.g. c.s.f. containing many pus cells. When a smear is too thick, the decolorization process is often incomplete which can result in Gram negative organisms being reported as Gram positive. The QC of reagents used in biochemical diagnostic tests is described in subunit 7.5.

Control of equipment
For each item of equipment there should be clear operating and cleaning instructions, and service sheets. Regular cleaning, servicing and maintenance
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are essential if equipment is to remain in good working order and safe to use.

The operating temperature of a refrigerator, incubator, heat block and water-bath should be monitored and charted daily. Regular checks should also be made of all glassware and reusable plastic items to ensure that they are completely clean, not damaged, and being sterilized correctly. Specimen containers should be inspected regularly, especially the caps of bottles and tubes for missing or worn liners.

The use, care, maintenance, and performance checks of microscopes are described in subunit 4.3 and of other items of equipment in subunits 4.4–4.12 in Part 1 of the book. Hazards associated with the use of equipment and glassware are covered in subunit 3.6 in Part 1. The use and control of an autoclave are described in subunits 3.4 and 4.8, also in Part 1. The use and control of anaerobic jars are covered in subunit 7.4.

Post-analytical stage
SOPs need to include:

- Reporting and verifying of microbiological test results.
- Taking appropriate action(s) when a result has serious patient or public health implications.
- Interpreting test reports correctly.

Reporting results
The terminology and format used in reporting microscopical preparations, cultures, and antimicrobial susceptibility tests should be standardized and agreed between laboratory personnel, clinicians, and public health officers. Any preliminary report of microscopical findings or isolation of a pathogen from a primary culture must be followed by a full written report.

All reports must be concise and clearly presented. The use of rubber stamps can be helpful in standardizing the report and making it easy to understand, e.g. stamps that list the presence or absence of recognized pathogens or that list the antibiotics against which an isolate has been tested. When using a stamp, care must be taken to position it correctly and sufficient ink must be used to reproduce clearly the entire stamp. The reporting of cultures is discussed in subunit 7.4.

Verifying and interpreting reports
Before leaving the microbiology laboratory, all reports must be checked for correctness and clarity and signed by the person in charge of the department. Reports which are urgently needed for patient care or the management of an epidemic must reach the clinician or public health officer/epidemiologist as soon as possible. Those receiving the reports should consult the laboratory when any part of the report is unclear. Improvement in the quality and usefulness of microbiological reports will only be achieved by effective communication between those requesting tests and laboratory staff.

A record of the results of all investigations must be kept by the laboratory, e.g. as carbon copies, work sheets, or in record books. Copies of work sheets should be dated and filed systematically each day.

External quality assessment
Whenever possible the regional public health laboratory should organize an external quality assessment (EQA) scheme to help district microbiology laboratories. An EQA scheme should include testing for major pathogens. It should not be too complicated, costly, or time-consuming for district laboratories.

The main objective of an EQA scheme is to confirm that a laboratory's SOPs and internal QC procedures are working satisfactorily. EQA schemes help to identify errors, improve the quality of work, stimulate staff motivation, and assure users of the service that the laboratory is performing to the standard required to provide reliable results.

WHO advises that an EQA scheme should operate monthly or at least four times a year. Instructions and a report form (to be returned with results after 1 week) should be sent with the specimens to each participating laboratory. Each specimen should be examined in the same way as routine clinical samples (not recognized as a QC specimen). The District Laboratory Coordinator should investigate and assist any poor performing laboratory and where indicated, arrange for the further training of staff. Refresher courses should be held periodically to maintain competence and motivation and to introduce new tests.

Note: An excellent chapter on quality assurance in microbiology can be found in the WHO publication Basic laboratory procedures in clinical bacteriology.1

Collection of Microbiological Specimens
The value and reliability of microbiological reports are directly affected by the quality of the specimen received by the laboratory and the length of time between its collection and processing.

The collection of specimens must form part of the department’s SOPs (see previous text), and the laboratory should issue written instructions to all
those responsible for the collection of specimens from inpatients and outpatients. Such instructions should include:

- The amount and type of specimen required, container to use, and need for any preservative or transport medium.
- Best time to collect a specimen.
- Aseptic and safe methods of collection to avoid contamination and accidental infection.
- Labelling of the specimen container.
- Conditions in which specimens need to be kept prior to and during their transport to the laboratory.
- Arrangements for processing specimens that are urgent and those collected outside of normal working hours, e.g. blood cultures collected by medical staff.

**Type of specimen**
The correct type of specimen to collect will depend on the pathogens to be isolated, e.g. a cervical not a vaginal swab is required for the most successful isolation of *N. gonorrhoeae* from a woman. Sputum not saliva is essential for the isolation of respiratory pathogens.

**Time of collection**
Specimens such as urine and sputum are best collected soon after a patient wakes when organisms have had the opportunity to multiply over several hours. Blood for culture is usually best collected when a patient’s temperature begins to rise. The time of collection for most other specimens will depend on the condition of the patient, and the times agreed between the medical, nursing, and laboratory staff for the delivery of specimens to the laboratory.

**Important:** Every effort must be made to collect specimens for microbiological investigation before antimicrobial treatment is started and to process specimens as soon after collection as possible.

**Collection techniques**
Detailed instructions on how to collect different specimens can be found in the subsequent subunits of this chapter.

The following apply to the collection of most microbiological specimens:

- Use a collection technique that will ensure a specimen contains only those organisms from the site where it was collected. If contaminating organisms are introduced into a specimen during its collection or subsequent handling, this may lead to difficulties in interpreting cultures and delays in issuing reports.

A strictly sterile (aseptic) procedure is essential when collecting from sites that are normally sterile, e.g. the collection of blood, cerebrospinal fluid, or effusions. An aseptic technique is necessary not only to prevent contamination of the specimen but also to protect the patient.

- Avoid contaminating discharges or ulcer material with skin commensals. The swabs used to collect the specimens must be sterile and the absorbent cotton-wool from which the swabs are made must be free from antibacterial substances.

- Collect specimens in sterile, easy to open, leak-proof, dry containers, free from all traces of disinfectant. Containers must be clean but need not be sterile for the collection of faeces and sputum.

To avoid breakages, whenever possible, containers made from autoclavable plastic should be used providing these are leak-proof.*

*Autoclavable plastics used in the manufacture of bottles include polypropylene, copolymer, polycarbonate, and polymethylpentene.

The containers given to patients must be easy for them to use. Patients should be instructed in the aseptic collection of specimens and asked to avoid contaminating the outside of containers.

When contamination occurs, wipe the outside of the container with a tissue or cloth soaked in disinfectant before sending the specimen to the laboratory.

- Report any abnormal features, such as cloudiness in a specimen which should appear clear, abnormal coloration, or the presence of pus, blood, mucus, or parasites.

The appearance of urine, pus, vaginal discharge, faeces, effusions, and cerebrospinal fluid should be described routinely.

**Labelling specimens**
Each specimen must be clearly labelled with the date and time of collection, and the patient’s name, number, ward or health centre.

Slides with one end frosted (area of opaque glass on which to write) should be used for making smears so that a lead pencil can be used to label the slides clearly.

Each specimen must be accompanied by a
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Correctly completed request form (see previous text).

**Specimens containing dangerous pathogens**

Those delivering, receiving, and examining specimens must be informed when a specimen is likely to contain highly infectious organisms. Such a specimen should be labelled HIGH RISK, and whenever possible, carry a warning symbol such as a red dot, star, or triangle which is immediately recognized as meaning that the specimen is dangerous and must be handled with extra care.

Specimens which should be marked as HIGH RISK include:

- Sputum likely to contain *M. tuberculosis*.
- Faecal specimen that may contain *V. cholerae* or *S. Typhi*.
- Fluid from ulcers or pustules that may contain anthrax bacilli or treponemes.
- Specimens from patients with suspected HIV infection, viral hepatitis, viral haemorrhagic fever, or plague.

Immediately after collection, a HIGH RISK specimen should be sealed inside a plastic bag or in a container with a tight-fitting lid. The request form must not be placed in the bag or container with the specimen.

Note: Because any specimen may contain infectious pathogens, it is important for laboratory staff to handle all specimens with adequate safety precautions and to wash their hands after handling specimens (see also subunits 3.2–3.4 in Part 1 of the book).

**Preservatives and transport media for microbiological specimens**

In general, specimens for microbiological investigations should be delivered to the laboratory without delay and processed as soon as possible. This will help to avoid the overgrowth of commensals.

When a delay in delivery is unavoidable, for example when transporting a specimen from a health centre to a hospital laboratory, a suitable chemical preservative or transport culture medium must be used. This will help to prevent organisms from dying due to enzyme action, change of pH, or lack of essential nutrients. A transport medium is needed to preserve anaerobes.

Amies transport medium is widely used and effective in ensuring the survival of pathogens in specimens collected on swabs, especially delicate organisms such as *Neisseria gonorrhoeae*. Amies medium is a modification of Stuart’s transport medium. Its preparation is described in No. 11 (Appendix I). An example of a preservative is boric acid which may be added to urine.

Cary-Blair medium is used as a transport medium for faeces that may contain *Salmonella*, *Shigella*, *Campylobacter* or *Vibrio* species (see No. 22).

Note: Preservatives that contain formaldehyde solution, such as merthiolate iodine formaldehyde (MIF) and formol saline, must not be used for microbiological specimens because formaldehyde kills living organisms.

**Transport of microbiological specimens collected in a hospital**

As mentioned previously, specimens should reach the laboratory as soon as possible or a suitable preservative or transport medium must be used.

Refrigeration at 4–10°C can help to preserve cells and reduce the multiplication of commensals in unpreserved specimens. Specimens for the isolation of *Haemophilus*, *S. pneumoniae*, or *Neisseria* species, however, must never be refrigerated because cold kills these pathogens.

Smears collected by ward staff or in outpatient clinics for subsequent Gram staining, must be placed in a safe place to dry, protected from dust, ants, cockroaches, and flies. The laboratory should provide wards and outpatient clinics with petri dishes (unsterile) or other containers in which to place and transport slide preparations.

**Dispatch of microbiological specimens collected in health centres or district hospitals without culture facilities**

Specimens for dispatch must be packed well and safely. When specimens are to be mailed, the regulations regarding the sending of ‘Pathological Specimens’ through the post should be obtained from the Postal Service and followed exactly. When dispatching microbiological specimens the following apply:

- Keep a register of all specimens dispatched. Record the name, number, and ward or health centre of the patient, type of specimen, investigation required, date of dispatch, and the method of sending the specimen (e.g. mailing, hand-delivery, etc). When the report is received back from the microbiology laboratory, record the date of receipt in the register.
- Check that the specimen container is free from cracks, and the cap is leak-proof. Seal around the container cap with adhesive tape to prevent loosening and leakage during transit.
● Use sufficient packaging material to protect a specimen, especially when the container is a glass tube or bottle (use a plastic container whenever possible). Place the packaged container in a strong protective tin or box, and seal completely. When the specimen is fluid, use sufficient absorbent material to absorb it should a leakage or breakage occur.

● Mark all specimens that may contain highly infectious organisms, ‘HIGH RISK’ (see previous text). Do not mail such specimens.

● Dispatch slides in a plastic slide container or use a strong slide carrying box or envelope.

● Label specimens dispatched by mail, ‘FRAGILE WITH CARE – PATHOLOGICAL SPECIMEN’.

When a specimen is likely to deteriorate unless kept cool, transport it in an insulated container, such as a polystyrene box or thermos flask containing ice cubes. The specimen must be sealed inside a waterproof bag or tin to prevent the label being washed off when the ice cubes melt. Precautions must also be taken to keep the request form dry.

Note: Details on international transport regulations can be found on p. 66 in Part 1.

Collection of individual specimens

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Note: Detailed and helpful guidelines on the collection and dispatch of microbiological specimens can be found in the WHO publication *Specimen collection and transport for microbiological investigations.*

Practice of virology in district laboratories

Viruses, particularly HIV, arboviruses, measles virus, and viruses that cause respiratory and diarrhoeal disease in young children, are major causes of death and illness in tropical and developing countries. At district level most virus diseases are presumptively diagnosed clinically or remain undiagnosed. It is usually only at central level that facilities exist for the laboratory investigation of virus diseases based on virus isolation, direct demonstration of virus or viral components, and the serological diagnosis of virus infections.

In recent years, however, rapid, simple to perform immunological assays have become available to diagnose virus diseases such as dengue (see subunit 718.53), HIV infection (see subunit 718.55), and viral hepatitis (see subunit 718.54). Where appropriate, affordable, and available, these rapid techniques are being increasingly used in district laboratories and regional blood transfusion centres.

When needing to investigate a serious epidemic caused by Ebola fever virus or other highly infectious virus causing viral haemorrhagic fever, testing must be performed in a virology laboratory or public health laboratory having adequate containment facilities with a specialist public health team (appropriately protected) collecting the samples.

Practice of mycology in district laboratories

The medically important fungi are listed in subunit 7.2 and the investigation of common fungal infections in district laboratories is described in subunits 718.38–718.52.

SAFE WORKING PRACTICES

Health and safety in district laboratories, including full coverage of microbial hazards, safe working practices, and the decontamination of infectious material and disposal of laboratory waste are described in Chapter 3 in Part 1 of the book.

The following are some of the important points which apply when working with infectious materials:

- Never mouth-pipette (see p. 63 in Part 1). Use safe measuring and dispensing devices as described in subunit 4.6 in Part 1.
- Do not eat, drink, smoke, store food, or apply cosmetics in the working area of the laboratory.
- Use an aseptic technique when handling specimens and culture (see subunit 7.4).
- Always wash the hands after handling infectious material, when leaving the laboratory and before attending patients. Cover any open wound with a waterproof dressing.
- Wear appropriate protective clothing when working in the laboratory. Ensure it is decontaminated and laundered correctly (see p. 59 in Part 1).
Wear protective gloves, and when indicated a face mask, for all procedures involving direct contact with infectious materials. When wearing gloves, the hands should be washed with the gloves on, particularly before using the telephone or doing clerical work.

Minimize the creation of aerosols. The commonest ways infectious aerosols are formed are detailed on pp. 61–62 in Part 1.

Centrifuge safely to avoid creating aerosols. Know what to do should a breakage occur when centrifuging (see p. 63 in Part 1).

Avoid practices which could result in needle-stick injury.

Do not use chipped or cracked glassware and always deal with a breakage immediately and safely (see p. 89 in Part 1).

Avoid spillages by using racks to hold containers. Work neatly and keep the bench surface free of any unnecessary materials.

Decontaminate working surfaces at the end of each day’s work and following any spillage of infectious fluid. Know what to do when a spillage occurs (see p. 63 in Part 1).

Report immediately to the laboratory officer in charge, any spillage or other accident involving exposure to infectious material.

Know how to decontaminate specimens and other infectious materials (see pp. 66–74 in Part 1).

Use and control an autoclave correctly (see p. 67 and subunit 4.8 in Part 1).

Dispose of laboratory waste safely (see pp. 66–71 in Part 1).

Do not overfill discard containers. Use appropriate disinfectants (see pp. 67–70 in Part 1). Use separate containers for ‘sharps’.

Do not allow unauthorized persons to enter the working area of the laboratory.

Ensure technical and auxiliary staff working in the laboratory receive appropriate immunizations. Those at increased risk of acquiring infections, e.g. immunocompromised persons, should not work in a laboratory handling infectious material.

REFERENCES


7.2 Features and classification of microorganisms of medical importance

Most microorganisms are free-living and perform useful activities that benefit animal and plant life. Microorganisms that have the ability to cause disease are called pathogens. They include:

- Bacteria (singular, bacterium)
- Viruses (singular, virus)
- Fungi (singular, fungus)
- Protozoa (singular, protozoon)*

*The protozoa of medical importance are described in Chapter 5 in Part 1 of the book.

Infection occurs when a pathogen is able to establish itself in a person. Not all infections, however, result in clinical infection, i.e. a person falling ill. Frequently a person displays no symptoms of disease (asymptomatic). Such an infection is referred to as subclinical.

Virulence is the term used to describe the degree of pathogenicity of an organism. It is mainly dependent on the invasiveness and, or, the ability of the organism to produce toxins (poisonous substances). The infectiousness or communicability of an organism refers to its capacity to spread. Epidemiology is the study of the spread, distribution, prevalence, and control of disease in a community.

Endemic, epidemic and pandemic disease

Endemic: This refers to the constant presence of a disease or agent of disease in a community or region. A sporadic disease is one which breaks out only occasionally.

Epidemic: This usually means an acute outbreak of disease in a community or region, in excess of normal expectancy, and derived from a common or propagated source. Many endemic diseases can rapidly become epidemic if environmental or host influences change in a way which favour transmission.

Pandemic: This refers to a disease which spreads to several countries and affects a large number of people. HIV disease, influenza and cholera are examples of diseases that have caused or currently are the cause of pandemics. The control and prevention of outbreaks of infectious disease depend on knowing the reservoirs,
Factors that contribute to the spread of communicable diseases in developing countries

Most microbial diseases are transmitted by:

- ingesting pathogens in contaminated food or water as in cholera, typhoid and paratyphoid fever, bacillary dysentery, hepatitis A, or ingesting pathogens in unpasteurized milk and dairy products as in brucellosis, or Campylobacter infections.

- inhaling pathogens in air-borne droplets as in tuberculosis, whooping cough, measles, influenza, pneumonia, meningitis, SARS.

- pathogens being transferred by direct contact from one person to another as in HIV infection, syphilis, gonorrhoea, ringworm infection.

- pathogens entering the blood and tissues through the bite of an arthropod vector as in bubonic plague, rickettsial infections, dengue, rift valley fever.

- pathogens entering wounds, cuts, or burns by way of contaminated hands or unsterile instruments as in infections of the skin such as boils and abscesses and tetanus (via contaminated soil or dust).

- transfer of pathogens in contaminated blood or blood products as in HIV infection, viral hepatitis (HBV, HCV).

- pathogens transmitted from mother to child during pregnancy or childbirth as in HIV infection, congenital syphilis, Chlamydia infection, herpes infection, congenital rubella, gonococcal conjunctivitis, cytomegalovirus neonatal infection.

In persons with inadequate immune responses, infections can also be caused by the body’s normal microbial flora (organisms that naturally colonize certain areas of the body, see later text).

Important factors which influence the transmission and spread of communicable diseases in tropical and developing countries include:

- Inadequate surveillance, preventive and control measures, and lack of health care facilities in rural areas to detect and treat patients with communicable diseases.

- Socioeconomic factors including increasing urbanization, poverty, unemployment, poorly constructed houses, overcrowding, malnutrition (particularly protein and vitamin deficiencies), and starvation brought about by drought, crop failure, flooding, war, and mass migration.

- Inadequate and contaminated water supplies, inadequate sewage disposal and unhygienic practices.

- Climatic factors including extreme rainfall and flooding leading to pollution of water supplies and greater numbers of insect vectors. During the dry season, an increase in dust-borne particles can lead to increased transmission, e.g. meningococci.

- Ineffective control of mosquitoes and other insect vectors.

- Geographical factors including the difficulties of vaccination teams and health workers in reaching remote villages.

- Unavailability of drugs and non-compliance by patients.

- Ineffective health education or lack of access to health education.

- Particularly involving young children: disruption to vaccine programmes, malnutrition, and co-existing infection, e.g. malaria.

Human carriers: A carrier is a person who is colonized by a pathogen but experiences no disease or only minor symptoms from it. Such a person can excrete the pathogen he or she is carrying over a long period and be a source of infection to others without realizing it. The carrier state is particularly important in the transmission of typhoid fever and also occurs in a proportion (about 10%) of those with hepatitis B infection.

Body’s defence mechanisms

Although the human body continually comes into contact with potentially pathogenic microorganisms, infection and disease are usually prevented or minimized because a healthy body has a range of defence mechanisms to protect it. These consist of:

- Non-specific defences

- Specific immune responses

Non-specific defences: Although referred to as natural or innate immunity, these defences are non-immunological. They include the body’s natural barriers to infection (skin, mucous membranes, antimicrobial secretions), phagocytosis of pathogens involving polymorphonuclear neutrophils (poly-