CHAPTER 4

SELECTION OF APPROPRIATE LABORATORY TESTS

4.1 General

Many medical laboratory diagnostic tests are suitable for use in the emergency laboratory. Most of these are the same as those used in conventional established laboratories, but some tests have to be modified for use in the field. Diagnostic laboratory tests can be classified as either direct or indirect. Direct tests are intended to specify the exact cause of the disease. Indirect tests examine the host's reaction to the infection or medical condition, as in haematology or urinalysis tests.

Both types of diagnostic tests are appropriate for emergency laboratories. Since infectious diseases are the primary concern in most disaster situations, direct tests are often the most important.

4.2 Direct diagnostic tests

4.2.1 Rapid diagnostic tests for infectious diseases

Rapid diagnostic tests are defined as laboratory tests that can yield a specific diagnosis within 24 hours, preferably within 10 minutes. Rapid diagnostic tests that are appropriate for use in emergency situations must be simple, easy to read, and clear to interpret.

Standard brightfield light microscopy provides direct, rapid diagnosis for many diseases, for example, malaria, bacterial meningitis, and intestinal parasites. Since microscopic examinations provide rapid diagnosis for many other infections, microscopy is considered a first-line rapid diagnostic technique.

Rapid diagnosis can also be made by serodiagnostic or immunodiagnostic tests. Serodiagnostic tests can be done rapidly by a variety of methods. Since these tests are very specific, pre-existing knowledge of potential disease problems in any geographical area is required to choose appropriate serodiagnostic reagents

The most suitable serodiagnostic tests for field diagnostic testing in disasters are.

- serological immobilization
- inert particle aggregation (passive agglutination)
- enzyme-linked immunosorbent assay
- fluorescent antibody (immunofluorescence).

Serological immobilization is the interruption of bacterial mobility by treatment of the

specimen with specific antiserum It is most commonly used for rapid diagnosis of cholera This test can often be done directly on liquid stool specimens.

Inert particle aggregation tests are very simple and useful. The two most commonly used are latex agglutination (LA) and coagglutination (COAG). Latex reagents are tiny styrene spheres covered with latex to which a specific antibody or antigen has been attached. COAG reagents are made from killed and stabilized cells of certain strains of *Staphylococcus aureus* that are rich in a substance called 'protein A'. Specific antibodies are attached to these cells through a natural affinity of the immunoglobulin-G of certain species for protein A. The resulting antibody-coated staphylococcal cells, tiny spheres themselves, are COAG reagents. When either LA or COAG reagents react with a homologous antigen such as bacteria in the spinal fluid of meningitis patients, a serological reaction occurs that causes clumping of the LA or COAG particles. This reaction can be observed easily with the naked eye, providing a very rapid andpecific diagnosis.

LA and COAG reagents are about equal in sensitivity Some of the important infectious diseases that can be diagnosed rapidly with these reagents are:

- cholera
- bacterial meningitis
- bacterial pneumonia
- salmonella gastroenteritis
- typhoid and paratyphoid fevers.

The difference between LA and COAG reagents lies in the ease of reagent preparation. The latex particles must be purchased. The COAG reagents can be easily prepared in almost any laboratory Commercially available LA and COAG reagents are listed in Annex 4

Enzyme-linked immunosorbent assay (ELISA) is becoming widely used for rapid diagnosis of infectious diseases, autoimmune diseases and normal conditions such as pregnancy. It can identify both antigens (microorganisms or their by-products) and antibodies ELISA tests are very sensitive and easy to use. Many are commercially available (see Annex 2).

Fluorescent antibody (FA) tests are used to detect and identify both antigens and antibodies The reagents are antibodies to which a fluorescent dye, a 'conjugate', has been attached chemically There are two types of FA test, direct and indirect. In direct FA test the specific fluorescent reagent is made to react with a specimen that may contain the target microorganism. If the target microorganism is present, for example, the meningococcus in spinal fluid, the individual (meningococcus) cells will glow brightly against a dark background when viewed with a fluorescence microscope. For indirect FA tests, an antibody is first made to react with the specimen, and then a fluorescent antibody specific for the protein of that antibody or species serum is made to react with it. The latter process yields great versatility to the indirect method. Only one fluorescent conjugate is needed, and many antisera (polyclonal and monoclonal) are available for detecting a wide variety of infectious disease agents

The primary disadvantage of performing FA tests in the field is the need for a fluorescence microscope. Recent technical developments in fluorescence microscopy equipment, however, have made it quite practical to do FA tests and fluorescent acid-fast stains in the emergency laboratory. This technological advance is due to the development of fluorescence objectives which can turn any standard microscope into a fluorescence microscope, at very reasonable cost

4.2.2 Communicable diseases: bacteria, parasites, rickettsia and viruses

General

The principal tests required for diagnosis of communicable diseases that may occur in disasters are listed in **Table 4.1**. The tests are divided into those that give presumptive diagnoses and those that are confirmatory.

Presumptive tests for some diseases are sufficiently sensitive and/or specific that further confirmatory tests are not appropriate or needed. Presumptive tests for other diseases are only a guide, having low sensitivity and/or specificity, and confirmatory tests should be done if at all possible.

TABLE 4.1 **Diagnosis of communicable diseases in disasters**

Disease	Specimen examined	Presumptive diagnostic test	Done on site	Confirmatory test	Done on site
AIDS/HIV	**Serum**	**ELISA**	**Yes**	**Serology**	**No**
		Fluorescent antibody	**Yes**		
		Latex agglutination	**Yes**		
Anthrax	**Lesion**	**Gram stain**	**Yes**	**Culture**	**No**
	Sputum			**Serology**	**No**
Bacterial dysentery/ gastro-enteritis	**Faeces**	**Culture/ coagglutination**	**Yes**	**Culture**	**Yes[1]**
		Microscopy, polymorphic and red cells	**Yes**		
Cholera	**Faeces**	**Serum immobilization**	**Yes**	**Culture**	**Yes[1]**
		Coagglutination	**Yes**		
		Latex agglutination	**Yes**		
Dengue	**Serum**	**None**	**No**	**Serology**	**No**
Diphtheria	**Throat swab**	**Microscopy, Gram stain**	**Yes**	**Culture**	**No**

TABLE 4.1 Diagnosis of communicable diseases in disasters (continued)

Disease	Specimen examined	Presumptive diagnostic test	Done on site	Confirmatory test	Done on site
Enteric fevers	Blood Faeces Urine	Culture/ coagglutination	Yes	Culture	Yes[1]
Hepatitis	Urine	Test strip	Yes	Serology	No
Intestinal helminths and protozoae	Faeces	Microscopy	Yes	NA	No
Leishmaniasis	Serum	DAT ELISA Fluorescent antibody	Yes No Yes	Culture Serology	No No
Leptospirosis	Blood Serum	Culture Darkfield	No Yes	Culture Serology	No No
Malaria	Blood	Microscopy	Yes	NA	
Measles	Serum	ELISA	Yes	NA	
Meningitis	Cerebrospinal fluid	Coagglutination ELISA Latex agglutination Microscopy	Yes Yes Yes Yes	Culture	No
Plague	Bubo aspirate Sputum	Gram stain	Yes	Culture	No
Pneumonia	Serum Sputum	Coagglutination ELISA Fluorescent antibody Latex agglutination	Yes Yes Yes Yes	Culture	No
Poliomyelitis	Serum	None	No	Culture, Serology	No
Protozoan dysentery	Faeces	Microscopy	Yes	NA	No
Relapsing fever	Blood	Field's stain/ Giemsa's stain Microscopy Wet mount (brightfield or darkfield)	Yes Yes Yes	Animal inoculation	No
Streptococcal disease	Throat swab	Coagglutination Latex agglutination Microscopy, Gram stain	Yes Yes Yes	Culture	No
Tetanus	None	None	No	NA	No
Trench fever	None	None	No	Serology	No

TABLE 4.1 Diagnosis of communicable diseases in disasters (continued)

Disease	Specimen examined	Presumptive diagnostic test	Done on site	Confirmatory test	Done on site
Trypanosomiasis	**Serum or plasma**	**CATT**	**Yes**	**ELISA Giemsa's stain**	**No**
Tuberculosis	**Sputum**	**Microscopy, acid-fast stain**	**Yes**	**Culture and sensitivity**	**No**
		Fluorescent acid-fast stain	**Yes**		
Typhus	**Serum**	**Indirect fluorescent antibody**	**Yes[1]**	**Serology**	**No**
		Weil Felix stained antigens	**Yes**		
Viral diarrhoeas	**Faeces**	**Latex agglutination**	**Yes**	**Culture**	**No**
Viral encephalitis	**Serum**	**None**	**No**	**Culture, serology**	**No**
Viral haemorrhagic fever	**Serum**	**None**	**No**	**Culture, serology**	**No**
Whooping cough	**Naso-pharyngeal swab**	**Fluorescent antibody**	**Yes**	**Culture**	**No**

[1] Dependent on the level of sophistication of the emergency laboratory
For example, many mobile laboratories can do this
CATT Card agglutination test
DAT Direct serum agglutination test
NA Not applicable

Notes to Table 4.1

1 A presumptive test involving bacterial immobilization with anti-*V cholerae* OI antiserum solution can be used in the field If OI antiserum containing preservatives is used, it must be diluted 1 10 with saline solution Interpretation of the test is subjective, and prior experience is important COAG and LA agglutination tests have been developed, and these have been field tested but not in disaster situations

2 If shigellosis is suspected, stool specimens should be transported in Cary-Blair medium or on filter paper for culture and susceptibility testing at a regional bacteriology laboratory

3 Experience with COAG and LA tests on selective enrichment broth cultures is limited, and confirmatory culture tests may be helpful

4 LA and COAG tests on clinical specimens have been used under field conditions, particularly in endemic meningitis areas They may be considered an acceptable alternative to microscopy with comparable sensitivity

5 The CATT is a useful screening test for trypanosomiasis DAT is a useful screening test for visceral leishmaniasis Representative serum samples should be sent to a reference laboratory for more sensitive and specific serology Marrow or splenic aspirates should only be done by suitably experienced medical personnel

6 Where specific viral fevers are endemic, and may become epidemic in disasters, on site serological tests should be considered (e g dengue)

Cholera

Diagnosis of cholera is, in most cases, essentially clinical. Presumptive laboratory diagnosis is important for epidemiological and outbreak investigations. Culture confirmation is essential for public health notification and to determine antimicrobial susceptibility. *V. cholerae* isolates or stool specimens should be sent to a reference laboratory for biotyping and serotyping. Stools may be tested directly on site with simple COAG or LA slide agglutination tests. Use of alkaline peptone water for enrichment culture incubation followed by LA or COAG tests gives more sensitive diagnostic results.

Enteric fever/salmonellosis

Typhoid/paratyphoid fever outbreaks are relatively uncommon in disasters, but all types of *Salmonella* infection are increasingly common in HIV endemic areas. No satisfactory non-culture diagnosis test for salmonellae exists for field use, with the possible exception of FA. Widal serological tests are of no value in tropical areas. Rapid COAG tests on stool and blood selective enrichment broth cultures have been reported. Confirmatory tests require culture of blood or faeces followed by biochemical and serological identification of suspect isolates.

Shigellosis

A presumptive diagnosis of shigellosis can be made by the presence of blood and polymorphonuclear leucocyte cells demonstrated by direct microscopy of the stool specimen. However, in the absence of amoebic trophozoites, this is rarely necessary and is of low specificity for epidemiological purposes. Culture confirmation and serotyping is necessary for epidemic investigation. Antibiotic susceptibility testing should be done in an extensive outbreak.

Amoebic dysentery (amoebiasis)

Stool microscopy demonstration of amoebic trophozoites is sufficiently sensitive and specific for diagnosis. Faecal specimens for this test must be very fresh.

Other diarrhoeal diseases

Investigations additional to those for shigellosis and amoebic dysentery are rarely indicated in disasters. In a diarrhoeal disease outbreak in which the above investigations are negative, a sampling of faecal specimens can be sent to a reference centre.

Stool microscopy for other enteric pathogens

Intestinal helminths and protozoan infections are common in communities where water and sanitation facilities are deficient. Their diagnosis may be important in individual patient management, but they are rarely implicated in disease outbreaks. Therefore, examinations for intestinal helminths should take low priority. Although they are easy to do and hold interest for those inclined towards parasitology, these examinations will have little impact on the health of the community at risk.

Bacterial meningitis

In an outbreak of suspected meningitis, lumbar puncture should be performed on a representative number of cases using aseptic technique. It should only be done by an experienced health worker. Stained cerebrospinal fluid (CSF) specimens should be examined with a microscope. Antigen detection, using COAG or LA tests has proved to be a useful field diagnostic technique. Culture confirmation will define the causative agent and allow serotyping and antimicrobial susceptibilities to be determined.

Tuberculosis

Presumptive diagnosis of tuberculosis can be made by microscopic examination of sputum smears stained by either the Ziehl Neelsen or Kinyoun methods. Fluorescent acid-fast staining provides very sensitive diagnosis. Mycobacterial culture facilities are often limited at regional or national levels. If tuberculosis is a major health problem, dispatch of a limited number of sputum specimens to a reference centre for culture and antimicrobial susceptibility testing may be considered. Microscopy is an essential component of tuberculosis control programmes. The idea is to detect active cases so that they can be treated and immediate contacts can be immunized with BCG vaccine.

Blood parasites

Microscopic examination of Giemsa-stained, Wright-Giemsa-stained, or Field-stained blood films for malaria, relapsing fever, and trypanosomiasis should be done. A card agglutination test (CATT) is available for serological screening, but microscopy remains the first line of diagnosis.

Rickettsial and viral fevers

For investigation of presumed viral or rickettsial fevers, filter paper blood specimens should be sent to an appropriate reference centre. Such specimens should be taken by a special investigation team, and adequate safety precautions must be taken for shipment (see the special shipping precautions described in Chapter 11).

Leishmaniasis

The direct serum agglutination test (DAT) can be used in the field as a screening test for visceral leishmaniasis. Serum specimens should also be sent to a reference centre for more specific serological tests.

Viral diseases

Increasingly, rapid diagnostic tests for viral diseases are becoming commercially available (see Annex 4). They are designed for detection and identification of either antigens or antibodies, but the most rapid and specific serodiagnostic tests detect and identify virus antigens. These simple rapid diagnostic tests are particularly significant because conventional tests for detecting and identifying viruses are time-consuming, difficult, cumbersome and expensive.

Rapid diagnostic test systems for viruses are available in a variety of formats including filters, micro-wells, slides and dipsticks. Testing methods include LA, COAG, ELISA, DNA probe-antigen immobilization, immunoperoxidase staining, and FA.

Some of these methods are very suited to use in the field The equipment required for some tests is more elaborate than that required for others For example, FA tests require a fluorescence microscope. As indicated above, some of these microscopes are field instruments and require very little electrical power. However, other methods require no electricity at all. Among these, the most simple are COAG or LA tests that can detect antigens or antibodies, for example the antibodies to HIV or infectious mononucleosis. The tests are simple, producing a reaction on a slide that can be read by the naked eye.

Dipstick methods have been produced for multiple testing at one step using one dipstick. They can be operated by hand, and reactions are read by eye. The technologies in these dipstick tests are ELISA and DNA probes that yield colour reaction endpoints. Dipstick technology is available to test for rubella, rubeola, HBs antigen and antibody, and HIV antibody. This technology can be easily adapted to other viral diseases

4.3 Indirect diagnostic tests

4.3.1 Haematology

Haematology tests for the emergency laboratory situation are much the same as in any medical laboratory. They are used to define the blood picture to give physicians and other medical care providers clues to the nature of the patient's disease. The tests include:

- total white cell (WBC) count
- differential WBC count
- abnormal red blood cells (RBC)
- platelet count
- reticulocyte count
- anaemia tests.

Total RBC counts are not normally done because they tend to be inaccurate. Better, easier methods exist to examine RBC content of the blood and the haemoglobin level.

All of the above tests can be easily done in the field Microtechniques are available for certain tests, like staining blood films and haematocrit. These permit limiting the quantities of certain stains and solutions required.

4.3.2 Rapid urine chemistry tests

Investigation techniques for common urine analytes are simple enough to be included in a portable or mobile laboratory The reagents can be purchased from scientific suppliers, or

they can be prepared locally. Convenient dry chemistry tests are commonly used, either by means of a test strip or with a powder mixture of chemicals. The stability of these dry reagents is greater than that of reagents in solution.

Urine tests for use in emergency laboratories are available for the following analytes:

- haemoglobin
- protein
- glucose
- nitrite.

While other urine analytes can also be measured easily, they are less important for diagnosis and monitoring of diseases in emergency situations. Test strips are produced by a number of companies. Test strips with more than these four tests on them are considerably more expensive.

Urine haemoglobin, glucose, protein, and nitrite can also be investigated by means of locally prepared mixtures of powdered chemicals. These tests are just as sensitive as the commercially available test strips but powdered chemical tests are far less expensive. Preparation of the reagent mixtures is described in Annex 5.

CHAPTER 5

TESTING WATER SUPPLIES

In most emergency situations, following a natural disaster or in population dislocations, water supplies should be assumed to be contaminated. Drinking water should be boiled or treated with commercial bleach or iodine before use. As the situation stabilizes, water supply systems return to normal. At that time, the emergency laboratory should begin to monitor drinking water supplies to ensure that the water delivery system is functioning properly.

Priority must be given to minimizing the risk of faecal contamination of water supplies. This may range from preventing defaecation in close proximity to the water source to construction of simple protected wells. When improvements in water supplies have been achieved by cleaning wells or system chlorination, water testing should be undertaken to monitor and ensure adequate sanitary water quality. In addition, water should be tested during investigation of enteric disease outbreaks.

The principal water quality test to assess the risk of enteric infection is the faecal coliform count. It is rarely appropriate to test water supplies for specific enteric pathogens because faecal coliform counts will detect faecal pollution. This test is more sensitive and specific than culture for enteric pathogens.

There are several test methods available. Either a selective lactose broth method or a membrane filter method can be used (see Annex 6 for manufacturers of water testing kits). The membrane filter method is rapid, provides a confirmed test, and contaminating bacteria in the water can be counted easily. The selective lactose broth method uses the production of gas by coliform bacteria in the water. It is less expensive than the filter method, but the investigator is left with a concentrated culture of bacteria requiring sterilization and disposal whereas membrane filters can be incinerated. If the broth method is chosen, one of the selective lactose broth culture media, e.g. lauryl sulfate tryptose broth, should be used. Presumptive positive results can be accepted in an emergency situation without doing confirmatory tests.

The preferred method for bacteriological monitoring of drinking water is the membrane filter method. Testing is easy and confirmed results are available in 18-24 hours. Exact numbers of coliform bacteria in a water sample can be determined. Compact portable laboratories for membrane filter water testing are available. They are very effective, but rather expensive. If an inexpensive field incubator is included in the emergency laboratory, an inexpensive plastic suction device can be used to sample the water, and the cost of membrane filter water testing equipment is considerably reduced. The monitors are disposable plastic, and the culture medium comes in a pre-packaged form, perfect for emergencies.

More details on collecting water samples for microbiological examinations and field tests for bacteriological analysis are given in Annex 7.

CHAPTER 6

LABORATORY SAFETY, DISINFECTION, AND WASTE DISPOSAL

6.1 Laboratory safety

6.1.1 Safe practices

The key to laboratory safety is good, careful technique. The practised skills of a qualified laboratory technician working in an emergency situation will promote efficient laboratory testing, good management, and personal laboratory safety.

The most frequent laboratory-acquired infections are:

- hepatitis B
- tuberculosis (very infectious by aerosol)
- salmonellosis
- shigellosis
- brucellosis (very infectious by aerosol)
- histoplasmosis (very infectious by aerosol)
- coccidiomycosis (very infectious by aerosol).

Less frequently reported laboratory infections are:

- anthrax
- plague
- tularaemia (very infectious by aerosol)
- leptospirosis
- relapsing fever
- melioidosis
- meningitis
- filoviruses
- arenaviruses.

Personal hygiene and good practices are important to ensure personal safety. The following rules apply.

1. Make sure that vaccinations for hepatitis B, tuberculosis, tetanus and typhoid fever are up to date before working in the emergency laboratory. In certain areas anthrax, plague, pneumococcal pneumonia, yellow fever, tularaemia meningococcal A and Japanese encephalitis vaccinations are also needed.
2. Do not eat or drink in the laboratory.

3 Wear a laboratory gown or coat in the laboratory, and leave it in the laboratory when going out
4. Wear disposable gloves when handling hazardous material
5 Wash hands before, during, and after laboratory work.

Safe laboratory practice includes the following.

- Specimen spillage can be reduced by using tube holding racks If specimens must be left standing on a workbench, they should be placed in a pan or on a disinfectant-soaked towel or cloth Spills should be disinfected After disinfection, the area should be washed with soap or detergent.
- Mouth pipetting must **never** be done. Safety pipetting devices should be included in the basic module.
- Infection can occur by inhaling aerosols containing pathogenic microorganisms Aerosols are formed when containers break in centrifuges, when samples are opened, when cultures or specimens are spilled, during pipetting, and when flaming bacteriological loops. To minimize aerosol infection, liquid specimens should always be kept covered, with the cap on the container, and opened carefully. When handling a sample suspected to be hazardous, a protective microbiological safety mask should be worn.
- Used needles and lancets should be discarded into a metal or sturdy plastic container, not paper or plastic bags Lancets may be sterilized by boiling or autoclaving
- The laboratory must be cleaned and decontaminated each day.

6.1.2 Proper use of centrifuge

Centrifuges are frequently the source of infectious aerosols The following rules should be applied when using centrifuges.

- The centrifuge must be placed on a flat and secure surface
- The centrifuge must be 'balanced' before operation by placing containers of equal volume opposite each other Clean tubes containing water can be used as balance tubes.
- Only a centrifuge with a cover should be used, and the cover must be closed before operation
- Special care must be taken when containers are broken in the centrifuge Plastic centrifuge tubes and bottles should be used The lid should be left closed for a few minutes to allow the aerosol to settle If particularly hazardous samples are involved, a microbiological safety mask and gloves should be worn when opening and disinfecting the centrifuge. The contents should be disinfected with 1% hypochlorite solution containing a little detergent and then discarded. The rotor and inside of the chamber should then be cleaned with a cloth soaked in a disinfectant such as glutaraldehyde, 70% (*V/V*) alcohol, or 10% formalin solution before further centrifugation. (Do not use bleach on metals)

6.2 Disinfection

The term 'disinfection' includes various physical and chemical procedures intended to prevent the spread of infective agents by inactivating or killing vegetative forms of harmful bacteria, fungi, protozoa and viruses. Waste, laboratory equipment, and disposables are commonly disinfected by chemical disinfectants or their aqueous or alcoholic solutions.

Table 6.1 **Disinfectants and their mode of application**

Disinfection target	Disinfectant	Application (disinfectant/ material V/V)	Minimum time of exposure of material	Stock preparation of disinfectant
Blood	5 % cresol (pH 9)	2:1	6 h	crystalline/ liquid
	1 % calcium hypochlorite[1]	2:1		powder
Stool	5 % cresol (pH 9)	2:1	6 h	crystalline/ liquid
	1 % calcium or sodium hypochlorite[1]	3:1		powder
	20 % calcium hydroxide	2:1		powder
	4 % chloramine T			powder
Urine	5 % cresol (pH 9)	1:1	4 h	crystallin/ liquid
Sputum	5 % cresol (pH 9)	1:1	4 h	crystalline/ liquid
Water	0.05 chloramine T	direct		powder
Skin	Lysol	direct	2 min	solution 50 % cresol in soap
	80 % ethanol	direct	2 min	95 % solution
	1 % iodine (Lugol) povidone iodine	direct	2 min	5 % solution
	70 % isopropanol	direct	2 min	pure
	60 % n - propanol	direct	2 min	pure
	1 % chloramine T	direct	2 min	powder
	quarternary ammonium compounds (QUATS)	direct	2 min	solution
Hypodermic needles	0.1 % hypochlorite[1]	direct		5, 10, or 15 % solution
Work benches	Lysol	direct	4 h	solution 50 % cresol in soap
	5 % cresol	direct	4 h	crystalline/ liquid
	5 % chloramine T	direct	4 h	powder
	1 % hypochlorite[1]			
Laboratory instruments	0.1 % hypochlorite[1]	direct	4 h	5, 10, or 15 % solution
	70 % isopropanol	direct		
Glassware	1 % hypochlorite[1]	direct	4 h	5, 10, or 15 % solution

With a small amount of detergent

Notes to Table 6.1

1 Cresols may be solid or liquid. Cresols are not very water soluble but a 5 % aqueous solution can be kept as stock solution. Cresols are more effective at alkaline pH (pH 9.0). Cresols are well emulsified in soap solutions.

Lysol is an emulsion of 50 % cresol in an aqueous solution of soap. Cresol can be replaced by phenol however phenol is a less powerful disinfectant. Therefore the time of exposure of material to phenol solution must be prolonged. Phenol and cresol solutions cause irritations of the skin and the eyes. Cresols and phenols, although they have strong odours, can be used where hypochlorites are corrosive.

2 Hypochlorite solutions (sodium or calcium) are very active disinfectants and are therefore used for a number of laboratory, household and industrial applications (in the form of household bleaches). Hypochlorites are active disinfectants against the hepatitis and human immunodeficiency viruses. Hypochlorites are rapidly inactivated by particles such as dust and organic materials. Hypochlorite solution must be prepared from stock solutions once a day. Hypochlorites cause irritation of the skin, eyes and lungs. They require contact time of 10 to 30 minutes. They are corrosive against metals, have toxic properties and are inactivated by organic matter.

3 Calcium hypochlorite (70 % available chlorine) is a solid (powder, granules). It decomposes at a slower rate than sodium hypochlorite. A solution of 1 % available chlorine is obtained by dissolving 14 g of calcium hypochlorite in 1 litre of water.

4 Chloramine-T is a crystalline powder which releases chlorine as the active disinfectant agent, although at a slower rate than the hypochlorites. It is also used for disinfecting water; chlorinated water has a concentration of 0.05 % chloramine-T. However, it should be remembered that chlorinated water can interfere with some laboratory tests in which case distilled or deionized water must be the diluent used.

5 Calcium hydroxide solution is prepared from the powder or granules of quicklime dissolved in water (1 part + 3 parts [W/V]). Calcium hydroxide solution is not suitable for disinfecting stools from patients with tuberculosis.

Quaternary ammonium compounds (QUATS) are effective against vegetative bacteria and some fungi. They are not effective against spores, viruses, and mycobacteria. QUATS are not toxic and are harmless to the skin.

7 Alcohols (ethanol, isopropanol, n-propanol) are fast acting but relatively expensive disinfectants and are usually used for skin disinfection. They are bactericidal, partially viricidal but not fungicidal. The penetrative power of alcohols is poor so they are not recommended for use with organic material. Because they need water to be absorbed by bacteria, alcohols should be diluted with water to be used as disinfectants; normally these disinfectants consist of 70 % to 90 % ethanol or 50 % isopropanol. Their shelf life is greater than one week. They are not corrosive and not inactivated by organic material.

8 Iodine is an excellent fast acting disinfectant with a wide range of action. It kills bacteria, many spores, viruses and fungi. At lower temperatures iodine is more active than other disinfectants. Some persons may have a sensitivity to iodine which will appear as a rash on the skin. This can be avoided by using iodophores (polymer solutions binding iodine such as povidone iodine).

Depending on the type of disinfectant, the principles for inactivating or killing microorganisms are different. The conditions for disinfection must be strictly followed in order to

a) achieve maximum inactivation of the microorganisms,
b) prevent injury to laboratory workers who may come in contact with the disinfectant during their work.

A number of chemical disinfectants are commercially available. Many of these disinfectants can also be prepared locally. The selection of one or another disinfectant will depend on the specific use. Inactivation of different microorganisms by a disinfectant may vary. For example, some disinfectants inactivate protozoa and bacteria but not viruses, some disinfectants effectively inactivate certain types of viruses but not other types, some disinfectants or

their solutions can cause irritation or inflammation of the skin, eyes, or the respiratory tract

The proper use of disinfectants depends on the time and temperature of exposure Some disinfectants are rapidly inactivated under certain conditions. Disinfectants for waste disposal and work surfaces are only active for a short time (up to several hours). Increasing temperature accelerates disinfection, but it can also accelerate the inactivation of the disinfectant by other materials Therefore, the temperature for chemical disinfection should be moderate (20°C to 40°C) Another important factor for optimal disinfection is the concentration of the disinfectant and the presence of water For example, 60 to 80 % aqueous solutions alcohol are good disinfectants whereas the pure alcohols have little or no effect. Some disinfectants, such as chlorine solutions (household bleach), should contain a small amount of non-ionic detergent to ensure complete wetting of the surface or item being disinfected.

Information about selected disinfectants can be found in **Table 6.1.**

REMEMBER : always disinfect used equipment and supplies **before** cleaning with soap and water.

6.3 Decontamination and disposal of specimens and infectious material

6.3.1 General

IMPORTANT The specimens examined in the laboratory (stools, pus, sputum, blood, urine, etc.) are often infectious After examination they must be treated in such a way that further risk of infection is avoided.

The specimens may be in

- cardboard cartons or plastic pots that can be destroyed (stools, sputum); or
- glass jars and bottles that can be cleaned, sterilized and used again.

Most disposable containers should be used once only, but polypropylene pots and tubes can be sterilized, cleaned, and reused

Specimen containers should be discarded into special disinfectant-filled containers (such as buckets), plastic disposal boxes, or hazardous waste bags Reusable plastics, slides, and other materials should be kept separate from disposables for later decontamination and cleaning before reuse.

6.3.2 Methods of sterilization or decontamination

Incineration

An incinerator is a device designed to completely burn up combustible materials, rendering them sterile ash Simple but effective incinerators can be made on site in emergency situations

Making such an incinerator and incineration are shown in **Figures 6.1** and **6.2** Incineration should be done at least once a week, or as often as necessary.

1. **Use an old metal petrol drum (200 litres/ 40 gallons).**
2. **Fix a strong metal grating (g) firmly about 1/3 of the way up the drum. Steel rods will keep it in place.**
3. **Cut a wide opening or vent (v) below the level of the grating.**
4. **Find a removable lid (l) for the drum.**

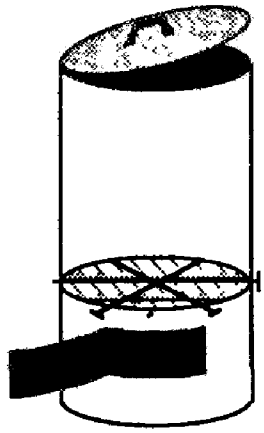

Figure 6.1 Making an incinerator

1. **At the end of each morning's and afternoon's work, place all used stool and sputum boxes on the grating of the incinerator.**
2. **Always keep the metal drum tightly closed (both lid and vent) except during incineration.**
3. **Fill the bottom of the drum with paper, sticks wood shavings, etc.**
4. **Remove the lid. Light the fire and keep it burning until all the infected material has been reduced to ash.**
5. **The ash produced is not dangerous and can be thrown on the refuse heap. The ash should be buried in a deep pit if it contains needles, lancets, etc.**

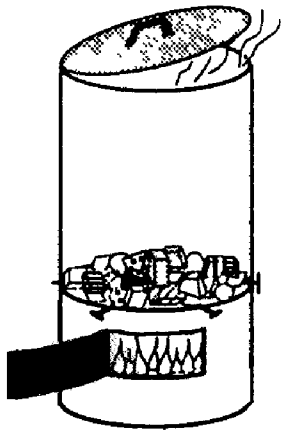

Figure 6.2 Incineration

Autoclaving

Anything can be sterilized in an autoclave. Heat-stable items such as glass containers, polypropylene tubes and cups, polycarbonate tubes and cups, cloth, instruments, etc will survive autoclaving intact. These can be emptied, washed, and put back into service Autoclaving is done at 121°C with pure steam (not a steam-air mixture) for 30 minutes

Sputum pots, urine bottles, and blood sample containers cann be autoclaved before being cleaned A number of field autoclaves are commercially available (**Figure 6.3**). They can be

heated by electricity, solar energy, gas burner, primus stove or cooking fire Electrically-heated models consume a large amount of electricity and so are not usually suited to field laboratories A pressure cooker designed for food preparation can also be used

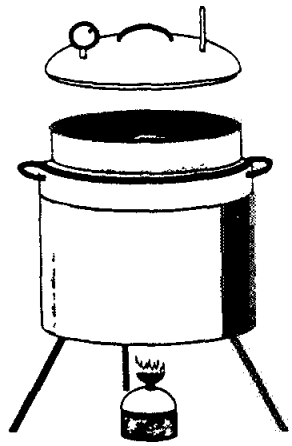

Figure 6.3 Autoclave

Boiling in detergent

When an autoclave is not available, boiling in detergent (**Figure 6.4**) is a satisfactory method of decontaminating most specimen containers. However, it does not kill spores and does not inactivate certain viruses Boil specimen containers for 30 minutes in a large pail containing a strong solution of washing powder or sodium carbonate crystals (60 grams per litre of water).

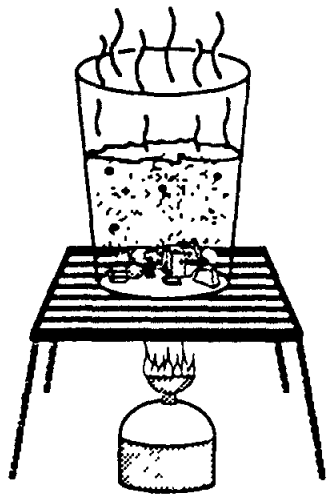

Figure 6.4 Boiling in detergent

Burial

Burial (**Figure 6.5**) does not decontaminate infectious material but it does prevent the material from being a hazard.

1. **Dig a pit 4-5 metres deep and 1-2 metres wide.**
2. **Make a lid that fits tightly over the pit. It is advisable to strengthen the upper rim of the pit by lining it with bricks and stones.**
3. **Throw stool or sputum boxes and other infected material into the pit twice a day. Replace the lid immediately.**
4. **Once a week, cover the refuse with a layer of quicklime. Alternatively, if quicklime is not available, cover the refuse with a layer of dried leaves (10 cm thick) once a week.**

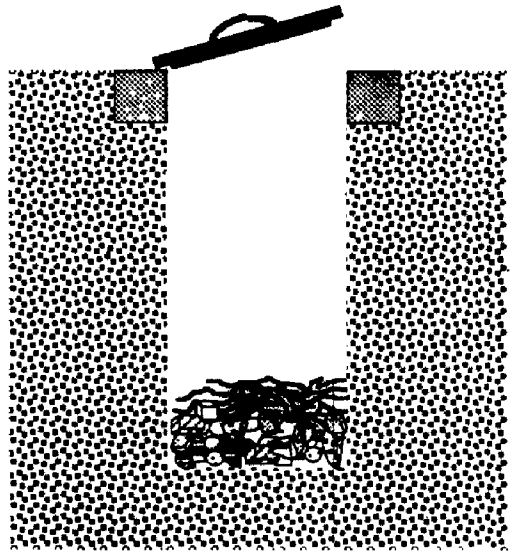

Figure 6.5 Burial of contaminated material

6.3.3 Disinfection of specific equipment

Reusable stool containers can be decontaminated by autoclaving or with strong disinfectant before cleaning. Autoclave at 121°C for 3 minutes or fill the jars containing stools with a 5 % solution of phenol or similar disinfectant. Leave for 24 hours. Empty into the lavatory

Reusable sputum pots and tubes of pus and CSF can be decontaminated by several methods before cleaning. In order of preference these are

- autoclaving;
- boiling in detergent

Follow the directions given for autoclaving or boiling

Urine bottles should be emptied into the lavatory Autoclave the bottles or fill them with a 1 % solution of commercial bleach, or 2 % solution of phenol Leave them for at least 4 hours before cleaning them with detergent.

Blood sample containers should be autoclaved Alternatively, they should be soaked overnight in a strong disinfectant, 5 % cresol or 1 % calcium hypochlorite, 1 2 V/V

Containers of fresh blood collected the same day should be rinsed in cold water and left to soak in a soap or detergent solution

Containers containing blood which has been kept for several days and in which organisms may have multiplied should be filled with a 1 % solution of commercial bleach and left for at least 6 hours before rinsing and cleaning.

Glass microscope slides used for tuberculosis should be soaked overnight disinfectant and then discarded

Slides used for Gram stain and Romanovsky stain should be soaked overnight in disinfectant The immersion oil should then be removed with xylene, washed in detergent and rinsed in several changes of clean water The slides should then be air dried, or dried using a lint-free towel

[1] **NOTE** If the lavatory is connected to a septic tank phenol or other antiseptic should not be put into the lavatory Clean the jars with detergent and water (see **Table 6.1**) They can also be autoclaved before cleaning

CHAPTER 7

LABORATORY KITS AND MODULES

7.1 Introduction to the use of modules and kits

A laboratory kit will allow a selected number of investigations to be carried out Equipment and supplies will be required, and these can be made up and provided in modules. (Annex 8 lists some non profit-making suppliers of tropical laboratories Annexes 9 to 16 list manufacturers of equipment and supplies.) The following definitions apply

Kit
everything required to carry out selected laboratory investigations.

Module
the separate components making up a kit; a module is a sub unit of a kit

Selected items provided in a kit will allow the technician to perform tests for a single disease, a group of related diseases, or indirect tests such as those for haematology or urinalysis

The concept of kit assembly is illustrated in **Figure 7.1**. In this example a kit has been assembled from appropriate modules to detect and diagnose malaria cases Note that not all modules are required and only part of the energy module is required, since a centrifuge and haemoglobinometer are not needed

Experience has shown that it is better to select equipment and supplies according to the particular need This will lead to a rapid and appropriate response The initial assessment will provide data leading to an indication of the specific needs in an emergency or disaster Prior preparation of a laboratory kit makes the immediate provision of the essential possible.

7.2 Use of kits and modules

The initial field laboratory will be established by using relevant modules to make up a kit At a later date there may be a need to send additional modules The contents of the modules can be considered as independent items Twenty-two modules are described The composition of each module is listed in **7.3.**

1 Basic module

Contains the essential equipment and supplies to establish a laboratory Simple and robust items have been chosen.

2 Energy module

Will provide a constant and reliable source of electrical energy

3 Water testing module

Will allow the testing of water for turbidity, chlorine faecal coliform bacteria and pH. It should be portable and may include a built-in battery

4 Microscope module

Consists of an ordinary microscope with a complete set of accessories suitable for work in harsh conditions, such as high temperature, humidity and dust It can be connected to a reliable and constant source of electricity, run on a battery which may be recharged by solar energy, or use direct sunlight.

5 Tuberculosis module

Contains the materials for staining acid-fast bacilli in sputum smears, using hot or cold techniques

6. Bacteriology module

Contains media, equipment and material for bacterial culture, if appropriate laboratory facilities are already available in the field (incubator, heat source, alcohol or gas burner, etc.).

7. Urinalysis module

Contains test strips or chemical powder reagents allowing the detection of proteins, blood, glucose and nitrite in urine specimens

8. Faecal parasite module

Contains reagents for staining specimens for detection of ova and parasites.

9. Blood parasite module

Contains material for staining thick and thin blood smears (Giemsa, Field, RAL 555).

10. Haematology module

Contains reagents for the determination of haemoglobin or packed red cell volume (PCV), white blood cell count, platelet count and differential white cell count.

11 Centrifuge module

Provides the means to separate blood serum or plasma or to concentrate deposits of cells from urine or cerebrospinal fluid (CSF).

12 Portable incubator module

Can be used for on-site bacteriological culture and, in conjunction with the water testing module, to assess water sanitation.

13 Refrigerator module

Necessary for the safe storage of reagents, transport media and blood.

14 Specimen transport module (except stools)

Contains equipment and material required to transport specimens.

15. Water purification module

Contains equipment and material required to provide clean water for the preparation of stains and reagents.

16 Electrolyte analyser module

Contains the instruments and calibration solutions for analysing electrolytes

17 Cleaning, disinfection, sterilization and specimen disposal module

Contains equipment and supplies to sterilize and safely dispose of specimens It also contains the material necessary to clean containers, slides, cover-glasses, etc for reuse.

18 Bacteriology-Gram stain module

Contains Gram stains for microscopical identification of bacteria

19. Stool specimen transport module

Contains devices and material for the transportation of faecal specimens

20 Serodiagnostic test module

Contains rapid diagnostic kits or reagents, except for fluorescence reagents The kits will have to be chosen according to the available knowledge of the endemic diseases in the area concerned and of anticipated epidemic diseases.

21 Fluorescence microscopy module

Contains either a fluorescence objective lens or a portable fluorescence microscope with associated supplies. This will permit using fluorescent antibody and fluorescent acid-fast rapid diagnostic tests in the field

22 Blood transfusion module

Contains devices, reagents and material for blood testing and transfusion

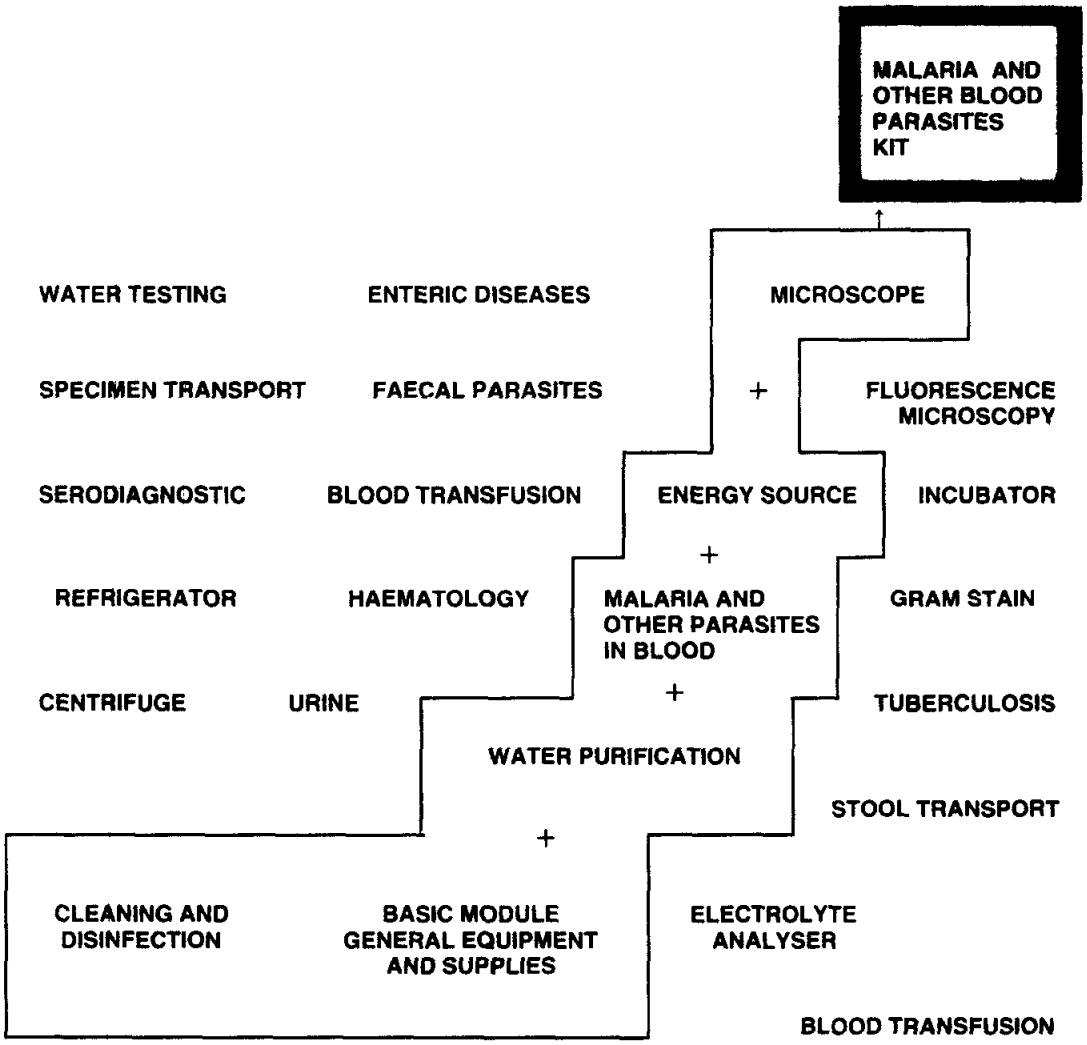

Figure 7.1 Assembling modules to make a kit

(Example kit for malaria detection)

7.3 Contents of modules

Module 1: Basic

Consumables, (renewable supplies)	Quantity	
Small, re-sealable plastic bags for specimens	1000	
or		
Whirl-Pac bags, polyethylene		
Containers, polypropylene (not polystyrene), screw-cap, leakproof, 60 mL capacity	20	
Xylene	250	mL
Microscope slides, frosted end, 76mm x 26 mm, 1 mm thick	1000	
Cotton wool swabs		
or		
Gauze squares, 5 cm x 5 cm	1	pack of 200
Ethyl alcohol, denatured technical grade	500	mL
Pasteur bulb pipettes, polyethylene, 3 mL (transfer pipettes)	100	
Toilet paper	4	rolls
Ball point pen, black	6	
Ball point pen, red	2	
Pencil with eraser	2	
Pencil sharpener	2	
Felt-tip pen, large, waterproof, black or blue	2	
Pot cleaner, non-abrasive	2	
Exercise books, A4, hard cover, squared paper	3	
Laboratory cloths	2	
Standard items		
Slide box, wood (for 100 slides)	1	
Beaker, polypropylene, 250 mL	2	
Beaker, polypropylene, 500 mL	1	
Beaker, glass, low form, spouted, 500 mL	2	
Rubber gloves, heavy duty	1	
Balance	1	
Plastic bags, polyethylene		
Containers, polypropylene, screw-cap leakproof, 60 mL capacity	20	each
Filter paper, Whatman No.1 circles, 12.5 cm or sheets	300	
Funnels, polypropylene, 90 mm diameter	2	
Funnels, polypropylene, 65 mm diameter	5	
Label, self-adhesive, removable in water, 65 mm x 45 mm	150	
Drying rack, plastic (for slides)	1	
Rods, stainless steel, minimum length 290 mm (with levelling screws)	2	
Metal spirit burner with screw-cap, with spare wick	1	
Diamond marker, preferably with aluminum handle	1	
Timer, mechanical, 1 minute steps, 60 minutes	1	

Standard items (continued)	Quantity
Basin, plastic, diameter 285 - 290 mm (for staining)	1
Basin, plastic, diameter 300 - 310 mm (for washing)	1
Slide mailer, polyethylene, with integral push-in lid	10
Pipettes, graduated, polypropylene, 1 mL	5
Pipettes, graduated, polypropylene, 10 mL	5
Containers, polypropylene (for blood collection), flat bottom, leakproof, screw-cap, 10 mL	50
Dropper bottle, translucent, polyethylene with lid,15 mL	3
Pipette filler (safety)[1]	1
Test tube rack, 24 place, white nylon coated-wire, for tubes 100 - 125 mm x 15 - 20 mm diameter	1
Measuring cylinder, graduated, polypropylene, 100 mL	1
Measuring cylinder, graduated, polypropylene, 500 mL	1
Microbiological safety mask	2 boxes
Reagent bottles, high density polyethylene, leakproof cap, 250 mL	20
Forceps, stainless steel, flattened bent and blunt-ended, 105 mm long	1 pair
Hazard safety labels with official symbols on yellow background, measuring 38 mm x 380 mm	1 pack
Glass stirring rods	5
Spatulas, polypropylene, length 100 mm	2
Tripod, metal	1
Gauze, stainless steel, ceramic (for tripod)	1
Paint brush 12 mm, soft bristles	1
or	
Camera brush (for cleaning microscope)	
Ruler 30 cm	1
Test-tube brushes, small 12 mm diameter	1
medium 18 mm diameter	1
large 50 mm diameter	1
Scrubbing brush	1
Scissors, sharp tip	1 pair
Thermometer, 0-100°C (for water and refrigerator) with holder to protect it from breaking.	2
Wash bottle, polyethylene, integrated tube spout, 250 mL	2
Eyewash bottle (safety), 500 mL	1
Eye-shield (goggles, clear shatter-resistant polycarbonate and fitted with side shields)	1
Glutaraldehyde or formalin to decontaminate centrifuge	1 L
Protective clothing e.g. plastic apron	3
Rubber gloves (washable) size 7 or 8	1 box

[1] A bulb pipette filler can be used, but a Pi-pump is much easier to control

Module 2: Energy

	Quantity	
Solar module, 57 W maximum peak power	1	
Charge regulator, 12 V	1	
Battery, 100 Ah, 12 VT	1	
Solar battery charger NiCd, size AAA-D	1	
Battery charger NiCd, size AAA-D (11 - 36 V d.c. input)	1	
Batteries, size AA [2]	8	
Batteries, size C[2]	8	
Cable 20 m, 1.5 mm[2]	2	
Light 11 W low consumption bulb	1	
Spare bulb 11 W	1	
Distilled water	5	L
Crocodile clips	2	pairs
Spare charge regulator	1	

[1] Sufficient for a microscope centrifuge haemoglobinometer and light

[2] Alkaline or lithium are recommended Although more expensive they last longer

Module 3: Water testing (for 200 tests)

	Quantity
Del Agua kit, consisting of	
Del Agua kit, complete (state 110 V or 220 V)	**1 kit**
plus	
- Spare fuse (110 v or 220 v)	**2**
- Forceps	**1**
- Bottles, very high density polypropylene, 60 mL	**10**
Consumables for 200 tests:	
- Membrane lauryl sulfate broth[1] 38.1 g	**2 packs**
- Filter membranes and pads, 200	**1 pack**
or	
Millipore kit, complete (switchable for 110 V 220 V, 6 V, 12 V, 24 V)	**1 kit**
or	
Inexpensive alternative (requiring separate incubator[2] and external battery, vehicle battery or mains) and the following:	
- Sample cup, graduated, polypropylene 100 mL	**1**
- Hand vacuum pump assembly, plastic with 2 way valve and tube)	**1**
- Microbiological analysis monitors, filters 37 mm, 0.45 mm pore size	**1 pack of 200**
- BROM MF, ENRO medium, ampoules, 0.8 mL	**200 ampoules**

[1] The broth is best kept in a well sealed container in the refrigerator

[2] An inexpensive portable incubator, e g GQF will be required as a separate module

Module 4: Microscope

	Quantity	
Complete binocular microscope with an all-metal stand with stable base, coaxial fine and coarse focus control on both sides, built-in transformer, built-in illumination diaphragm, conversion filter; and including the following:	1	
Achromat objectives x10, numerical aperture 0.25	1	
Achromat objectives x40, numerical aperture 0.65, spring-loaded	1	
Achromat objectives x100 (oil immersion, numerical aperture 1.25, spring-loaded	1	
Eyepieces x10 (wide field)	1	**matched pair**
Condenser (bright field), pre-adjusted	1	
Iris diaphragm	1	
Filter holder (with centering screws)	1	
Optional:		
Dark-field condenser (or condenser stops for x10 and x40 objectives)	1	
Eyepiece micrometer with calibrated graticule or micrometer/small glass measuring disc (to measure cysts and ova)	1	
Binocular head	1	
Mirror, one side flat and the other concave	1	
Lamp, built-in (Halogen, 6 V, 10 W)	1	
Mechanical stage with specimen holder	1	
Jeweller's screwdrivers, set	1	**set**
Dust cover	1	
Lamp and fuse, spare	1	
Lens paper, box	1	
Manual on the function and maintenance of the microscope	1	
Optional:		
Stage micrometer (engraved with 100 of 0.01 mm divisions)	1	
Oil immersion	50	**mL**
Silica gel (humid areas)[1]	100	**g**

[1] Under conditions of high humidity it may be necessary to pack (using a cloth bag or sock) silica gel with the microscope. This will help to reduce the growth of fungi on the lenses.

Note A binocular microscope with a light system which can be connected to a rechargeable battery may be useful under field conditions. For such a microscope a battery adapter suitable for a 6 V or 12 V battery must be provided.

Module 5: Tuberculosis (for 200 tests)

	Quantity
Carbol fuchsin liquid stain[1]	**1 L**
or	
Basic fuchsin powder	**25 g**
Methylene blue or malachite green	**25 g**
Hydrochloric acid (concentrated) or sulfuric acid (25%)	**500 mL**
Loop wire holder	**2**
Nickel chromium loop, 24 gauge	**5**
Ethanol (if fuchsin powder used)	**500 mL**
Phenol (if fuchsin powder used)	**500 mL**
or	
Phenol crystals	**250 g**
Optional:	
Tween 80 or Teepol	**500 mL**

[1] In emergencies it might be better to use ready-made stain available in bulk (5 L)

Module 6a: Cholera bacteriology[1] (for 50 tests)

	Quantity
Rectal swab	100
Cary-Blair medium (See Module 19 Stool specimen transport)	50 g
TCBS medium, 500 g	2
Nutrient agar	100 g
Bacto Peptone, 500 g	1
Disposable plastic Petri dishes, diameter 9 cm	100
Test tube 13 mm x 100 mm	300
Test tube cap, 13 mm test tube, plastic	300
Disposable Bijou bottles with screw-caps	100
Polyvalent or O-group V. cholerae-antiserum, 2 mL	5
Agglutination slide, 10 wells	6
Grease (wax) pencil	1

[1] Incubators may be available in nearby laboratories or the local ambient temperature may be high enough for incubation

Module 6b: Cholera and enteric bacteriology (for 50 tests)

	Quantity
DCLS agar or SS agar or Desoxycholate agar	500 g
Kligler iron agar	300 g
Lysine iron agar	200 g
Motility indole ornithine medium	200 g
Acetate agar	100 g
Test tube rack, 40 tube, foam	20
Bacteriologic needle and handle, nichrome	2
Bacteriologic loop and handle, 3 mm, nichrome	2
Alcohol or gas burner	1
Fuel (alcohol)	500 mL
or	
Gas (may be local Butane)	1 tank
Antiserum, *Salmonella* polyvalent	5 mL
Antiserum, *Salmonella* Vi	3 mL
Antiserum, *Shigella* A	2 mL
Antiserum, *Shigella* B	2 mL
Antiserum, *Shigella* C	2 mL
Antiserum, *Shigella* D	2 mL
Test tube, 16 mm x 125 mm	100
Cap, test tube, plastic, 16 mm	100
Test tube, 13 mm x 100 mm	350
Cap, test tube, plastic, 13 mm	350

Module 7: Urinalysis (for 100 tests)

		Quantity
Biochemistry		
Protein test strip for protein	} or all on one strip[1]	100 strips
Glucose test strip	} or all on one strip[1]	100 strips
Strip test strip (bacteria)	} or all on one strip[1]	100 strips
Haemoglobin test	} or all on one strip[1]	100 strips
Microscopy		
Hand centrifuge 4 x 15 mL		1
Tubes, conical, polypropylene, 15 mL		10
Coverslips 20 mm x 20 mm		500
Pregnancy test kit		1 kit
If required		
Schistosoma haematobium urine microscopy:		
Swinnex filter holder 13 mm diameter (Cat No. SX00 02500, Millipore Ltd.)		2
Polycarbonate membrane (15 m or 20 m pore size), Cat No. 110616 Sterilin Ltd		100
Polycarbonate filter, 12 m pore size		100

[1] Alternatively reagent powders can be assembled as described in Annex 5

Module 8: Faecal parasites (for a large number of tests)

	Quantity
Microscope slides	10 boxes of 72
Microscope coverslips, 20 mm x 20 mm	5 boxes of 100
Potassium iodide, analar	100 g
Iodine, analar	100 g
Sodium chloride	500 g
Eosin Y	25 g
Applicator sticks	1 box of 1000
Dropper bottle, polyethylene, opaque, 15 mL	2
Dropper bottle, polyethylene, translucent, 15 mL	1
Reagent bottle, high density polyethylene, opaque fitted with leakproof cap, 250 mL	2
KATO[1] test kit (for *Schistosoma* ova)	1 kit
Cellophane coverslips (22 cm x 40 cm)	15 sheets
or	
Water wettable cellophane, 22 mm width	1 roll
Glycerol	500 mL
Malachite green	10 g
Metal sieve, 105 mesh	1
or	
105 nylon mesh sieve or nylon mesh (102 cm x 25 mm roll)	1 roll
Stage micrometer (specially engraved slide) total scale measures 1 mm, each of the 100 engraved dimensions is 10 m	1

[1] Recommended for surveys

Module 9: Blood parasites (for 1000 tests)

	Quantity
Giemsa's stain[2]	500 mL
Field's A stain, powder	25 g
Field's B stain, powder	25 g
or	
RAL 555 I stain, pre-weighed, 250 mL	1 bottle
II stain, pre-weighed, 250 mL	1 bottle
Methanol AR or other pure grade, 250 mL	1000 mL
Blood lancet	1000
Buffer tablets, pH = 7.2	1 bottle
or	
Buffer RAL pre-weighed for 1 litre	1 bottle
or	
Buffer ampoule 500 mL for 1 litre (for Giemsa use only)	1 ampoule
Strips indicator pH paper 1-10	50 strips
Optional:	
Sodium azide (0.1 % for Field's stain preservation)	25 g
For Microfilariae:	
Swinnex filter holder 25 mm diameter (Cat No. SX00 02500, Millipore Ltd.)	2
Polycarbonate membrane (3 m pore size), 25 mm diameter Cat. No. 110612 Sterilin Ltd.	1000
Plastic container to hold 2.5 mL of EDTA anticoagulated blood	20
Polycarbonate membrane (15 m or 20 m pore size), Cat. No. 110616 Sterlin Ltd.	1000

[1] For the powder form it is recommended that suppliers provide pre-weighed reagents

[2] Alternative Wright-Giemsa rapid stain

Module 10: Haematology
(for 100 tests)

	Quantity	
White blood cells		
Counting chamber (improved Neubauer)	2	
Coverslip, optically plain, 20 mm x 26 x 0.4 mm	5	
Pipette, 50 L	2	
or		
Pipette, 20 L (using safety bulb)	2	
Hand tally counter (1 parameter)	1	
Turk solution	100	mL
Blood lancet	110	
Safety pipetter	4	
Differential count		
Same items as Module 9: Blood parasites		
Tubes to make WBC dilution (10 mL)	110	
Platelets		
Same items as Module 9: Blood parasites	1	
or		
Ammonium oxalate	10	g
Haemoglobin		
Haemoglobinometer (see equipment)	1	
Haematocrit		
Haematocrit centrifuge (see equipment)	1	
Capillary tubes (heparinized) for the centrifuge	120	
Sealing compound for capillary tubes	1	pack

Module 11: Centrifuge

	Quantity	
Portable centrifuge with fixed rotor (battery driven)	1	
Portable haematocrit centrifuge with fixed rotor (battery driven)	1	
Haematocrit capillaries (size as advisable by the manufacturer)	1000	
Centrifuge tube, plastic, screw-cap	50	
Sealing compound	1	**pack**

Module 12: Portable incubator

	Quantity
GQF portable laboratory incubator, 12 V d.c.	1
Transformer, 110 V a.c. or 220 V a.c. to 12 V d.c.	1
or	
WHO incubator	1
or	
Millipore portable incubator (not suitable for use with Module 6: Enteric bacteriology)	1
or	
Portable waterbath incubator (tubes only)	1

Module 13: Portable refrigerator

	Quantity
Portable refrigerator, minimum volume 40 L (photovoltaic or gasoline)	1
Cool box, minimum volume 5 L	2
Ice bricks or similar with cool box	2

Module 14: Collection and transport of sera/blood specimens (for 100 specimens)

	Quantity
Whatman filter paper No.4[1]	100
Silica gel	250 g
Plastic bag, re-sealable	100
Blood lancet	100
Dry tube, screw-cap (sera)	100
Anticoagulant tube (blood) EDTA or sodium citrate or Li-heparin	100
Needles 21 G/19 G	100
Syringe disposable, 5 mL	100
Labels, self adhesive 35 mm x 20 mm	100

[1] Supplied in paper sheets or paper discs. One basic unit will be at least 3 cm diameter. Instructions for use see Annex 4

Module 15: Water purification

	Quantity
Gravity water filter, used with sterasyl (self-sterilizing) ceramic elements (candle filter)	1
Spare sterasyl candle, 18 cm	2
Stiff bristled brush (to clean filters)	1
Aluminium sulfate (or alum cake)	1 g
pH paper, pH 1 - 11	2 sets
or	
Deionizer using ion exchange cartridges (bench unit or hand unit)	4
or	
Simple still	1

Module 16: Electrolyte analyser[1]

	Quantity
Portable, photovoltaic ion selective electrolyte analyser	1
Spare ion selective electrodes	1
Reference buffer, 200 mL	1 bottle
Capillary tubes, anticoagulant	2 boxes of 100

[1] Optional

Module 17 : Cleaning, disinfection, sterilization and specimen disposal

	Quantity
Field autoclave (pressure cooker), top loading; volume of 20 litres	1
Primus stove	1
Bucket (10 - 12 litres), may be obtained locally[1]	2
Incinerator (may be made locally)	1
Detergent containing enzyme	2 L
Detergent, ordinary	
Brush, scrub	2
Bag for hazardous waste	200
Bowl, plastic, may be obtained locally	4
Iodophor (Povidione)	1 L
Calcium hypochlorite	1 kg
Isopropanol	1.5 L

[1] Already included in the basic module

Module 18: Bacteriology - Gram stain
(For 200 tests)

	Quantity
Crystal violet, powder	**75 g**
Ammonium oxalate	**50 g**
Carbol fuchsin (1/10 diluted),	**500 mL**
Safranin or neutral red	**20 g**
Iodine, analar	**20 g**
Potassium iodide, analar	**20 g**
Acetone	**1000 mL**
Methanol	**2000 mL**
Mortar and pestle	**1 set**
Grease (wax) pencil [1] or	**2**
lead pencils	**5**
Frosted slides	**220**
Dispensing containers for staining 100 mL, polyethelene, leakproof	**4**
Gram stain pack (for 50 tests)	**1 pack**

[1] It is better to use frosted slides and lead pencils, as grease pencil is soluble in acetone and adheres poorly in hot climates under field conditions

Module 19: Stool specimen transport[1] (For 20 tests)

	Quantity	
Transport medium (Cary-Blair), 3 mL		
in 5 mL screw-capped tubes	**20**	**tubes**
or		
Filter paper disc, non-impregnated, 6 mm diameter	**20**	
Tube, 5 mL, polypropylene, screw-cap, sterile	**20**	
Saline	**50**	
Forceps	**1**	
or		
Dacron swab	**20**	
Tube, screw-cap	**20**	
Saline solution	**50**	**mL**
Labels, self-adhesive 35 mm x 20 mm	**100**	

[1] Maximum transportation time for reliable results is 8 days at ambient temperature

Module 20: Sero-diagnostic test module

	Quantity
Diagnostic kits (LA, COAG, ELISA), as required for prevalent diseases	**2 kits**
Microscope slides	**2 boxes of 72**
Bacteriologic loop and handle	**1**
or	
Applicator sticks	**1 box of 1000**
Dropper pipette	**10**
Wax pencil	**1**
Beaker, 250 mL	**2**
Disinfectant, (calcium hypochlorite)	**25 g**
Detergent	**25 g**

Module 21: Fluorescence microscopy

	Quantity
Fluorescence objective, x50 oil or x60 oil	1
or	
Portable fluorescence microscope with x5 ocular	1
Power supply for either of the above	1
Battery cables and clips	1 set
Immersion oil, non-fluorescing	28.5 g
Buffered glycerine, pH 8.6	50 mL
Microscope slide	2 boxes of 72
Coverslip, 22 mm x 22 mm	28.5 g
Coverslip, 22 mm x 40 mm	28.5 g
Auramine O	25 mL
Fluorescent conjugate, anti-rabbit serum	25 mL
Rabbit antisera to selected infectious agents, each agent	5 mL
Phosphate buffered saline, pH 7.2, powder	25 g
Bottle, plastic, 100 mL	2
Beaker, 100 mL	3

Module 22a: Blood transfusion
(For simple testing, storage, and administration of 50 units of whole blood, or red cells)

	Quantity
Equipment	
Tiles, porcelain	4
Test tube rack, 30-hole, plasticized wire	4
Refrigerator module	1
Access to:	
Centrifuge	
Incubator (or waterbath)	
Microscope	
Consumables	
Adhesive tape, zinc oxide, 75 mm x 5 m	2 roll
Aprons, disposable plastic	100
Beakers, polystyrene, 250 mL	4
Disinfectant, hypochlorite granules, 70 %	5 kg
Gloves, disposable	50 pair
Marker pens, permanent	5
Microscope slides, plain, 25 mm x 75 mm	500
Pipettes, plastic, reusable, 3 mL	100
Pipettes, plastic, reusable, 5 mL	100
Sample tubes, 10 mL, plain	100
Test tubes, 75 mm x 12 mm	1000
Transfusion sets, with filter	60
Wash bottles, polystyrene, 500 mL	4
Reagents	
Anti-A, monoclonal, 10 mL	4
Anti-B, monoclonal, 10 mL	4
Anti-D, monoclonal, 10 mL	4
Anti-human globulin reagent, monoclonal, 10 mL	4
Sodium chloride, reagent grade	1000 g
Saline, low ionic strength, ready-made	500 mL
Access to:	
Deionized water	
Stationery	
Labels for blood bags	200
Laboratory register	1
Request forms	100

Module 22b: Blood transfusion
(For collection, testing and transfusion of 50 units of whole blood)

	Quantity	
Equipment		
Forceps, artery	1	
Scales, spring balance	1	
Scissors	1	
Tiles, porcelain	4	
Test tube racks, 30-hole, plasticized wire	4	
Tourniquet	1	
Refrigerator module	1	
Access to:		
Centrifuge		
Incubator (or waterbath)		
Microscope		
Consumables		
Adhesive tape, zinc oxide, 75 mm x 5 m	4	rolls
Anticiseptic, Hibitane, 5 % per litre	1	L
Aprons, disposable plastic	1	
Beakers, polystyrene, 250 mL	4	
Blood bags, single, CPDA-1, 450 mL	50	
Capillary tubes, 75 mm heparinized	100	
Cotton wool, absorbent, non-sterile	500	g
Disinfectant, hypochlorite granules 70 %	5	kg
Gloves, disposable	50	pairs
Lancets, disposable	100	
Marker pens, permanent	5	
Microscope slides, ground-edge, plain 25 mm x 75 m	500	
Needles, disposable, 22 gauge x 40 mm	100	
Pipettes, plastic, reusable, 3 mL	5	packs of 20
Pipettes, plastic, reusable, 50 l	5	packs of 20
Sample tubes, plain, 10 mL	200	
Sharps containers, disposable (10 L volume)	10	
Syringes, plastic, disposable, 10 mL	100	
Test tubes, 75 mm x 12 mm	2000	
Transfusion sets, with filter	60	
Wash bottles, polystyrene, 500 mL	4	

Module 22b: Blood transfusion (continued)
(For collection, testing and transfusion of 50 units of whole blood)

	Quantity
Reagents	
Copper sulfate solution, 1.053 s.g.	1 L
Copper sulfate solution, 1.055 s.g.	1 L
HIV test kits, rapid, simple	100
HBsAg test kits, simple	100
VDRL test kits, simple	100
Anti-A, monoclonal, 10 mL	2
Anti-B, monoclonal, 10 mL	2
Anti-D, monoclonal, 10 mL	2
Anti-human globulin reagent, monoclonal, 10 mL	2
Sodium chloride, reagent grade	500 g
Saline, low ionic strength, ready-made	500 mL
Access to :	
Deionized water	
Stationery	
Donor cards	100
Labels for blood bags	200
Laboratory register	1
Request forms	100