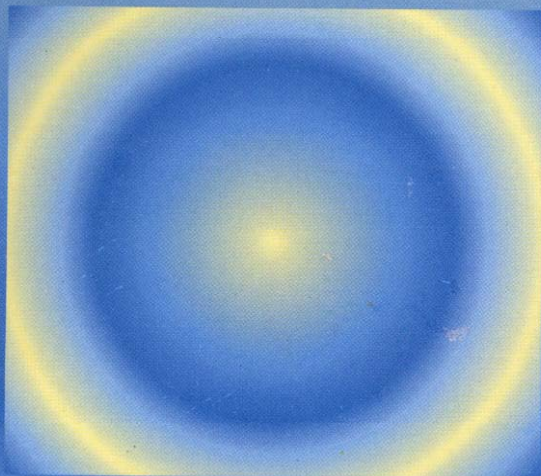
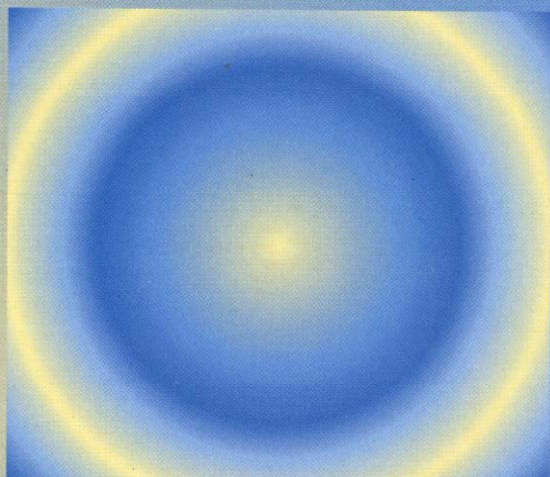


Specimen collection and transport for microbiological investigation



**WORLD HEALTH
ORGANIZATION**
Regional Office
for the
Eastern Mediterranean

Specimen collection and transport for microbiological investigation

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FOREWORD

Laboratory results have become increasingly important for the diagnosis, treatment, prevention and surveillance of infectious diseases at all levels of the health care system. The World Health Organization has long been actively involved in improving the quality and the clinical usefulness of microbiological laboratory investigations, particularly in those parts of the world where resources are limited and qualified staff still scarce. To improve laboratory services, WHO has organized training courses, established international centres for external quality assessment, and published several manuals for use by workers in health laboratories. The adoption of standardized techniques and uniform testing methods has been actively promoted.

The pre-analytical phase, including specimen ordering, collection and transport, has been a rather neglected area in laboratory medicine. Many laboratory directors still do not believe that their responsibility also extends to that part of the laboratory process which is performed outside the laboratory. This involved specimen collection, the use of proper containers, the collection itself, correct labelling, and the transport to the laboratory within a specified time. Also essential is the submission of a requisition form with patient data and elementary clinical information. It is our conviction that proper attention to these details may have a greater influence on the test results than some control steps in the internal laboratory process. Quality assurance, therefore, extends beyond the analytical procedure and involved good communication with the clinical staff, in order to guarantee optimal collection and transport of specimens.

It has repeatedly been emphasized that no result of a laboratory test can be better than the specimen submitted by the clinician. The head of the laboratory should therefore make sure that clinicians and nursing staff are informed on the best methods for handling specimens. Not only should written instructions be prepared, but appropriate containers should also be provided. This is particularly important for

the diagnosis of infections, which generally requires sterile containers, blood culture bottles, and transport media. The laboratory should also monitor the quality of incoming specimens and there should be a policy for rejecting inappropriate specimens.

Throughout the collection and transport process, safety measures should be taken to protect clinical and other staff against the accidental transmission of HBV, HIV and other infectious agents. Most of the earlier WHO publications in the field of microbiology have concentrated on the analytical procedure itself, with less emphasis on proper collection and prompt transportation. The present document brings together in one manual a series of guidelines for the optimal collection and transport of specimens destined for different types and levels of microbiological laboratory. They should be consulted not only by the laboratory staff, but also by the users of laboratory services. It is our sincere hope that this manual will soon find its way to these intended users and that it will contribute to better cooperation between laboratory and clinical staff.

Together with the already existing WHO laboratory manuals, this new document has a specific role to play in attaining WHO's goal of health for all by the year 2000.

Prof Dr J. Vandepitte

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Clinical Microbiology, Belgium

PREFACE

Owing to the heterogeneity of health facilities in different countries within the Eastern Mediterranean Region and sometimes even within the same country, this manual deals with a wide variety of specimens and their methods of collection; some of these might be more applicable to certain laboratories than to others.

The aim of these guidelines is to encourage the successful identification of bacterial and viral infections in humans, either by isolation or by immunoassays. The diagnosis of these infections requires knowledge of the pathogenicity of the suspected agents regarding the state of infection, as well as the history and immunocompetence of the patient involved. The identification of the agents involved depends on the proper choice of specimens, careful collection, time of collection and transport. The viability of agents decreases over time; the rate of decay is also a function of temperature. Thus the stability of the agent is enhanced in most cases by cooling; exposing to harmful freezing-thawing cycles should also be avoided.

Improper collection does not only lead to waste of financial resources but may also lead to misleading diagnosis and to potential danger to the patient.

The importance of other microbiological specimen collection related to the environment, such as drinking water, for example, should also be considered. As other WHO publications* have already addressed this subject, no attempt has been made to include these topics in this publication, with the exception of specimen collection of poliovirus and *Vibrio cholerae* from sewage and water sources.

The authors are grateful for the valuable continuous support received from Dr. Hussein A. Gezairy, Regional Director for the Eastern

**Guidelines for drinking-water quality*, second edition, 1993, WHO, Geneva.

Mediterranean Region, and Dr. M. H. Khayat, Director, Programme Management, for his continuous encouragement and support. Our thanks are also due to Prof Dr. J. Vandepitte, Louvain, Belgium, for reviewing the draft and for constructive suggestions.

INTRODUCTION

The proper selection, collection and transport of specimens to the laboratory is an essential part of the quality assurance of the microbiology laboratory. Improper collection not only leads to waste of financial resources, but also may cause an incorrect diagnosis and lead to potential danger to the patient. Much of the success of the laboratory investigation depends on things that happen to the specimen before it arrives in the laboratory. Was the specimen properly selected? Was it collected properly, so as to avoid or minimize contamination with the normal flora of the skin and mucous membranes? Was it protected from high temperature or drying out during transportation? Was it rapidly transported to the laboratory for processing?

EDUCATION AND WRITTEN GUIDELINES

Though these events occur before the specimen arrives in the laboratory, it does not imply that they are beyond the control of laboratory personnel. Laboratory professionals must promote, advise and collaborate in taking measures to ensure proper selection, collection and transport of specimens. They must disseminate written guidelines regarding these procedures, which should be revised regularly. Such guidelines should also contain information regarding the location of the microbiology laboratory, the name of doctors and chief technicians, the telephone numbers of the laboratory, duty hours of doctors and/or technicians, how specimens are brought to the laboratory, both during and outside working hours, and where the specimens should be placed. The laboratory personnel should ensure the availability of appropriate supplies, containers and request forms for the collection and transport of specimens.

THE REQUEST FORM AND LABELLING OF SPECIMENS

It is the duty of the clinician to ensure that the specimen container and request form are correctly identified with the patient's name and

record number before the specimen is taken. Incorrect patient identification of either specimen or request form can have disastrous consequences for the patient who may be deprived of proper treatment or receive inappropriate treatment.

The request form should contain information such as the patient's name, identification number or insurance number (if any), date of birth or age, specimen type and source, date and time of collection, main clinical signs and symptoms and most likely diagnosis, the physician to whom the results are to be sent, and any antimicrobial agents the patient is receiving. As a minimum, the specimen container must be labelled with the patient's name and identification number, source of specimen, and date and time of collection. The time of collection is especially important because it will be used to determine the suitability of the specimen for processing.

TRANSPORTATION OF SPECIMENS

Except body fluids and urine, most other specimens for culture should be sent in bacterial or viral transport media if the transportation is to be delayed for more than 1 hour. Body fluids may be transported in sterile tubes if the material is purulent and the volume is more than 1 mL. Swabs should be placed deep into the agar of the transport medium, the swab stick broken off where it was held, and the cap replaced quickly. Aspirated material should not be transported in the syringe. Instead, 1-2 mL of the material should be ejected into a tube with the transport medium and placed deep into the agar butt with a swab. Small tissue samples collected at the time of surgery may be treated in the same way. Larger tissue specimens may be transported in a sterile Petri dish or urine cup if the processing occurs within 1-2 hours. If specimens are not immediately transported to the laboratory, they should be placed in the refrigerator (2-8 °C). High temperatures will cause differential bacterial overgrowth or loss of some strains, and very low temperatures will increase the diffusion of oxygen which may be detrimental to anaerobic bacteria. In contrast to bacterial transport media, the viral transport media should contain antibiotics to prevent overgrowth with bacteria. Freezing the specimen for viral culture should be avoided unless frozen to -70 °C. Rapid transport of viral specimens is essential.

If specimens are sent to a laboratory outside the institution, special requirements must be fulfilled. See Appendix 7.

SPECIMEN EVALUATION

Every specimen must be evaluated for its suitability for processing. The criteria for rejection of specimens can be found in Appendix 6.

ISOLATION OF RICKETTSIA

Isolation of *Rickettsia* can be extremely hazardous for some of these organisms and should only be attempted by experienced personnel in well equipped laboratories. Therefore, serological assays are recommended for identification of these infections.

ISOLATION OF VIRUS

Only specimens routinely examined for virus are included in this manual. See Appendix 3. Although most of the other specimens mentioned in this book can be used for virus isolation, this is only done occasionally. In these cases, analysis is carried out by serological investigation.

BLOOD

- Collection time:** Before the start of antibiotic therapy. If time permits, it is generally recommended that the first two sets of blood cultures be taken one hour apart and the third set after another 3-6 hours. If this is not possible due to the seriousness of the patient's illness, two sets of blood cultures should be drawn from two different sites before the antibiotic is administered.
- Equipment:** Request form, labels, blood culture bottles for aerobic and anaerobic cultivation, antiseptic solution (Appendix 5), cotton wool sponges, needles, syringes and tourniquet.
- Procedure:**
1. Fill in request form, identify the patient and explain what will happen.
 2. Collect and assemble the equipment. Remove the protective cap from the culture bottles, if present.
 3. Apply tourniquet and select an appropriate vein for the collection, usually the antecubital vein.
 4. Disinfect the skin: apply the antiseptic solution to a cotton wool sponge and rub a 5 cm square area around the selected site for one minute. Leave the antiseptic to dry and discard the sponge. Make a new sponge and cleanse the site again, beginning at the centre and scrubbing in a circular motion outwards. Let it dry.
 5. If necessary, palpate the site before the venepuncture. Disinfect the finger with the same procedure as described for the skin of the patient. Do not touch the prepared site with fingers that are not disinfected.
 6. Disinfect the diaphragm of the blood culture bottles as described for the skin (not needed if there is a protective cap).
 7. Insert the needle of the syringe into the vein and draw the volume needed for the culture.
 8. Remove the tourniquet and withdraw the needle from the vein.
 9. Immediately apply pressure to the puncture site with a clean cotton sponge.
 10. Inoculate the culture bottles carefully, adding the correct amount of blood. Be careful that no air is injected.
 11. Put a label on the bottle, indicating the patient's identification, ward number and time of collection, so as not to cover the area occupied by the medium.

- Safety precautions:** Do not puncture the same site twice as this may cause infection. Blood from a patient is potentially infected (hepatitis, AIDS) and when injecting it into the culture bottles be careful not to prick your fingers. Needles should not be recapped, but discarded in a safety container.
- Storage:** According to the manufacturer's instructions. Do not store inoculated bottles in the refrigerator.
- Transportation:** Bring the blood culture bottles to the laboratory immediately, and place the culture bottles immediately in the incubator.
- Reporting:** In case of positive culture, the result should be conveyed immediately to the treating physician or doctor on duty. Negative results are only reported after 7 days of incubation. If slow growing organisms are suspected - *Brucella* spp., *Francisella tularensis* - it should be clearly indicated on the requisition form, and the culture bottles should be further incubated for another 1 to 2 weeks before being reported out as negative.
- Comments:** When bacteremia is detected, the apparent primary infection focus (bone, joint, lung, kidney, intravenous line, etc.) should be verified by culture of specimens relevant to the situation. This is important for the duration of the antimicrobial treatment. Five to 30% of positive blood culture represents contamination with skin bacteria. Both gram-positive and gram-negative bacteria are found on the skin of healthy persons, but normally the gram-negatives are found in low numbers. In hospital patients and in patients receiving antibiotics the gram-negatives increase considerably in numbers. Though many of these contaminants can be recognized on the basis of their identity, others are very difficult to recognize. Therefore, to keep the number of contaminants low, proper skin disinfection is extremely important before doing a blood culture.

BLOOD FILM

- Objectives:** Actiological diagnosis of malaria, trypanosomiasis, filariasis, and borreliosis by microscopic examination.
- Test material:** Capillary blood.
- Collection time:** No special requirements.
- Equipment:** Request form, labels, cotton wool sponges, alcohol, clean grease-free microscope slides, sterile lancet, pencil. Slides should be cleaned in detergent, and washed with 70% alcohol before use.
- Procedure:** **Thick and thin blood film on the same slide**
1. Fill in the request form.
 2. Turn the patient's left hand so the thumb turns upwards. Clean the ball of the third finger with a cotton wool sponge soaked with alcohol, using firm strokes to remove dirt and grease.
 3. Dry the finger with clean cotton wool, using firm strokes to stimulate blood circulation.
 4. With the lancet, prick the lateral side of the ball, not too close to the nail bed.
 5. Wipe away the first drop of blood with dry cotton wool, making sure that no strands of cotton remain on the finger to be later mixed with the blood.
 6. Working quickly, and handling clean slides only by the edges, collect the blood as described in the next steps.
 7. Apply gentle pressure to the finger and collect a single small drop of blood, about the size of a seed, on to the middle of the microscope slide for the thin film.
 8. Apply pressure again and collect two or three larger drops on to the slide about 1 cm from the drop intended for the thin film. The blood should flow freely and should not be "milked" as this will dilute the blood with tissue fluid.
 9. Spread the films.

Thin film

Let the slide with the blood drops rest on a flat, firm surface, place the spreader just in front of the drop at an angle of 45° , draw the spreader backwards until it touches the drop of blood and allow the blood to run along the backside of the edge. Firmly push the spreader along the slide, keeping the spreader at an angle of 45° . In this way, the blood is drawn after the spreader in a thin smear which, if the original drop is small enough, ends in a drawn-out tail well before reaching the end of the slide. Make sure the spreader is in even contact with the surface of the slide all the time the blood is being spread.

Thick film

Spread the blood evenly in a film about 10 mm in diameter with the corner of a slide, needle, toothpick, or applicator, or by slowly rotating the slide. The film should be spread quickly and have the correct thickness (one should be able to see the hands of a wristwatch but not the letters through the smear). **Thick film should not be fixed!**

10. Allow the film to dry in a flat, level position (on a sunny bench, under a lampshade or electric fan) protected from flies, dust and extreme heat. Label the dry film with a pencil by writing across the thicker portion of the thin film the patient's name or number and date.
11. Wrap the dry slide in a piece of paper and dispatch it to the laboratory as soon as possible. Don't use the request form for wrapping. The slide used for spreading the blood films can now be used for the next patient and another clean slide from the pack will be used as a spreader.

Common faults of thin blood film**Cause**

The end of the film is lost	drop of blood was too big
The film ends in a thick line	spreader has been lifted too early
The end of the film is ragged	edge of the spreader was uneven
Lines along the film	blood was clotting when spreading
Lines across the film	spreader was pushed forward jerkily
Hollows in the film	the slide was greasy

- Safety precautions:** The film should not touch the edges of the slide to prevent infection of the investigator.
- Storage:** At room temperature, not for more than 24 hours. **Never in the refrigerator!**
- Transportation:** No special requirements, except when serious falciparum malaria is suspected; then the specimen should be sent immediately to the laboratory.
- Reporting:** All positive falciparum malaria results are conveyed immediately to the treating physician. Other malaria species and negative results are sent through the routine reporting system.
- Comments:** Demonstration of the organism by direct microscopy confirms the diagnosis. If no organisms are found and the diagnosis is still doubtful, the examination should be repeated after 1 or 2 days.

SERUM LEVEL OF ANTIBIOTICS

- Objectives:** To determine the level of aminoglycosides and vancomycin in a patient with compromised renal function. Two levels are generally measured - valley or trough level and peak level.
- Test material:** Venous blood (4 mL) or capillary blood (4 capillaries).
- Collection time:** The valley or trough level is the level of the antibiotic obtained in a serum before the next dosage is administered.
- The peak level is obtained 15-30 minutes after an intravenous administration and 45-60 minutes after intramuscular administration of the antibiotic. In the case of renal failure, the peak level is delayed until 120-150 minutes after the intramuscular dose.
- Equipment:** Specimen tube with round bottom, or glass capillaries.
- Procedure:**
1. Identify the patient and check when the last dosage of the antibiotic was given. If a valley level is requested, the sample should be taken just before the next dosage. If a peak has been requested it should be taken 30 minutes after the last dosage when given by the intravenous route.
 2. Reassure the patient and position the patient properly.
 3. Verify paperwork and tube identification.
 4. Assemble syringe and needle.
 5. Select vein site (do not draw the blood from an intravenous line).
 6. Apply tourniquet.
 7. Disinfect the venepuncture site.
 8. Perform venepuncture and draw the blood.
 9. Release the tourniquet.
 10. Remove the needle and bandage the arm.
 11. Squirt the blood into the specimen tube.
 12. Dispose of the needle and syringe.
- Storage:** Refrigerated (2-8 °C).

Transportation: No special requirements.

Reporting: With enzyme immunoassays, the result is available after 2 hours, and with the biological method 6-9 hours after the specimen is received.

All increased valley levels should be reported immediately to the treating physician.

Comments: If the valley level is increased over its normal level, the dosage should be reduced either by increasing the interval but maintaining the dose or by reducing the dose and maintaining the interval.

	Valley level μg/mL*	Peak level μg/mL
Gentamicin	< 2	6-8
Tobramycin	< 2	6-8
Netilmicin	< 3	8-10
Vancomycin	< 15	< 30

* The usual daily dose divided into 8-hourly doses. If the daily dose is given undivided as a single dose, the valley level should be below 1 μg/mL 18 hours after the last injection.

SEROLOGY

- Objectives:** Aetiological diagnosis by determining the specific amount of antibody against bacterial or viral agents in patients in order to distinguish between a current or previous infection or by antigen detection of the respective agents.
- Test material:** Sufficient serum for most serological tests is obtained from 3–4 mL of venous blood. Some tests can be performed with less than 0,5 mL of blood which makes them suitable for newborns and small children.
- Collection time:** The first sample should be taken as early as possible after the onset of illness and the second at least 1 week later.
- Equipment:** Specimen tubes, syringes, needles, tourniquet, cotton wool pads, 70% alcohol, Tensoplast.
- Procedure:**
1. The patient should sit on a chair so that he can comfortably stretch his left or right arm in the horizontal position. This also applies to a patient lying flat on a bed. The elbow may be supported by an armrest.
 2. Gather the necessary tubes and label them with the patient's identification, ward and time of collection. Ask the patient to state his full name and other personal data and compare it with that on the request form and tube labels.
 3. Attach a sterile syringe firmly to a sterile needle.
 4. Select the vein site. The collection site is most often the median elbow (antecubital) vein, but other veins may also be considered, depending on their prominence.
 5. Wrap a tourniquet around the patient's arm about 10 cm above the elbow with the end stuck under the last round. Preferably the working arm should be selected (which most often is the right arm), since veins are more accessible on this site.
 6. Palpate the filled vein with your finger and cleanse the site before venepuncture with a sterile cotton wool sponge soaked with alcohol.

7. Grasp the patient's arm and use the thumb to draw the skin tight.
8. Hold the needle at a low angle (about 15-25 °C) to the skin and enter the vein with the bevel pointed upwards and directed towards the tourniquet.
9. Fill the syringe by pulling the plunger slowly to maintain a continuous blood flow, but avoid development of too low a pressure in the syringe chamber, which otherwise may cause collapse of the vein and haemolysis of the red blood cells.
10. After the syringe is filled, place a sterile dry cotton wool sponge over the puncture wound while the needle is still in the vein, and open the tourniquet to allow the blood to flow freely. Do not pull the needle out prior to opening the tourniquet.
11. Withdraw the needle from the vein with a rapid movement; immediately press the cotton wool sponge on the puncture wound and bend the elbow of the patient to prevent bleeding. Bandage the puncture wound.
12. Remove the needle from the syringe (but for safety reasons do not put on the protective cap) and slowly squirt the blood from the syringe into the specimen container. Fast expulsion of the blood may cause haemolysis.
13. Cover the tube with a stopper for further transportation.
14. Dispose of the needle and syringe in a safety box.

Preventing haematoma during venepuncture

1. Preferably use veins in the elbow area, and only major veins.
 2. Be careful that the bevel of the needle is fully inside the vein.
 3. Be careful not to pierce the vein.
 4. Loosen the tourniquet and make haemostasis with a dry cotton wool sponge before pulling out the needle.
-

Safety precautions: Be careful not to prick your finger when you remove a needle from the syringe. **Never replace a protective cap on a needle!**

Storage: Refrigerated (2-8 °C).

Transportation: No special requirements.

Reporting: The results should be available within 24 hours.

Comments: For certain infections the laboratory may request a second sample collected during the convalescence phase so as to examine the two samples together. Since antibody may persist long after recovery and may also be present in healthy individuals, the examination of a single sample of serum may not yield diagnostic information. In such circumstances a four-fold rise in IgG antibody during the period between the onset of the illness and convalescence is regarded as significant. Detection of IgA or IgM specific antibodies in a single serum sample of non-persistent viral infections may confirm the clinical diagnosis. A number of commercial immunoassays are available for the diagnosis of infectious diseases.

VIRUS ISOLATION FROM BLOOD

Objectives:	Suspected cases of haemorrhagic fever and other arbovirus infections by cell culture.
Test material:	Heparinized venous blood, 3-5 mL.
Equipment:	Request form, labels, sterile blood tubes, antiseptic solution, cotton wool sponges, needles, syringes and tourniquet.
Procedure:	<ol style="list-style-type: none">1. Fill in the request form, identify the patient and explain what will happen.2. Collect and assemble the equipment.3. Apply the tourniquet and select an appropriate vein for the collection, usually the antecubital vein.4. Disinfect the skin.5. Insert the needle on the syringe into the vein and draw the amount of blood.6. Remove the tourniquet and withdraw the needle from the vein.7. Immediately apply pressure to the puncture site with a clean cotton sponge.8. Apply a label, indicating patient identification, ward number.
Safety precautions:	Blood from a patient is potentially infectious. Needles should not be recapped, but discarded in a safety container.
Storage:	Should be transported immediately to a specialized laboratory for processing.
Transportation:	In a cooling box (2-8 °C).
Reporting:	The culture result cannot be obtained before 1 week of receipt of the specimen. The specimen used for cell culture may also be used for serological investigation by separating the serum from the clot. The results of serological investigation may be provided within 24 hours.
Comments:	The diagnosis of HIV infection is usually made on the basis of the detection of antibody or antigen of HIV, and sampling for culture is not discussed.

BODY FLUIDS

BILE

- Objectives:** Aetiological diagnosis of biliary tract infection with quantitative isolation, identification and susceptibility testing of isolated pathogen(s).
- Test material:** Duodenal bile or gall bladder bile collected at the time of surgery, approximately 5 mL.
- Collection time:** No special requirement; for the duodenal collection the patient should have fasted overnight.
- Equipment:** Sterile containers, double-lumen duodenal tube (Diamond, Dreiling), pancreozymin.
- Procedure:**
1. Following an overnight fast, a sedative dose of pentobarbital is administered parenterally.
 2. A double-lumen Diamond tube is inserted into the mouth and passed a distance of 45 cm, which brings the tip approximately to the cardia.
 3. Place the patient on his left lateral side on the examination table with the head elevated 40 cm.
 4. Ask the patient to swallow the tube for another 15 cm, which results in its being positioned along the greater curvature of the stomach.
 5. Ask the patient to sit on the edge of the table with his body bent forward at the waist as far as possible.
 6. Make the patient take several deep breaths. This will facilitate entrance of the tube into the antrum.
 7. Ask the patient to change his position to his right lateral side with his face elevated for about five minutes; the peristalsis will move the tube into the duodenum.
 8. Place the patient on his back for another five minutes while the tube is slowly advanced another 10-15 cm.
 9. Adjust the tube with fluoroscopic visualization so that the tip is located in the middle of the third portion of the duodenum.

10. Secretions are collected by suction either by a vacuum pump or other suitable apparatus which can deliver a pressure of at least 25 mm Hg.
11. If no secretion is obtained stimulate with pancreozymin.
12. Collect a sample of the aspirated material in the specimen container and send it to the laboratory.

Storage:	Refrigerated (2-8 °C).
Transportation:	Immediately, preferably in a cooling box to prevent overgrowth of the specimen with indigenous bacteria.
Reporting:	Culture result will be available in 2-3 days.
Comments:	As the duodenum normally will contain bacteria swallowed from the mouth, the culture should be made quantitatively, and only isolations of more than 10^3 bacteria per mL of known pathogenicity should be taken into consideration.

CEREBROSPINAL FLUID

- Objectives:** Diagnosis of bacterial or fungal meningitis by microscopic examination and culture with identification and susceptibility test of the isolated organism. Diagnosis of viral meningitis (polio, rabies, some arboviruses) by isolation and identification of the virus in cell culture.
- The aspiration of the cerebrospinal fluid (CSF) is contraindicated in the following situations: elevated cerebrospinal fluid pressure (except if there is a strong suspicion of meningitis or subarachnoid haemorrhage); suspicion of a space-occupying lesion (e.g. abscess) in the posterior fossa; bleeding diathesis or anticoagulant therapy (relative contraindication); and infected lumbar area (relative contraindication).
- Test material:** Cerebrospinal fluid (CSF), subarachnoid fluid, subdural fluid, cisternal fluid, ventricular fluid, ventricular shunt fluid.
- Collection time:** As soon as the suspicion arises, preferably before antibiotic treatment is started.
- Equipment:** Request form, labels, surgical gloves, sterile cotton wool sponges, antiseptic solution, 1% lidocaine with 1% epinephrine, syringe, needle, spinal puncture needle, manometer, sterile specimen tubes, plaster.
- Procedure:**
1. The collection should only be performed by a physician.
 2. Inform the patient about the procedure and get his or her acceptance. Fill in the request form.
 3. Do a fundoscopic examination and look for signs of elevated cerebrospinal fluid pressure (bilateral disc elevation, distended retinal veins with absent pulsation). If this is present, use a small-bore (25 gauge) lumbar puncture needle and be prepared to treat herniation (see below).
 4. Place the patient on his side with his back at the edge of the bed. Ask the patient to bend his knees, hip, back, and neck as much

as possible to decrease the normal lumbar lordosis and spread the spinous processes.

5. Wash your hands and put on surgical gloves. The puncture site is normally between the spinous processes of the 4th and 5th lumbar vertebrae and can be found where a line joining the iliac crest crosses the columna. The space between the 3rd and 4th vertebrae may be used in adults, but not in infants and children.
6. Disinfect the skin around the puncture site two times with the antiseptic solution and let it dry in between. Infiltrate the interspinous area with local anaesthetic to the level of the dura.
7. Insert the spinal puncture needle between the spinous processes in the midline with the obturator in place, parallel to the axis of the spine, but angle the needle 10° in a cephalic direction to minimize tearing of the dura.
8. Advance slowly until a definite resistance followed by a “give” is felt as the dura is passed. Remove the obturator stylet. If no “give” is felt when the needle has reached a depth of 4 cm (adult), remove the obturator stylet at each 2 mm advancement of the needle.
9. Ask the patient to straighten the legs and neck and connect the manometer to the needle. Use the needle as a zero point and measure the initial cerebrospinal fluid pressure in millimetres. The normal pressure is 60-200 mm and more than 300 mm is definitely increased. Disconnect the manometer.
10. Collect a total of 5-6 mL of CSF in three sterile specimen tubes and observe for colour, viscosity and translucency. Connect the manometer to the needle again and measure the pressure again. A final cerebrospinal fluid pressure above 200 mm is abnormal.
11. If the patient develops signs of herniation, stop the collection and start treatment (see below).
12. Remove the lumbar puncture needle gently and if iodine has been used as an antiseptic, wash it away with alcohol. Apply a plaster over the puncture site.
13. Send the collected sample at once to the laboratory for cell count, glucose and total proteins, Gram stain, culture for bacteria and antibiotic susceptibility test and/or viral isolation.
14. Instruct the patient to remain flat in bed for at least 4 hours.

Other investigations: If the CSF is cloudy: blood culture, haemoglobin, blood leukocytes, serum electrolytes, serum creatinin, plasma glucose.

Complications: Transient headache

Aetiology: occurs in 15-30% of all punctures, more in younger males, usually suboccipital, lasts 1-10 days.

Prevention: Use a small-gauge spinal needle; instruct the patient to remain flat in bed for at least 4 hours; do not remove an excessive amount of spinal fluid.

Bloody tap

Aetiology: Laceration of epidural venous plexus with the spinal needle.

Transient backache

Aetiology: Multiple puncture attempts.

Prevention: Infiltrate adequately with local anaesthetic; avoid multiple or traumatic pass.

Dry tap

Aetiology: Excessive lateral location of needle tip.

Prevention: Accurately determine midline and intraspinal position.

Meningitis or epidural or subdural empyema

Aetiology: Contaminated needle, inadequate skin disinfection, local sepsis of the skin.

Prevention: Use meticulous aseptic technique, avoid puncture in presence of local infection in the lumbar area, avoid lumbar puncture in septicemic patients without signs of meningitis.

Subdural haematoma

Aetiology: Removal of too large a volume of spinal fluid in an elderly patient, resulting in tearing of the perforating vein.

Prevention: Check prothrombin (Quick) time or prothrombin-proconvertin test (thrombotest) in patients on anticoagulant therapy.

Herniation (unconsciousness, bradycardia, respiratory depression, bilateral pupil dilatation).

Aetiology: Removal of spinal fluid from below a site of impaction of neural tissue.

Prevention: Do not tap in the presence of papilloedema or focal neurological signs unless a mass lesion is ruled out.

Treatment:

- Do not remove the lumbar puncture needle.
- Attach the manometer to the lumbar puncture needle.
- Administer mannitol (20%) intravenously (10 mL/kg over 30-60 minutes in adults), until the cerebrospinal fluid pressure is below 200 mm Hg.
- Remove lumbar puncture needle and immediately give dexamethasone phosphate 10 mg intravenously or intramuscularly.
- Control pressure with dexamethasone phosphate, 4 mg every 6 hours intramuscularly, or mannitol intravenously.

Exacerbation of paraparesis

Aetiology: Removal of spinal fluid from below a complete intraspinal block.

Prevention: Do not remove excessive amounts of CSF in a patient suspected of intraspinal mass lesion.

Transient radicular pain

Aetiology: Nerve root hit by spinal needle.

Prevention: Advance a spinal needle with bevel parallel to the axis of the spine.

Storage: Refrigeration at 2-8 °C.

Transportation: Short transportation time is essential. If the transportation time is more than one hour, the specimen should be sent in a cooling box (2-8 °C).

Reporting: Results of the microscopy and all positive cultures of CSF are reported immediately to the treating physician. Negative bacterial results are sent out 72 hours after the CSF is received. Viral isolation may take at least 7 days.

Comments:

Total and differential white cell count is essential, particularly in the differentiation of bacterial and nonbacterial meningitis. In bacterial meningitis the glucose level is usually low and protein level is high, whereas in viral meningitis the glucose is within normal value and might increase slightly. In partially treated bacterial meningitis, e.g. in patients who have received oral antibiotic therapy before meningitis was considered, the differentiation between bacterial and viral meningitis can be extremely difficult, but a low glucose level should be taken as an indication of the need for the start of intravenous antibiotic therapy.

Early in the infection of bacterial meningitis one may find an abundance of bacteria with only a few leukocytes. This is particularly true in pneumococcal and staphylococcal infections. Initially polymorphonuclear leukocytes predominate, but as recovery takes place these are gradually replaced by lymphocytes. Polymorphonuclear leukocytes may also be present, along with lymphocytes, in tuberculous meningitis, cerebral abscess, listeria meningitis, and in the early stages of poliomyelitis. A predominance of lymphocytes is the typical picture in most viral meningitis and encephalitis, active neurosyphilis, and tuberculous meningitis cases. Eosinophilic pleocytosis is seen with certain helminthic infections of the central nerve system (angiostrongyliasis, gnathostomiasis, paragonimiasis and cestode larval disease).

In tuberculous meningitis "classical signs" of meningitis may be missing in a substantial number of cases. Choroid tubercles, miliary tubercles in the lung or a primary complex may give the clue. To identify acid-fast bacilli by microscopy, adequate volumes of CSF should be investigated. On a single specimen there is a less than 40% chance of identifying acid-fast bacilli, but this increases to nearly 90% on four specimens, drawn daily for four days.

If the CSF is collected it may also be used for immunoassays (antigen detection) for certain infections.

JOINT, BURSA AND TENDON SHEATH

Objectives: Aetiological diagnosis of joint, bursa or tendon sheath infections by microscopic examination and culture with identification and susceptibility test of isolated organism(s).

Contraindication: Skin infection overlying joint or bursa (relative contraindication).

Test material: Pus; bloody, serous or purulent effusion.

Collection time: Preferably before any antibiotics are administered.

Equipment: Request form, label, sterile cotton swabs, antiseptic solution, surgical gloves, lidocaine 1%, syringe, needle, sterile specimen tube, plaster.

Procedure:

1. This collection should only be performed by a physician. Obtain X-rays in two planes and correlate with easily palpable bone landmarks.
2. Have the patient placed comfortably, select the approach and adjust the position of the joint. Extensor surfaces are generally preferred; avoid major nerves, vessels and tendons.
3. Disinfect the skin two times and let it dry in between, use gloves and drape the site.
4. Infiltrate with local anaesthetic from the skin to the synovial membrane.
5. Reassemble the syringe and needle, perform the centesis without injuring the cartilage, and aspirate 2-5 mL of the fluid.
6. Remove the needle and apply a plaster to the puncture site.
7. Send the synovial fluid for investigation: cell count, glucose, Gram stain, culture, crystals, and rheumatological tests.

Approaches: **Shoulder**

1. Arm at site and hand across abdomen.
2. Insert needle dorsally 2 cm inferior to the posterior angle of the acromion and direct the needle anteriorly and 15 degrees medially towards the glenoid cavity.

Subacromial bursa

1. Arm at side, flex and pull the elbow distally.
2. Insert the needle laterally 1 cm below the tip of the acromion and direct medially.

Elbow joint

1. Flex the elbow to 90 degrees, turn the forearm so the palm is down.
2. Insert the needle between the lateral humoral epicondyle and the radial head, direct the needle medially.

Olecranon bursa

1. Flex elbow to 90 degrees.
2. Insert the needle at the distal tip of the olecranon and direct the needle along the shaft of the ulna.

Wrist joint

1. Pull the hand distally and flex the wrist 30 degrees.
2. Insert the needle distal to the dorsal tubercle of the radial condyl and medial to the extensor pollicis longus tendon.
3. Direct the needle volarly to the joint.

Fingers

1. Flex the finger 15-30 degrees and pull distally.
2. Insert the needle dorsally, medially or laterally to the extensor or abductor pollicis tendon.

Hip

1. Place the patient on his back with the hip unflexed and unrotated.
2. Insert the needle at the intersection of a parasagittal line through the anterior superior iliac spine and transverse the line through the pubic symphysis.

Greater trochanteric bursa

1. Place the patient on his back with the hip straight and internally rotated.
2. Insert the needle at and direct it to the point of maximum tenderness.

Knee

1. Extend the knee.
2. Insert the needle 1 cm medially to the patella and direct the needle to intercondylar notch.

Ankle

1. Plantar flex the foot.
2. Insert the needle medially to the anterior tibial tendon, direct to hollow at the anterior margin of medial malleolus.

Toes

1. Flex the toe to 15-30 degrees and pull distally.
2. Insert the needle dorsally, medially or laterally to the extensor tendon.

Storage: Refrigerated (2-8 °C).

Transportation: Preferably in a cooling box (2-8 °C), if transportation time is more than one hour.

Reporting: Positive results both by microscopy or by culture will be reported immediately to the treating physician.

Comments: Isolation of bacteria is generally regarded as diagnostic, but there is a risk of contamination during sampling and inoculation.

PERICARDIAL EFFUSION

- Objective:** Actiological diagnosis of pericardial effusion by microscopic examination and culture and susceptibility test of isolated organism.
- The aspiration may be contraindicated in the following situations: bleeding diatheses or anticoagulant therapy, and infection in the puncture site.
- Test material:** Pericardial effusion.
- Collection time:** Before institution of antibiotic therapy.
- Equipment:** Request form, labels, sterile sponges, antiseptic solution (Appendix 5), mask, gown and gloves, towels, towel clips, syringe, needle, lidocaine 1%, syringes, spinal needles, sterile alligator clip, ECG machine electrically isolated, culture tubes, haematocrit tubes, cytology tubes, adhesive tape.
- Procedure:**
1. This collection should only be performed by a physician. Use mask, gown, and gloves.
 2. Attach the ECG limb leads to the patient.
 3. Disinfect and drape the puncture site (left xiphocostal angle).
 4. Infiltrate deep with local anaesthetic at the left xiphocostal angle to the costal arch.
 5. Connect the spinal needle to a 50 mL syringe and the sterile alligator clip of the V lead to the metal needle hub.
 6. Insert the needle in the anesthetized tract and advance carefully.
 7. When the needle tip is deep in to the costal arch, depress the hub and advance the needle towards the left shoulder, aspirating during advancement. A sudden "give" or "pop" may be felt with pericardial puncture.
 8. Continually monitor the V lead on the ECG for injury current. An elevation of the S-T segment appears with ventricular epicardial contact. Epicardial contact is felt through the needle as a grating sensation. With epicardial contact, withdraw the needle slightly and redirect the needle towards the head or right shoulder.

9. Confirm the intrapericardial position by fluid withdrawal. Ventricular puncture is suggested if the fluid is bloody and clots, or if the haematocrit of the fluid is the same as peripheral venous blood.
10. Remove the pericardial fluid.
11. Send the fluid for protein, cell count, cytology, Gram stain, and culture tests. Outside the laboratory's working hours 2 mL of fluid may be injected into a blood culture bottle and placed in the incubator.
12. Withdraw the needle and apply dressing.

Complications:**Ventricular puncture** (most have no sequelae)

Aetiology: Advancement of needle into the ventricular cavity.

Prevention: Monitor injury current on ECG; advance needle slowly, rotating hub and aspirating.

Therapy: Withdraw needle until blood is no longer obtained and/or S-T changes disappear; monitor the patient and observe for tamponade.

Arrhythmia

Aetiology: Irritation of ventricular or atrial myocardium by needle.

Prevention: Withdraw needle when arrhythmia or S-T segment deviation occurs, using electrically isolated ECG machine; older machines can carry a significant shock hazard and may cause ventricular arrhythmias, including ventricular fibrillation.

Storage:

Specimens collected in tubes should be stored in the refrigerator, whereas specimens in blood culture bottles should be placed in the incubator immediately.

Transportation:

Specimen tubes should preferably be shipped in a cooling box (2-8 °C), if transportation time is more than one hour, whereas specimens in blood culture bottles should be transported uncooled and placed in the incubator as soon as possible.

Reporting:

Positive results are reported immediately. The final report is sent out two days after receipt of specimen.

Comments:

Isolation of bacteria usually confirms the diagnosis, but there is risk of contamination of the specimen during sampling and inoculation.

PERITONEAL EFFUSION

- Objectives:** Actiological diagnosis of peritonitis with microscopic examination and aerobic and anaerobic culture of the peritoneal fluid with identification and susceptibility test of isolated organism(s). If a tuberculous aetiology is suspected, it should be stated on the request form.
- Contraindication:** Multiple previous abdominal operations (relative).
- Material:** Peritoneal effusion.
- Equipment:** Request form, sticker, sterile cotton wool sponges, antiseptic solution, mask, gown, gloves, lidocaine 1% , syringes, needles, scalpel with blade, haemostats, suture (2-0 chromic and 3-0 nylon), sterile specimen tube or blood culture bottle set.
- Collection time:** Before start of antibiotic therapy.
- Position:** Supine, bladder empty.
- Procedure:**
1. The collection should only be done by a physician. Select the centesis site:
4 cm below umbilicus in midline (infraumbilical) or
4 cm above umbilicus in midline (supraumbilical) or
right or left lower quadrant area lateral to the rectus sheath.
 2. Prepare, disinfect and drape the area.
 3. Infiltrate local anesthetic from skin to peritoneum.
 4. Make a 3 cm long incision through the skin and subcutaneous tissue.
 5. Incise the fascia.
 6. Lift up each side of the incision with haemostats and incise peritoneum with a 2 cm long incision in the midline.
 7. Insert a peritoneal dialysis catheter through the incision, rotate towards the pelvis to avoid catching the omentum.
 8. Place a pursestring suture (2-0 chromic) in the fascia and peritoneum and tie around the catheter to reduce leakage.
 9. Connect a 20 mL syringe to the catheter and aspirate.

10. Remove the catheter.
11. Close incision: 2-0 chromic catgut for fascia, and 3-0 nylon for skin, apply sterile sponge dressing and fix with occlusive tape.
12. Transfer the peritoneal fluid to a sterile specimen tube or inject 5 mL of the effusion into each of two blood culture bottles for aerobic and anaerobic cultivation.

Complications:**1. Pneumoperitoneum**

Aetiology: Introduction of air through the catheter.

2. Bowel perforation

Aetiology: Loop of bowel adherent to anterior peritoneum, ileus.

Prevention: Do not place catheter through surgical scars; do not use stylet; advance the catheter under direct vision.

3. Intraperitoneal bleeding

Aetiology: Laceration of omental or mesenteric vessel.

Prevention: Insert catheter gently, without stylet

Storage:

Specimens collected in a tube should be stored in the refrigerator, whereas specimens in blood culture bottles should be placed immediately in the incubator.

Transportation:

Preferably in a cooling box (2-8 °C) if transportation time is more than one hour.

Reporting:

Negative results are sent out 2 days after the specimen is received.

Comments:

Peritonitis is usually secondary to diseases in the abdominal organs or to infection elsewhere in the body which has extended to the peritoneal cavity. In a small number of cases it appears to be primary, as in some cases of pneumococcal or streptococcal peritonitis in children. So-called aseptic peritonitis is seen when sterile blood, urine, bile, pancreatic juice or the contents of certain cysts leak into the peritoneal cavity. The most common source of peritonitis is the intestinal tract. Perforations, as in appendicitis, peptic ulcers, diverticulitis or carcinoma, at once contaminate the peritoneal cavity, bringing about a localized or generalized peritonitis. Peritonitis may result from biliary tract disease, usually secondary to an acute cholecystitis with perforation. It is also a common manifestation in

the terminal stage of cirrhosis of the liver. The female pelvic organs are also a common site for peritonitis as in gonorrhea or septic abortions or delivery. Uncommon causes are tuberculous and pneumococcal peritonitis secondary to bacteremia.

PLEURAL EFFUSION

Objectives: Aetiological diagnosis of pleural effusion by microscopic examination and culture with identification and susceptibility test of the isolated organism.

Aspiration of the pleural effusions may be contraindicated in the following circumstances: bleeding diathesis or anticoagulant therapy, and infection over the puncture area.

Test material: Pleural effusion.

Collection time: Before institution of antibiotic therapy.

Equipment: Request form, labels, surgical gloves, sterile cotton wool swabs, skin disinfectant, syringe, needle, 2 sterile conical specimen tubes with stoppers, plaster.

Procedure:

1. The collection should only be performed by a physician.
2. Do an X-ray or confirm the fluid level by comparison of dullness to percussion over the two lung fields. Select the puncture site one or two interspaces below the fluid level in a posterior axillary line, but not lower than eight intercostal spaces.
3. Place the patient on the edge of the bed, either with the arms lifted by an attendant or placed on two bulky pillows over the back of a chair.
4. Wear gloves and disinfect the puncture site. Local anaesthetic is usually not necessary for a diagnostic puncture.
5. Draw down the skin and insert the needle along the superior margin of the rib to avoid the intercostal nerves and blood vessels; aspirate regularly to check for the location in pleura.
6. Aspirate the specimen (5 mL).
7. Remove the needle, and as the skin has been pulled down during the puncture, the channel will close by itself. If iodine has been used as a skin disinfectant, remove it with alcohol, apply a plaster.

8. Send the specimen for cell count, protein, glucose, Gram stain and culture tests.
9. Check for pneumothorax either by an X-ray or percussion.

Complications:**Pneumothorax**

Aetiology: Laceration of the lung, opening of 3-way stopcock so that pleural cavity is open to the atmosphere.

Prevention: Insert needle no further than necessary to collect the fluid; use a short-bevelled needle; be familiar with the stopcock.

Haematoma or haemothorax

Aetiology: Laceration of the intercostal vessel.

Prevention: Insert the needle at the superior margin of the rib.

Accidental liver or spleen puncture

Aetiology: Puncture site too caudal or too deep.

Prevention: Avoid puncture posteriorly lower than eight intercostal spaces, mark the proper needle depth with a clamp.

Storage:

Refrigerated (4-8 °C).

Transportation:

If the transportation time is more than 2 hours, it should be sent in a cooling box (2-8 °C).

Reporting:

A positive microscopy result is reported immediately to the treating physician. Cultures without growth are reported 2 days after receipt, except when culture for slow organisms (*Brucella*, *Legionella* or *Mycobacterium tuberculosis*) has been requested.

Comments:

Isolation of bacteria usually confirms the diagnosis, but there is a risk of contamination of the specimen during sampling and inoculation. Except mycoplasma and virus, the organism can usually be cultured, unless inhibited by antibiotics. Pleural effusion may complicate any bacterial pneumonia. Tuberculosis is the most common cause of a unilateral pleural effusion in the tropics. Bilateral effusion is suggestive of tuberculosis of the thoracic spine. The chest X-ray is not suggestive, except when there is pleural calcification. Occasionally, pleural effusion is also a prominent

manifestation of parasitic infections, such as amoebiasis, malaria, strongyloidiasis, and paragonimiasis. In amoebiasis, a pleural effusion may either represent a sympathetic transudate over an unruptured liver abscess or may reflect a rupture of the abscess into the pleural cavity. A pyogenic infection should be suspected if the neutrophilic cell count is high, the ratio effusion protein to serum protein is greater than 0,5, effusion LDH is above 200 IU, the ratio of effusion LDH to serum LDH is greater than 0,6, and the pH is less than 7,3.

A tuberculous aetiology should be suspected if the lymphocytic cell count is high, the ratio of effusion protein to serum protein is greater than 0,5, the glucose content normal or decreased. Acid-fast bacilli are rarely demonstrated, unless a pleural biopsy is obtained. Cultures take up to 6 weeks and only 60% are positive.

GENITAL SPECIMENS

(male and female)

GENITAL ULCERS, GRANULOMA AND BUBO

Objectives: Aetiological diagnosis of genital ulcer, granuloma and bubo by microscopic examination and culture with identification and susceptibility test of isolated organism.

Test material: Tissue fluid or pus.

Collection time: During the laboratory's opening hours, as the specimen should be examined and processed immediately.

Equipment: Request form, labels, disposable gloves, gauze, normal saline, antiseptic solution, sterile thin inox spatula or sterile bacteriological loop, microscope slides, cover slips, sterile cotton wool or calcium alginate swabs, tubes with transport medium, Petri dish for transportation, selective enriched chocolate agar, diluted Chlamydia transport medium, scalpel.

Procedure: **Syphilitic chancre**

1. Wear gloves.
2. Cleanse the lesion carefully with gauze and saline and wipe dry.
3. Squeeze the ulcer carefully to produce a serous exudate.
4. Transfer the exudate with a spatula, bacteriological loop, or by touching the slide directly to the surface of the wound.
5. Add a small drop of saline to the specimen and cover the preparation with a cover slip.
6. Immediately dispatch the specimen to the laboratory in a Petri dish.

Chancroid (ulcus molle or soft chancre)

1. Cleanse the surface of the ulcer with dry gauze to remove crust and superficial debris; extensive cleaning is not required.
2. With a cotton or dacron wool swab sample the exudate from the base of the ulcer.

3. Place the swab in the transport medium. It is recommended to inoculate a plate of selective enriched chocolate agar before the swab is placed in the transport medium, and to immediately send the swab and chocolate agar to the laboratory.
4. Specimens from a fluctuating lymph node may be obtained by aspiration, but are often negative on culture.

Lymphogranuloma venereum (LGV)

1. Wear disposable gloves, cleanse the skin over a fluctuating lymph node with antiseptic solution. If a small genital ulcer is present, cleanse the surface with saline.
2. Assemble the syringe and needle.
3. Insert the needle through healthy looking skin laterally into the centre of the fluctuating lymph node.
4. Aspirate the pus into the syringe.
5. Place the syringe in a Petri dish and send it immediately to the laboratory. **Do not put on the protective cap!**
6. If an ulcer is present squeeze out a little exudate and collect it on a thin calcium alginate or cotton wool swab.
7. Place the swab in 1 mL diluted transport medium in a sterile glass vial.

Granuloma inguinale or donovanosis

1. Wear disposable gloves.
2. Cleanse the ulcerated area with gauze and saline.
3. With a scalpel scrape off a small piece of clean granulation tissue from the border of the lesion.
4. Crush the specimen between two slides.
5. Air dry the smear and fix in methanol for 2-3 minutes.

Storage: The specimen should be brought to the laboratory immediately.

Transportation: As rapid as possible to assure a high isolation rate.

Reporting: Positive results will be reported immediately to the treating physician.

Comments: Syphilis is caused by *Treponema pallidum*, which has worldwide distribution. Lymphogranuloma venereum is caused by *Chlamydia trachomatis*, serotypes L-1, L-2 and L-3. The disease is endemic in many tropical and subtropical countries. Granuloma inguinale or donovanosis, presumed to be caused by *Calymmatobacterium*

granulomatis, is common in many tropical countries, particularly in southern India, Indonesia, Viet Nam, Papua New Guinea and parts of tropical Africa.

URETHRA, RECTUM, ENDOCERVIX, PHARYNX FOR GONOCOCCAL INFECTION

- Objectives:** Aetiological diagnosis of gonorrhea by microscopic examination and culture with identification and susceptibility test.
- Test material:** Discharge (pus) from the male urethra or the female endocervix. Urine sediment is not recommended for the diagnosis of gonococcal urethritis, although it may be used for the microscopic detection of *Trichomonas vaginalis*. Prostatic massage does not increase the recovery of gonococci from the urethra.
- Collection time:** Preferably during the laboratory's opening time.
- Equipment:** Request form, labels, cotton wool, dacron or calcium alginate swab, microscopic slides, transport medium (Stuart or Amies), speculum, warm water.
- Procedure:** Fill in the request forms and labels for the specimen containers.

Urethra

1. In case of abundant exudate, a drop of the purulent discharge is collected with a sterile swab or sterile bacteriological loop and immediately used to prepare a thin film on a clean microscopic slide.
2. If no discharge is evident, the urethra is stripped towards the orifice to express some pus, a thin sterile swab is inserted 2-3 cm into the urethra and rotated before being withdrawn.
3. Place the swab in the transport medium. Under such conditions, gonococci will remain viable for up to 24 hours at room temperature.

Rectum

1. Insert a cotton swab 3 cm into the anal canal and rub the swab up and down to collect exudate from the crypts.
2. Place the swab in the transport medium.

Endocervix

1. Moisten the speculum with warm water.
2. Insert a sterile swab 2-3 cm into the cervical canal.
3. Rotate the swab for 5-10 seconds to permit absorption of the exudate.
4. Place the swab in the transport medium.
5. If the woman has had a hysterectomy, the swab may be taken from the posterior fornix of the vagina.

Pharynx

1. Swab the tonsillar crypts and the posterior pharynx with a cotton or dacron swab.
2. Place the swab in the transport medium.

Storage: To assure a high isolation rate the specimen should be inoculated with as little delay as possible, and not later than 24 hours after the sampling was made.

Transportation: Preferably in a cooling box (2-8 °C), if transportation time is more than one hour. **Refrigeration should be avoided.**

Reporting: A result will be available 2-3 days after the specimen is received.

Comments: Gonorrhea should never be diagnosed without the aid of microscopy or culture. In the majority of males, diagnosis of acute gonorrhoea can be made quite easily. A microscopic examination of a Gram-stained urethral smear will lead to a correct diagnosis in 80-90% of the cases. Only in asymptomatic cases can the diagnosis be missed by microscopy and then cultural verification will be essential.

In the female, acute gonorrhoea usually involves the endocervical canal, the urethra and the Bartholin's and Skene's glands. When symptoms are present, they will be referable to the structures involved. However, in the majority of females there will be no or only slight unspecific symptoms, and cultural examination becomes much more important for correct diagnosis.

VAGINAL AND ENDOCERVICAL SWABS

Objectives:	Aetiological diagnosis of vaginal discharge, vesicular genital lesions and warts. Identification of <i>Gardnerella vaginalis</i> , <i>Trichomonas vaginalis</i> and <i>Candida</i> species is mainly determined by microscopy, as cultivation will detect a large number of asymptomatic carriers. Herpes virus is identified by direct antigen detection and cell culture, whereas human papilloma viruses are usually detected by the hybridization technique.
Test material:	Vaginal discharge collected from the posterior fornix.
Equipment:	Request form, labels, speculum, cotton wool tipped swabs.
Procedure:	<ol style="list-style-type: none">1. Fill in the request form.2. The patient is placed in the dorsal recumbent position on the examining table with flexed thighs and knees, the feet resting on the stirrups, and the limbs and lower abdomen draped with a sheet.3. The exudate exposed in the posterior fornix is collected on a sterile cotton tipped swab and placed in the respective transport media.4. The speculum is removed and the appearance and smell of the discharge on the speculum is noted.
Storage:	Specimens for trichomonas should be kept at room temperature, whereas the other specimens should be stored in the refrigerator.
Transportation:	Specimens for bacterial and viral culture should preferably be sent in a cooling box (2-8 °C).
Reporting:	The results of direct examination of specimens can be expected within 24 hours after receipt of the specimen, whereas results of herpes simplex culture may take at least 3 days.
Comments:	Increased vaginal or endocervical discharge may reflect a vaginitis caused by <i>Candida albicans</i> (or other <i>Candida</i> spp.), <i>Trichomonas vaginalis</i> or bacterial vaginosis, a syndrome caused by the synergistic

action of *Gardnerella vaginalis* and some strict anaerobic bacteria. Genital vesicular lesions are mainly caused by HSV-2, and genital warts by papillomavirus.

Bacterial vaginosis is the most common vaginal infection characterized by increased discharge with a fishy odour. Aetiology is unknown. Previously *Gardnerella vaginalis* was regarded as the aetiological agent, but recent investigations have shown that more than 50% of healthy women with normal vaginal examination are colonized by this organism. Routine culture for *Gardnerella vaginalis* is therefore not recommended. In typical cases the concentration of anaerobes is higher and *Lactobacillus* lower than in the normal vagina. A noncultural diagnosis can be made by detecting at least three of the following four characteristics:

Bacterial vaginosis

1. thin, homogeneous discharge adhering to the vaginal wall;
 2. pH greater than 4.5;
 3. fishy odour intensified on addition of 10% potassium hydroxide;
 4. presence of clue cells by microscopic examination of the discharge.
-

Candida vaginitis usually presents as vaginal itching, a curdy white discharge (cottage cheese) without odour. Diagnosis is confirmed either by a simple wet mount, or better, a 10% potassium hydroxide (KOH) wet mount microscopy and culture. *Candida albicans* is the major cause accounting for 85% of the isolates. *Candida glabrata* is the second common cause, while *Candida krusei*, *C. tropicalis* and *C. stellatoidea* rarely cause infection.

Trichomonas vaginitis induces purulent frothy discharge, vaginal itching or irritation, dysuria and malodour. Some patients are asymptomatic. Diagnosis is made by microscopic examination of a wet mount of the discharge or culture which is more sensitive.

Streptococcus agalactiae (group B streptococci). Approximately 10-30% of pregnant women harbour this organism in the genital tract. Around 1% of their offspring develop symptomatic infections;

NASOTRACHEAL SUCTION

- Objectives:** Actiological diagnosis of lower respiratory tract infection by microscopic examination and culture with identification and susceptibility test and antigen detection and culture for virus.
- Test material:** Sputum, 1-2 mL.
- Equipment:** Request form, labels, suction catheter with finger-occluded suction vent, suction source, sputum trap, gloves, sterile water, specimen container, water-soluble lubricant.
- Procedure:**
1. Inform the patient about what will happen.
 2. Use gloves.
 3. Interpose the sputum trap between the catheter and the suction source.
 4. Lubricate the distal end of the catheter and insert the catheter into the nose and advance horizontally along the floor of the nose and aiming for the ear lobe until it curves into the hypopharynx.
 5. Instruct the patient to assume a "sniffing position" of the head and neck, and ask the patient to breathe deeply.
 6. Keep the suction vent open and advance the catheter into the trachea during deep spontaneous inspiration. If intubation is difficult, place a gauze sponge around the tongue and pull the tongue forward; this will move the epiglottis forward and helps uncover the glottis.
 7. If the catheter is in the trachea, the patient will start coughing and he will have difficulties in phonation.
 8. Intermittently occlude the suction vent for a maximum of 5 to 10 seconds at a time.
 9. Wait 30-60 seconds before resuctioning to allow the patient to get his breath.
 10. Remove the catheter.
 11. If the sputum is retained in the catheter, wash it out by suctioning a little sterile water through the catheter.

Complications:	Cardiac arrhythmia Aetiology: Vagal stimulation in presence of hypoxemia. Prevention: Avoid suctioning longer than 10 seconds. Bleeding Aetiology: Trauma to mucous membranes. Prevention: Avoid forcing the catheter; a small amount of bleeding is common and of no concern.
Storage:	Refrigerated (2-8 °C).
Transportation:	In a cooling box (2-8 °C), if transportation time is more than one hour.
Reporting:	Culture for bacteria will be sent out after 2-3 days, culture for virus after 7-10 days.
Comments:	<p>The specimen is evaluated microscopically according to the same principles as for sputum. Finding of organisms which are presumed to be resistant to penicillin should be given in a preliminary report.</p> <p>Only organisms demonstrated as originating from the lower respiratory tract are identified and tested for antibiotic susceptibility.</p> <p>If examination for <i>M. tuberculosis</i> is requested, the specimen is examined by both microscopy and culture.</p>

SPUTUM, EXPECTORATED

Objectives:	Aetiological diagnosis of lower respiratory tract infection by microscopic examination and culture with identification and susceptibility test of the isolated organism(s). In some countries sputum may also be used for the microscopic demonstration of bronchopulmonary parasites, such as the ova of <i>Paragonimus</i> spp. and larvae of <i>Strongyloides stercoralis</i> .
Test material:	Secretion from the lower respiratory tract produced by coughing
Collection time:	Before the patient has received antibiotic treatment.
Equipment:	Clean, wide-mouthed screw-capped container (minimum content 25 mL).
Procedure:	<p>Instructions should be given to the patient beforehand by a nurse or a laboratory technician.</p> <ol style="list-style-type: none">1. The patient should be standing, if possible, or sitting upright in bed.2. He or she should take a very deep breath to fill the lungs, and empty them in one breath, coughing as hard and as deeply as possible.3. The sputum brought up should be spit into the container.4. If the quantity of sputum is not sufficient (1-2 mL), the procedure may be repeated. A single well collected specimen, however, is better than a specimen collected over several hours.5. Before being sent to the laboratory, the specimen should undergo a brief visual evaluation and its appearance should be recorded on the request form.6. Tighten the cap on the container and send the specimen immediately to the laboratory.
Sample quantity:	1-2 mL.
Storage:	Refrigerated (2-8 °C).

- Transportation:** In a cooling box (2-8 °C) except when the transport time is less than one hour.
- Reporting:** Isolation of a possible pathogen can be expected after 2-3 days. Negative cultures will be reported out 1-2 days after receipt of the specimen.
- Comments:** The sputum will be examined by microscopy before it is cultured. If the specimen only or mainly consists of saliva, it will not be processed further, and the request form will be returned to the department with the statement "Improper specimen, only saliva, please resubmit". If the specimen contains lower respiratory secretion with signs of inflammation (more than 10 polymorphonuclear neutrophils per squamous epithelial cell), the pathogenic organisms will be identified and tested for antibiotic susceptibility.
- All expectorated sputum is contaminated to some degree with secretion of the oropharyngeal cavity, which contains a wide variety of commensal bacteria, some of which are potential pathogens of the lower respiratory tract (pneumococci, *Haemophilus influenzae*). Since the sputum should reflect the infection in the bronchi and the lung, contamination with oropharyngeal secretions should be kept to a minimum. A good technique for expectoration and collection of the specimen is essential.
- The examination of sputum is not indicated in patients with fever of unknown origin.

TRANSTRACHEAL ASPIRATION

- Objectives:** Aetiological diagnosis of lower respiratory tract infection by microscopic examination and culture of “transtracheal” sputum. Isolated pathogenic bacteria will be tested for antibiotic susceptibility. The specimen is uncontaminated by oropharyngeal organisms.
- This type of collection should only be performed if: the expectorated sputum is unrevealing or confusing, the patient cannot raise sputum, or the patient is likely to be infected with anaerobic bacteria or unusual organisms (immunodeficiency, lung abscess).
- Contraindication:** Bleeding diathesis, uncontrollable cough, uncooperative patient, untreated hypoxemia.
- Specimen:** Sputum.
- Collection time:** Preferably before any antibiotic is prescribed (may not be relevant if fungal or parasitic infection is suspected).
- Equipment:** Sterile sponge, skin disinfectant, gloves, syringe, needle, lidocaine 1% without epinephrine, Intracath®, syringe, sterile saline, transport container, plaster.
- Procedure:**
1. The collection should only be done by a physician experienced in the procedure.
 2. Locate the cricothyroid space.
 3. Put on gloves, disinfect the skin, and drape.
 4. Infiltrate the skin with lidocaine in the midline down to the cricothyroid membrane (avoid injecting lidocaine into the trachea).
 5. ~~Revel~~ the Intracath needle on the syringe and hold the needle 1,5 cm from the point to avoid plunging it too deeply.
 6. Aim the Intracath needle 45° caudal to the skin and firmly thrust it through the membrane; confirm the position by aspiration of air.
 7. Remove the syringe and thread the catheter down into the trachea.

8. Remove the stylet from the catheter, slide the needle out of the trachea over the cannula.
9. Attach the 10 mL syringe to the catheter and aspirate during cough. If the specimen is inadequate or the patient does not cough, inject 2-3 mL of sterile saline and aspirate again.
10. Withdraw the catheter, apply pressure to the puncture site, cover with a plaster, and order bed rest for 8 hours.

Complications:**1. Bleeding into trachea**

Aetiology: Bleeding diatheses, entry into infracricoid venous plexus.

Prevention: Check bleeding studies before procedure, ensure puncture is between thyroid and cricoid cartilage.

Treatment: Immediate endotracheal intubation, if ventilation is compromised.

2. Subcutaneous or mediastinal emphysema

Aetiology: Entry of air into subcutaneous tissue or mediastinum through puncture site.

Prevention: Should not be done in patients with uncontrollable cough. Apply pressure to the puncture site for 5 minutes, prescribe bed rest for 8 hours after the procedure.

3. Catheter aspiration

Aetiology: Catheter cut off, when the catheter is pulled back through the needle.

Prevention: Never withdraw the catheter through the needle.

4. Cardiac arrhythmia or arrest

Aetiology: Vagal stimulation in hypoxemic patient.

Prevention: Ensure adequate oxygenation.

Storage:

Refrigerated (2-8 °C).

Transportation:

Preferably in a cooling box (2-8 °C), if delayed for more than 2 hours

Reporting:

A culture report will be available in 2-3 days.

Comments:

The specimen is uncontaminated by oropharyngeal flora. If *Mycobacterium tuberculosis* or other fastidious organisms are needed these should be specified on the request form.

UPPER RESPIRATORY TRACT

EAR DISCHARGE

Objectives:	Aetiological diagnosis of external or media otitis by aerobic and anaerobic culture with identification and susceptibility test of the isolated organism(s).
Test material:	Pus from the external or middle ear.
Collection time:	Before start of antibiotic therapy.
Equipment:	Cotton wool or dacron swab, test tube with transport medium.
Procedure:	<ol style="list-style-type: none">1. Collect a specimen of the discharge on a thin, sterile cotton wool or dacron swab.2. Place the swab in a container with the transport medium, breaking off the swab stick to allow the stopper to be replaced tightly.3. Label the specimen and send it to the laboratory.
Storage:	Refrigerated (2-8 °C).
Transportation:	Preferably in a cooling box (2-8 °C), if delayed for more than one hour.
Reporting:	Results can be expected within 48 hours.
Comments:	<p>For external ear infections only <i>Staphylococcus aureus</i>, <i>Streptococcus pyogenes</i>, <i>Pseudomonas aeruginosa</i>, <i>Vibrio</i> spp. and <i>Aspergillus</i> will be looked for and reported. For middle ear infections only pneumococcus, <i>Streptococcus pyogenes</i>, <i>Haemophilus influenzae</i> and <i>Staphylococcus aureus</i> will be reported with a susceptibility test. For the chronic discharging ear, <i>Bacteroides</i> species and fungi will also be reported in addition to the organisms reported for middle ear infections.</p> <p>Cultures of the external auditory canal generally do not reflect the bacteria causing middle ear infection unless there has been a recent rupture of the tympanic membrane. Tympanic membrane aspiration is rarely performed.</p>

SALIVA AND CONJUNCTIVAL SWAB FOR RABIES VIRUS

Objectives:	Aetiological diagnosis of rabies virus by direct antigen detection or cell culture in suspected patients with an exposure history. Diagnostic work with rabies virus should only be carried out in laboratories designated for this purpose.
Test material:	Saliva collected on swabs.
Collection time:	As soon as suspicion is raised.
Equipment:	Sterile cotton swab, sterile specimen tube, viral transport medium (see Appendix 2).
Procedure:	<ol style="list-style-type: none">1. Wear protective gloves, face mask and protective glasses.2. Collect saliva from the anterior floor of the mouth, near the parotid duct and from the lower fornix of the conjunctiva with 1-2 sterile swabs from each place.3. Place the swabs into the viral transport medium.
Storage:	Should immediately be sent to the diagnostic laboratory and processed.
Transportation:	In a cooling box (2-8 °C).
Reporting:	The results (negative or positive) will be conveyed immediately by telephone.

SWAB FROM UPPER RESPIRATORY TRACT

Objectives:	Aetiological diagnosis of infections of the pharynx by microscopic examination and culture for bacteria and for virus by direct antigen detection and cell culture. Routine culture will only be done for <i>Streptococcus pyogenes</i> . If other aetiologies (diphtheria, Vincent's angina, thrush, gonococci, whooping cough) are suspected, it should be stated on the request form. The most common viruses to look for are measles, mumps, rubella, herpes, influenza A and B, parainfluenza, adeno- and rhinoviruses.
Test material:	Throat swabs should be collected for streptococcal sore throat, diphtheria, Vincent's angina, thrush, and gonococcal pharyngeal infection. Nasopharyngeal swabs are recommended for respiratory viruses and suspected cases of whooping cough, as <i>Bordetella pertussis</i> is found more commonly in this part of the upper respiratory tract.
Collection time:	In the acute phase of the disease and for bacterial infections before start of antibiotic therapy.
Equipment:	Cotton swab, bacterial and viral transport medium (see Appendix 2).
Procedure:	<p>Throat swabs</p> <ol style="list-style-type: none">1. Turn the patient's face against the light, ask the patient to open his mouth wide and phonate an "ah"; gently depress the patient's tongue with a tongue blade so that the throat is well exposed and illuminated.2. Guide a swab over the tongue into the posterior pharynx.3. Rub the swab firmly over the back of the throat, both tonsils and any areas of inflammation, exudation or ulceration. Care should be taken to avoid touching the tongue, cheeks or lips with the swab.4. Place the swab in the transport medium and push it down to the bottom.5. Place the swab in the transport medium.

Nasopharyngeal swabs**First method**

1. Have the patient's head firmly supported.
2. Insert a nasal speculum.
3. Gently insert a wire swab through the nostrils to the posterior nasopharynx.
4. Rotate the wire swab gently and allow it to remain in that position for 20-30 seconds, and then withdraw deftly.
5. Place the swab in the transport medium.

Second method

1. Bend the cotton tip of the wire swab against the inside of the transport tube at a right angle.
2. Turn the patient's face against the light, and depress the patient's tongue so that the throat is well exposed and illuminated.
3. Insert the swab through the mouth and move it upwards behind the uvula and soft palate into the nasopharynx.
4. Swab the nasopharynx firmly and withdraw the swab, being careful not to contaminate the swab with throat and mouth secretions.
5. Place the swab in the transport medium.

Storage: Refrigerated (2-8 °C), if the specimen is not processed the same day.

Transportation: A cooling box is not necessary if the specimen reaches the laboratory within one day.

Reporting: Negative bacterial cultures are reported 24 hours after receipt of the specimen. Positive cultures are reported after 2-3 days.

To date there are facilities available to provide results for viral antigen detection within 24 hours of receipt of the specimen. Tissue culture results may take from 5 to 10 days.

Comments: None of pneumococci, *Haemophilus influenzae*, *Staphylococcus aureus* or enteric bacilli cause sore throat and therefore are not reported. Isolation of *Streptococcus pyogenes* in low numbers is sometimes done in otherwise healthy carriers.

If examination for other organisms is required, this should be stated on the request form. In patients with acute epiglottitis, an examination for *Haemophilus influenzae* is made.

For viral infections the laboratory result should be interpreted in conjunction with the clinical findings. A negative result may be confirmed by serological analysis.

EYE

CONJUNCTIVAL DISCHARGE

- Objectives:** Aetiological diagnosis of bacterial conjunctivitis by aerobic cultivation with identification and susceptibility test of the isolated bacteria and of viral conjunctivitis by direct antigen detection and cell culture.
- Test material:** Discharge from the eye(s).
- Collection time:** Before start of antibiotic therapy.
- Equipment:** Sterile cotton wool sponge, cotton tipped swab, test-tube with transport medium, clean microscope slide.
- Procedure:**
1. Pull down the lower eyelid so that the lower conjunctival fornix is exposed.
 2. Swab the fornix without touching the rim of the eyelid with the sterile cotton swab.
 3. Place the swab immediately in a bacterial or viral transport medium or, if the specimen is brought to the laboratory immediately, in a sterile test tube with 0.5 mL of buffered saline (pH 7).
- Storage:** Refrigerated (2-8 °C).
- Transportation:** Preferably send in a cooling box (2-8 °C).
- Reporting:** If suspicion of gonococcal infection in a newborn is suspected upon microscopic examination, the result should be conveyed to the treating physician immediately. All bacteria isolated in fair amounts and not resembling contaminants will be identified and tested for antibiotic susceptibility, including susceptibility to chloramphenicol. Culture results will be available in 1-2 days.

The main bacterial causes of conjunctivitis are pneumococci, *Staphylococcus aureus*, *Haemophilus influenzae*, *Streptococcus pyogenes*, *Moraxella lacunata*, gonococci, and enterobacteria. The main viral causes are adenovirus, picornavirus, measles, Molluscum contagiosum and Herpes simplex. The rim of the eyelids are heavily

colonized with bacteria, so if the sample is not taken correctly, the culture result can be quite misleading. Common contaminants are coagulase-negative staphylococci, viridans streptococci, diphtheroids, nonpathogenic neisseriae, and *Acinetobacter*.

Comments:

Bacterial conjunctivitis is characterized by a sticky mucopurulent discharge, whereas viral conjunctivitis has a watery discharge, pseudomembranes, follicles, petechial haemorrhages and superficial punctate keratitis.

Chlamydia trachomatis starts as a follicular conjunctivitis which may progress to punctate keratitis and occasionally to trachoma with follicles, papillae, and corneal pannus. Examination for *Chlamydia* is dealt with in the section “eye scraping for *Chlamydia*” (page 69-70).

Neonatal conjunctivitis is caused by many organisms. The gonococcus is the most serious cause producing an acute conjunctivitis within the first few days of birth, which may be followed by corneal ulceration, scarring and eventually blindness. *Chlamydia trachomatis* produces a more mild conjunctivitis within the first 2 weeks of birth. Bacteria from the mother’s birth canal may also infect the newborn child’s conjunctiva. Nongenital bacteria such as *Staphylococcus aureus* and *Streptococcus pyogenes* may also cause conjunctivitis in the newborn.

Granulomatous conjunctivitis is a rare unilateral conjunctivitis with local inflammatory granuloma and regional lymphadenopathy. It has many aetiologies, e.g. tuberculosis, syphilis, tularaemia, actinomycosis, sporotrichosis, lymphogranuloma venereum, eye worms and fly larvae.

Viral conjunctivitis is mainly caused by herpes-, paramyxo-, and enterovirus.

CONJUNCTIVAL SCRAPINGS FOR CHLAMYDIA TRACHOMATIS

Objectives: Aetiological diagnosis of inclusion conjunctivitis and trachoma by demonstrating the agent *Chlamydia trachomatis* by light microscopy or by immunological methods. Light microscopy lacks sensitivity, and the immunological methods are recommended for a definitive diagnosis.

Test material: Conjunctival discharge.

Collection time: Early in the infection when the number of organisms is high.

Equipment: Request form, cotton wool sponge, anaesthetic eye drops, spatula or sterile cotton or dacron tipped swabs, microscope slides or heat resistant glass vial, methanol or acetone.

Procedure: **Giemsa- or iodine-stained preparation**

1. Anaesthetize the conjunctiva with anaesthetic eye drops.
2. Carefully remove excess exudate from the surface of the eye before sampling.
3. Using a spatula with a thin, blunt end, scrape the whole of the conjunctiva.
4. Spread the specimen evenly on to the central area of a microscope slide.
5. Air dry the specimen, fix it with methanol for 2-3 minutes.

Immunofluorescent staining technique

1. Anaesthetize the conjunctiva with anaesthetic eye drops.
2. Carefully remove excess exudate from the surface of the eye before sampling.
3. Using a spatula with a thin, blunt end, scrape the whole of the conjunctiva.
4. Spread the specimen evenly on to the central area of a microscope slide.
5. Air dry the specimen, fix it with acetone for 2-3 minutes.

Enzyme immunoassay technique

1. Anaesthetize the conjunctiva with anaesthetic eye drops.
2. Carefully remove excess exudate from the surface of the eye before sampling.
3. Vigorously apply an appropriate swab to the lower lid conjunctiva of the affected eye.
4. Place the swab in 1 mL diluted transport medium in a heat resistant glass vial and send this immediately to the laboratory.

Storage: Send the sampling immediately to the laboratory.

Transportation: Preferably in a cooling box (2-8 °C), if transportation time is more than one hour.

Reporting: Specimens from newborns are examined immediately by microscopy and the result is conveyed to the treating physician. Specimens from older children and adults are considered “non urgent” specimens.

Comments: Neonatal inclusion conjunctivitis is distinguished clinically from the more serious gonococcal ophthalmia neonatorum by the latter’s shorter incubation period (3 days).

STOOL

CELLOPHANE TAPE METHOD FOR PINWORM

Objectives:	Aetiological diagnosis of pinworm (<i>Enterobius vermicularis</i>) infection by microscopic demonstration of the ova.
Material:	Perianal impression.
Collection time:	In the morning, before visiting the toilet or having a bath.
Equipment:	Request form, sticker, microscopic slide, clear cellophane tape, a wooden tongue depressor, paper for wrapping the slide.
Procedure:	<ol style="list-style-type: none">1. Fill in the request form.2. Cut off a piece of cellophane tape (10 cm long) and place one end on a microscope slide.3. Use gloves.4. Place the microscope slide with the tape partially attached to it on a wooden depressor and fold the tape over the end of the depressor to expose the gummed surface.5. With one hand press the buttocks apart to expose the perianal region.6. Press the gummed surface against several areas of the perianal skin.7. The gummed surface which has been in contact with the perianal skin is placed with the sticky side down on to the slide.8. Label the specimen.9. Wrap the microscope slide in a piece of paper.10. Wash hands.
Storage:	Room temperature.
Transportation:	No special requirements.
Reporting:	The result is send out the same day that the specimen is received.
Comments:	Three negative investigations taken on successive mornings are necessary to rule out infection. Eggs can be demonstrated in faeces in only 5% of cases.

STOOL FOR ENTEROPATHOGENIC BACTERIA

- Objectives:** Aetiological diagnosis of acute gastroenteritis due to *Salmonella*, *Shigella*, *Aeromonas*, *Campylobacter*, enterotoxigenic *E. coli*, *Vibrio cholerae*, non-cholera *Vibrio* and *Yersinia enterocolitica* by culture with identification and susceptibility test of the isolated organism.
- Pronounced and long-lasting diarrhoea in connection with antibiotic treatment should provoke an investigation for *Clostridium difficile*.
- Test material:** Diarrhoeal stool. Formed stool should be rejected for examination.
- Collection time:** Faecal specimens should be collected in the early stages of the disease when pathogens are usually present in the stool in large numbers, and preferably before antibiotic treatment is begun. The specimen should be collected in the morning to reach the laboratory before noon, so that it can be processed the same day.
- Equipment:** A suitable specimen container provided with a lid: a clean glass cup, a plastic or a waxed cardboard box, or a special container with a spoon attached to the lid or stopper; two sticks to transfer the specimen to the container. The use of penicillin bottles, match boxes and banana leaves, should be discouraged as they expose the laboratory staff to risk of infection.
- Procedure:**
1. Instruct the patient on how the specimen should be collected and transferred to the container; provide him/her with sticks and containers.
 2. The stool should be collected on a piece of toilet tissue or old newspaper.
 3. A sample is transferred with the sticks to the container. The specimen should contain at least 5 g of faeces and, if present, those parts that contain blood and/or mucopus should be selected. The specimen should not be contaminated with urine. Close the lid.

4. The specimen should be transported to the laboratory and processed within a few hours.
5. If a delay in shipment of the specimen to the laboratory is anticipated, stool specimens should be placed in a container with transport medium (Cary-Blair, Stuart or Amies) or glycerol-phosphate buffer 0,033 M. For cholera and other *Vibrio* spp. alkaline peptone water is an excellent transport (and enrichment) medium. In such media pathogens may survive for up to one week even at room temperature, although refrigeration is preferable. Transfer faeces repeatedly with an applicator stick to a container with the appropriate stool preservative.

Storage:	Refrigerated (2-8 °C).
Transportation:	Preferably in a cooling box (2-8 °C). If shipped through the mail, the transport tubes should be carefully wrapped and packed in a suitable, durable container that does not break during shipment.
Reporting:	Negative results are sent out 48 hours after receipt of the specimen. Results of positive cultures can be expected in 2-3 days.
Comments:	<p>Stool samples should be examined and cultured as soon as possible after collection. As the stool specimen cools, the drop in pH will inhibit the growth of most <i>Shigella</i> spp. and some <i>Salmonella</i> spp.</p> <p>The examination of the cellular exudate of diarrhoeal stools may give an indication of the organism involved. Clumps of polymorphonuclear leukocytes (>50 cells per high power field), macrophages and erythrocytes are typical of shigellosis. Polymorphonuclear leukocytes in smaller numbers (< 20 cells per high power field) are found in salmonellosis, typhoid fever, invasive <i>Escherichia coli</i>. Few leukocytes (< 2-5 cells per high power field) are present in the stools of cholera, viral diarrhoea and enterotoxigenic <i>Escherichia coli</i>. Degenerated ghost cells are typical for amoebic dysentery. Leukocytes and erythrocytes are also found in about half of the cases of campylobacter diarrhoea.</p>

STOOL FOR PARASITES

Objectives: Aetiological diagnosis of parasitic infection of the intestinal tract by microscopic examination of stool specimens and identification of the parasite(s).

Test material: Faeces.

Equipment: Specimen container: a clean glass cup, a plastic or waxed cardboard box, or a special container with a spoon attached to stopper or lid. The use of penicillin bottles, match boxes and banana leaves should be discouraged, as they expose the nursing and laboratory staff to risk of infection.

Procedure: **Direct smear and concentration method**

1. Faecal material should be delivered in a clean bedpan, or on a paper towel or a piece of toilet tissue.
2. It should then be transferred to a suitable container provided with a lid. The specimen should contain at least 5 g and, if present, those parts that contain blood and/or mucopus should be selected. The specimen should not be contaminated with urine or contain barium, bismuth or oil.

Trophozoites of *Entamoeba histolytica*

1. The specimen should be kept warm and examined within 1 hour after defecation without previous refrigeration.
2. It is generally recommended that the examination be performed immediately and on the spot. A wet mount in saline of the mucopus from liquid or bloody stool is prepared and examined immediately under the microscope.

Storage: Refrigeration is generally not recommended; instead the stool specimen can be preserved for several weeks by mixing the specimen with at least 3 volumes of preservative fluid. A 10% formalin (3,4% formaldehyde) solution is recommended and is prepared by diluting 50 mL of commercial formalin (34%) with 450 mL of distilled water.

- Transportation:** Formed stool specimen may be sent by normal shipment. Liquid specimens or specimens with mucopus and blood should reach the laboratory within one hour and protected from cooling during transport. In a cold climate the specimen should either be collected at the laboratory itself or sent in a thermos bottle.
- Reporting:** The results will usually be sent out within 24 hours of receipt of the specimen.
- Comments:** No technique claims to be 100 per cent successful for the detection of parasites by a single stool examination. Cysts of *Giardia lamblia* and *Entamoeba histolytica* and larvae of *Strongyloides stercoralis* tend to be excreted in "showers" and it is generally accepted that five serial stools must be examined before an individual is considered free from these infections. For helminths more than one specimen is seldom necessary for a correct diagnosis. For the diagnosis of pinworm (*Enterobius vermicularis*) infection, the cellophane tape method is recommended.
- Formed stools are examined microscopically after concentration for cysts, ova and larvae.
- Loose stools without blood or mucopus are examined microscopically in saline for trophozoites of protozoa and after concentration for cysts, ova and larva.
- Loose stools with blood and mucopus are examined microscopically in saline for trophozoites of *Entamoeba histolytica*, *Balantidium coli*, but not for cysts. Ova of *Schistosoma mansoni* or *S. japonicum* may be found.
- Taenia segments and adult pinworm, if present, are also reported.
- Special staining methods are needed (modified acid-fast stain, safranin-methylene blue) for the detection of oocysts of *Cryptosporidium* and *Isospora*.

STOOL FOR VIRUS

Objectives:	Aetiological diagnosis of poliovirus and other <i>enteroviruses</i> , rotavirus and adenovirus infections by demonstration of the organism in faeces by serological techniques and cell culture.
Test material:	Faeces, 4-8 gm (approximately 5 mL).
Collection time:	The excretion of virus in faeces is maximal in the first four days of illness, but the virus may still be excreted for several days or weeks (poliovirus). For poliovirus it is recommended that two samples be taken from each patient 24-48 hours apart.
Equipment:	Clean specimen tube, viral transport medium (see Appendix 2).
Procedure:	<ol style="list-style-type: none">1. The stool should be collected either directly into the container, or in a clean bedpan.2. It should then be transferred to a suitable specimen tube provided with a leak-proof lid. The specimen should not be contaminated with urine.3. If a rectal swab has to be used, the swab should be suspended in 2 mL of saline buffer.
Storage:	Refrigerated (2-8 °C).
Transportation:	Preferably in a cooling box (2-8 °C).
Reporting:	In case direct antigen detection methods are available (poliovirus, rotavirus, adenovirus) the results will be ready within hours. The results of cell culture will take at least 7-10 days.
Comments:	<p>The antigen detection tests are less sensitive than the cell culture technique. Culture and direct antigen detection for poliovirus should preferably be confirmed by serology. Positive isolation of poliovirus will be sent for intratypic differentiation.</p>

Rotavirus is seen mainly in toddlers and small children, between six months and 2 years of age. The disease decreases in prevalence up to 10-12 years of age. It is rare in newborns and adults.

SURFACE SPECIMENS

SKIN SCRAPINGS, NAILS AND HAIRS (DERMATOPHYTES)

Objectives: Aetiological diagnosis of dermatophytosis by microscopic examination, cultivation with identification of the isolated organism.

Test material: Skin scales, crusts, nails and hairs.

Equipment: Cotton swabs, disinfectant, sterile scalpel, sterile scissors, sterile forceps, clean paper pieces (6 x 6 cm).

Procedure: **Skin scrapings**

1. Cleanse the affected area with an alcohol swab.
2. Scrape or remove crusts close to the margin with a sterile scalpel.

Nails

1. Cleanse the nail with an alcohol swab.
2. Take snipping of the infected part of the nail with sterile scissors or a scalpel.

Hairs

1. Examine the patient's hair with a Wood's light.
2. Remove fluorescent or broken, dull hairs with sterile forceps for examination.
3. Collect the skin scales, crusts, nail snipping and hair bits on a clean piece of paper.
4. Fold the paper to form a flat packet and close the packet with a paper-clip. Label with the the patient's name, specimen number, source of material and the date of collection.
5. Fill in the request form and send the specimen and the form to the laboratory.

Storage: Room temperature.

Transportation: No special requirements.

- Reporting:** Direct microscopic examination of the specimen is the preferred method, as between 10-20% of the specimens, which show fungi by this method, are negative on culture.
- Comments:** *Malassezia furfur*, the aetiological agent of pityriasis versicolor, requires special media for cultivation and is generally not cultivated in clinical laboratories.

SKIN SMEARS AND NASAL SCRAPINGS (LEPROSY)

- Objectives:** Aetiological diagnosis of leprosy and estimation of the number and morphology of acid-fast bacilli by microscopic examination of stained skin smears and scrapings of nasal mucosa.
- Test material:** Skin tissue pulp, bits of nasal mucosa.
- Equipment:** Cotton wool sponge, ether, scalpel, microscope slides, spirit lamp, curette size 1 or 2 (Down Surgical HV-210-O1-D) or a paper-clip straightened out and the end hammered flat and fixed into a piece of wood or a piece of bicycle spoke, tissue towel.
- Procedure:**
- Skin smears**
1. Select the sites for the smears: 6-8 smears are taken from suspected lesions as well as from sites commonly affected in lepromatous leprosy, usually the forehead, ear lobes, chin, extensor surfaces of forearms, buttocks and trunk.
 2. Cleanse the area to be examined with a cotton wool sponge wetted with ether.
 3. Pick up the skin in a fold between the thumb and index finger of the left hand.
 4. Compress the fold hard between the fingers to drive out the blood.
 5. With a small-bladed sterile scalpel make an incision between the fingers of the left hand about 5 mm long and 3 mm deep, while the pressure of the fingers is maintained. If blood or juice appears, wipe it off. Make sure that the incision is deep enough to include the deepest portion of the dermis.
 6. The blade is then turned at a right angle to the cut and the wound is scraped several times in the same direction so that the tissue pulp collects on one side of the blade.
 7. The pulp is gently smeared on to a premarked area on a microscope slide. Multiple smears from the same patient are best smeared on to the same slide.

8. The smear is dried and fixed over a flame before being sent for examination.
9. The patient is given a piece of cotton wool to compress the cut until oozing stops. No bandage is necessary.

Nasal scraping

1. Have the patient positioned properly in good light.
2. With the aid of a torch and nasal speculum, scrapings are taken from the anterior part of the inferior turbinates, where they jut into the nasal cavity, one scraping from each side.
3. The material, which is usually slightly blood-stained, is picked up with the tip of a scalpel blade and smeared onto a premarked area on a microscope slide.
4. The smear is dried and fixed over the flame.

“Nose-blow” smear

1. Have the patient blow his nose in a tissue towel.
2. Immediately smear the mucous on to a separate microscope slide.
3. The smear is dried and fixed over the flame.

Storage: Room temperature.

Transportation: No special requirements.

Reporting: The result is available within 24 hours of receipt of the specimen.

Comments: As the laboratory diagnosis of leprosy depends solely on the microscopic examination, an estimation of the number of acid-fast bacilli in skin lesions is required to assess the severity of infection, its classification, response to treatment, contagiousness, as well as a verification of cure. However “the bacterial index” is slow to respond to alterations in the state of the bacilli due to treatment. For these reasons, a study of the morphology of bacilli becomes a valuable supplement. The degree of disintegration of bacilli is expressed as “the morphological index” or “the granularity index or SFG index (Ridley)”.

Routine nasal scrapings are not recommended; in fact they can be quite misleading for the diagnosis of leprosy. However they can be of great importance in determining whether a leprosy patient is

infectious or not. They are always positive in untreated lepromatous leprosy, but are negative in most cases of borderline lepromatous (BL) and in all cases of mid-borderline (BB), borderline tuberculoid (BT) and polar tuberculoid (TT). Furthermore, acid-fast bacilli disappear more rapidly from the nose as a result of chemotherapy, than they do from the skin.

SKIN SNIPS FOR *ONCHOCERCA VOLVULUS*

- Objectives:** Aetiological diagnosis of onchocerciasis by microscopic demonstration, identification and quantification of the microfilaria in skin snips.
- Test material:** Skin snips.
- Collection time:** No special requirements as the microfilariae of *Onchocerca* are non-periodic.
- Equipment:** Cotton swab, antiseptic solution, needles, sclerocorneal punches or razor blade or scalpel, distilled water, microscopic slides and cover slips or a microtiter plate.
- Procedure:**
- The cut method**
1. Select the sites with the highest numbers of microfilariae for examination. In central America the best site is over the scapula or iliac crest; in Africa the pelvic girdle, buttocks and external thigh; in Yemen the lower calf. However, in early infections and in localized light infections, the site of selections should be that in which the dermatitis is most marked. At least two snips should be taken, and in chronic cases five or six, if possible.
 2. The skin is cleansed with an alcohol sponge and allowed to dry.
 3. Insert a fine sterile needle almost horizontally into the skin, raise the point of the needle, lifting with it a small piece of skin measuring about 2 mm in diameter and height.
 4. Cut off the piece of skin with a sterile razor blade or scalpel.
 5. Immerse the skin snip in water or normal saline solution and place it on to a microscopic slide or in the well of a microtitration tray.
 6. After taking a skin snip, disinfect the instruments.
 7. Cover the preparation with a cover glass and place the slide or tray on a piece of damp tissue in a Petri dish or plastic box to prevent the preparation from drying out

8. Bring the specimen immediately to the laboratory.

The scarification method

1. The skin is cleansed with an alcohol sponge and allowed to dry.
2. The skin is scarified with a sterile needle.
3. A small amount of tissue fluid is carefully squeezed out between the thumb and index finger.
4. The fluid is collected on to a slide, dried, fixed and then sent to the laboratory.

The corneoscleral punch method

1. With the punch small pieces of skin are snipped off.
2. Disinfect the punch after use.

Safety precautions A suggested method is to disinfect needles, razor blades, scalpels or punches in cold 2% activated glutaraldehyde for 10 minutes.

Storage: At room temperature.

Transportation: Preferably within 5 minutes, as the movement of the microfilaria decreases and eventually ceases with time.

Reporting: The results will be available within 24 hours.

Comments: The species and number of microfilaria emerging from the skin snip are reported. The number will be reported as 1-4, 5-14, 15-49, 50-100 or more than 100 per snip.

Besides the microfilariae of *Onchocerca volvulus*, those of *Mansonella streptocerca* in Africa and *M. ozzardi* in South America may also inhabit the human skin. Microfilaria in the eye may also be looked at by using a slit lamp. The adult worms are found in the subcutaneous tissue or in nodules.

WOUND AND ULCERS

Objectives: Aetiological diagnosis of infected wounds and ulcers by culture with identification and susceptibility test of pathogenic bacteria.

Test material: Purulent material or tissue.

Equipment: **Swabs:** Forceps, cotton wool sponges, sterile cup with sterile saline, sterile cotton wool swab, test-tube with transport medium (Stuart, Cary-Blair or Amies), bandage.

Aspiration: Forceps, cotton wool sponges, 70% alcohol, syringe, needle, sterile cotton wool swab, test-tube with transport medium, bandage.

Procedure: **Swabs**

1. Inform the patient.
2. No-touch technique: remove bandage with the forceps.
3. With the forceps take a sponge, dip it in the saline and wash the surface of the wound or ulcers free from exudate.
4. Remove the swab from its covering and extend the tip of the swab deep into the wound, taking care not to touch the adjacent skin margins.
5. Remove the stopper from the test-tube with transport medium, plunge the swab into the transport medium and replace the stopper. If the wooden stick of the swab is too long, break off the end over the rim of the test-tube.
6. Apply new bandage.
7. Wash hands and fill in the request form.

Aspiration

1. Inform the patient.
2. With the forceps take a sponge, wet it with alcohol and decontaminate the margins of the wound or ulcer. Let the alcohol dry in between, and repeat the decontamination once more.
3. Insert the needle through the decontaminated margin and aspirate the material from the depth of the wound.

4. Squirt some of the material slowly on to a sterile cotton wool swab and plunge the swab into the transport medium. The rest of the material is deposited on a microscope slide and a smear is made with the needle.
5. Apply new bandage.
6. Wash hands and fill in the request form.

Storage: Refrigerated (2-8 °C).

Transportation: In a cooling box (2-8 °C), preferably.

Reporting: A preliminary result is reported 2 days after the specimen is received. The final report is often delayed for 4-5 days.

Comments: Cultures from wounds and ulcers are frequently contaminated with colonizing and environmental bacteria, and swab samples often do not reflect the true cause of the infection. For this reason, the most profitable method of collecting wound specimens is aspirating loculated purulent material from the depths of the wound with a sterile needle and syringe. The wound margins should be decontaminated as much as possible with soap and alcohol before the material is aspirated.

Except for certain ulcers peculiar to the tropics, the most common skin ulcers and wound infections are the same as those seen in temperate climates.

Tropical ulcer is a specific acute ulcerative skin disease limited to tropical and subtropical regions with a characteristic slough containing, in its early stages, numerous fusiform bacilli and spirochaetes. If neglected, it may develop into a chronic non-specific ulcer which is indistinguishable from indolent ulceration resulting from other causes.

Cancrum oris is an infective gangrene of the tissues of the cheek or mouth occurring in undernourished children, generally as a complication of Vincent's Disease or ulcerative gingivitis. As in these diseases, fusiform bacilli and spirochaetes play an important role in the early stage of the disease, though their presence in the slough is often later obscured by heavy mixed secondary bacterial flora.

Cutaneous diphtheria is an acute infection of the skin mainly seen in children due to *Corynebacterium diphtheriae*. It is more common in tropical countries where pharyngeal diphtheria is rare. The lesions are extremely variable (ulcerative, eczematous, impetiginous, vesicular, pustular, bullous, or gangrenous) and cardiac and nervous manifestation due to absorption of toxin may appear.

Buruli ulcers is an indolent ulcerative skin lesion appearing in foci in Australia (Bairnsdale), Central Africa (Kakerifu, Kasongo in East Zaire, lower Zaire, Congo-Brazzaville, Gabon, Nigeria, Buruli in Uganda, Tanzania) South-East Asia (Malaysia, Sumatra, New Guinea) and Mexico, due to *Mycobacterium ulcerans*. The lesion starts as a nodule which breaks down to an ulcer with undermined edges and an abundant yellowish necrotic gelatinous slough. Satellite lesions are often seen.

Cutaneous leishmaniasis is an ulcerative skin disease endemic in several countries around the Mediterranean littoral, the Middle East, West Asia and South and Central America due to various species of *Leishmania*. The lesion starts as a nodule which may remain indolent or become painful and ulcerate; it may be single, multiple or diffuse, self-limiting or chronic and may involve the nasopharyngeal tissue (espundia) as well.

SURGICAL SPECIMENS

BONE MARROW

- Objectives:** Aetiological diagnosis of visceral leishmaniasis by microscopic examination and identification of the isolated organism. Bone marrow examination may also be useful in the diagnosis of African and American trypanosomiasis, congenital toxoplasmosis, malaria, infectious granulomatous infections such as brucellosis, tuberculosis, and histoplasmosis, when other investigations are negative.
- Contraindication:** Haemophilia and major bleeding diathesis.
- Test material:** Bone marrow.
- Collection time:** During laboratory hours.
- Equipment:** Request form, labels, cotton wool swabs, antiseptic solution, sterile gloves, lidocaine 1% with epinephrine 1%, syringe, needle, sternal needle with obturator and shield (Salah, Klima) or Radner needle, Luer-Lok syringes, culture tubes with NNN and Schneider's drosophila medium, microscope slides, Pasteur pipette, glass slide spreader, plaster.
- Procedure:**
- Sternal puncture**
1. The collection should only be made by a physician experienced in the procedure. Inform the patient about what will happen and get his acceptance.
 2. Fill in the request form, mark the microscope slides with name, date, department and "sternal", and arrange them in a slanted position.
 3. Wash hands carefully.
 4. Draw up the local anaesthetic (3-5 mL) with needle and syringe.
 5. Place the patient on his back.
 6. Identify the aspiration site in the midline of the sternum at the height of the second intercostal space.
 7. Disinfect the site two times and let it dry in between; shave, if hairy.

8. Infiltrate the site with the local anaesthetic from the skin to the bone. It is important that some of the local anaesthetic is forced under the periosteum, which is best done by changing over to a stouter hypodermic needle with a very short bevel, after making the preliminary intradermal bleb; otherwise very severe pain will be experienced while penetrating the bone and particularly while sucking out the sample with the syringe.
9. Adjust the shield of the sternal needle to a 5-10 mm depth.
10. Hold the needle perpendicular to the skin, insert down to the skin and advance through the anterior table of the sternum with a rotating movement. Decrease in resistance indicates entry into the marrow cavity.
11. Remove the obturator and attach a tightly fitting syringe to the needle and gently aspirate 0.5 mL of marrow contents. Remove the syringe from the needle, replace the obturator.
12. When sufficient specimen is obtained, remove the needle, apply pressure with a sponge until bleeding stops and cover with a plaster.
13. Deposit two drops of the aspirate at the bottom of a sterile specimen tube for culture or directly into the culture media.
14. Place a drop of the aspirate on each slide and let it run down the slide. Immediately suck away the excess blood with the Pasteur pipette, leaving the marrow particles behind. Spread the specimen with the slide spreader. Air dry the films and fix in methyl alcohol.

Crista puncture

1. Place the patient on his side with the hips and knees slightly bend and the back flexed.
2. Locate the aspiration site:
Anterior iliac crest: the centre of prominence of anterior superior iliac spine, just below the lip of crest.
Posterior iliac crest the centre of the posterior superior iliac spine.
3. Disinfect the aspiration site two times and let it dry in between. Place the sterile equipment on a sterile tray.
4. Infiltrate with local anaesthetic from the skin to the bone.
5. Collect the Radner needle and place the obturator correctly, so that the cutting edge on both the needle and the obturator turns the same way.

6. Hold the Radner needle nearly perpendicular to the superior anterior/posterior iliac spine and with a firm turning force penetrate the bone compacta.
7. When the compacta are bored through, the resistance is suddenly reduced and a brittle crackling sound is heard. Continue to bore the needle half a cm longer into the spongiosa.
8. Remove the obturator and fix a 10 mL syringe with the connector to the needle.
9. Gently aspirate 0,5 mL of bone marrow. Vigorous aspiration is painful and increases the dilution with peripheral blood.
10. Remove the syringe, replace the obturator. If sufficient specimen is obtained, loosen the Radner needle from the bone with a rotating movement and then slowly withdraw it.
11. Deposit two drops of the aspirate at the bottom of a sterile specimen tube for culture or directly in the culture media.
12. Place a drop of the aspirate on each slide and let it run down the slide. Immediately suck away the excess blood with the Pasteur pipette, leaving the marrow particles behind. Spread the specimen with the slide spreader. Air dry the films and fix in methyl alcohol.

Complications:**1. Pro- and retrosternal haematomas**

Prevention: Evaluate haemostasis if bleeding diathesis is suspected, use atraumatic technique.

2. Bleeding from puncture site

Prevention: Evaluate haemostasis if bleeding diathesis is suspected; apply pressure after puncture.

3. Perforation of aorta

Prevention: Use depth guard on bone marrow needle.

Storage:

Room temperature.

Transportation:

The slides and specimens for culture should immediately be brought to the laboratory.

Reporting:

Microscopic examination is made the same day and the result is reported immediately to the treating physician. The culture may take up to 3 weeks or more before a negative result is reported.

Comments:

Demonstration of leishmania by direct microscopy or by culture confirms the diagnosis. Bone marrow aspiration is less rewarding compared to spleen puncture, and the organisms may be extremely few although abundant in the other organs, particularly in the initial stage of the disease. However, bone marrow aspiration is usually safe, and as fatalities have been reported from splenic puncture, this examination should be reserved for patients in whom the marrow has proved negative. The examination may also be used to establish successful chemotherapy.

LIVER ABSCESS

- Objectives:** Aetiological diagnosis of amoebic and pyogenic liver abscess by microscopic examination and culture with identification and susceptibility test of the isolated organism.
- The aspiration should not be done if the patient is uncooperative, has a bleeding disorder, an infection in overlying skin, pleura, lung or peritoneum, or a severe extrahepatic obstruction.
- Test material:** Creamy to yellowish-green pus or reddish-brown gelatinous material.
- Equipment:** Sterile gauze sponge, antiseptic solution, medicine cup, sterile towels, sterile gloves, syringe, needles, lidocaine 1%, scalpel with blade, wide-bore needle or Potain's aspirator, plaster.
- Procedure:**
1. The aspiration should be done in the operating theatre taking strict aseptic precautions, and only by a physician experienced in the procedure.
 2. Place the patient in a left dorso-lateral position with the right arm abducted over the head.
 3. If available, needle aspiration should be carried out using ultrasound guidance. If ultrasound examination is not available, the exploratory puncture should be carried out at the site of localized tenderness, pain, oedema, pneumonic crepitus, pleuritic or peritoneal friction. If no localizing sign is present, the needle should first be inserted in the anterior axillary line in the eight or ninth costal interspace, but not more than 8 cm deep, as the distance of the inferior vena cava from any part of the chest wall in adults is only 10 cm.
 4. Abscess may be aspirated with a wide-bore needle fitted on to a 50 mL syringe, but it is much more comfortable for the patient to remove large abscesses with a Potain's aspirator.
 5. The Potain's aspirator consists of a graduated glass bottle fitted with a rubber cork pierced by a T-shaped tube with two stopcocks (no. 1 and no. 2). Both stopcocks are connected via rubber tubing - no 1 is connected to a syringe pump and no. 2

to the aspirating needle. The air from the glass bottle is exhausted by the metal pump, while stopcock no. 1 is kept closed. After the needle has been introduced into the abscess cavity, the stopcocks no. 1 and no. 2 are opened and the necrotic material enters the exhaust bottle by suction.

6. Put on sterile gloves, drape with sterile towels, and disinfect the puncture site two times with skin antiseptic solution and let it dry in between.
7. Infiltrate with local anaesthetic from the skin through the intercostal muscles to the liver capsule, keeping the bevel of the needle at the upper edge of the rib. Do not advance the needle through the capsule into the liver.
8. Make a 4 mm skin incision with the scalpel blade.
9. Affix the needle on the syringe, or assemble the Potain's aspirator.
10. Introduce the aspirating needle through the skin incision and through intercostal muscles. The patient is asked to breathe superficially while the needle is advanced gently into the liver pointing against the abscess, or medially and cephalad if the abscess is not identified. The needle swings with the respiration when the needle is inside the liver.
11. When it is felt that the needle is inside a space, the needle is inside the abscess cavity and the aspiration can begin. As much of the necrotic material as possible should be removed, but not more than 1000 mL in each trial.
12. 2-5 mL of the necrotic material should be collected in a sterile specimen tube and sent for culture and sensitivity test, and the last few mL of aspirated material should be collected separately and sent for examination for amoeba trophozoites.
13. When the aspiration is finished, the needle is removed and the puncture site is covered with a broad bandage.
14. If no material is found at the initial trial, one or two other directions may be tried, but it is unwise to persist with more trials. It is better to repeat the attempt one or two days later making use of ultrasound or scintigraphic examination to aid localization.

If more than 250 mL of material is withdrawn initially, or if swelling or local tenderness recurs, aspiration should be repeated at two- or three-day intervals.

15. If the material has a foul odor and is cream-, yellow- or green-stained, it indicates a pyogenic abscess or a secondary infected amoebic abscess. Bile-stained material indicates communication with the biliary system; the response in these patients is notoriously slow and large quantities of fluid are sometimes obtained even after repeated aspirations.
16. If no pus is found, a decision must be made to carry out open drainage or to continue conservative therapy.

Open drainage is indicated, if

1. pus is too thick or contains slough
 2. pus is too much (500-1000 mL per day)
 3. pus is infected
 4. spontaneous rupture
-
-

17. If there is more than 200 mL of aspirate, further aspiration will be needed and this may be repeated daily.

Complications:

1. Haemorrhage into abscess cavity, haemobilia, bile peritonitis, hepato-portal AV fistula

Aetiology: Laceration of blood and bile vessels due to trauma, bleeding disorder, bile leak, respiratory motion.

Prevention: Check coagulation parameters, pass needle gently into the liver, have patient hold breath during aspiration.

2. Secondary bacterial infection

Aetiology: Introduction of organisms into cavity.

Prevention: Use meticulous aseptic technique.

3. Pneumothorax

Aetiology: Lung laceration due to respiratory motion.

Prevention: Have the patient hold breath during aspiration.

4. Haemothorax

Aetiology: Laceration of intercostal vessels or lung.

Prevention: Pass the needle through the intercostal space at the upper edge of the rib.

5. Injury to other viscera

Aetiology: Improper needle placement.

Prevention: Precise determination of liver and abscess before aspiration.

Storage: Specimens for bacteriological investigation may be stored in the refrigerator (2-8 °C). Specimens for parasitological investigation should be kept warm (32-35 °C).

Transportation: The specimen for parasitological investigation should immediately be brought to the laboratory, preferably in a thermos flask (32-35° C).

Reporting: Positive findings of amoebae in the aspirate will be reported immediately. A negative culture report will be sent out two days after the specimen is received.

Comments: The indications for aspiration are:

1. a palpable mass or ultrasonic demonstrated mass;
2. persistent localized tenderness;
3. clinical and radiographic evidence of a markedly raised hemidiaphragm;
4. failure of signs and symptoms to remit with specific therapy.

Aspiration of "pus" in which amoebae can be identified is diagnostic. Intestinal symptoms are often absent, and stools show amoebae in only 10 per cent of cases. Secondary infection may occur, especially after surgical intervention. Pyogenic liver abscess is usually secondary to bacteremia, septicemia or suppurative cholangitis, but there is a number of cases in which no cause is found. The main organisms isolated are *Escherichia coli*, anaerobic streptococci, *Staphylococcus aureus*, *Streptococcus faecalis*, and sometimes *Salmonella typhi*. In the Far East, suppurative cholangitis and liver abscess may be associated with liver flukes or immature lung flukes. *Ascaris* may invade the bile duct and cause cholangitis and liver abscess, but this is rare.

Most amoebic liver abscesses are situated in the upper and back part of the right lobe. Small liver abscesses will probably resolve on specific therapy alone. Large abscesses require drainage which is best achieved by closed aspiration with a wide-bore needle. Unless specific and adequate therapy is given, the condition will recur.

LIVER BIOPSY

Objectives: Aetiological diagnosis of liver disease by microscopic examination and culture with identification of the isolated organism.

The biopsy should not be done in uncooperative patients, in patients with a bleeding disorder, an infection of overlying skin, pleura, lung or peritoneum, in patients with suspected liver abscess, severe extrahepatic obstruction, or in patients where the liver is difficult to localize due to ascites.

Test material: Liver tissue.

Equipment: Request form, labels, sterile gauze sponges, antiseptic solution, sterile gloves, sterile towels, lidocaine 1 %, syringe, needles, Menghini needle, scalpel blade, sterile saline, plaster.

Procedure

1. The biopsy should only be performed by a physician experienced in the procedure.
2. Check haematocrit, bleeding time, platelet count, prothrombin time, and partial thromboplastin time.
3. Place the patient on his back, right side near the edge of the bed, right hand under the head, and the head turned to the left.
4. Percuss maximal liver dullness between anterior and mid-axillary lines after end of expiration and mark one intercostal space below.
5. Disinfect the skin, drape with sterile towels, and put on sterile gloves.
6. Let the patient practice holding his breath for 10 seconds after full expiration.
7. Infiltrate with local anaesthetic from the skin through the intercostal muscles to the liver capsule, keeping the bevel of the needle at the upper edge of the rib. Do not advance the needle through the capsule into the liver.
8. Assemble the Menghini needle and syringe: insert the “nail” into the proximal end of the needle to prevent aspiration of

- biopsy specimen into the syringe; fill the syringe (20 mL) with 10 mL sterile saline and attach the needle to the syringe.
9. Make a 4 mm skin incision with the scalpel blade.
 10. Insert the Menghini needle through the skin incision and advance the needle parallel to the bed towards the xiphoid into, but not through, intercostal muscles. Flush the needle with 0,2 mL saline.
 11. Have the patient hold his breath at full expiration. Apply constant suction on the syringe and with a rapid, smooth motion, advance the needle 4-5 cm into the liver and withdraw. The total duration of this movement should not exceed 1 second.
 12. Apply a plaster.
 13. Expel the biopsy specimen into a sterile Petri dish.
 14. Divide the specimen into two smaller (0,5 cm long) and one larger sample. Inoculate the culture tubes with the smaller samples.
 15. Roll the larger sample on to one or two microscopic slides. Place the sample on to filter paper and suspend it in 10% formalin.
 16. Have the patient lie on the right side for 2 hours and remain in bed for 8 hours, check pulse and blood pressure frequently, and have a haematocrit taken 4 and 12 hours after the biopsy.

Complications:

1. Haemorrhage, intrahepatic haematoma, haemobilia, bile peritonitis, hepato-portal AV fistula

Aetiology: Laceration of blood or bile vessels due to direct trauma, bleeding disorder, bile leak, respiratory motion.

Prevention: Use biopsy needle 1,2 mm in diameter or less, keep needle in liver less than 1 second, have patient hold breath during biopsy.

2. Pneumothorax

Aetiology: Respiratory motion causing lung laceration, when needle passes through the lung.

Prevention: Use biopsy needle 1,2 mm in diameter or less, keep the needle in the liver less than 1 second; full expiration minimizes chances of the needle passing through the lung.

3. Haemothorax

Aetiology: Laceration of intercostal artery or vein, lung laceration.

Prevention: Pass the needle through the interspace at the upper edge of the rib to avoid the intercostal vessels.

4. Injury to other viscera

Aetiology: Improper needle placement.

Prevention: Precisely determine the liver location before biopsy.

5. Needle fracture

Aetiology: Striking rib with needle.

Prevention: Before rapid needle thrust, assure intercostal placement.

Storage: *Leishmania* protozoa do not survive long outside the human body, and if the specimen is to be cultured for this organism, it must be processed without delay.

Transportation: The transportation time should be as short as possible, and the specimen should not be kept in a cooling box.

Reporting: The results of the microscopic examination will be available the same day, and, if positive, are reported immediately. Cultures for *Leishmania* and *Histoplasmas* are examined twice weekly for 21 days before being reported as negative. Culture for *M. tuberculosis* may take 2-4 weeks.

Comments: If the laboratory diagnosis is delayed, look for other clinical signs and epidemiological features. In miliary tuberculosis, tubercles may be found in the ocular fundus and on a chest radiogram. *Histoplasma capsulatum* produces a respiratory illness which rarely progresses into the disseminated form. *Histoplasma duboisii* is restricted to West, East, and Central Africa and usually has lesions in other organs (lung, skin, bone). Visceral leishmaniasis is widespread and occurs in the eastern part of India, Burma, countries surrounding the Mediterranean littoral and the Red Sea, Iran, Iraq, southern Russia, northern China, East Africa, northeast Brazil, Paraguay, and sporadically in Central America and some of the northern countries of South America.

LYMPH NODE PUNCTURE

- Objectives:** Aetiological diagnosis of septic lymphadenitis, chancroid, lymphogranuloma venereum; secondary syphilis; mononucleosis, tuberculosis, brucellosis, plague- or anthrax lymphadenopathy; visceral leishmaniasis; bartonellosis; African and American trypanosomiasis and toxoplasmosis by microscopic examination and culture with identification and susceptibility test.
- Test material:** Lymph node pulp.
- Collection time:** Preferably during the laboratory's open hours.
- Equipment:** Request form, labels, antiseptic solution (Appendix 5), cotton wool sponge, tight-fitting syringe, needle, specimen tube, microscope slides.
- Procedure:**
1. Should only be done by a physician. Disinfect the skin two times and let it dry in between and before performing the puncture.
 2. Immobilize the lymph node between two fingers.
 3. Pierce the skin and capsule with the needle attached to the syringe.
 4. Massage the lymph node over the tip of the needle with the left hand while creating strong suction with the syringe.
 5. Withdraw the needle.
 6. Detach the syringe from the needle; pus or tissue may remain within the needle.
 7. Fill the syringe with air and squirt the specimen:
 - into a sterile tube and close with sterile stopper for culture;
 - on to a glass slide and make a smear for light field microscopy; and
 - on to a glass slide, cover with cover slip and examine under a dark field microscope.
 8. Bring the specimens to the laboratory immediately.

- Storage:** Specimens for culture of bacteria may be stored in the refrigerator; specimens for culture of protozoa should be stored at room temperature.
- Transportation:** Send the specimen to the laboratory immediately, not in a cooling box.
- Reporting:** The microscopic examination is made the same day and the results reported immediately to the treating physician. All positive findings are conveyed to the treating physician. Cultures may take several weeks before a negative result is sent out.
- Comments:** To restrict the number of investigations and cultures on each specimen, it is important to state on the request form which organ is involved, other symptoms, where and how the infection was contracted, and the most likely diagnosis.

In Europe, **tuberculosis** of the cervical glands was previously not an uncommon condition and usually caused by *Mycobacterium bovis*. The infection developed as a result of consumption of milk containing the organism. Investigations in India and in other tropical countries have demonstrated that in these countries cervical tuberculous lymphadenitis is not caused by *M. bovis*, but by *Mycobacterium tuberculosis*.

Syphilitic glands are almond-shaped, firm, painless and not adherent. The lymph nodes close to the chancre are the first to be enlarged; later all lymph nodes become involved, including the occipital nodes which are only rarely affected in other diseases, except in impetigo capitis, kerion, pediculosis capitis, German measles and African trypanosomiasis.

In **infectious mononucleosis** it is mainly the cervical glands which are involved, though generalized enlargement sometimes occurs. Tonsillitis, pronounced lymphocytosis and a petechial rash on the soft palate usually provides the diagnosis.

Cat-scratch fever is mainly seen in children and presents with an inflamed pustule and enlarged nodes which later suppurate.

Localized enlargement of the lymph nodes is mainly caused by septic infections of the skin, infections of the throat and rarely by

chancroid, lymphogranuloma inguinale, lupus vulgaris, plague or anthrax.

Enlargement of the mesenteric lymph nodes can be seen with any inflammatory condition of the bowel, including salmonellosis, shigellosis, yersiniosis, tuberculosis and brucellosis.

PELVIC ABSCESS AND PERITONITIS

- Objectives:** Aetiological diagnosis of pelvic abscess or peritonitis by microscopic examination and aerobic and anaerobic culture with identification and susceptibility test of the isolated organism (gonorrheal, pyogenic or tuberculous).
- Material:** Pus, seropurulent effusion, sometimes bloody.
- Collection time:** Preferably before any antibiotics are administered.
- Equipment:** Request form, labels, sterile cotton sponges, antiseptic solutions, long dressing forceps, sterile drapes, lidocaine 1%, syringes, spinal needle, Graves vaginal speculum, cervical tenaculum, sterile specimen tube.
- Procedure:**
1. The collection should be made by a physician. Inform the patient about the procedure and get her acceptance.
 2. Have the patient lie in the dorsal recumbent position with the feet resting on the stirrups of the examination table.
 3. Perform a rectovaginal and pelvic examination and note the position of the uterus, and look for fullness of cul-de-sac and the presence of adnexal or pelvic masses.
 4. Insert the Graves bivalve speculum, locate the posterior fornix, grasp the posterior lip of the cervix with the tenaculum and pull the cervix forward and upward.
 5. With the 22-gauge spinal needle infiltrate the posterior fornix in the midline with local anaesthetic, just below the vaginal reflection on the cervix.
 6. Maintain traction on the uterus and insert the 18-gauge spinal needle on the 3-ring syringe, and thrust the needle through the posterior fornix parallel to the axis of the uterus.
 7. Aspirate any fluid and remove the needle.
 8. Inject it into a sterile specimen tube for Gram stain, culture and susceptibility tests.
 9. Fill in the request form and label the specimen tube.

Complications:	<p>1. Aspiration of air or faeces (needle in rectum) Aetiology: Needle directed posteriorly into rectum. Prevention: Insert needle in the midline of posterior fornix parallel to axis of the uterus.</p> <p>2. Aspiration of blood that clots on standing Aetiology: Needle directed laterally with puncture of mesenteric or pelvic veins. Prevention: Direct needle exactly in the midline.</p>
Storage:	Refrigerated (2-8 °C).
Transportation:	Preferably in a cooling box (2-8 °C) if transportation time is delayed for more than 1 hour.
Reporting:	Negative culture results are reported 2 days after the specimen is received. Positive culture results may take more than 3 days before they are ready.
Comments:	If blood is aspirated, inject it into a stoppered glass tube. Absence of clotting confirms the presence of intraperitoneal blood. If pus is obtained, this verifies a pelvic inflammatory disease. If serous fluid is aspirated, the investigation is inconclusive.

PLEURAL BIOPSY

Objectives: Aetiological diagnosis of pleural effusion or pleural thickening due to pleural tuberculosis, by microscopic examination and culture with susceptibility test of the isolated organism.

There is a relative contraindication against the biopsy in patients with infection in overlying skin, bleeding diathesis or on anticoagulant therapy.

Test material: Parietal pleural tissue.

Equipment: Request form, labels, surgical gloves, mask, drape, sterile cotton wool swabs, antiseptic solution, syringe, needle, 1% lidocaine with 1% epinephrine, scalpel blade, Cope needle (Abraham Harefield or Vim Silvermann), syringe, sterile conical specimen tubes with stoppers, plaster.

Procedure:

1. The collection should be made by a physician. Fill in the form and identify the patient.
2. Review a chest X-ray not more than 24 hours old and localize the biopsy area.
3. Use a puncture site one or two interspaces below the fluid level, but not lower than eight intercostal space in the angular, posterior or anterior axillary line.
4. Place the patient either in a sitting position on the edge of the bed, with the arms placed on two bulky pillows over the back of a chair or lying in a supine lateral position.
5. Use mask and gloves, disinfect the selected puncture area and drape.
6. Pull the skin down and infiltrate local anaesthetic from the skin to the parietal pleura. Aspirate fluid to confirm the position and mark the needle depth with a clamp. Withdraw the needle.
7. Make a 1-2 mm incision of the skin with the scalpel on the puncture site.
8. Assemble the Cope needle: place the obturator in the needle, place the needle in the cannula and adjust the movable sleeve

to the measured depth from the local anaesthetic and fix the screw.

9. Insert the Cope needle through the incision to the measured depth; withdraw the obturator from the needle, and replace it with the 10 mL syringe. Confirm the presence in the pleural cavity by aspiration of fluid.
10. Withdraw the needle and cannula slightly until gentle aspiration does not yield any fluid; this places the cannula just outside the pleura. Remove the needle from the cannula.
11. Place the syringe on a curette and insert the curette into the cannula until it is within the pleural cavity, which is demonstrated by aspiration of fluid.
12. Perform the biopsy: slant the cannula with the curette 15-20° C from the perpendicular position to catch the parietal pleura firmly with the hook. With the curette held stationary, advance the cannula with a rotating motion to transect the specimen held in the hook.
13. Withdraw the cannula and curette slightly, until the cannula is just outside the pleura. Remove the curette, and extract the specimen with a hypodermal needle and place it in sterile saline in a specimen tube.
14. Insert the curette again into the cannula, repeat the procedure biopsying the other three quadrants of the puncture site.
15. Suture the incision and apply a sterile bandage.

Complications:

Pneumothorax

Aetiology: Aspiration of air through cannula, laceration of lung.

Prevention: Never remove syringe from needle or leave the cannula open to air when in the pleural space; insert needle no further than necessary to collect the fluid.

Haematoma or haemothorax

Aetiology. Biopsy of intercostal vessel, bleeding diathesis.

Prevention: Insert the needle at the superior margin of the rib, check the clotting status before performing the biopsy.

Inadequate specimens:

Aetiology: Bent hook on curette.

Prevention: Inspect the hook at the end of the curette before use.

Storage: Refrigerated (2-8 °C).

Transportation: No special requirements.

Reporting: The results of the microscopy will be available the same day the specimen is received. A negative culture result will first be reported after 4-6 weeks. Positive findings will be reported immediately.

Comments: Demonstration of acid-fast bacilli by microscopy or culture confirms the diagnosis.

PUS FROM ABSCESS AND MYCETOMA

Objectives: Aetiological diagnosis of skin abscess, visceral abscess and mycetoma by microscopic examination and culture of the pus with identification and susceptibility test of the isolated organism(s).

Test material: Pus.

Collection time: Preferably before administration of antibiotic therapy.

Equipment: Request form, labels, syringe, needle, sterile specimen tube, sterile cotton wool swab, transport medium.

Procedure: Fill in request forms and labels for each specimen tube.

Subcutaneous abscess

1. Disinfect the skin over and around the abscess.
2. With syringe and needle aspirate the abscess, or incise the abscess and swab the bottom of the abscess wall with a sterile swab.
3. Remove the needle and squirt the pus into a specimen tube or place the swab into the transport medium.

Mycetoma

1. Remove the crust over the sinus opening.
2. With a sterile Pasteur pipette aspirate pus and draining granules from the sinus.

Visceral, intra-abdominal, pelvic and retroperitoneal abscess

1. Locate the abscess by ultrasound, gallium scans or CT scan.
2. Aspirate the abscess under ultrasound guidance.
2. Squirt the pus into a sterile specimen tube.

Storage: Refrigerated (2-8 °C).

Transportation: Preferably in a cooling box (2-8 °C) if delayed for more than two hours.

- Reporting:** Negative results or preliminary positive results without susceptibility test are sent out after two days. Positive culture may take more than 3 days.
- Comments:** Identification and sensitivity testing is generally made on all organisms of clinical importance.

SIGMOIDOSCOPY

- Objectives:** Aetiological diagnosis of large bowel infections, such as bacillary dysentery, amoebic dysentery, tuberculous colitis and gonorrheal proctitis, by microscopic examination and culture with susceptibility test of isolated organism(s).
- Test material:** Pus, necrosis or biopsy material of the mucous membrane.
- Equipment:** Request form, bed or sigmoidoscopic table, lubricants, gloves, 25-30 cm sigmoidoscope with eyepiece, light, obturator and inflating bulb, suction tip cannula with tubing, suction source, biopsy forceps, insulated suction tip and electrocautery, specimen tubes.
- Procedure:**
1. Give the patient a laxative the night before and an enema $\frac{1}{2}$ -1 hour before the examination. Laxative and enema is contraindicated in patients with massive gastrointestinal haemorrhage, diarrhoea, or inflammatory bowel disease.
 2. Explain to the patient what will happen. Fill in request form.
 3. Position:
 - Knee-shoulder:** The patient rests on the surface of the bed with both knees, left shoulder, left side of the face, and right arm on the bed. The head should be turned toward the right with left side of the face resting on the table; the left arm extends towards the right beneath the chest; thighs must be perpendicular to the surface.
 - Left Sim's:** The patient is placed on the left side with the head resting on a pillow over the left arm and the buttocks placed slightly over the edge of the bed.
 - Head down:** Requires a proctoscopy table. The patient is asked to kneel down on the knee-rest, bend forward and place his forearms against the armrest with his forehead resting in his hands.

4. Wear gloves and inspect the anus and perineum.
5. Place the obturator in the sigmoidoscope and lubricate the distal end and obturator.
6. Separate the buttocks and gently insert the sigmoidoscope about 6-8 cm into the anus aiming for the umbilicus.
7. Remove the obturator and attach the cecopiece. Advance towards the os sacrum under direct vision, never advance the scope blindly or unless lumen is clearly visible ahead. Insufflate air if necessary to distend the bowel and facilitate passage. Gentle pressure against an area of spasm is permissible only if the lumen is visible ahead, and gentle angulation is permissible to negotiate the rectosigmoid flexure.
8. When the sigmoidoscope is advanced as far as is comfortable for the patient, withdraw the scope while examining the bowel. Use one hand at the anus as a support and sweep the instrument around the entire circumference of the bowel to visualize all areas. Keep bowel lumen distended with air to prevent redundant mucosa from obscuring lesions.
9. Note the appearance of the mucous membrane: look for ulcerations, bleeding, polyps, tumours, exudates, mucosal injection, haemorrhoids, fissures, and infection. Note location of lesions: distance from anus and position on circumference of rectum.
10. Biopsy suspicious lesions: remove cecopiece, insert the biopsy forceps, and biopsy the lesion. Avoid full-thickness biopsy of the bowel wall. Cauterize the bleeding point with the insulated suction tip.
11. Material from the mucosal surface should be obtained by aspiration or scraping.
12. Withdraw the sigmoidoscope and finally make a digital exploration of the ano-rectum, the pelvis and the pelvic organs.
13. Mucopus and biopsies are transferred to a specimen tube and immediately sent to the laboratory for direct examination.

Complications:**Bowel perforation**

Aetiology: Excessive force in advancing sigmoidoscope, full-thickness biopsy of bowel wall or necrosis

from excessive electrocautery, insufflation of excessive air.

Prevention: Do not advance the scope unless the bowel lumen is clearly visible; do not use force in negotiating bowel angulation; insufflate only enough air to produce slight bowel distension; biopsy only lesions that are clearly visible; avoid excessive depth of biopsy; do not tear biopsy specimen from the bowel wall; use electrocautery for brief periods only.

Excessive bleeding

Aetiology: Biopsy of vascular lesion, tear of bowel wall, coagulation defect.

Prevention: Avoid biopsy of obviously vascular lesion; carefully cauterize biopsy site that continues to bleed; do not force sigmoidoscope; do not perform biopsy in presence of coagulation defect.

Storage: Fresh warm specimen is mandatory for the detection of amoebic trophozoites.

Transportation: If amoebiasis is suspected, the specimen should be protected from cold during the shipment, e.g. by placing the specimen in a thermos bottle.

Reporting: A positive finding of amoebic trophozoites will be reported immediately. Negative results for enteropathogenic bacteria and gonococci are reported after 2 days, positive culture results after 3 days.

Comments: Examination of sigmoidoscopy material does not invalidate the routine faecal examination, and a series of at least three faecal specimens should be submitted for each patient undergoing this investigation.

TISSUE BIOPSY

Objectives:	Aetiological diagnosis by microscopic examination and aerobic and anaerobic cultivation of biopsy or tissue specimen removed at surgery with identification and susceptibility test of the isolated organism(s).
Specimen:	Tissue biopsy removed by surgery.
Collection time:	Preferably before the patient is treated with antibiotics.
Equipment:	Mask, gown, gloves, sterile forceps, sterile specimen container.
Procedure:	<ol style="list-style-type: none">1. Large biopsy or tissue specimens are divided into smaller pieces 0,5 cm in diameter.2. With aseptic technique transfer the specimen to a sterile container and immediately bring it to the laboratory.3. If transportation is prolonged for more than one hour, place the biopsy in a tube with transport medium and push it to the butt of the agar with a sterile swab.4. Fill in a request form and label the container with patient's identification.
Storage:	Refrigerated (2-8 °C).
Transportation:	Preferably in a cooling box (2-8 °C) if transportation time is delayed for more than 1 hour.
Reporting:	Negative results are reported two days after the specimen is received, positive findings may take from two to five days or even longer.
Comments:	If the tissue has been removed with aseptic technique, the container is sterile and the storage and transportation are as described, false positive isolations are rare. False negative isolations may occur when the patient has received antibiotics before collection or when transportation has not been optimal.

SPLENIC ASPIRATE

Objectives: Aetiological diagnosis of visceral leishmaniasis (kala-azar) by microscopic examination and culture and demonstration of the organism.

Aspiration may be contraindicated in patients with haemorrhagic disorders, local infection (skin, peritoneum, liver abscess, lung, pleura) close to the puncture site, or hydatid disease.

Test material: Splenic aspirate.

Collection time: Preferably in the morning, as the patient should be under close supervision for the next 10-12 hours.

Equipment: Two glass slides, tubed culture media (NNN and Schneider's *Drosophila* media), syringe, needle, antiseptic solution, cotton wool sponges, plaster.

Procedure:

1. Before the examination have the following laboratory results checked:
 - thrombocyte (platelet) count $> 10^5/\text{mm}^3$ ($10^{11}/\text{L}$);
 - coagulation time, whole blood (Lee and White) < 10 min.;
 - bleeding time (Duke method) < 5 min.;
 - prothrombin time (Quick) $> 40\%$ of normal value;
 - thromboplastin coagulation time < 10 sec. longer than control value.
2. Clean two glass slides and label with patient's name, number, date, and "splenic aspirate." Have ready culture media (1 tube each of NNN and Schneider's *Drosophila* media) labelled like the slides. Allow the culture media to warm to room temperature. Attach the needle to the syringe. Place all items on the table at bedside.
3. Explain the procedure to the patient. Palpate the spleen and outline its margins on the patient's abdomen with a pen. For safety, the spleen should be palpable at least 3 cm below the costal margin on expiration. Disinfect the skin at the site of aspiration two times and allow it to dry in between.

4. With the 21-gauge needle attached to the 5-mL syringe, just penetrate the skin, midway between the edges of the spleen, 2-4 cm below the costal margin. Aim the needle cranially at an angle of about 45° to the abdominal wall.
5. To perform the actual aspiration, pull the syringe plunger back approximately 1 mL to apply suction, and with a quick in and out movement push the needle into the spleen to the full needle depth, and then withdraw it completely, maintaining suction throughout. The insertion should be timed with breathing so that the diaphragm is not moving.
6. In uncooperative young children, have two assistants hold the child (arms folded across the chest with shirt raised in the patient's line of vision and pelvis held firmly). Carry out the aspiration as a single stage procedure, using the same landmarks, angles, and suction as in steps 3 to 5, all in one, quick motion.
7. Only a tiny amount of splenic material is obtained, but this is adequate for culture and smear. Slowly pull the plunger back to 2-3 mL, and, using the sterile technique insert the needle into a tube of culture medium. Briskly push the plunger into the barrel to expel the contents of the needle into the side walls of the tube. If necessary, repeat once or twice until the splenic material is visible in the tube. Replace cap on tube and invert to wash the splenic material off the side of the tube. Repeat this procedure with the second tube of culture medium. A sterile technique is essential.
8. Expel additional material gently onto glass slides, holding the needle tip on the surface of the slide. Immediately spread evenly with the needle using a linear (not circular) motion. The smear should be not quite as thick as a thick blood film for malaria. Remove the needle and use it to obtain additional material from the tip of the syringe and spread it on slides. Further material may be found on the end of the plunger and dabbed directly on to a slide and spread. Allow the slides to dry.
9. Write time of aspirate on the patient's chart and instructions: "Record pulse and blood pressure half-hourly for 4 hours, then hourly for 6 hours. Patient to remain in bed for 12 hours." See that the patient understands these instructions. Enter the procedure in the notes and sign.

- Storage:** Room temperature, should not be refrigerated.
- Transportation:** The slides and media should be brought to the laboratory immediately.
- Reporting:** The microscopic examination is available within 24 hours; the culture may take up to 14 days.

URINE

URINE

Objectives: Aetiological diagnosis of bacterial urinary tract infection by quantitative cultivation of the urine with identification and susceptibility test of the isolated bacteria(s), and of cytomegalovirus infections by cell culture.

Test material: Clean-catch, midstream urine specimen.

Collection time: For urine culture a morning urine is recommended or, in case this is not possible, then a specimen collected 2 hours after last micturation. For the demonstration of eggs of *Schistosoma haematobium*, a random urine sample should be collected preferably in a Petri dish between 10 a.m. and 2 p.m., as the concentration of eggs is greater during this period, particularly in the last drops of the passed urine. Exercise just before collection will increase the excretion of eggs.

Equipment: A clean, preferably sterile container of appropriate size (50 mL or more), cotton wool or gauze sponges, soap, handwarm water, bedpan.

Procedure: Collection of urine for bacterial and viral investigation:

Patient not needing assistance:

- Give the patient a suitable container.
- Instruct the patient before the collection, preferably with illustration.
- Tell the patient not to touch the inside or rim of the container.

Male

1. If not circumcised, draw back the foreskin.
2. Begin to urinate, but pass the first portion into the toilet.
3. Collect the mid-portion of urine into the container, and pass the excess into the toilet.

Female

1. Squat over the toilet and separate the labia with one hand.

2. Void the first portion of urine into the toilet.
3. Collect the mid-portion of urine into the container and pass the excess into the toilet.

Patient needing assistance

Have ready: Clean, preferably sterile container of appropriate size, cotton, wool or gauze sponge, handwarm soapy water, bedpan.

- Explain to the patient what will happen.

Male

1. Draw back the foreskin and clean the glans with cotton wool sponges soaked in handwarm soapy water while working away from the urethra.
2. Ask the patient to urinate into the bedpan.
3. Collect the mid-portion of urine into the specimen container.

Female

1. Separate the labia with one hand and clean around the urethra with cotton wool sponges soaked with handwarm soapy water while wiping from front to back.
2. Ask the patient to urinate into the bedpan and collect the mid-portion of urine in the container.

Infants

- Have ready: Clean, preferably sterile container of appropriate size or a plastic bag, cotton wool or gauze pads, handwarm soapy water.
1. Clean the external genitals as described above.
 2. Give the child as much liquid as possible just prior to the collection.
 3. Seat the child on the lap of the mother, nurse or ward attendant.
 4. Collect as much urine as possible in the container or plastic bag when the child urinates.

Collection of urine for eggs of *Schistosoma haematobium*

1. Ask the patient to pass urine into a large bottle between 10 a.m. and 2 p.m.
2. Shake the bottle well and fill a conical specimen glass with the urine.

3. Let the urine in the specimen glass stand for at least 30 minutes.
4. By that time any schistosome eggs will have settled at the bottom of the specimen glass. Without disturbing the deposit, carefully pour away the supernatant urine so that only 10 mL are left.
5. Mix up the deposit by shaking and pour this into a specimen tube.
6. Send the specimen tube to the laboratory. If the urine must stand for an hour or longer, add 2 drops of undiluted formalin (formaldehyde) to the specimen.

Important: It is important that the urine is collected “clean”, as any discharge or pus from the vagina or external genitals added to the urine will invalidate the examination.

Storage: All urine specimens should be brought to the laboratory within 1 hour of collection. If this is not possible, the urine sample should be refrigerated just after collection and then be brought to the laboratory to be processed within 24 hours.

Transportation: In a cooling box (2-8 °C) except when the transport time is very short.

Reporting: Negative bacterial cultures are reported 1 day after receipt of specimen and positive cultures after 1-2 days. Viral culture may take from 5-7 days.

Comments: In healthy persons the urine in the kidney, ureter and bladder is sterile, while the lower parts of the urethra and the genitalia are normally colonized by different bacteria, many of which may also cause urinary tract infection. All passed urine is therefore contaminated to some degree. However, it is possible to predict if a patient has a urinary tract infection or not by counting the number of bacteria present in a mid-stream urine specimen. Patients having $\geq 10^5$ bacteria per mL urine will have a symptomatic infection in over 80% of the cases, whereas patients with 10^4 bacteria per mL urine will have a symptomatic infection in $< 30\%$ and patients with $\leq 10^4$ bacteria per mL urine will have a symptomatic infection in $< 4\%$ of the cases.

Urinary tract infections may be primary or associated with congenital anomalies, stasis, stones, or foreign bodies. Bacteriuria may occur in symptomless individuals; quantitative counts of more than 100 000 (10^5 /mL) bacteria per mL of urine in freshly voided clean-catch specimens are usually indicative of infection.

Escherichia coli is most commonly cultured, but other Gram-negatives of the *Enterobacteriaceae* family are also regularly isolated. *Salmonella* infection of the urinary tract is usually seen in patients infected with *Schistosoma haematobium*.

Sterile pyuria in a patient with chronic fever, frequency, dysuria, or haematuria should suggest the possibility of renal tuberculosis.

Cytomegalovirus (CMV) should be looked for in immunocompetent patients with mononucleosis-like illness negative for EBV, immunoincompetent patients with atypical pneumonia, fever and jaundice after blood transfusion and organ transplantation, and in newborns with congenital illness. As CMV may be found in both symptomatic infections and asymptomatic carriers, verification of the infection should be made by demonstrating a rise in antibody titer. Potential transplantation donors should be negative for CMV antigen.

ENVIRONMENT

ISOLATION OF *VIBRIO CHOLERA*E FROM WATER SOURCE

Objectives: Isolation and identification of *Vibrio cholerae* in environmental water samples and sewage.

Test material: water (approximately 1 litre) and sewage, 4-8 g (approx. 5 mL).

Collection time: During epidemics of suspected cases of cholera.

Equipment: Clean containers, sodium chloride, pH-indication paper, (Moore swabs, alkaline peptone water).

Moore swabs can be made by cutting pieces of cotton gauze (15 cm wide and approximately 100 cm long), folding them lengthwise several times to form tight cylindrical rolls, and tying the centre with strong wire. The swabs are wrapped in heavy brown paper and autoclaved.

Procedure:

1. For water: samples should be at least 1 litre and collected in a sterile 1 litre bottle. Add 2 teaspoonfuls of sodium chloride to the 1 litre water sample and adjust the pH to 9 (approximately). Transport to the laboratory immediately.
2. For sewage: the sample is collected in a container and diluted with sterile saline. Transport to the laboratory immediately.
3. Another method is to use Moore swabs. The wire holding the Moore swab is tied to nylon fishline. the swab on the line is suspended in the sewage or water to be tested, and left in place for 1 or 2 days. Remove the swabs, separate them from the wire, and immediately submerge them in 250-500 mL of alkaline peptone water (pH 8,5-9) in a jar. Transport the jar to the laboratory.

Storage: Refrigerated (2-8 °C).

Transportation: If delay is unavoidable, either inoculate the transport medium or refrigerate the specimen to suppress overgrowth by other bacteria.

Reporting: Culture result will be available in 3-4 days.

ISOLATION OF POLIOVIRUS FROM THE ENVIRONMENT

Objectives:	Isolation and identification of poliovirus in environmental samples. This is usually achieved by sampling sewage, but may also require examination of drinking and bathing water.
Test material:	Typical household sewage, drinking water (approximately 1 litre).
Collection time:	During epidemic or paralytic cases.
Equipment:	Clean vessel, cooling box.
Procedure:	<ol style="list-style-type: none">1. One litre of sewage or drinking water is taken into a clean vessel.2. Place the vessel in a cooling box (2-8 °C).3. Bring the cooling box to the laboratory without delay.
Storage:	Refrigerated (2-8 °C).
Transportation:	In a cooling box (2-8 °C).
Reporting:	The results of cell culture will take at least 7-10 days.
Comments:	Positive isolation of poliovirus will be sent for intratypic differentiation at a reference laboratory.

APPENDICES

APPENDIX 1

Example of request form for microbiological investigation

REQUEST FORM FOR MICROBIOLOGICAL INVESTIGATION		
Name and address of referring hospital/health centre:		Treating physician:
Full name of patient:		Address:
Age:	Sex:	Insurance name and number:
Essential clinical information:		Clinical diagnosis/tentative diagnosis:
Date of onset of symptoms/signs:		Antimicrobial therapy:
Specimen source/nature:		Time of collection:

APPENDIX 2

Examples of transport media

Bacterial transport media:

1. Stuart transport medium (Oxoid)
2. Amies transport medium
3. Cary-Blair transport medium

Viral transport media:

1. Swab-tube combinations:

Culturette	IIBSS	Decton Dickinson
Virocult	HBSS	Medical Wire & Equipment Co.
	Glucose	1 g/litre
	Lactalbumin Hydrolysate	3 g/litre
	Chloramphenicol	2,5 µg/mL
	Cycloheximide	10 µg/mL

2. Liquid media:

	Viral/chlamydial	Carr-Scarborough
	Sucrose	74,6 g
	Potassium hydrogenphosphate	0,52 g
	L-glutamic acid	0,72 g
	Bovine serum albumin	5,0 g
	Gentamicin	50 mg
	Potassium dihydrogenphosphate	1,25 g
	L-15 medium	9,9 litre
	Water fill up to	10 litre
Cell culture	MEM	
	Fetal Bovine serum	2-10%
	Penicillin	50 µg/mL
	Streptomycin	50 µg/mL
	Gentamicin	10 µg/mL
	Amphotericin B	4 µg/mL
Skimmed milk	PBS, pH 7,0	0,1M
	Skimmed milk	50%

APPENDIX 3

Recommended sites for specimen collection for virus and *chlamydia* isolation

DISEASE AND AGENTS	COLLECTION SITE
Meningo-encephalitis	
Mumps	Throat, CSF, urine
Enterovirus	Stool, throat, CSF
Herpes simplex	Vesicle fluid
Rabies	Saliva, conjunctiva
Arbovirus	Blood
Respiratory disease	
Myxovirus	Throat
Paramyxovirus	Throat
Rhinovirus	Throat
Adenovirus	Throat, stool
Exanthems and enanthems	
Rubella	Throat
Measles	Throat, blood (lymphocytes)
Varicella-zoster	Vesicle fluid
Herpes simplex	Vesicle fluid, throat
Enterovirus	Stool, throat
Myocarditis-Pericarditis	
Enterovirus	Stool, throat
Myxovirus	Throat
Paramyxovirus	Throat
Haemorrhagic fever	
Arbovirus	Blood
Hantavirus	Blood
Other	
Cytomegalovirus	Urine, throat
<i>Chlamydia trachomatis</i>	Cervix, urethra, eye
<i>Chlamydia psittaci</i>	Respiratory tract
<i>Chlamydia pneumoniae</i>	Respiratory tract

APPENDIX 4

Specimens not to be cultured anaerobically

1. Throat or nasopharyngeal swabs
2. Gingival swabs
3. Conjunctival swabs
4. Swabs from auditory canal (except in malignant external otitis)
5. Expectored sputum
6. Sputum obtained by nasotracheal or orotracheal suction
7. Bronchoscopy specimens not collected by a protected, double-lumen catheter
8. Gastric and small bowel content (except in blind loop syndrome)
9. Large bowel contents, ileostomy, colostomy, faeces (except in *Clostridium difficile* or *C. botulinum* infections)
10. Voided and catheterized urines
11. Vaginal and cervical swabs
12. Surface swabs from decubitus ulcers, wounds, eschars and sinus tracts.

APPENDIX 5

Skin disinfectants

There are a number of alternatives for skin disinfectants, but the following are recommended for microbiological specimens:

1. One or 2% of tincture of iodine is effective and convenient as one can visually see the area of skin which has been disinfected. Some patients have an iodine hypersensitivity and it is recommended that the iodine be removed with alcohol after the collection of the specimen.
2. Ten percent povidone-iodine (iso-betadine) causes less irritation to the skin, but has the disadvantage that the disinfected area is not well visualized.
3. 0,5% chlorhexidine (Hibitane) in 70% alcohol causes less irritation and sensitivity to the skin, but has the same disadvantage as the povidone-iodine.

All three solutions should be kept in airtight bottles as evaporation of the alcohol substantially reduces the activity and efficacy of the antiseptic.

No disinfectant works instantly but requires a contact time with the microorganisms for at least 1-2 minutes. A further reduction of the skin flora is achieved, if the disinfectant is well rubbed on to the skin.

APPENDIX 6

Criteria for rejection of specimens

A specimen may be rejected, if

- it does not have proper patient identification;
- it is received in an inappropriate condition; or
- an unrewarding investigation is requested.

However, no specimen should be rejected before the reason for rejection has been explained to the physician, and he has sanctioned the rejection. After informing the doctor, a short statement explaining the same is written on the request form, which is returned to the ward.

The following statements are approved to be written on the request form after the specimen has been rejected:

Fault of specimen	Statement to be written on form
Discrepancy between identity on specimen and request form	Incorrect patient identification on form or specimen container.
No identification on container	Container not identified.
Specimen source or type of culture not marked on form	Try to solve the fault over the phone; if this is not possible, reject specimen, and state the reason.
Specimen received in formalin	Specimen in fixative, please submit new specimen.
Dry swab	Specimen unsatisfactory, dry swab, please resubmit.
Specimen in unsterile container	Improper specimen, container unsterile, please resubmit.
Container leaking	Improper specimen, container leaking, please resubmit.

Fault of specimen	Statement to be written on form
Multiple specimens from similar source on the same day	Multiple specimens received, one will be processed. Notify lab within 24 hours, if the remaining specimens should be processed and <i>explain the circumstances.</i>
One specimen only for bacterial, fungal and TB culture	Call doctor to submit additional specimen or to state priorities.
Anaerobic culture requested of specimen from throat, nose, sputum, urine, vagina, ulcers, wounds, skin and environment	'Anaerobic culture request' is crossed off.
Blood culture with request for microscopy or culture for TB	'Microscopy' or 'culture for TB' is crossed off.
Sputum specimen consisting saliva only	Improper specimen, only saliva, of please resubmit.
24 hour's collection of sputum for TB or fungi	24-hour collection is unacceptable, please resubmit 3 consecutive, samples of early morning sputum.
Urine held at room temperature for more than 2 hours	Improper specimen, urine more than 2 hours at room temperature, please resubmit.
24 hour's collection of urine for TB or Schistosomiasis (Bilharzia)	24-hour collection is unacceptable, please resubmit 3 consecutive, early morning specimens.
Urine catheter tip for culture	Unsatisfactory specimen, contaminated. send urine sample.
Microscopy for gonococci of specimen from anus, rectum or vagina	Cross 'microscopy' off the form.
Excess barium or oil in stool samples for parasites	Specimen unsatisfactory, barium or oil in excess, please resubmit.
Cold stool sample for amoeba trophozoites	Improper specimen, please resubmit warm, freshly passed stool sample.

APPENDIX 7

SHIPMENT OF SAMPLES



Packing must be in three layers as detailed below.

a) Primary container

This contains the specimen. The primary container must be leakproof with a screw-cap. To avoid cracking or bending this container, never use mechanical devices to tighten the cap. Make sure that the specimen is correctly labelled.

b) Secondary container

This must be a durable waterproof container, made of metal or polycarbonate plastic with a screw-cap. It must be large enough to hold the primary container and made of sufficiently absorbent material (absorptive paper, cotton, or cloth) to absorb all the fluid in the primary container, should it be accidentally broken.

Several primary containers can be enclosed in the secondary container under the following conditions:

- The total volume in the primary containers should not exceed 50 mL.
- Each primary container must be individually protected to reduce shock, prevent breakage, and provide absorption.
- Enough space must be left between the inner side of the secondary container and the primary containers in case of accidental leakage or breakage.

Tape one copy of the specimen data form and information about the specimen on the outside of each secondary container.

c) Outer (tertiary container)

The outer package is the outer shipping container. It should be of corrugated fibreboard, cardboard, wood, or other material strong enough to withstand the weight and shock commonly associated with handling and shipment.

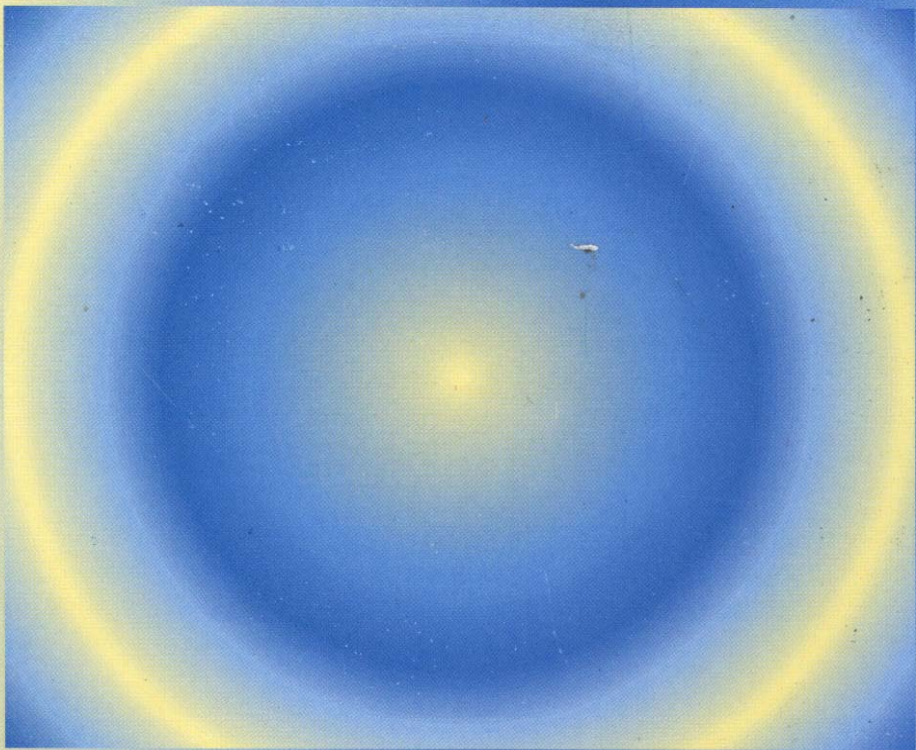
When packing specimen volumes of 50 mL or more, a shock-absorbent material should be added (a volume equal to the sample volume) between the outer sides of the secondary container and the outer shipping container.

If dry ice is used for shipping frozen and refrigerated specimens the following should be remembered.

- Dry ice must be placed between the secondary container and the outer shipping container.
- Shock absorbent material should be placed so as not to permit the secondary container to become loose inside the outer container as dry ice sublimates and disappears.
- The outer container must permit the release of carbon dioxide gas in order to prevent the build-up of pressure leading to rupture of the container.

In emergency situations, all specimens should be considered potentially dangerous. All packages shipped must bear biohazard labels. For more details on this subject, please refer to the recent Eastern Mediterranean Regional publication.*

* *Health laboratory facilities in emergency and disaster situations*. WHO Regional Publications, Eastern Mediterranean Series No 8. WHO Regional Office for the Eastern Mediterranean, Alexandria, 1994.



Specimen collection and transport for microbiological investigation

The correct collection and transport of specimens for microbiological investigation plays an essential role in the efficient and safe operation of laboratory services. Improper collection not only leads to waste of financial resources but may also provoke a misleading diagnosis, causing potential danger to the patient.

The publication brings together in one manual a series of guidelines for the optimal collection and transport of specimens destined for different types and levels of microbiological laboratory. It will be of interest not only to laboratory staff, but also to the users of laboratory services.