



NHLS TYGERBERG

**MICROBIOLOGY SPECIMEN SAMPLING
MANUAL**

January 2006

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SPECIMEN SAMPLING MANUAL

Please remember that all diagnostic information from our laboratory is dependent on the quality of specimen received.

GENERAL INSTRUCTIONS:

1. Please check the patient's identity before taking any samples.
2. Samples **will not** be processed by the laboratory if they are not labelled correctly
3. Please ensure that laboratory specimens are stored out of direct sunlight.
4. Please ensure that the correct sample container is used.
5. Please ensure that samples are stored safely for transport and handling.
6. Please ensure that samples are not at risk to leak out or break, as the laboratory **will not** process these samples.
7. Please ensure that safety and infection control procedures are followed at all times.
8. If a test requested is not covered in the sampling manual, please phone the laboratory for special instructions regarding correct specimen containers, special sampling procedures and requirements/precautions to be taken.
9. Any after-request tests (tests not requested on original request form) must be telephonically requested with the laboratory. The laboratory will inform you if the after request can still be carried out.
10. If in any doubt regarding any aspect of our service, please feel free to contact the Laboratory at all times.

COMPLETING THE REQUEST FORM:

A request form must accompany each sample submitted to the laboratory. This request form must contain the proper information in order to process the specimen. The essential elements of the request form are:

- Patient's surname and first name
- Patient's hospital number
- Patient's date of birth and sex
- Requesting physician's complete name
- Contact number if urgent
- Date and time of collection
- Source of specimen
- Diagnosis
- Specify antibiotic therapy
- Indicate the test(s) requested

LABELLING THE SAMPLE

Please note: the laboratory will not process unlabelled specimens

A properly labelled sample is essential to ensure that the results of the test match the patient. The essential elements in specimen labelling are:

- Patient's surname and first name.
- Patient's hospital number, clinic number or ID number.

Following these instructions will ensure that a high level service can be maintained by the NHLS to the benefit of our customers as well as to the patients.

MICROBIOLOGY LABORATORY

General Instructions:

1. It is of critical importance that the laboratory provides clinicians with clear guidelines for the proper collection and transport of specimens. All diagnostic information from the microbiology laboratory is contingent on the quality of specimen received. Consequences of a poorly collected and/or poorly transported specimen include: i) failure to isolate the causative micro-organism, and ii) recovery of contaminants or normal microbial flora which may be misleading and result in improper treatment of the patient.
 2. Safety considerations with regard to the handling of specimens:
 - Treat all specimens as potentially hazardous
 - Do not contaminate the external surface of the collection container and/or its accompanying paperwork
 - Minimize direct handling of specimens in transit from the patient to the laboratory. Ideally, specimens should be placed in plastic sealable bags with a separate pouch for the specimen request form.
 3. Please ensure that samples are correctly labelled and that the request form is filled in with all the relevant data.
 4. The points listed below each specimen type is to enable clinicians, nursing staff and patients to be able to take a good quality specimen.
 5. Clinicians, nursing staff and patients are responsible for ensuring that these guidelines are followed.
 6. Please contact the laboratory if in any doubt as to the collection or transport of a specimen.
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1. FAECAL SPECIMENS - COLLECTION AND TRANSPORT

SPECIMEN COLLECTION

1. **Acceptable specimens:** Specimens should be submitted to the laboratory in a sterile screw-cap jar as soon after collection as possible (i.e. within 1 to 2 hours). Care should be taken to ensure that the specimen is not contaminated with urine. Rectal swabs should be submitted to the laboratory in a suitable transport medium (e.g. Cary-Blair, Amies' or Stuart's transport medium). It is advisable to submit 2 or 3 specimens on separate days to increase the probability of isolating a pathogen. The single most important requirement is a freshly passed stool specimen, since acid metabolites in stored specimens may be detrimental to enteropathogenic bacteria. In some instances, the collection of a rectal swab rather than stool specimens may be necessary, particularly in neonates or in severely debilitated adults. Rectal swabs are also indicated when screening food handlers for enteropathogens. Specimens collected for occult blood: It is recommended that stools for testing be collected for at least three consecutive days. Patients should be on a meat-free diet prior to testing. Stools for Amoebae detection must be sent at 37°C.
2. Select portions containing pus, blood or mucus when processing the specimen. A 1- to 2-g quantity is sufficient for bacteriological processing.
3. If there is a long delay in transit to the laboratory, stools submitted for *Clostridium difficile* testing should be kept refrigerated. Specimens must reach the lab within 48 hours.
4. Submit rectal biopsy specimens in a sterile screw-cap jar with a small amount of sterile water to prevent desiccation. Specimens for microbiological processing **must not be submitted in formalin**.
5. **Guidelines for preparing and placing of stool specimen in transport medium:** A small amount of stool can be collected by inserting a sterile cotton swab into the stool and rotating it. If mucus is present, it should be sampled with the swab. Immediately insert the swab into transport medium. The swab should be pushed completely to the bottom of the tube of transport medium and the top portion of the stick touching the fingers, broken off and discarded. Recap and tighten firmly. Place the tube in a refrigerator or cold box. Adhere strictly to the request form supplied by NHLS and ensure that all particulars are completed.

6. Collection of rectal swabs

Rectal swabs may be collected as follows: moisten the swab in sterile transport medium, insert through the rectal sphincter 2-3cm (1-1.5 inches) and rotate, withdraw and examine to make sure there is some faecal material visible on the swab. Immediately insert the swab into cold transport medium as described in above paragraph. Place the tube in a refrigerator or cold box.

Specimens of doubtful value:

- 1) Unpreserved stool samples >2 hours old.
- 2) Dry rectal swabs or biopsy samples.
- 3) Multiple specimen collections on the same day.

Routine MC&S includes microscopy of a wet mount preparation, culture for *Salmonella*, *Shigella* and *Campylobacter* and sensitivity testing. The wet mount preparation is examined for red and white blood cells and parasites.

Cultures for *Yersinia enterocolitica* will only be routinely done if white and red blood cells are seen in the wet preparation. If *Vibrio cholera* or *E. coli* 0157.H7 (hemolytic uremic syndrome) is suspected, please indicate so on the request form.

A modified acid-fast stain is performed to identify *Cryptosporidium parvum*. It is routinely done only on specimens from children under 3 years and on specimens from immunocompromised patients. Indicate if this investigation is specifically required.

2. URINE SPECIMENS - COLLECTION AND TRANSPORT

Urine is normally a sterile body fluid. However, unless it is collected properly, it can become contaminated with microorganisms from the perineum, urethra or vagina. The following guidelines are provided to ensure proper specimen collection and subsequent, prompt, delivery of urine samples to the laboratory.

A. SPECIMEN COLLECTION

1. MIDSTREAM URINE SPECIMENS (MSU):

- a. The person obtaining the urine specimen should wash their hands with soap and water, rinse, and dry. If the patient is collecting the specimen, he/she should be given detailed instructions, including diagrams or a pictorial display.

Females: Cleanse the urethral opening and the vaginal vestibule area with clean gauze pads soaked with sterile saline or sterile water. Do not use disinfectants to clean the genitalia. Hold labia apart during voiding.

Males: Cleanse the penis, retract the foreskin (if not circumcised), and wash with sterile saline.

Keep foreskin retracted during voiding (to minimise contamination with skin flora).

- b. **Both females and males:** Allow a few millilitres of urine to pass (DO NOT STOP THE FLOW OF URINE) and collect the midstream portion of urine in a wide-mouthed sterile container. In circumcised men, cleansing of the peri-urethral area does not improve the detection of bacteriuria and is therefore not necessary.
- c. Collect voided urine directly into a sterile container; do not use a urinal or bedpan for collection.

2. CATHETER URINE

- Avoid contamination during urine collection from indwelling catheters.

Indwelling urinary catheter specimens are the most unsatisfactory of all urine specimens, because these catheters are often colonized and therefore bacterial cultures are difficult to interpret.

Do not collect sample from drainage bag

Collect sample from the sampling port with a syringe and needle using aseptic technique.

Method:

Clamp catheter tubing below port

Clean sampling port with at least 2 separate 70% alcohol swabs
 Insert needle obliquely into port and aspirate urine.
 Transfer to sterile container and mark correctly: "indwelling catheter urine specimen".

- A straight (non-indwelling) catheter can be used by a physician to obtain urine directly from the bladder.
 - This procedure is not routinely recommended because there is a risk of introducing microorganisms into the bladder. It should be done aseptically if necessary.
3. Urine from an ileal conduit must be collected after removal of the external device and insertion of a catheter into the cleansed stoma.
 4. Urine collected by suprapubic needle aspiration of the bladder avoids contamination associated with the collection of voided urine. This is the preferred method for infants and for patients for whom the interpretation of results of voided urine is difficult.
 5. Foley catheter tips are **unacceptable** for culture.
 6. In addition to routine information it is essential that the patients' specimen label accurately reflects:
 - The *mode* of specimen collection (eg MSU, suprapubic aspirate etc.). Please note that urine samples obtained by suprapubic aspiration and at cystoscopy are processed differently in the laboratory compared to, for example, conventional MSU specimens and it is therefore essential, so as to not compromise the accuracy of results, to inform the laboratory about the mode of specimen collection.
 - The patient's diagnosis or other underlying factors that may influence laboratory decisions on how to process the specimen further (e.g. prolonged incubation for fastidious organisms) should be indicated.

B. TIMING OF SPECIMEN COLLECTION

1. Obtain early-morning specimens whenever possible because of increased bacterial counts after overnight incubation in the bladder.
2. Do not force fluids in order to have the patient void urine. Excessive fluid intake will dilute the urine and may decrease the colony count to $<10^5$ CFUs/ml.
3. For *Schistosoma haematobium* (Bilharzia), send 3 terminal urine specimens for identification of ova.

C. SPECIMEN TRANSPORT

1. Transport urine to the laboratory as soon as possible after collection.
2. Urine specimens must be submitted for culture within 2 hours after collection, or refrigerated and cultured within 24 hours whenever possible.

All specimen containers must be closed tightly to prevent leaking. If sample has grossly leaked from the container, the specimen will be rejected for processing. If specimen has leaked slightly, decontaminate the outside of the container with 70% alcohol prior to processing.

3. STERILE BODY FLUIDS INCLUDING CSF - COLLECTION AND TRANSPORT

A. CEREBROSPINAL FLUID (CSF)

Please note: CSF MUST BE COLLECTED PRIOR TO ANTIMICROBIAL THERAPY!

Collection considerations for Cerebral Nervous System (CNS) specimens:

Culture	Optimal volume (ml) ^a	Comments
Bacteria	1	Send cloudiest CSF specimen to microbiology laboratory immediately.

Fungi	5 - 10	Culture for <i>Cryptococcus</i> spp. is more sensitive if a higher volume of CSF is processed
Mycobacteria	5-10	<i>Mycobacterium tuberculosis</i> , <i>Mycobacterium avium-intracellulare</i> complex.
Anaerobes	NA	Brain abscess pus or central nervous system (CNS) biopsy specimens.
Parasites	NA	Brain abscess or CNS biopsy specimens for <i>Entamoeba histolytica</i> , <i>Toxoplasma gondii</i> , <i>Naegleria</i> species, ^b <i>Acanthamoeba</i> spp. ^b
Virus	1-2	Send to laboratory on ice.
^a Amounts are guidelines. Greater volumes increase the chance of organism recovery. ^b CSF can be submitted. NA, not applicable.		

- The laboratory irrespective of the volume received must process all CSF specimens. Ideally at least 3 separate sterile tubes (1 of which should be a glucose vacutainer tube (grey top) if available and the other 2 must have no anticoagulant added (red/yellow tops)) must be submitted so that the CSF can be analysed in Microbiology and Biochemistry Laboratories.
- Material aspirated from a brain abscess should be placed in an anaerobic transport medium. Alternatively, such material should be immediately transported to the laboratory in the collection syringe after the doctor collecting the specimen has removed the needle and capped the syringe.
- CSF specimens should be transported to the laboratory promptly. Failure to do this may result in the non-viability of fastidious organisms and in overgrowth by more hardy bacteria.
- If prompt delivery is not possible specimens should be kept at room temperature, but never refrigerated. Organisms such as *Neisseria meningitidis* and *Haemophilus influenzae* are sensitive to chilling.

INTERPRETATION OF CSF RESULTS:

Condition	Macroscopic appearance	Cell count (per mm ³)	Erythrocytes	Protein (g/l)	Glucose (mmol/l)
Normal	Clear	0-5 lymphocytes (0 – 30 cells in neonate, mainly neutrophils)	None	0.15 – 0.4 (0.15 – 1.5 in neonate)	2.2-3.3 (60% of blood glucose)
Bacterial meningitis	Turbid	100-2000 neutrophils	None	0.5 – 3 ↑↑	0 – 2.2 ↓
Viral meningitis	Clear or slightly turbid	15 – 500 lymphocytes	None	0.5 – 1 ↑	Normal
Tuberculous meningitis/ Cryptococcus*	Clear or slightly turbid	30 – 500 lymphocytes plus neutrophils	None	1 – 6 ↑↑↑	0 – 2.2 ↓
Bloody tap or recent haemorrhage	Bloody or xanthochromic	Variable	High	↑ due to blood	Normal

*All parameters may be completely normal in the severely immunocompromised patient with Cryptococcal meningitis

B. OTHER STERILE FLUIDS

Commonly submitted fluids

1. Joint or synovial fluid
2. Pleural fluid
 - a. Thoracentesis fluid
 - b. Empyema fluid
3. Peritoneal fluid
 - a. Ascites fluid
 - b. Paracentesis fluid
4. Pericardial fluid
5. Culdo-centesis fluid

SPECIMEN COLLECTION

1. Specimens should be collected with as little contamination from indigenous microbial flora as possible to ensure that the sample will be representative of the infected site.
2. Sterile equipment and aseptic technique must be used to collect specimens to prevent introduction of microorganisms during invasive procedures.
3. If a specimen is to be collected through intact skin, cleanse the skin first. For example, use 70% alcohol followed by iodine solution (1-2% tincture of iodine or 10% solution of povidone-iodine). **Prevent burn by tincture of iodine by removing excess after the specimen has been collected.**
4. In addition to routine information it is essential that the patients' specimen label accurately reflects:
 - The specific body site from which the specimen was taken
 - Provisional diagnosis
5. Collect specimens in sturdy, sterile, screw-cap, leak-proof containers with lids that do not create an aerosol when opened.
6. Although occasionally small clots will form in some fluids, addition of anticoagulant is not recommended; citrate or EDTA inhibits some organisms. If anticoagulant must be used, heparin should be the choice.
7. Although in the past the use of blood bottles for fluid collection has not been recommended, recent studies suggest that the larger the sample volume that can be cultured the more likely the recovery of low numbers of organisms in fluids such as ascitic fluid will be. As with any broth system, however, the fastest growing organism is often the only one isolated, jeopardizing the recovery of slow growers. When a broth is used, no direct smear information is available and, therefore, no assessment of the initial distribution of organisms or inflammatory cells can be made. A smear can be prepared, however, at the time of specimen collection and submitted with the broth medium.

TRANSPORT

1. Syringes:

Specimens obtained by a doctor using needle aspiration should be transferred to an anaerobic transport medium prior to transport to the laboratory. Alternatively, and only if transferring it from the syringe will compromise the specimen, the **doctor should remove the needle**, using a protective device to avoid injury, and cap the syringe with a sterile cap prior to transporting it to the laboratory. If the latter procedure is followed it is essential that the specimen be submitted to the laboratory immediately after collection.

2. Sterile tubes

Fluid specimens can also be transferred into a sterile tube without preservative. The specimen should be submitted to the laboratory without delay so as not to compromise the recovery of anaerobic organisms.

3. Blood culture bottles

Using aseptic techniques, inoculate bottles with fluid in the same medium/sample ratio as is recommended for blood (follow blood culture bottle manufacturers recommendations), especially when anticoagulants are required.

4. Swabs

Swabs are the least desirable sample for culture of body fluids and their use should be discouraged. Protection of anaerobes from ambient oxygen is impossible. A good direct smear cannot be made, and the quantity of sample may not be sufficient to ensure recovery of a small number of organisms. If a swab is taken it is essential that it be placed in an anaerobic transport medium.

4. BLOOD CULTURES - COLLECTION AND TRANSPORT

We recommend that a minimum of 2 bloodcultures from different sites should be submitted in order to acquire the optimal volume of blood and to facilitate the interpretation of results. Anaerobic blood cultures are not available routinely. This test is not cost effective due to low number of requests and high cost.

1. PROCEDURE

Site selection

The phlebotomist should:

- Select a different site for each blood sample.
- Avoid drawing blood through indwelling intravenous or intra-arterial catheters. However if blood cultures have been obtained from intravascular catheters, they should be labelled as such and one set of blood cultures should also be obtained by venipuncture at the same time in order to help assess positive blood cultures from catheters.

Site preparation

- Vigorously cleanse the venipuncture site with 70% isopropyl or ethyl alcohol and wait till dry.
- Apply 2% tincture iodine or povidone-iodine in ever increasing circles starting at point where venipuncture is to be made. Note: A contact time of 1.5-2 minutes after swabbing is necessary for optimal disinfection.
- Do not touch the venipuncture site after preparation and prior to phlebotomy.

Disinfecting blood culture bottles

- Disinfect the top of the bottle or tube with alcohol and allow top to dry.

Collection of blood

- Using syringe and needle insert the needle into the vein, and withdraw blood. Do not change needles before injecting the blood into the culture bottle due to risk needlestick injury.
- After the blood is inserted into the blood culture system mix well to avoid clotting.
- Use a new needle if vein is missed initially.
- Add sufficient volume of blood to attain a 1:10 ratio of blood to medium (the volume of blood required is indicated by the manufacturer on the bottle).
- After phlebotomy, cleanse the site with 70% alcohol to remove remaining iodine, which can cause irritation in some patients and cover puncture wound appropriately.

2. SPECIMEN VOLUME

Note: The volume of blood is critical because the number of organisms in the majority of bacteremias is low, especially if the patient is on antimicrobial therapy. In infants and children, the number of microorganisms during bacteraemia is higher than in adults; therefore, less blood is required for culture.

Recommended volume per bottle: see label on bottle

Children: Ideally, 3 to 5ml of blood should be added to bottle

Neonates: 1-3ml of blood per bottle

Adults: Ideally 10ml blood per culture bottle (aerobic). .

3. RECOMMENDATION ON NUMBER AND TIMING OF BLOOD CULTURES

- a. A minimum of 20ml (one set consisting of two aerobic bottles) is recommended in order to get an optimal yield from blood cultures. It may be desirable to collect sets over 3 consecutive days in patients who have been on antimicrobial therapy.
- b. Fever of unknown origin (occult abscess, typhoid fever, or brucellosis): Obtain two separate samples initially. It is recommended that a further 2 samples be obtained during temperature spike ideally after 24-36 hours of the initial samples. The increase in positive cultures beyond four cultures is very minimal.
- c. Suspected endocarditis – collection of blood cultures do not have to coincide with fever spikes due to continuous bacteraemia.

BOTTLE TYPES:

BOTTLE	USE	BLOOD VOLUME
Standard Aerobic Culture Bottles (Blue caps) – available in wards	These bottles are generally used in most of the bacteraemia and fungaemia cases	The optimal blood volume per bottle for culture is 8-10ml.
Paediatric Culture Bottles (Pink caps) – obtainable from lab	These bottles are aerobic and are used for low volume specimens; such as in neonates	The optimal blood volume per bottle for culture is 1-3ml
Resin containing Aerobic Culture Bottles (Grey caps) – obtainable from lab	The resin bottles absorb antibiotics and the inhibiting components out of the blood; enhancing the recovery of micro-organisms.	The optimal blood volume per bottle for culture is 8-10ml..
Myc/F lytic (Mycobacteria, fungi) (red cap) – obtainable from lab	These bottles are generally used in cases of disseminated TB; M. avium-intracellulare and systemic fungal infections. Candida and Cryptococcus will grow well in standard aerobic bottles.	The optimal blood volume per bottle for culture is 1-5ml.

DURATION OF INCUBATION :

TBH incubates blood cultures for 5-7 days; using an automated system.

When fastidious organisms are suspected as a cause of sepsis or infectious endocarditis, e.g. HACEK organisms (Haemophilus aphrophilus/paraphrophilus, Actinobacillus actionmycetemcomitans, Cardiobacterium hominis, Eikenella corrodens and Kingella kingae), the laboratory should be notified of this possibility, so that the blood culture can be incubated for a longer time (14 days). Suspected Brucella is incubated for 28 days and suspected TB and fungi are incubated for 42 days, before the culture is regarded as negative.

QUALITY CONTROL:

Media

- Check expiry dates of each batch of blood culture bottles used.
- Uninoculated blood culture bottles should be stored in a cool dark place
- Examine bottles for turbidity and/or change of colour before adding any blood.
- Discard any bottles showing abnormal characteristics.

Labelling and transport.

Please ensure that all blood culture bottles are labelled correctly (not over bar code) and that the request form is completed with all the relevant required data. All specimens should be transported to the laboratory promptly. Failure to do this may result in the death of fastidious organisms and in overgrowth by more hardy bacteria.

INTRAVASCULAR CATHETER TIP CULTURES

Cleanse skin around catheter site with alcohol.

Aseptically remove catheter, and clip 5 cm distal tip of catheter directly into sterile tube.

Transport directly to microbiology laboratory to prevent drying

Acceptable IV catheters for culture: central, CVP, Hickman, Broviac, peripheral, arterial, umbilical, hyperalimentation, Swan-Ganz

5. PUS SWABS INCLUDING BURN SWABS - COLLECTION AND TRANSPORT

SPECIMENS

Specimens should be collected prior to the administration of antimicrobial therapy.

1. SUPERFICIAL WOUNDS:

Aspirates:

- a. Syringe aspirates (3- to 5- ml syringe with 22- to 23- gauge needle) are preferable to swab specimens.
- b. After adequately decontaminating the surface of the wound (e.g. with 70% alcohol and then with 10% povidone-iodine solution) and allowing the disinfectant to dry, collect the specimen.
- c. The deepest portion of the lesion should be aspirated. If a vesicle is present, collect both fluid and cells from the base of the lesion.
- d. If the initial aspiration fails to obtain material, inject sterile, non-bacteriostatic 0.85% NaCl subcutaneously and repeat the aspiration attempt. If no material is obtained, rinse the needle and syringe with broth by drawing the culture medium through the needle into the syringe. In the past, it has been permissible to use the aspirating syringe as the transport container provided the needle was capped. This practice is no longer acceptable because of the increased possibility of needle-stick injuries. If a delay in processing of more than 30 minutes is anticipated, the specimen should be transferred to an anaerobic transport container.

Pus swabs:

- a. If material cannot be obtained with a needle and a syringe, and a swab must be used, it may be necessary either to separate the wound margins with the thumb and forefinger of one hand (wearing a sterile glove) or make a small opening in a closed abscess with a scalpel blade before extending the tip of the swab deeply into the depths of the lesion with the other hand. Care should be taken not to touch the adjacent skin margins. The swab should then be inoculated onto appropriate culture media as soon as possible after collection; alternatively, it can be placed immediately into a suitable transport medium (eg. Amies or Stuart's). **Dry swabs are unacceptable.**

2. ULCERS AND NODULES:

- a. Clean the area with 70% alcohol and then a 10% povidone-iodine solution.
- b. Remove overlying debris.
- c. Curette the base of the ulcer or nodule.
- d. If exudate is present from the ulcer or nodule, collect it with a syringe or a sterile swab.

3. BURN SPECIMENS:

The surfaces of burn wounds will become colonised by the patient's own microbial flora or by environmental organisms. When the organism load is large, infection of underlying tissue may occur, and bacteraemia may ensue. Cultures of the surface alone are misleading; therefore biopsies of deeper tissues are often indicated. Clean the surface of the wound with sterile saline/water before collecting

specimens. Blood cultures should be taken if septicaemia is suspected.

4. DEEP WOUNDS, ASPIRATES, AND TISSUE SPECIMENS:

a. Bite wounds:

Aspirate pus from the wound, or obtain it at the time of incision, drainage, or debridement of the infected wound.

b. Bone:

Obtain bone specimen during surgery. Submit in sterile container without formalin. Specimen may be kept moist with sterile 0.85% NaCl.

c. Deep wounds or abscesses:

Disinfect the surface with 70% alcohol and then with an iodine solution (eg. 10% solution of povidone-iodine). Aspirate the deepest portion of the lesion, avoiding contamination by the wound surface. If collection is done at surgery, a portion of the abscess wall should also be sent for culture.

d. Punch skin biopsies:

Disinfect the skin surface with 70% alcohol and then with 10% povidone iodine solution. Collect a 3- to 4-mm sample with a dermal punch. Submit for microbiological analysis in a sterile container without formalin.

e. Soft tissue aspirate:

Disinfect the surface with 70% alcohol and then with 10% povidone iodine solution. Aspirate the deepest portion of the lesion or sinus tract. Be careful to avoid contamination by the wound surface.

f. Throat (Pharyngeal specimens):

1. Do not obtain throat samples if epiglottis is inflamed, as sampling may cause serious respiratory obstruction.
2. Depress tongue gently with tongue depressor.
3. Extend sterile swab between the tonsillar pillars and behind the uvula. (Avoid touching the cheeks, tongue, uvula, or lips).
4. Sweep the swab back and forth across the posterior pharynx, tonsillar areas, and any inflamed or ulcerated areas to obtain sample.

g. Nasal swabs:

- Submitted primarily for the detection of staphylococcal carriers.
- After moistening the swab with sterile water or saline, insert the swab into the nose until resistance is met at a level of the turbinates (2cm).
- Rotate the swab against the nasal mucosa.
- Repeat the process on the other side.

h. Nasopharyngeal swabs:

- Submitted to diagnose *B. pertussis*.
- Carefully insert a flexible-wire calcium alginate-tipped swab through the nose into the posterior nasopharynx, and rotate the swab. (Keep the swab near the septum and floor of the nose).

i. Swabs for the culture of *B. pertussis*:

Contact lab to obtain special agar medium (charcoal-cephalexin plates) and “calcium-alginate” swabs for the collection of pernasal specimens.

How to take a pernasal swab: Insert the swab in the nasal passage, aiming towards the midline and down. Follow the floor of the nasal passage for ~5 cm (depending on the age of the patient) until progress is blocked by the posterior wall of the nasopharynx. Take > 1 swab on consecutive days for optimal results. Plates are incubated for 7 days. Alternative: nasopharyngeal swab or rinse, sputum if produced.

j. Oral cultures:

- Used to prepare smears for the detection of yeast or fusospirochetal disease.
- Rinse mouth with sterile saline.
- Wipe with lesion with dry sterile gauze.
- Swab or scrape areas of exudation or ulceration.

Other upper respiratory tract specimens that may be submitted to the laboratory by a clinician are sinus aspirates and tympanocentesis fluid.

5. GENERAL RECOMMENDATIONS FOR SPECIMEN COLLECTION FOR SEXUALLY TRANSMITTED DISEASES:

Cervical swabs: The cervix should be visualized via speculum examination and normal or inflammatory discharges should be removed with swabs. For chlamydia and gonorrhoea, the collection swab should be inserted 2-3cm into the endocervical canal and rotated against the walls of the canal to dislodge columnar epithelial cells. The swab is rolled onto a slide for microscopic examination or placed into appropriate transport/storage medium (Amies transport medium for GC and Chlamydial transport medium) for the subsequent diagnostic test required. Swabs for Herpes Simplex Virus (HSV) should be collected from the exocervix.

Genital Ulcer: Specimen collection requires different approaches depending upon whether diagnosis of syphilis, chancroid and/or herpes infection is required. In general, scabs and crusts of superficial pus should first be wiped away with gauze pads. Where syphilis is being evaluated, the base of the lesion should be squeezed until fluid exudates can be collected from the lesion surface with sterile capillary pipettes. For chancroid or herpes, swabs should be used to obtain specimens from the ulcer base and placed into appropriate transport medium. If vesicles are also present in the same area, vesicle fluid may be collected after lancing the vesicle.

Rectal swabs: Insert the swab 2-3cm into the anal canal, press laterally then rotate to obtain columnar epithelial cells with minimal faecal contamination. Process as for cervical swabs.

Urethral swabs: A thin cotton or Dacron swab on a wire shaft is inserted 2-4cm into the urethra, rotated and used to prepare smears for microscopic examination or placed into appropriate transport media.

Eye specimens: Conjunctival scrapings to detect *C trachomatis* should be collected by a medical officer or an experienced nurse. Using a dry sterile cotton swab, collect a specimen of the discharge or lower conjunctival surface and inoculate blood agar, chocolate agar or put in appropriate transport media. A smear of the discharge is made on a frosted-ended slide for staining with Gram or Giemsa technique.

TRANSPORT

1. All specimens should be transported to the laboratory promptly. Failure to do this may result in the death of fastidious organisms and in overgrowth by more hardy bacteria.
2. If prompt delivery is not possible specimens should be refrigerated at 4-8°C
3. Syringes:
Specimens obtained by a doctor using needle aspiration should be transferred to an anaerobic transport medium prior to transport to the laboratory. Alternatively, and only if transferring it from the syringe will compromise the specimen, the **doctor should remove the needle**, using a protective device to avoid injury, and cap the syringe with a sterile cap prior to transporting it to the laboratory. If the latter procedure is followed it is essential that the specimen be submitted to the laboratory immediately after collection.

6. COLLECTION AND TRANSPORT OF SPECIMENS FOR FUNGAL CULTURE

SKIN - Skin scales are collected by scraping the affected areas with a blunt scalpel. Material from the active margin of lesions is taken without drawing blood. In paronychia infections, the nail fold should be moistened with sterile water and a dental probe used to remove material from under the nail fold. Roofs of vesicles are snipped off with sterile scissors for examination. Clean skin with 70% ethanol particularly if ointments or other topical medications have been recently applied.

NAIL - Whole thickness of affected nails are clipped off using nail clippers. Subungual debris is scraped out with a blunt scalpel or dental probe and often contains much fungus.

HAIR - Scalp and other hair-bearing areas should be examined under a Wood's lamp. Fluorescent hairs (bright green in *Microsporum* infections, dull green in favus) or hair stumps should be plucked out with sterile forceps. If no fluorescence is noted, lustreless hairs or stumps of hairs broken off at follicular level should be plucked out.

Skin scrapings should also be taken from suspect areas (hair stumps are often extracted by this method). Scalp samples (especially for mass screening) can be obtained using individually bagged plastic massage brushes, velvet squares or even swabs.

TRANSPORT

Specimens should be sent DRY in clean specimen jars to prevent overgrowth of contaminating fungi.

Spores of fungi in these specimens will remain for many weeks to several years when maintained in a dry condition.

SUBCUTANEOUS FUNGAL LESIONS:

Send biopsy tissue or aspirated pus in sterile container.

SPUTUM, BRONCHIAL WASHINGS, TRANSTRACHEAL ASPIRATES etc.

Collect into sterile containers and transport to the laboratory without delay. Refrigeration will kill the yeasts of *Histoplasma capsulatum* rapidly, therefore this is not advised when histoplasmosis is suspected.

BONE MARROW

Bone marrow should be aspirated into Myco/F lytic blood culture bottles for fungal culture. At the time of collection, 2 smears should be made for special fungal staining and submitted to the laboratory with the material for culture.

7. INFECTIONS OF THE RESPIRATORY TRACT

UPPER RESPIRATORY TRACT

PHARYNGITIS, PERTUSSIS AND LARYNGITIS - see pus swab section

EPIGLOTTITIS

Culture of the throat is *not* indicated. Touching the inflamed epiglottis may precipitate complete obstruction of the airway

SINUSITIS

The specimen of choice is a needle aspirate of the sinuses.

Do not submit a swab. No specimen other than an aspirate is recommended

SPUTUM AND LOWER RESPIRATORY TRACT - COLLECTION AND TRANSPORT

INTRODUCTION:

Infections of the lower respiratory tract are a major cause of morbidity and mortality. Diagnosis of these infections frequently is complicated by the contamination of specimens with upper respiratory tract secretions during collection.

SPECIMEN COLLECTION:

Specimens include sputum, tracheal aspirates, bronchial washings, bronchial brushes, bronchial biopsy specimens, bronchoalveolar lavage fluid, transtracheal aspirate, lung aspirate and lung biopsy specimens.

- It is best to obtain a sputum specimen early in the morning, before the patient has eaten or taken medication.
- Collecting a good sputum specimen is not easy and requires that the patient be given clear instructions.
- It is important to remember that aerosols containing TB bacteria may be produced when the patient produces a sputum specimen.
- It is best for the patient to produce a specimen either outside in the open air or away from other people.
- Patients should not produce sputum in confined spaces such as toilets.
- The person supervising the sputum collection should stand behind the patient to avoid breathing in any aerosols that may be created when the patient coughs.

THE FOLLOWING INSTRUCTIONS SHOULD BE GIVEN TO THE PATIENT, WHEN COLLECTING SPUTUM SAMPLES:

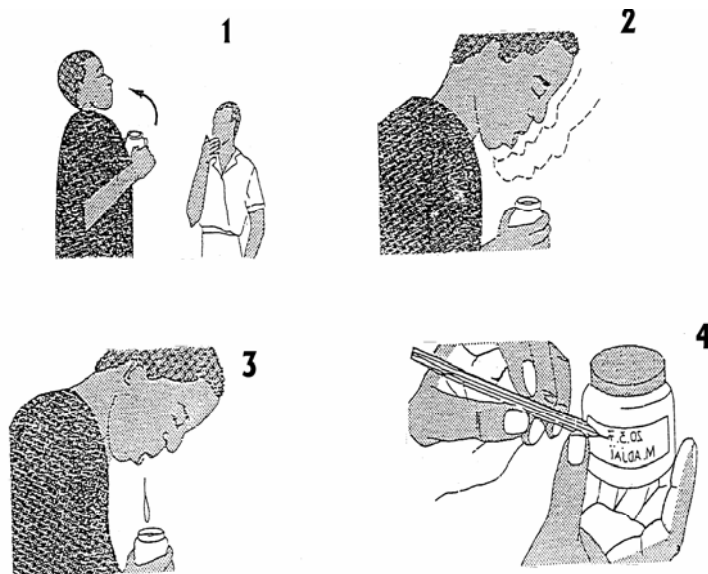
1. The patient should rinse his/her mouth with water, then take two deep breaths, holding the breath for a few seconds after each inhalation and then exhaling slowly.
2. The patient should hold the specimen container to the lower lip and gently release the specimen from the mouth into the container and avoid spills. An adequate specimen should be about 5 to 10 ml of sputum.
3. The specimen container is then capped and clearly labelled. **THIS SHOULD NOT BE DONE BY THE PATIENT.**
4. The specimens should be transported to the laboratory as soon as possible after collection. **DO NOT FREEZE SPECIMENS!**

PROCEDURE FOR INDUCTION OF SPUTUM for the isolation of *Pneumocystis jirovecii*:

1. Patient should preferably not have eaten for 8 hours.
2. Patient should brush teeth with water, rinse thoroughly and gargle several times.
3. Patient inhales 20-30 ml of hypertonic saline (3-5%) in a fine mist generated by an ultrasonic nebuliser over 10-20 minutes.
4. Patient is encouraged to take several deep breaths and cough deeply.
5. Collect sputum in sterile containers.
6. Sputum collected initially should be sent for TB and AFB, fungal culture and MC&S.
7. Later specimens are more likely to be representative of distal respiratory tract secretions and should be sent for *Pneumocystis jirovecii* examination.

GUIDELINES FOR PROPER SPECIMEN TRANSPORT:

All specimens should be transported to the laboratory promptly. Failure to do this may result in the death of fastidious organisms and in overgrowth by more hardy bacteria. If prompt delivery is not possible specimens should be refrigerated at 4-8°C.



TB LABORATORY

Proper collection procedures are imperative for accurate laboratory analysis. The quality of specimens collected and the proper transport of those specimens to the laboratory are critical to successful isolation of etiological agents.

General guidelines for specimen collection for TB analysis:

- Use **only** sterile, screw cap, leak proof, disposable plastic containers for specimen collection.
- Specimens taken and sent in syringes (without needles still attached) etc. **will not be processed**.
- Do not use waxed containers as they may produce false-positive smear results.
- Label the container with the patient's name, specimen type and date and time of collection.
- Collect initial specimens before antimicrobial therapy is started.
- Collect specimens aseptically, minimising contamination with indigenous micro-organisms.
- Swabs are not recommended for the isolation of mycobacteria.
- Collect sufficient material for the tests requested (see table below)
- Do not use any fixatives or preservatives
- The specimen should be transported to the lab as soon as possible after collection. If this is not possible, the specimens should be refrigerated to inhibit the growth of unwanted micro-organisms.
- **Do not freeze specimens.**
- Specimens should be dispatched in a box surrounded with absorbent packing and should be kept cool during transport.
- Mycobacteria are killed by ultraviolet light, therefore specimens should **not** be placed anywhere where they may be exposed to direct sunlight or become too hot.

Please note: the laboratory will not process leaking specimens!

Table: Specimen requirements for mycobacterial isolation and acid-fast stains

SPECIMEN TYPE	SPECIMEN REQUIREMENTS	SPECIMEN INSTRUCTIONS	UNACCEPTABLE SPECIMENS
Abscess contents, aspirated fluid	As much as possible in sterile container	Cleanse skin with alcohol before aspirating sample. Collect sample on swab, and place in transport medium only if volume is insufficient for aspiration by needle and syringe.	Dry swab
Blood	10ml heparin blood tube or 5ml inoculated directly into BACTEC Myco-F-lytic bottle	Disinfect site as for routine blood culture. Mix tube contents immediately after collection. SPS is preferred anti-coagulant, as it enhances growth of mycobacteria. Heparinised blood is also acceptable.	Blood collected in EDTA, Citrate, Oxalate or fluoride tubes – these inhibit mycobacterial growth even in trace amounts
Body fluids (pleural, pericardial, peritoneal etc.)	As much as possible (10-15 ml minimum) in sterile container. Collect bloody specimens into heparin blood collection tubes.	Disinfect site with alcohol if collecting by needle and syringe. Conical polypropylene bottles (200 ml) for centrifugation of large volumes are commercially available. Since many of these fluids may contain fibrinogen, it may be necessary to add anti-coagulant (heparin) to collection containers.	
Bone	Bone in sterile container without fixative or preservative		Specimen submitted in formalin
Bone marrow	As much as possible in heparin blood tube or inoculate directly into BACTEC Myco-F-lytic	Collect aseptically. Mix tube contents immediately following collection.	

SPECIMEN TYPE	SPECIMEN REQUIREMENTS	SPECIMEN INSTRUCTIONS	UNACCEPTABLE SPECIMENS
	bottle		
Broncho-alveolar lavage or bronchial washings	≥ 5ml in sterile container	Avoid contaminating bronchoscope with tap water. Saprophytic mycobacteria may produce false-positive culture or smear results.	
Bronchial brushing	Sterile container		
CSF	≥ 2ml in sterile container	Use maximum volume attainable	
Fine needle aspirate	Submit dry, unfixed slide and aspirate in a sterile container or directly inoculated into a specific culture bottle (only supplied to cytology clinic)	Make smear of aspirate on a clean dry slide. Air dry. Do not use any fixative.	Slide sprayed with fixative
Gastric lavage fluid	≥ 5-10ml in sterile container. Collect in the morning soon after patient awakens in order to obtain sputum swallowed during sleep.	Collect fasting early-morning specimen on 3 consecutive days. Use sterile saline. Adjust to neural pH with 100mg of sodium carbonate immediately following collection.	Specimen that has not been neutralised
Lymph node	Node or portion in sterile container without fixative or preservative	Collect aseptically, and avoid indigenous microbiota. Select caseous portion if available. Do not immerse in saline or other fluid or wrap in gauze	Specimen submitted in formalin
Skin lesion material	Submit biopsy specimen in sterile container without fixative or preservative. Submit aspirate in sterile container.	Swabs in transport medium (Amies or Stuarts) are acceptable only if biopsy sample or aspirate is not obtainable. For cutaneous ulcer, collect biopsy sample from periphery of lesion, or aspirate material from under margin of lesion. If infection was acquired in Africa, note on request form, because <i>Mycobacterium ulcerans</i> may require prolonged incubation for primary isolation.	Dry swab
Sputum	5-10ml in sterile, wax-free, disposable container. Collect an early morning specimen from deep, productive cough on at least 2 consecutive days. Do not pool specimens.	For expectorated sputum, instruct patient on how to produce sputum specimen as distinct from saliva or nasopharyngeal discharge. Have patient rinse mouth with water before collecting specimen to minimise contaminating specimen with food particles, mouthwash, or oral drugs, which may inhibit the growth of mycobacteria. For induced sputum, use sterile hypertonic saline. Avoid sputum contamination with nebulizer reservoir water. Saprophytic mycobacteria in tap water may produce false-positive culture or smear results. Indicate on request if specimen is induced sputum, as these watery specimens resemble saliva and risk rejection as inadequate.	24 hour specimens
Stool	≥ 1g in sterile, wax-free, disposable container	Collect specimen directly into container, or transfer from bedpan or plastic wrap stretched over toilet	Frozen specimen. Utility of culturing stool for acid-fast bacilli

		bowl. Wax from container may produce false-positive smear.	remains controversial.
SPECIMEN TYPE	SPECIMEN REQUIREMENTS	SPECIMEN INSTRUCTIONS	UNACCEPTABLE SPECIMENS
Tissue biopsy sample	1g of tissue, if possible, in sterile container without fixative or preservative.	Collect aseptically, and avoid indigenous microbiota. Select caseous portion if available. Do not immerse in saline or other fluid or wrap in gauze. Freezing decreases yield.	Specimen submitted in formalin.
Trans-tracheal aspirate	As much as possible in sterile container		
Urine	As much as possible (minimum – 40ml) of first morning specimen obtained by catheterisation or of midstream clean catch in sterile container. Send 3 consecutive early morning specimens. For suprapubic tap, as much as possible in sterile container.	Collect first morning specimen on 3 consecutive days. Accept only one specimen/day. Organisms accumulate in bladder overnight, so first morning void provides best yield. Specimens collected at other times are dilute and are not optimal.	24 hour pooled specimens, urine from catheter bag. Specimens of <40ml unless larger volume is not obtainable.
Wound material	See biopsy or aspirate	Swabs are acceptable only if biopsy or aspirate is not obtainable. If used they must be placed in transport medium (Amies or Stuarts). Negative results are not reliable.	Dry swab