

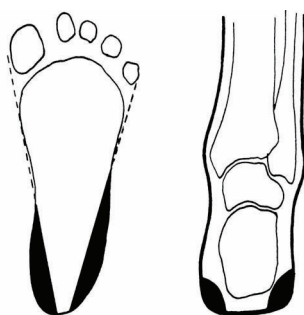
9 Procedures

9.1 Neonate Skin Puncture

Skin punctures on children must be performed on appropriate areas. Puncturing inappropriate areas can cause complications including osteochondritis, sepsis and gangrene.

Heel Puncture should be made on the most lateral or medial portions of the plantar surface (shaded areas). The puncture should NOT be performed:

- On the posterior curvature of the heel
- Through previous puncture sites
- In any area that has bruises or abrasions
- In the arch area of the foot as puncture in this area may result in injury to nerves, cartilage and tendons



Heel Puncture Sites

Fingerstick should be performed on the palmar surface of the distal phalanx (fleshy part of the end of finger) of the middle, ring or index finger. The middle or ring finger is the site of choice since the index finger has more nerve endings and is more sensitive. The sides and tips of the distal phalanx should not be used as the tissue on this area of the finger is only half as thick as that on the palmar surface. Likewise, the fifth finger should not be used as the tissue is too thin.

Fingersticks should be performed perpendicular to the fingerprints. If the puncture is made parallel to the fingerprints the blood will channel down the finger instead of forming a drop at the puncture site making collection much more difficult.



Fingerstick Sites

9.2 Kleihauer Test

This test detects foetal red cells in the maternal circulation. It is performed on Rh(D) negative mothers post delivery of an Rh(D) positive infant, or after any procedure or incident during pregnancy which may have provoked a placental bleed. The result is reported as the estimated number of mL of foetal red cells in the mother's circulation and will determine the dose of anti-D Immunoglobulin needed for effective prophylaxis. Advice on dosage will be given by the local Pathology Queensland laboratory.

9.3 Factor Assays

When collecting for factor assays, collect 3 x 3.5 mL citrated specimens. Spin tubes at approximately 1200 g for approximately 15 minutes and separate plasma immediately. Freeze plasma as soon as possible after collection. Specimen will deteriorate after 2 hrs at room temperature.

Factor assays are not to be confused with a request for Factor V Leiden, which is a test to detect the **presence of a genetic mutation** and uses EDTA whole blood, whereas Factor assays are tests to measure the **level** of a normal coagulation factor.

9.4 Double Spinning Method for Coagulation

If there is a delay in testing, some specimens need to be separated by using the double spinning method.

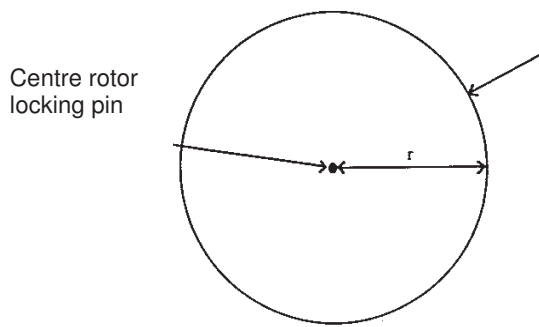
Procedure

1. Immediately after collection, the specimen is spun for 10 minutes at 3000 rpm.
2. The spun plasma should be removed from the cells as soon as centrifugation has completed.
3. Take care not to pipette up cellular debris.
4. Take off as much plasma as possible without disturbing the red cells, white cells & platelet layer.
5. Label this tube No.1.
6. Place separated plasma into a 5 mL sterile specimen tube and spin this sample for another 15 minutes to remove any residual cells or platelets.
7. After the second spin has been completed, remove the top 80% of the plasma into a second 5 mL collection tube.
8. Avoid touching the bottom of the tube which will contain any residual cellular debris spun down.
9. Label this tube No. 2.

Separated plasma (both tubes) should be stored and transported frozen. Plasma tubes should be wrapped in foil and taped to the ice brick during transport noting on the ice brick that there are specimens underneath. Some clotting factors are unstable and deteriorate quickly at room temperature. The original tube containing the red cells should be sent as well, but should not be frozen.

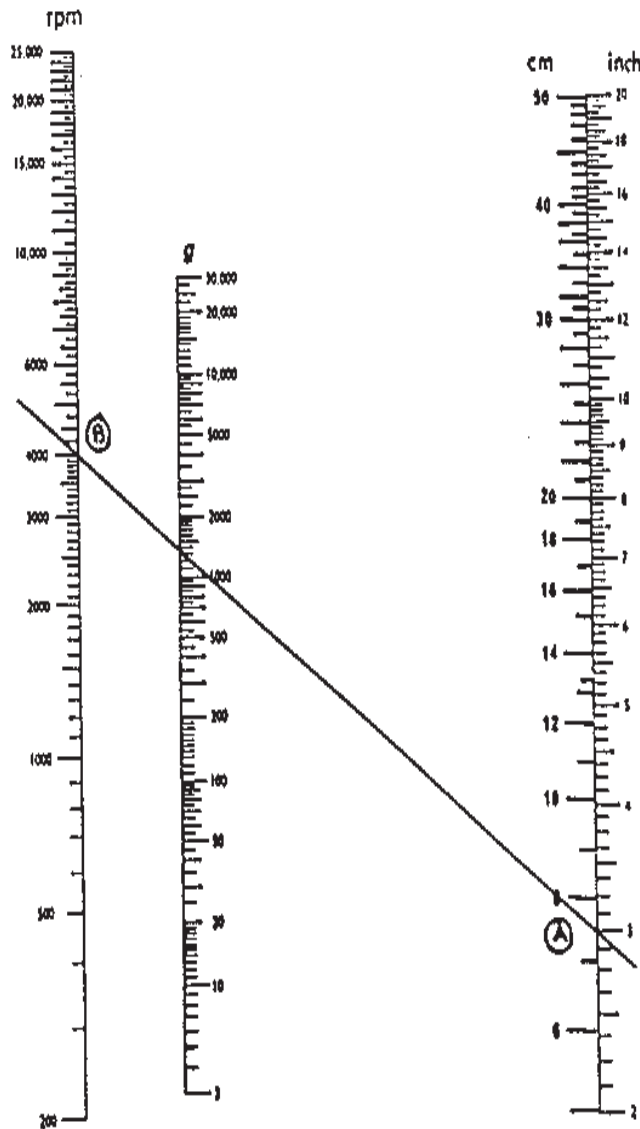
In order to obtain accurate coagulation results, it is essential to spin citrated tubes at approximately 3000 rpm for approximately 10 minutes as soon as possible after collection. See 9.5 for method of calculating accurate centrifugal force, g.

9.5 Nomogram for Centrifuging Blood



Outer bowl of centrifuge

If radius, r , of a particular centrifuge bowl is just over 3 inches (point A) and centrifuge rpm is set at 4000 rpm (point B) then centrifugal force g is at 1200 g . Since the radius of the centrifuge bowl is fixed by adjusting rpm (point B) the centrifugal force can be changed accordingly.



Conversion of this figure to rpm depends upon the radius of the centrifuge; it can be calculated by reference to the illustrated monogram.

r = radius (cm) and N = speed of rotation (rpm)

The following centrifugal forces are recommended:

Coags = 'High-spun' plasma 1200-1500 g (for 15 mins)

Clotted tubes = 3000 g for 10 min

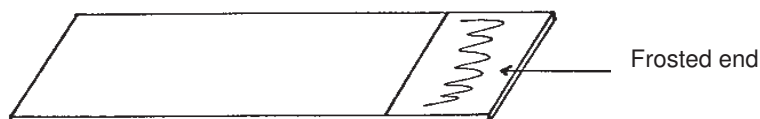
Nomogram for computing relative centrifugal forces. (By courtesy of MSE Ltd.)

9.6 Preparation of Blood Films for Routine Full Blood Count (FBC)

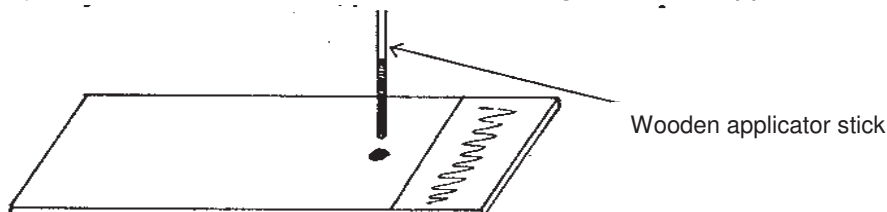
Equipment

- gloves
- safety glasses
- glass slides
- wooden applicator sticks
- glass spreader
- sharps container
- contaminated waste container
- slide carrier

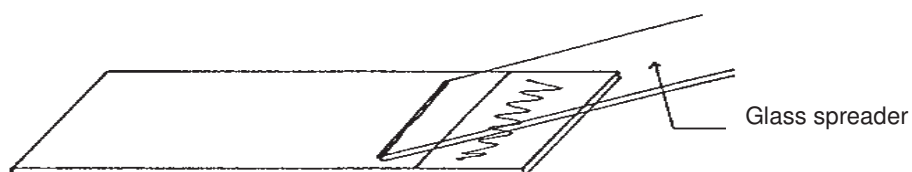
1. Place a clean dry slide on a flat surface, frosted side up.



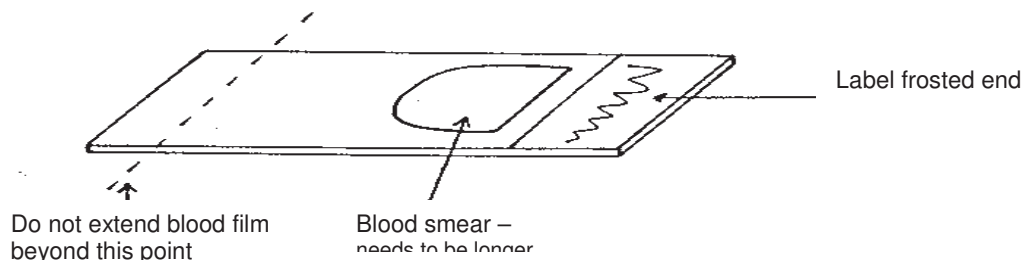
2. Wearing gloves and safety glasses collect the blood, place in an EDTA tube, mix by inversion for a minimum of 30 seconds, then transfer a small drop (no bigger than a match-head) close to the frosted end of the slide using a wooden applicator stick.



3. Without delay, take a special glass spreader and place it down in front of the drop of blood and draw the short edge of the spreader backwards until it makes contact with the drop of blood. The spreader should be held at about 45° from horizontal. To achieve thicker films if blood is anaemic hold the spreader closer to the vertical. Conversely if blood is thick/polycythaemic, hold the spreader closer to the horizontal. Wait until the drop runs along the edge of the spreader.



4. With the spreader held at the appropriate angle push forward smoothly and quickly in one motion to spread the blood. Note that blood is being drawn behind the spreader and not pushed in front. If the drop is too big the blood film will be too long and may possibly continue off the end of the glass slide. If the drop of blood is too small, the blood film will be too short. Ideally the tail should be smooth at the end. The length of the smear is ideally 2/3 along the slide.

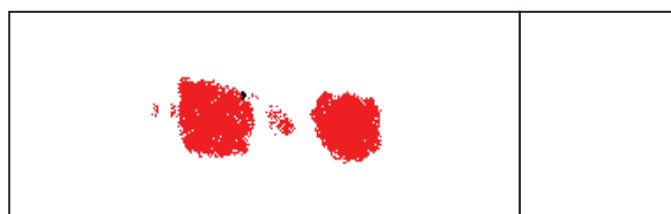


5. Dry film by waving it in the air. **DO NOT** blow dry with breath as this will haemolyse the blood. Label frosted end with the patient's details using a lead pencil (i.e. both names, DOB or UR No. if available).
6. Place the slide in a slide carrier once it is dry. Do not place inside an esky or refrigerator as this will cause haemolysis and render the film useless. Tape the slide carriers in an envelope and secure to the inside of the esky lid.
7. Dispose of all equipment in biological hazard waste bags. Ensure that all used glass slides are disposed of in sharps container.

9.7 Thick and Thin Slides for Malarial Parasites

Two thick and two thin films should be made from blood collected into an EDTA tube. For malarial studies the EDTA specimen must always be forwarded to the laboratory with the films as additional films may need to be made. The EDTA specimen will be sent on to the Malarial Reference Laboratory for confirmation if the patient has a positive malaria screen.

1. Place two large drops of blood 1cm apart onto a clean glass slide.
2. Evenly spread in a circular motion using a clean wooden applicator stick until each measures approximately 1cm in diameter and newsprint or a watch face can be read through the film of blood.
3. Allow to dry before placing in slide carriers.
4. The thin films are made in the same way as for a normal blood film.



Thick Film

9.8 Crossmatch, Blood Group and Antibodies

9.8.1 General Principles

For infants less than 3 months old with no previous transfusion history ABO compatible blood is used after crossmatching against the mothers blood. Blood for neonatal transfusion should be less than 5 days old. A leucocyte removal filter should be used. Contact the laboratory for details. Please give as much notice as possible to the laboratory as cleared blood may have to be sent from Brisbane when none is available from the local source.

Please phone the laboratory if blood is required urgently. An uncomplicated emergency crossmatch takes about 35 minutes bench time. Out-of-hours requests should be for emergency situations only. Requests for crossmatch for elective procedures should be received as early as possible the day before the blood is required. When fresh untested/uncleared blood is required in an emergency, the Red Cross Blood Bank must be contacted by the clinician for the release of blood for crossmatching.

Blood for transfusion must be stored in appropriate blood fridge at 2.5-5.5 degrees C. Only remove one unit at a time for immediate transfusion. Blood should not be returned to the fridge for possible reuse if it has been out of a controlled environment eg esky for more than 30 mins.

The laboratory will reclaim blood that has been crossmatched after 48 hours unless instructions to the contrary are given.

When fresh blood is required, it should be remembered that screening for Hepatitis B, hepatitis C and HIV is carried out in Brisbane and the blood is usually about two days old by the time it has been cleared.

Specimens for **crossmatching or group and hold** should be packed in a separate plastic bag for delivery to the **crossmatching laboratory**.

ALL EMPTY BAGS MUST BE KEPT AT THE HEALTH FACILITY FOR 48 HOURS AFTER TRANSFUSION AND THEN DISPOSED OF SAFELY IF THERE HAS BEEN NO REACTION.

9.8.2 Specimen Labelling and Request Form

Most acute haemolytic blood transfusion reactions result from clerical errors including wrongly labelled specimens. For this reason crossmatching and group and hold specimens must be legibly labelled and signed by the collector. See Section 4 for correct labelling.

The request form and specimen tube must carry identical patient information. Specimens, which are inadequately labelled, will have to be recollected. Unlabelled specimens will be discarded. **Pre-printed patient sticky labels must not be used for crossmatch or group and hold specimens.**

It is important to supply the information requested on the crossmatch form regarding previous transfusions, known antibodies. This information can be both vital to the safety of the patient and time saving to the laboratory. Crossmatch request forms without a required time or date collected will be treated as a group and hold.

9.8.3 Transfusion Reactions

All reactions that are considered potentially haemolytic must be reported immediately to the laboratory that supplied the blood. Possible haemolytic transfusion reactions must be investigated prior to further transfusion.

Procedure

1. The patient's identification and donor unit compatibility label should be rechecked at the bedside.
2. The following should be sent to the laboratory:
 - i. The blood pack, IV administration set and empty packs from previous units.
 - ii. 1 x 6 mL EDTA sample and 2 x 10 mL clotted (no gel) samples collected away from the transfusion site.
 - iii. Collect the first sample of urine passed by the patient after transfusion for haemoglobin and urobilinogen testing.

If the local laboratory is unable to investigate other types of transfusion reaction eg. allergic, leucocyte, platelet, HLA, further testing is conducted by the Red Cross Blood Transfusion Service in Brisbane to which the laboratory will refer specimens.

9.8.4 Group and Hold

Precautionary crossmatches can cause a large number of otherwise available blood units to be 'tied up' in the blood bank. As an alternative to precautionary crossmatching of donor units, a group and hold (i.e. a blood group and antibody screen and hold) is recommended. If the antibody screen is **POSITIVE** a crossmatch will be done against selected donor units and held for at least 48 hours.

If the antibody screen is **NEGATIVE** the patient's blood sample is held for 2 weeks. If the patient has had blood products in the previous three months, the sample will be held for 72 hours only. Experience in the United States has shown that the chance of a significant antibody being missed by the antibody screen is 1 in 17,000. A group & hold can be converted to a crossmatch by phoning the Crossmatch Laboratory.

9.9 Blood Cultures

9.9.1 Procedure for Collection

Materials:

1. Blood culture bottles and types may vary within regions. Usually a set will consist of one aerobic and one anaerobic bottle, or a single paediatric bottle, for a manual and automated system. A single bottle may be used in some smaller Pathology Queensland laboratories.
2. Sterile 20 mL syringe and 2-3 needles.
3. 5-6 Alco-wipes (70% isopropyl alcohol and 1% chlorhexidine).
4. Tourniquet & clean forceps for changing of needles.

Method:

1. Apply a tourniquet loosely to the arm and swab the skin over the chosen vein twice with two alcohol wipes. For maximum antisepsis, it is essential that the alcohol dries between applications and before insertion of the needle.
2. Flip the plastic cap off the blood culture bottle and cleanse the exposed rubber diaphragm with an alcowipe. Leave a clean swab on top of the bottle.
3. Wash hands and wear gloves.
4. Prepare the patient's skin over a suitable vein by re-swabbing with an Alco-wipe in a circular motion working outward and allow to dry.
5. Repeat step 4 once more.
6. Tighten the tourniquet and without palpating the vein again, perform the venepuncture and withdraw 10 mL **or** 20 mL of blood (10 mL of blood per bottle (adult), or 1-4mL (paediatric)).
7. Exchange the needle on the syringe with a fresh sterile needle using clean forceps.
8. Remove the alcohol wipe from the top of each bottle and inject 10 mL of blood into each bottle. Avoid injecting air into the bottle.
9. Mix the blood and the medium by gentle inversion a few times.
10. Clean the top of each bottle with an Alco-wipe to remove traces of blood.
11. Label the bottles checking name corresponds to request form. **Important: Do NOT** cover the barcode on the bottle as it is required for recognition of the bottle if an automated system is used by the laboratory.
12. **DO NOT** refrigerate the bottles and do not forward to the laboratory in an esky on ice bricks. See section 6.0 on transport.
13. Send the bottle(s) in a biohazard bag with request form to the laboratory.
14. Indicate on the request form any clinical details that might suggest to the laboratory that special culturing conditions are required, eg. SBE, meliodosis, fungi, Brucella, and other fastidious organisms.
15. Dispose of all sharps and blood stained swabs into appropriate sharps and biological waste containers.

9.9.2 Positive Blood Cultures

If a blood culture is positive the results are **telephoned** to the health facility **immediately** the causative organism is identified by the laboratory. The morphology +/- motility of the

organism will be reported. The laboratory may comment on the possible identification at a genus level and link the blood culture isolate with other positive cultures eg. urine, cerebrospinal fluid or wound swab. Identification and preliminary antimicrobial susceptibilities will be available usually in another 24 hours. This information will be entered onto the Pathology Information System and can be accessed by facilities with access to the system, minimising the need to telephone the laboratory for results. Written reports will be issued following validation of the identification and antimicrobial susceptibilities.

9.9.3 Leptospiral Blood Culture

Leptospirosis is caused by the spirochaete *Leptospira interrogans*. Infection in humans may result from contact with infected animals or an environment contaminated with their urine.

Leptospira usually only remain in the blood for 7-14 days during which period the patient may experience mild to severe fevers with myalgia. This is the ideal time to isolate the organism by culture.

Culture should be attempted prior to treatment with antibiotics. Culturing after 14 days is rarely successful, therefore if leptospirosis is suspected a culture specimen should be taken as soon as possible.

Procedure for Culture of Blood for *Leptospira*

1. Inoculate two tubes of *Leptospira* culture medium with one culture tube receiving 2 drops of whole blood and the other 5 drops.
2. A serum specimen should also be submitted to allow for serological investigations against *Leptospira* antibodies.
3. **DO NOT REFRIGERATE** specimens.
4. Specimens must be held and transported at ambient temperatures above 13°C. Overnight storage at 2°C will kill *Leptospira*.
5. If **culture medium** has been refrigerated incubate the **medium** to room temperature before use.
6. Specimens must be kept out of direct sunlight – ultraviolet light will destroy the organism.

9.10 Oral Glucose Tolerance Test (GTT) for Diabetes Mellitus

This test is used in the classification of patients as having impaired glucose tolerance, diabetes mellitus or gestational diabetes.

Special Notes

- For three days prior to the test the patient must be on a diet containing approximately 150 grams of carbohydrate daily.
- Drug therapy should be stopped if possible.
- Smoking is not permitted during the test.

Contraindications and Precautions

Prior to the commencement of the test, a random or fasting blood glucose must be sent to the laboratory for analysis. A glucose tolerance test must **NOT** be performed if:

- The patient is ill, as the test is invalid in the presence of intercurrent illness (e.g infection) or after recent surgery
- The patient is known to have diabetes mellitus
- The patient has symptoms suggestive of diabetes mellitus with either fasting plasma glucose > 7.8 mmol/L on two occasions or random plasma glucose > 11.1 mmol/L on two occasions

Procedure for GTT

1. Following a fast of at least 8 hours, but no more than 16 hours collect 5 mL fluoride oxalate blood. This should be analysed via a glucometer before the glucose dose is given.
2. If the fasting glucose is greater than or equal to 7.8 mmol/L the glucose dose is withheld until discussed with the laboratory.
3. If the fasting glucose is less than 7.8 mmol/L the oral dose of glucose is given. Dose: Adult = 75 g; Child = 1.75 g/kg body weight (75 g maximum).
4. Following the dose of glucose, collect 5 mL of fluoride oxalate blood 1 and 2 hours post dose with time of collection (actual time plus hours post glucose) clearly recorded.
5. Note if the patient has any nausea, pallor, or sweating during the test.

9.11 Urine Collection**9.11.1 Twenty Four Hour Urine Collection****Procedure**

1. Instruct the patient to discard the first specimen Day 1, marking the time on the label.
2. Collect all of the urine for the next 24 hours until the same time Day 2 when the bladder is emptied into the bottle supplied.
3. Specimen should be kept in a cool place during collection (eg. bottom of the refrigerator) and returned to the health facility on the morning of completion.
4. When accepting delivery of the specimen ensure the label is completed with the patient's details and exact commencement and completion times and dates.
5. Measure the total volume and document on the request form with added preservatives if any were used.
6. Send 50 mL of the collected urine in an aliquot container to the laboratory for analysis.
7. Urine should be stored at 4°C.

24 Hour Urine Collection Preservative Requirements

Analyte	Preservative
5 HIAA HMMA (VMA) Catecholamines Cystine Oxalate	15 mL of 6 M Hydrochloric Acid Always advise patients to be careful with the additive
Albumin Timed Urine (Microalbumin) Calcium Timed Urine Copper Cortisol Urinary Free Phosphate Protein Zinc Creatinine Clearance	4 g of boric acid A request for Creatinine Clearance requires plasma collection on the day the 24 hour urine collection is returned
Mercury Metabolic Screen Chloride Timed Urine Lead Magnesium Sodium Urate Urea and Electrolytes	No Preservative or additive Refrigerate Send Cold

9.11.2 Urine Specimens for Microbiological Examination

The best specimen for a microbiological examination of urine is an early morning specimen. If infected, urine bacterial counts are highest at this time due to overnight incubation in the bladder.

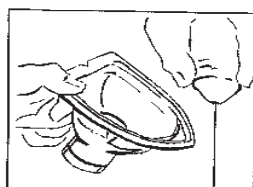
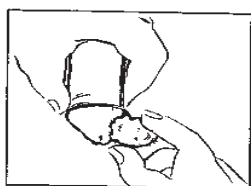
When requesting a micro-urine please indicate whether the patient has a catheter and whether the patient is on antibiotics or not. Also please indicate the date of the last request in cases for follow-up micro-urine.

9.11.3 Procedure for the Collection of Urine

1. Clean the urethral opening with water or saline to reduce microbial normal flora and contamination of the specimen.
2. For males, the glans penis should be swabbed using three separate sterile swabs with a downward motion and the foreskin, if present, retracted throughout the collection.
3. For females, the interlabial area should be swabbed using three separate sterile swabs with a downward motion and the labia parted throughout the collection.

9.11.4 Procedure for the Collection of a 'mid-stream' Urine Specimen

1. The first passage of urine should be voided into the toilet to flush out normal flora resident in the urethra.
2. The middle portion of the stream should be collected in the sterile container provided.
3. Additional urine should then be voided into the toilet.



9.11.5 Paediatric Urine Specimen

Procedure

1. Special sterile paediatric urine collection bags are used to collect urine specimens from infants.
2. Cleanse the external genitalia using sterile saline swabs and then dry the area to remove any normal skin or gastrointestinal flora contaminating the skin.
3. A paediatric collection bag can then be attached to a male or female infant via the adhesive portion of the bag. A nappy should then be placed on the child.
4. If no urine is passed within 30 minutes this procedure should be repeated and a **new bag** attached.
5. When urine has been collected remove the bag immediately. Use sterile scissors to snip the corner of the bag and transfer the urine to a sterile collecting container.

NB: There are disadvantages of infant urine collected in this way. These are:

- A 'mid-stream' urine is not collected
- The specimen will contain the normal urethral flora
- There is greater possibility of picking up normal skin flora or gastrointestinal flora during the collection
- These factors all make it difficult to interpret the results

Figure 1.11

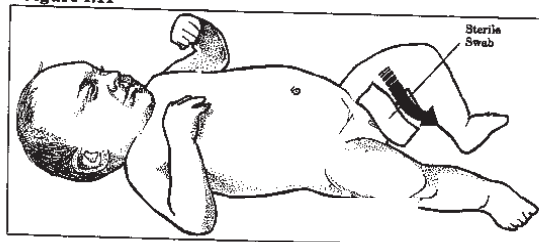


Figure 1.12

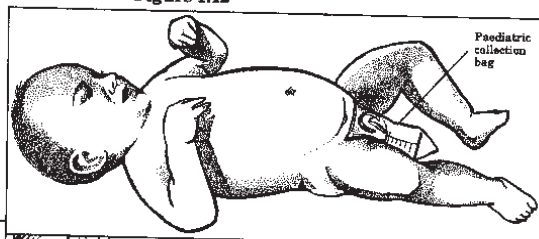
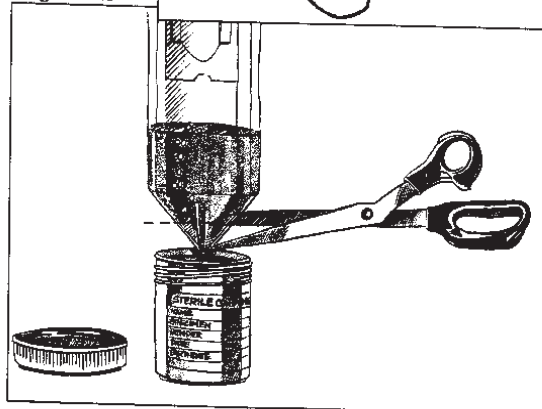


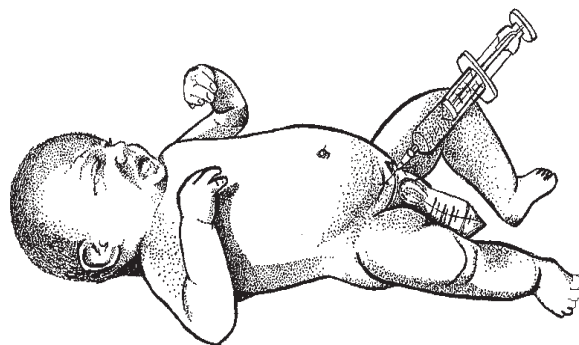
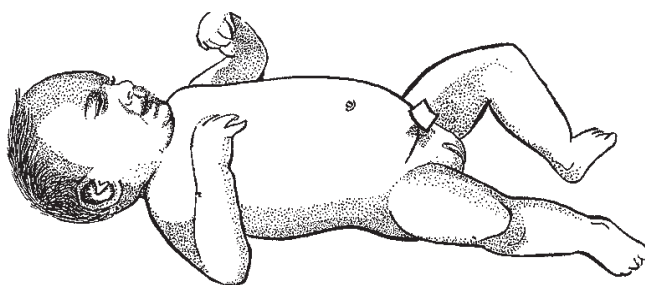
Figure 1.13



9.11.6 Collection of a Suprapubic Aspirate

Procedure

1. This is an aseptic procedure. The skin of the lower abdomen is swabbed using povidine iodine or an alcohol swab to prevent contamination or inoculation of organisms into the bladder.
2. The bladder should be full prior to performing this test.
3. A paediatric urine collection bag is attached.
4. A sterile needle is then inserted through the abdominal wall directly into the bladder at a site 2 cm above the pubic symphysis and urine is then aspirated.
5. The urine is then transferred aseptically to a sterile collecting container.
6. Any microorganisms isolated from this specimen must be considered significant because the specimen is taken directly from the bladder.



9.12 Sexually Transmitted Infections

9.12.1 Syphilis

A Rapid Plasma Reagin test (RPR) may be positive as early as five days from the start of symptoms. The RPR test is not specific for syphilis and is susceptible to biological false positives, eg. pregnancy, systemic lupus erythematosus (SLE) and viral illness. The RPR usually becomes reactive 4-6 weeks after infection or 1-2 weeks after the appearance of the primary lesion, and is a useful tool to monitor both progression and treatment of syphilis. If the RPR is positive, specific treponemal tests are performed - Treponema Pallidum Particle Assay (TPPA) Fluorescent Treponemal Absorbed Antibodies. These tests usually become reactive 4-6 weeks after contact. The specific treponemal tests usually stay reactive for life (despite treatment). The possibility of biological false positives still exists.

A request for RPR and TPPA must be written on the Pathology Request Form

Cerebrospinal Fluid – VDRL

The CSF-VDRL test is still the only serological test recognised as the standard for the diagnosis of neuro syphilis. If neurosyphilis is suspected, the CSF-VDRL test must be specifically requested.

A 6 mL clotted blood specimen must also accompany the CSF for confirmation of the result by serum RPR, Treponemal Pallidum Particle Assay and Fluorescent Treponemal Absorbed Antibodies.

9.12.2 Donovanosis (*Granuloma inguinale*)

GUMP (Genital Ulcer Multiplex PCR) Test (*Refer to Primary Clinical Care Manual for more information*)

The GUMP test is performed on a swab taken from a genital ulcer. It is funded in the Northern Area Health Service if the following criteria are present:

1. Client is Indigenous
2. Donovanosis is suspected
3. The cause of a genital lesion is unclear

Equipment

1. Sterile dry swab
2. 5mL yellow top specimen container OR a clear plastic transport container that does NOT contain an added medium. A container GUMP pcr
3. Clean glass slide and cardboard carrier
4. Normal Saline
5. Gauze Square

Specimen Collection

1. Clean lesion with Normal Saline
2. Swab the edge and centre of an open genital ulcer (crusted specimens must be gently de-roofed)
3. Aim to moisten most of the swab with ulcer exudate
4. Place swab in transport container (that contains no medium)
5. Press the glass slide firmly onto the open lesion, particularly around edges and allow to air dry

9.12.3 Collection of Genital Specimens

Genital specimens are collected to isolate or detect pathogens that cause sexually transmitted diseases including *Neisseria gonorrhoeae*, *Treponema Pallidum*, *Chlamydia*, *Trichomonas vaginalis*, herpes simplex virus and others.

Specimens are also required to investigate other infections such as puerperal sepsis, septic abortion, pelvic inflammatory disease, urethritis and vaginitis.

Different collection techniques are employed depending on the cause of the infection and the likely site infected. Often more than one type of collection may be required to investigate an infection (e.g urethral, high vaginal and/or endocervical).

In female patients, if sexual history reveals a strong suspicion of gonorrhoea and/or chlamydia, it is best practice to insert a speculum into the vagina to visualise the cervix to prevent contamination of swabs from the endocervical canal by the normal flora of the vagina.

However if a non-invasive examination is more appropriate due to patient preference, the PCR technology offers an opportunity of still testing reliably for gonorrhoea, chlamydia and Trichomonas with either a high vaginal swab taken by the patient themselves OR a first catch urine specimen.

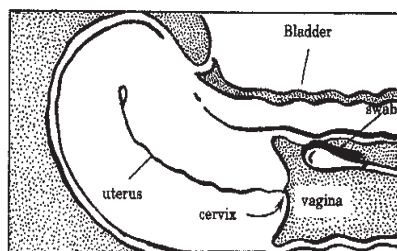
9.12.4 Collection of (High) vaginal Swabs (self or clinician collected)

Procedure for MCS swabs and smear (charcoal)

1. Two swabs should be taken from the vagina
2. The first should be used to make a smear on a microscope slide
3. The slide should then be allowed to air dry
4. The second swab should be placed in transport media

Procedure for PCR Swabs (female)

1. Two swabs should be taken from the vagina. One for Gonorrhoeae, Chlamydia, and the other for Trichomonas vaginalis.
2. Insert each swab into vagina
3. Wait for approx 10 seconds rotate swab stick and remove, place in transport tube.



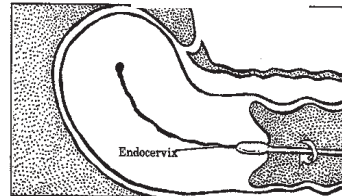
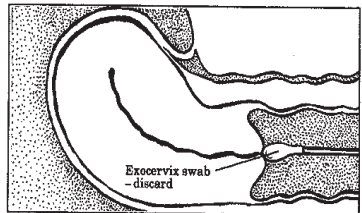
9.12.5 Collection of Cervical and Urethral Swabs/Smears

Procedure for MCS Charcoal (female)

1. Insert a vaginal speculum.
2. Remove excess discharge from the exocervix using a swab or cotton ball so that the cervix can be plainly seen. Infections often occur in the cervix rather than in the vagina.
3. Using two swabs collect discharge from the endocervix, being careful not to touch the walls of the vagina. A poorly collected specimen can be detected as the stain will demonstrate contamination with cells and normal flora of the vagina.
4. Use the first swab to prepare a smear on a microscope slide, roll it gently over the surface of the slide, and allow it to air dry.
5. Place the second swab in transport media for culture.

Procedure for PCR swab (female)

1. Collect cervical discharge on one PCR swab and high vaginal discharge on a second swab
2. Place swabs into two separate PCR transport tubes
3. Label the cervical specimen for “chlamydia/gonorrhoea PCR” and the vaginal specimen for “trichomonas PCR”



Procedure for MCS Swab Charcoal (male)

1. Do not insert swab stick into urethra
2. Milk the penis (if necessary) and collect the discharge at the urethral meatus
3. Roll swab onto a glass slide and allow to air dry
4. Collect a second specimen and place the swab in transport media for culture

Procedure for PCR Swabs (male)

1. Do not insert swab stick into urethra
2. Milk the penis (if necessary) and collect the discharge at the urethral meatus
3. Collect two specimens, and place each into the PCR transport tubes
4. Label one for “chlamydia/gonorrhoea PCR” and the second for “trichomonas PCR”.

9.13 Faeces

9.13.1 Investigation of Diarrhoea

Although diarrhoea is a clinical condition, the cause of the diarrhoea cannot be determined clinically. The following examinations are required because:

- a. multiple pathogens may be present at any one time
- b. clinical symptoms are not specific for a causative organism
- c. diagnosis is essential to ensure that appropriate therapy is instituted

Wet preparation will determine:

1. Presence of leucocytes (inflammation).
2. Presence of erythrocytes (ulceration).
3. Presence of Protozoan amoebae, Giardia trophozoites, Blastocystis hominis.
4. Specific motility of some intestinal bacterial flora (Vibrio cholera).

NOTE: Rotavirus infection is the commonest cause of diarrhoea in children under 5 years old. It is seasonal and may be tested routinely on loose specimens during the winter months or anytime on request.

9.13.2 Bacterial Culture

See Section 8.3 for specimen collection guidelines. Anti-microbial susceptibilities are not reported to ensure bacterial gastroenteritis is not inappropriately managed with anti-microbial agents that may lead to:

- a. persistent carriage of the pathogen
- b. bacterial resistance
- c. possible overgrowth or super infection with other microorganisms

9.13.3 Toxin Assay

Testing for *Clostridium difficile* will be performed if the patient has previously been on antibiotics. Submit the faeces in a separate container.

Cytotoxin B and Enterotoxin A requests are referred to laboratories that perform these tests. Please note that the toxin producing *C. difficile* may be found in up to 50 percent of infants.

9.13.4 Worms or Proglottids for Identification

Adult worms or proglottids of tapeworms may be passed with or without faecal material. These can be picked out of the faeces and placed in a sterile container of saline or water and sent directly to the laboratory. Where there is delay send specimens in 10% formalin.

9.13.5 Viral Examination

Rotavirus Viral culture is probably not useful except if the patient is immuno-compromised. This is because there are normally large numbers of viruses in the bowel and the two common causes of viral gastroenteritis, Rotavirus and Norwalk virus, cannot be cultured.

Stool culture for virus may be requested to determine the cause of suspected viral meningitis. If viral culture is required, submit a specimen in a separate container for forwarding to the nearest Pathology Queensland Laboratory.

9.14 Meningitis

In investigation of acute meningitis, blood cultures taken at presentation can be very useful in establishing the cause of the disease, especially if antibiotics have already been administered. For specific details of bottles, please refer to the blood culture protocol 9.9.1.

9.15 Peritonitis in Continuous Ambulatory Peritoneal Dialysis (CAPD)

Peritonitis is generally associated with a cloudy effluent containing more than $100 \times 10^6/L$ white cells. A cloudy bag may be caused by the presence of erythrocytes, chyle, fibrin and other factors.

Specimen Collection

If the patient notes a cloudy bag or has a tender abdomen at home, the bag should be brought to the hospital for investigation in the laboratory. Do not submit clear bags especially if they are mixed with antimicrobial agents.

The whole bag of effluent should be submitted to the laboratory for sampling under sterile conditions. The first or most cloudy bag (if it is not the first bag) should be sent.

Do not refrigerate the effluent bag prior to or en route to the laboratory as this inhibits the isolation of organisms.

Specimen and Request Forms

1. Note the date and time the bag was collected.
2. **LABEL** the **bag** (NOT the packaging) with the patient's details.
3. Note the presence or absence of antibiotics in the effluent.

Request

1. Quantitative cell counts and a differential count.
2. Gram stain of the centrifuged deposit of 50 mL of dialysate.
3. Culture for aerobic and anaerobic organisms, *Nocardia* and fungi.
4. Culture for *Mycobacterium* sp. on specific request.

9.16 Investigations of the Upper Respiratory Tract

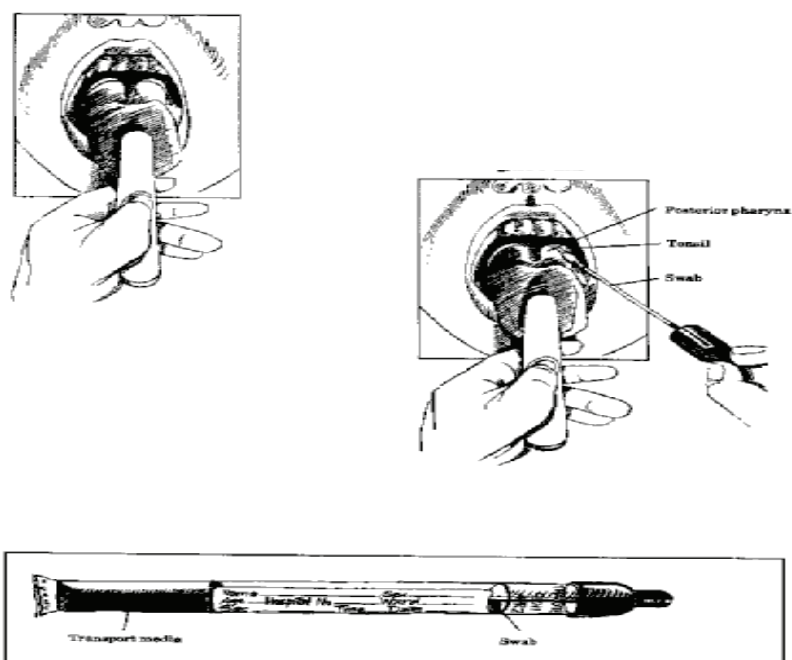
Throat swabs are examined for the presence of pathogens if there is a yellowish purulent exudate or if there is a greyish membrane. The most common cause of an upper respiratory infection is *Streptococcus pyogenes*, Group A beta haemolytic Streptococci, and the possibility of diphtheria due to *Corynebacterium diphtheriae* must always be considered. Many other bacteria can also be found in the throat but are considered normal flora. Screening for *Strep. pyogenes* carriage in communities is infrequently warranted. If this screening is being considered contact the microbiology department of your Pathology Queensland regional laboratory.

Most sore throats are due to viral infections. Only 5-10% of sore throats in adults and 15-20% in children are associated with bacterial infections.

Microscopic examination of smears from throat swabs are of little value because of the large variety of normal flora. Pathogens are generally isolated by culture.

9.16.1 Collection of a Throat Swab

1. Use a sterile swab and a tongue depressor for the collection.
2. Depress the tongue in order to visualize the collection site.
3. Swab the tonsils and the posterior pharynx.
4. Avoid the tongue and the uvula to reduce contamination of the specimen with normal flora and to prevent gagging.
5. Any visible exudate should also be swabbed.
6. Place the swab into transport media or viral transport media and send it to the laboratory.



9.16.2 Nasopharyngeal Mucus Aspirate

Many microorganisms can commonly be present or carried in the nasopharynx rather than in the nose or the throat, including *Strep. pneumoniae*, *Neisseria meningitidis*, *Haemophilus* species, *Chlamydia trachomatis*, *Chlamydia pneumoniae* and *Bordetella pertussis*.

Detection of respiratory viruses by rapid fluorescent antibody test requires an aspiration of nasopharyngeal secretions. Other specimens are unsuitable.

NOTE: Since this is a direct test against seven different viral antigens, a negative result would not be expected to become positive on repeat testing. Therefore a second specimen is not necessary unless requested by the laboratory.

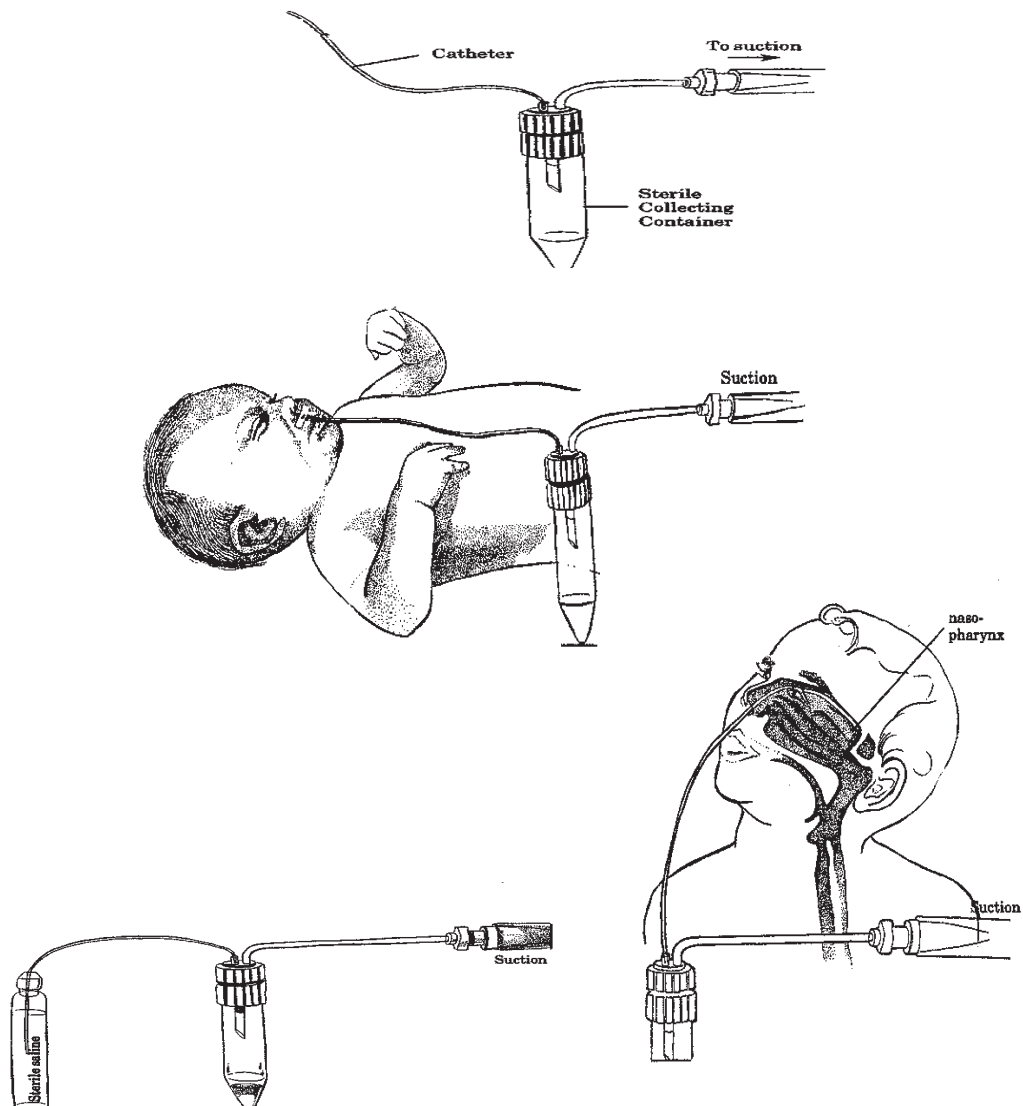
Arrange immediate delivery of the mucus extractor/suction set to the laboratory. This is necessary due to the rapid deterioration of the specimen even at 4 °C.

NB. – Viral Transport Media contains antimicrobials. Samples collected for viral or other antigen detection cannot be used for bacterial culture. If bacterial culture is required an additional aspirate sample must be collected and submitted.

Procedure

An aspirate is best collected early in the morning before breakfast because secretions have collected in the cavity overnight and any microorganisms present will have multiplied in this site.

1. Attach a sterile polythene feeding tube Size 8 to one of the two outlets of a sterile plastic mucus extractor or paediatric suction set.
2. A tube connected to the second outlet may be attached to a suction pump with a maximum negative pressure of 26 psi.
3. The catheter is then inserted into the posterior nasopharynx.
4. Approximately 0.2 to 0.5 mL of fluid is sucked into and trapped in the sterile collection container.
5. If the secretions are trapped in the catheter then sterile saline can also be suctioned into the catheter. This will wash any secretions into the sterile collecting container.



9.17 Investigations of the Lower Respiratory Tract

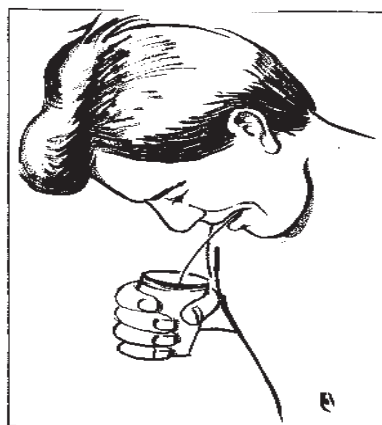
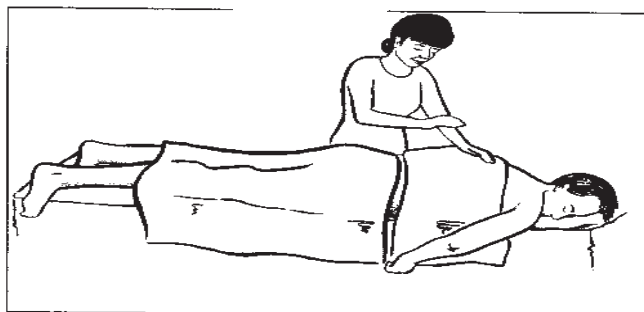
The lower respiratory tract can become infected by many of the same viruses and bacteria that infect or colonise the upper tract, and these can be diagnosed by examination of a sputum specimen. Organisms most frequently recovered from lower respiratory tract infections include *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Haemophilus influenzae*, *Legionella pneumophila* and *Mycobacterium tuberculosis*.

The specimen is cultured and a Gram stain is performed. If on staining the specimen contains many squamous epithelial cells, this suggests contamination with saliva. If a large number of both polymorphonuclear leucocytes and bacteria are present this is indicative of an infection.

The collection of a 'good' sputum specimen with minimal contamination from the normal flora of the upper respiratory tract is essential. Prior treatment by physiotherapy improves the collection. The specimen is best collected early in the morning before food, fluid and antibiotics are given. This will minimise the contamination of the specimen with foreign material and at this time antibiotic concentrations in the body are at their lowest. A saliva specimen is not suitable. Mucus should be thick and viscous and if infected, may be purulent, green or blood stained. Saliva appears as a thin watery frothy fluid and contains normal flora of the upper respiratory tract.

Procedure

1. Place the patient in a postural drainage position.
2. Pummel the chest region to loosen mucus.
3. The sputum is then coughed up from the lower tract into a sterile collection container.



9.18 Neonatal Gastric Aspirate

These are always processed as urgent specimens.

Specimen should be forwarded in a yellow-topped sterile jar.

Indications for collection from the newborn:

- where the mother has been febrile in labour
- where labour was premature
- where there was prolonged rupture of membranes >24 hrs
- no antenatal care

A urine specimen may be useful in screening cases of possible sepsis for Group B Streptococci in neonates.

9.19 Examination of Synovial Fluid

Routine Tests - Microbiology

1. Cell count with differential (only if heparinized tube provided).
2. Crystallography (clotted and anti-coagulated specimens will be examined).
3. Culture and gram stain.

Other Tests available on Specific Request

1. Biochemistry, eg. Glucose, protein, lactate dehydrogenase (specimen collected in heparinized tube).
2. Rheumatoid factor, antinuclear antibodies and other tests (specimen in sterile container only).
3. For other microbiology eg. virology, mycobacteria contact the nearest Pathology Queensland laboratory.
4. If septic arthritis is suspected, a blood culture should also be collected. Examination of synovial fluid specimens under these circumstances is an urgent test.

Note: If the syringe contains sufficient aspirate, carefully remove the needle and discard into a sharps container. Cap the syringe with a syringe cap and forward all samples to the laboratory.

Delay in transit of samples will adversely affect crystal examination and also the results.

9.20 Collection of Clinical Specimens for the Isolation of Fungi

Fungi are ubiquitous, eucaryotic microorganisms, and the majority of species grow well as saprophytes on nonliving organic materials. Most species that do infect humans are limited by nutritional requirements and by the host's defences to invasion of the superficial skin and subcutaneous tissues.

Some species are capable of invading the deeper tissues to cause serious, life-threatening infections. Human fungal infections may be classified as superficial mycoses, subcutaneous mycoses and systemic mycoses. Subcutaneous mycoses are caused by organisms that normally grow in soil and decaying vegetation, and are introduced by trauma into the subcutaneous tissue, causing disease. Systemic mycoses are caused by soil fungi and infection is acquired by inhalation. These rare infections may disseminate and infect any organ. Contact the laboratory for required tests.

Superficial Mycoses

Superficial mycoses are caused by organisms which include the dermatophytes (*Microsporum*, *Epidermophyton*, *Trichophyton*, *Candida* sp.) invading only the superficial keratinized tissues of skin, hair and nails. Dermatophyte infections include tinea, ringworm and athlete's foot.

Specimens collected are:

1. Skin scrapings - collected from areas of cracking, peeling and dry skin. There may be small vesicles that rupture and release clear fluid. Ringworm infections typically have a red advancing border with a centre area of dry, flaking, healing skin.
2. Hair - collection of hair specimens is indicated if the hair is fragile, or broken off just above the scalp leaving short stubs and a balding circular patch. There may also be scalp involvement indicated by redness, oedema, scaling and vesicle formation on the scalp.
3. Nails - infected nails are often brittle, flaky, discoloured, thickened or grooved.

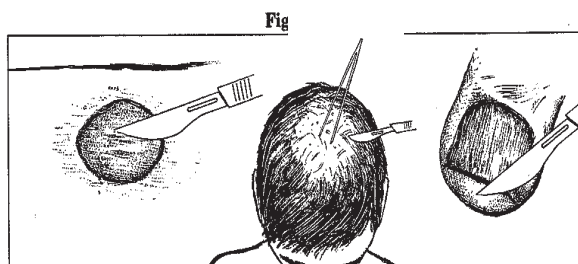
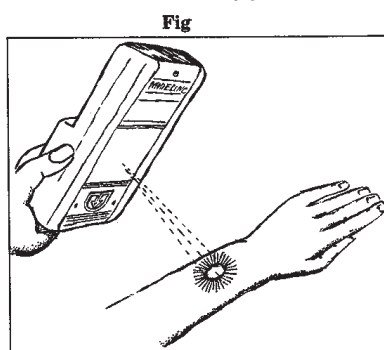
Procedure for Collecting Fungal Specimens from Skin, Hair and Nails

Skin

1. Shine an ultraviolet lamp onto the infected area. Some infections will fluoresce when irradiated with an ultraviolet lamp.
2. Swab the skin thoroughly with a 70% alcohol swab.
3. The red advancing border is the region with active fungal growth.
4. Using a sterile scalpel blade scrape scales of skin from the active edges of the lesion, into a sterile, dry container such as a urine collection jar. The specimen must be kept dry to prevent bacterial growth.
5. Use a fresh scalpel blade for each site tested to prevent the spread of the infection during the collection.
6. Place the used blade in the container after the collection. Sometimes minimal skin flakes are obtained and so they may be collected from the edge of the blade for culture.
7. Send dry to the laboratory for microscopy and culture.

Hair and Nails

1. Select infected hair and nails.
2. Swab the area with alcohol.
3. Pluck or cut hair using sterile scissors or forceps, or clip infected nails to obtain infected hair or nails.
4. Place the used blade in the container after the collection and forward to the laboratory for microscopy and culture.



9.21 *Mycobacterium ulcerans*

M. ulcerans infection classically develops as a subcutaneous nodule, particularly on the extremities which later breaks down to reveal an ulcer characterised by variable undermining of the edge, vascular necrosis, especially of the fatty tissue, and accompanied by round cell infiltration, oedema and variable fibrosis.

The disease although rare is endemic in some areas of Queensland. Cases have been reported from Beerwah, Maryborough, Rockhampton, Townsville, Cairns and the Mossman/Daintree area. Slow healing ulcers with suggestive appearance should be screened. The infective organism is an **acid-fast bacillus** which is usually found in great numbers in necrotic areas and beneath the undermined edge of the lesion.

Procedure

1. Take two swabs from deep in the undermined area as far as is possible, and place in a sterile container with a very small amount of **sterile water**, not saline.
2. A direct smear for Ziehl Neilsen (Z-N) staining is also required.
3. Label the swabs No.1 for PCR and No. 2 for mycobacterial culture. Send both swabs to the nearest Pathology Queensland laboratory for referral.

9.22 Leprosy

The protocol for screening is available from Pathology Queensland. If further details are required contact the nearest Pathology Queensland laboratory.

9.23 Viral and Bacterial Serology

Serology testing can be a valuable tool in the diagnosis of viral or bacterial infection. To maximise serological diagnosis, the collection of both acute and convalescent specimens is required. The acute specimen should be collected as soon as possible after the onset of symptoms and the convalescent specimen collected at least 14 days after the onset of symptoms.

The specific aetiological organism must be requested by a medical officer where possible.

It is also recommended that the Clinical Notes be as thorough as possible to ensure the most suitable screens are requested.

Another method of use in the diagnosis of viral illness is the Viral Culture. The use of both Serology and Viral Culture may provide a clearer picture when it comes to determining the specific causative organism involved and the progression of resultant infection. Please refer to appropriate sections for collection guidelines.

9.23.1 Streptococcal Serology

Anti Streptolysin O and anti-DNase B titres are used in the diagnosis of rheumatic fever and acute glomerulonephritis, the latter being associated with streptococcal pharyngitis.

Complement components C3 and C4 testing is a useful adjunct to Anti Streptolysin O and Anti DNase B testing in acute glomerulonephritis. Low levels of C3 or both C3 and C4 are consistent with this disease although low levels are also associated with other renal diseases, namely systemic lupus erythromatosis and idiopathic mesangiocapillary glomerulonephritis.

9.23.2 Cryptococcal Serology

The CRYPTO-LA Test is a simple and rapid latex particle agglutination test that is used for the qualitative and quantitative detection of *Cryptococcus neoformans* antigen in serum and CSF as an aid in the diagnosis of cryptococcosis.

9.23.3 Rubella Contact or Infection

- First or acute specimens should be collected as soon as possible after contact or onset of symptoms. See Section 8.1.1 for collection guidelines.
- The second or convalescent specimen should be collected 3-4 weeks after contact with Rubella or 10-14 days after the appearance of symptoms.
- Please provide full clinical history with dates of contact and onset of illness to permit selection of the appropriate test and interpretation of the results.
- Rubella specific IgM testing is available to confirm current infection.

9.23.4 Congenital Rubella Syndrome

Congenital rubella syndrome is diagnosed by the presence of Rubella specific IgM antibodies in the baby's blood or from recovery of the virus from urine or throat swabs. Specimens should be collected from the mother at the same time. See collection guidelines for collection guidance.

9.24 Infection Control

9.24.1 Methicillin Resistant *Staphylococcus aureus* (MRSA)

Screening may be undertaken as outlined depending on the policy of the regional infection control committee. If there appears to be an outbreak of MRSA infection please consult the local Infection Control Coordinator.

Collection: Swabs are taken routinely from:

- nose, groin, axilla and in addition, from the perineum and umbilicus of neonates
- wounds if applicable
- any dermatitis or skin lesions
- if the area to be swabbed is dry, dampen the swab in a sterile saline solution before using to collect a bacterial swab in transport medium

Who to swab:

- patients transferred from another hospital
- patients to be transferred to another hospital
- patients known to be harbouring MRSA
- re-admitted patients previously positive for MRSA if the same condition is being treated or exists

NB Swabs for MRSA are cultured **ONLY** for *Staphylococcus aureus*. If a full microbiological examination is required separate specimens and request forms should be submitted.

Staff Swabs

1. Routine screening of staff is not performed.
2. Contact the Infection Control Coordinator regarding staff inter-hospital transfer swabs as required. These usually consist of nose, groin, axilla and any skin lesions for MRSA only.

9.24.2 Other

If there is an outbreak of a particular bacterial or viral infection please consult the nearest Pathology Queensland laboratory or the local Infection Control Coordinator for the appropriate screening procedures.

9.25 Histology

An important feature on the request form is a clear indication of where the report should be sent. A brief clinical statement is often very helpful with diagnostic interpretation and this should highlight the main clinical problem (eg. massive unilateral pleural effusion or carcinoma).

The majority of specimens for histo-pathology should be sent in 10% neutral buffered formalin and this is generally available in most clinics and theatres. If this is unavailable, alcohol is appropriate in concentrations between 50-100%. If rapid transfer is available the specimen can be sent fresh or in normal saline notifying laboratory staff or confirming if this is appropriate.

9.25.1 Frozen Sections

In general, frozen sections are performed in hospitals where a pathologist can be accessed. Frozen sections must not have any additive, and must be handed immediately to a pathology staff member, identifying the specimen as a frozen section.

9.25.2 Lymph Nodes/Lymphoma Studies

As per Frozen Sections. These specimens may require marker studies, flow cytometry or culture (eg. TB) in addition to histology, and therefore should be delivered fresh and unfixed, as quickly as possible, with an appropriate form.

9.26 Cytology

Ideally all specimens should be fresh and delivered with minimal delay. If rapid transport is not available treat as described below.

9.26.1 Body Fluids

Body fluids include ascitic, pleural, and pericardial fluids, all cyst fluids and peritoneal washings. **If delivery to the laboratory is not on the same day:**

1. Halve the specimen.
2. Add an equal volume of 50% ethanol to one half, leaving the other half fresh.
3. Label the specimen jar to indicate which is fixed and which is fresh.
4. Store both at 4°C until they can be despatched.

9.26.2 Sputum

1. Encourage deep cough specimens. Collect 3 specimens over consecutive days.
2. **If delivery is delayed:** add approximately 10 mL of 50% ethanol.

9.26.3 Urine

1. Collect midstream, mid-morning specimens on three consecutive days.
2. Note on the request form whether the specimen is voided or catheterised.
3. **If delivery is delayed:** Add an equal volume of 50% ethanol.

9.26.4 Fine Needle Aspirate

Clinicians are encouraged to seek the advice of Pathology Queensland laboratory for fine needle aspiration procedure. When attendance of Cytology staff is not possible, the following protocol is recommended:

1. For each pass, or each body site, prepare 1 air dried slide and 1 spray fixed or ethanol fixed slide. Prompt fixation is essential.
2. Needles and syringes used in these procedures are often a useful source of diagnostic material, and should be washed out into a specimen jar with sterile saline. The needle washout is sent to the laboratory with the slides.
3. Sending of needles and syringes to the laboratory is strongly discouraged, for health and safety reasons.
4. Smears may be spread by either of the following techniques depending on the nature of the specimen:
 - i. Blood smear technique – using the edge of a glass slide or pasteur pipette, prepare a smear in the same fashion as used for haematology blood films.
 - ii. Squash method - if the specimen is mucoid or contains tissue fragments squash between two slides.
5. When preparing spreads ensure that the leading edge of the smear is on the slide. The edge often contains most of the epithelial cells, and if this is smeared off the slide, these are lost.
6. Label air-dried smears “AD”.

9.26.5 Cervical Smears

Routine wet fixed smears from the cervix and lower genital tract are referred to the Queensland Cytology Service at the Royal Brisbane Hospital. The reports are generally available within 5 working days. Should urgent reports be required please indicate by ticking the urgent box on the request form.

Using **lead pencil**, label frosted end of slide with:

- Patients name
- Date of Birth
- Specimen type (if vaginal, vulval etc.)
- **PLEASE LABEL SLIDE NOT MAILER**

Collection

- After visualising the cervix with the assistance of a speculum, take a sample from the squamo-columnar junction/transformation zone with spatula. A cytobrush may be used to sample the endocervical canal (**not recommended for pregnant women**)
- If using a Cervex Brush (broom) device, use as per manufacturer's instructions making sure that the squamo-columnar junction/transformation zone is adequately sampled
- Spread evenly on a slide and FIX IMMEDIATELY with pump action fixative or immerse in 95% ethanol for at least 15 minutes
- Allow slide to dry before securing lid of slide mailer. Include completed request form
- Post to the Cytology Department or send to the laboratory for forwarding
- Please DO NOT post single slides in plain envelopes as breakages can result