

2. Collection, Transport and Storage of specimens for laboratory diagnosis.

Why a Clinical Practice Guideline?

The specimen is vital for the laboratory diagnosis. To make a correct diagnosis the availability of an appropriate sample collected in a timely manner and sent under suitable conditions is essential. Very often a precious sample is lost due to inappropriate sample collection, transport and storage. The sample passes through different persons before it reaches the laboratory. If proper handling does not take place at these stages the quality of the sample suffers. This guideline aims to improve the quality of laboratory services by improving the quality of the samples received at the laboratory.

For whom is this guideline intended?

The guidelines are intended to be used by all health care providers in Sri Lankan health care institutions from primary care level to tertiary care level. Although they are targeted for the institutions functioning under the Ministry of Health, the guidelines are encouraged to be used in any private health facility where out-patient or in-patient care is provided.

Objectives

The objective of this guideline is to provide evidence based recommendations to laboratory users in sending specimens to the laboratory.

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Quick Reference guide

3.1	Guideline objectives & Target Groups	67
3.2	General Considerations and infection control issues	67
3.3	General guidelines for collection.....	69
3.4	Collection, transport & storage	71

Contents

Topic No	Topic	Page No
3.1	Guideline objectives & target groups	67
3.2	General considerations and infection control issues	67
3.3	General guidelines for collection.....	69
3.3.1	General specimens collected for virus detection	69
3.3.2	Transport and storage of specimens for Virology	70
3.4	Collection, transport & storage	71
3.4.1	Collection, transport & storage of Urine	71
3.4.2	Collection, transport & storage of stool	76
3.4.3	Collection, transport and storage of respiratory specimens	79
3.4.4	Collection, transport & storage of Respiratory specimens for bacteriology	82
3.4.5	Collection, transport & storage of Ear & Eye specimens	83
3.4.6	Collection and transport of specimens from the urogenital tract:	84
3.4.7	Collection, transport & storage of Skin & subcutaneous specimens	87
3.4.8	Collection, Storage & Transport of Cerebrospinal Fluid (CSF)	94
3.4.9	Blood culture	98
3.4.10	Virus isolation & antigen detection	99
3.4.11	Immunological investigations	110
3.4.12	Tissue & Biopsy specimens for microbiological investigations:	112
3.4	References	113

Collection, Transport and Storage of specimens for viral diagnosis

Introduction

3.1.Guideline objectives and target groups

1. Laboratory confirmation of infectious diseases.
2. Improve quality of specimens
3. Uniformity of sampling
4. Infection control issues

Target groups:

- Health care workers
- Medical administrators

3.2.General considerations and infection control issues

Important points to consider in collecting specimens for microbiological diagnosis include 1. What to collect? 2. When to collect? 3. How to collect?

Type of specimens to be collected depends on the infective disease. Specimens from localized infections are usually collected from the site of infection. When systemic spread is known to occur blood cultures are carried out in addition. If the organism is known to be excreted from another body site, specimens from these sites may also be sampled in an attempt to isolate the

pathogen. i.e. *Salmonella typhi* from urine. Stage of the illness and whether the patient is on antibiotics also influence on suitability of a specimen for microbiological diagnosis.

Specimens should be collected before starting on treatment with antibiotics. Further isolations from the primary site of infection are more successful if the specimen is collected very early in the disease. In some diseases, the organism may appear at different body sites at different times of the disease process. In certain infective diseases the pathogen appear intermittently and therefore, more than one specimen collected at intervals may be needed. In some diseases where serological diagnosis is used, paired serum samples are collected 10-14 days apart and both samples could be tested in order to compare the titres.

When it is necessary to collect a specimen, first inform the patient about the need of a sample for a particular test. It is also important to give instructions to the patient on the correct procedure of collecting the sample if it is done by the patient. (delete the part within the brackets. discuss on how to collect a proper specimen) Further tests should be coordinated in order to minimize the number of vene-punctures.

3.3. General guidelines for collection, transport and storage of specimens for viral diagnosis

3.3.1 General specimens collected for virus detection

- Swabings from lesion sites (i.e. skin, throat) with swab heads transferred to viral transport medium that include antibiotics. Dry swabs are not acceptable.
- Scrapings of lesions to obtain infected cells (e.g. bases of vesicles, corneal ulcers).
- Aspirates of secretions or exudates (e.g. from posterior nasopharynx, conjunctiva, cervix).
- Excreta such as urine or faeces.
- Biopsy specimens obtained by needle aspirations, open exploration or endoscopy from liver, kidney, heart, lung, brain or intestine.

Blood

- A -** For peripheral blood leucocytes, blood is collected into a preservative free heparin tube.
- B -** For serological tests, 10ml blood sample is collected into a dry sterile container.

3.3.2 Transport and storage of specimens for Virology

- a) Specimens for isolation of infectious virus should be transported to the laboratory without delay in order to maximize the recovery of the infectious agent.
- b) When short delays are anticipated due to unavoidable circumstances or during transport over long distances optimum preservation of infectivity is obtained by placing the container in melting ice or refrigerating the sample at 4°C.
- c) In general freezing the specimen should be avoided but if long delays are inevitable (>48 hours) specimen should be frozen at -70°C, not -20°C.
- d) In the hospital environment, taking specimens at night should be avoided. But when this practice is unavoidable, it is important to place the container in the fridge rather than the freezer compartment.
- e) Transport from a single point of origin of a large number of specimens inside a single plastic bag should be discouraged. Because, a single leaking container can lead to wastage of all specimens and potential source of infection to exposing staff in such situations.

Most satisfactory transport system is to provide racks to keep the blood /specimen -bottles upright and store the racks inside a sturdy insulated container provided with a carrying handle.

- f) Transport of pathological specimens by air is governed by strict guidelines laid down by the International Air Transport Association (IATA). It is the responsibility of the shipper to adhere to current IATA regulations as the penalties for contravening IATA regulations are severe.

3.4. Collection, transport & storage

3.4.1 Collection, transport & storage of Urine

Urine specimens:

Indications:

- To establish diagnosis, aetiology and antimicrobial sensitivity of an urinary tract infection.
- Diagnosis of enteric fevers, leptospirosis and legionellosis.
- Isolation/detection of viruses excreted in urine i.e. CMV, rubella virus, mumps virus and polyoma viruses.

General principles:

- Whenever possible, collect the urine sample for culture in the morning. Patient should be instructed the night before to refrain from passing urine until the specimen is collected in the morning.

- Patient should be given a sterile, dry, wide-necked leak proof screw- capped container.
- Explain to the patient the importance of collecting a specimen with minimum contamination as possible.
- Wash hands with soap and water before collecting the urine.
- Catching urine in ‘midstream’ is the goal. In the clean catch method, skin around the urethra is cleaned, and the patient urinates, stop urinating and then urinate into the collection container.

This method is difficult for young children.

A specimen of urine may sometimes have to be obtained by supra-pubic aspiration or by catheterisation from infants and very young children who cannot provide a good midstream specimen of urine

Collection of urine:

A. Mid -stream urine (MSU) samples from females:

- a) Clean the external genitalia with soap and water.
- b) Avoid contaminating the bottle mouth and the inside of the lid with hands while collecting the specimen
- c) Then holding the labia apart start passing urine. Discard the 1st part of the stream into the commode.

- d) Place the sterile container provided in the line of the flow of urine (still holding the labia apart) and collect about 20ml of urine from the midstream.
- e) When sufficient sample has been collected into the container void the rest of the urine into the commode.
- f) Replace the cap, wash hands and hand over the sample to the nursing or the laboratory staff immediately.

B. Mid stream urine (MSU) samples from males:

- a) Retract the fore skin in uncircumcised males and clean the glans with water or normal saline.
- b) Discard the first part of the stream into the commode.
- c) Place the sterile container provided in the line of the flow of urine (still holding back the foreskin) and collect about 20ml of urine from the midstream.
- d) Void the rest of the urine into the commode.
- e) Replace the cap, wash hands and hand it over to the nursing or the laboratory staff immediately

C. Bed-ridden patients:

Nursing personnel must assist the cleansing procedure and in males foreskin should be retracted and glans should be cleaned before passing urine.

D. Infants and young children:

- Mother is instructed to clean the external genitalia of the child, give plenty of fluid to drink.
- Mother can collect as much urine as possible avoiding contamination when the child urinates.
- In very small children urine bags may be used and sample should be collected as soon as the child urinates and send to the lab within 2 hours.
- Supra-pubic aspiration is occasionally necessary in infants.

E. Catheterised patients:

- Do not collect urine from the urinary bag.
- Clamp the catheter tube for about 2 hours.
- Open the drainage tube and allow few mls of urine to pass into the urinary bag.
- With the help of a sterile syringe and needle collect urine and directly from the rubber tubing of the catheter after the cleaning the surface of the catheter with antiseptic (70% alcohol).
- When introducing the needle point it downwards towards the bag, this will prevent seeping of urine from the rubber tubing.
- Collect 10-15ml of urine into sterile dry screw capped bottle.

F. Transport of urine specimens:

- Properly collected urine sample should be labeled and sent to laboratory with a request form without delay preferably within 2 hours.
- If a delay of more than 2 hours is anticipated refrigerate the specimen until dispatch. If no refrigerator available, keep the specimen on ice in an insulated flask.
- If the delay is longer add 0.5gram of borax for 20ml of urine (1.8% w/v.)

G. Collection of urine in suspected renal tuberculosis:

Urine may be collected as three early morning samples or 24 hour sample.

i. Three early morning urine samples:

Collect the first urine passed (entire specimen) on three successive days. This is better than 24 hour sample. Store in 4°C until all three samples are collected.

ii. 24 hour urine sample:

This method of collection should be avoided as far as possible

Collect all the voided urine over a period of 24 hours into a clean, sterile dry and leak proof and sufficiently large container. Store in 4°C until all 24 hour urine is collected.

H. Collection of urine in suspected chronic prostatitis:

- Collect a mid stream specimen as described for males and another 20ml of urine into a sterile dry, screw capped container after a prostatic massage. Prostatic massage should be avoided in patients with acute prostatitis to prevent the risk of potential bacteraemia. .
- Label both specimens accurately and send to the laboratory as soon as possible.
- Refrigerate the specimens until dispatch.

I. Urine for viral studies:

Collect 5-10ml of MSU sample and refrigerate until dispatch.

3.4.2 Collection, transport & storage of stool samples

A. Faecal specimens

Indications:

- Investigation of diarrhoeal disease
- To identify Salmonella carriers
- Investigation of viral meningitis, encephalitis, acute paralytic disease or hand foot and mouth disease.

Procedure:

- i. Request the patient to pass faeces into a clean, dry disinfectant free bedpan. Container need not be sterile. Advice the patient not to contaminate faeces with urine.

ii. Transfer a portion of the voided faeces preferably containing mucous, blood, pus blood shreds of epithelium into a clean dry disinfectant free leak proof container.

- Wide mouthed screw capped plastic disposable pot containing small plastic or wooden spoon which fits into the pot when closed is the most appropriate container.
- In the case of a glass container - it should be boiled or sterilized before use.
- In the case of liquid stools fill one third of the container
- If faeces is solid spoonful of faeces is collected using the spoon.
- If the specimen contains worms or tape worm segments, transfer these into a separate container and send them to the Parasitological investigations.

B. Rectal swabs

Indication:

- Only when it is not possible to obtain faeces
- A specimen of faeces is always better than a rectal swab.

Procedure:

- Use a sterile cotton wool swab moisten with sterile saline or transport medium. Do not use lubricating jelly.
- Insert the swab into the rectum through the anal sphincter, rotate and leave for about 10

seconds and withdraw. Swab should be stained with faecal material

- If the swab could be processed in 2 hours replace the swab in a sterile empty test tube. If it is to be kept longer it should be inoculated into a transport medium.

C. When cholera is suspected:

A rectal catheter could be used to collect watery stool. Should be sent in alkaline transport medium i.e. Venketrman Ramakrishnan medium or alkaline peptone water

If transport media is not available and if the specimen has to be send to a distant laboratory impregnate a pledget sterile cotton wool or sterile filter paper with stool specimen , dry it and place in a sterile screw capped container and sent to the laboratory

Note: take necessary safety precautions regarding collection and transport of this material.

D. Labeling and filling request forms for faecal specimens:

Label specimen and send to the lab with request form within 2 hours. Refrigerate until the dispatch. If amoebic dysentery suspected transport the specimen without delay.

E. Transport media for faecal specimens:

If further delay is anticipated use a suitable transport medium to increase the chances of recovery of organism.

i. Cary Blair medium:

Samonella and Shigella may survive up to 48 hours, Campylobacter for about 6 hours. Insert a faecal swab into this semisolid transport medium, break off the swab stick jutting out of the bottle and replace the cap tightly.

ii. Venketrman Ramakrishnan medium or alkaline peptone water:

This is used for transport of faeces from suspected case of cholera. Transfer about 1ml of specimen into 10ml of medium.

F. Collection of faeces for laboratory analysis to exclude the possibility of polio.

- Two specimens taken 24-48 hours apart for virus studies.
- Should be collected within 2 weeks of onset of paralysis.
- Carefully seal the container and refrigerate or pack between frozen ice packs at 4-8°C in a cold box and transport to the virus lab. Specimen kept in 4-8°C should reach the lab in 72 hours of collection. If it is not possible pack in dry ice.

3.4.3 Collection, transport and storage of respiratory specimens

A. Respiratory specimens for virology:

- Throat swab
- Nasopharyngeal aspirates (NPA)
- Bronchoscopy specimens

i. Throat swab for virus isolation

Indications:

- a) To establish microbial cause of pharyngitis
- b) Isolation of respiratory viruses, herpes group of viruses, mumps, rubella, and enteroviruses from throat.

Procedure for collecting a throat swab:

- a) Patient must not be treated with antiseptic mouth washes for 8 hours before swabbing
- b) Throat swab should be collected by medical or other trained personnel.
- c) Explain the procedure to the patient.
- d) The patient should sit in front of a light source.
- e) While the tongue is kept down with a tongue depressor, a sterile cotton wool or alginate swab moisten with VTM or sterile physiological saline is rubbed vigorously over each tonsil and posterior pharyngeal wall.
- f) Care should be taken not to touch the tongue or buccal surfaces.
- g) Break off the swab head into a sterile glass or

plastic container containing VTM and send to the laboratory as soon as possible.

ii. Nasopharyngeal aspirates (NPA) for virus isolation

Indications:

- Bronchiolitis
- Investigation of other respiratory virus infections in a small child.

Procedure:

- a) The specimens should be collected by a physician or other trained personnel
- b) Explain the procedure to the patient.
- c) The specimen is collected through the nose
- d) Gently pass a sterile fine-bore catheter into patient's nostril and hence into upper pharynx (usually easier through left nostril but use right side if unsuccessful)

In adult insert the catheter 7-8 cm; in children length inserted depend on age. Hold babies in a sitting-up position because they are less likely to vomit.

- e) After catheter is inserted into nostril, apply intermittent suction by placing thumb or finger over end of free arm of Y suction catheter.
- f) Continue to apply intermittent suction while slowly withdrawing catheter and collecting mucous.

Whole process should take approximately 5-10 seconds depending on amount of mucous.

- g) Dispense the specimen into a sterile container and deliver to laboratory as soon as possible with a completed request form.

- h) Wash the specimen into the trap by sucking 4-5ml virus transport medium (VTM) through the catheter.

iii. Bronchoscopy specimens for Virology:

Indications:

- For the diagnosis of cytomegalovirus pneumonitis
- Biopsy, brushings and lavage specimens may be contaminated with normal upper respiratory tract flora that is carried into the lower respiratory tract with the passage of the bronchoscope.
- The specimens may also be contaminated with the local anesthetic solutions instilled into the upper airway during the bronchoscopy procedure.
- A special sheathed bronchial brush (microbiology specimen brush) enclosed within a telescopic inner and outer catheters, when available largely overcomes the problem of contamination.

3.4.4 Collection, transport & storage of Respiratory specimens for bacteriology

A. Lower

i. Bronchoalveolar Lavage, Brush or Wash Endotracheal Aspirate

Collection

- a. Collect into a sputum trap
- b. Place brush in sterile container with 1 ml saline

Transport device

Sterile container > 1 ml

Transport time and temperature

=< 2 hours at room temperature

Storage time

=< 2 hours at room temperature

Replica time

1 per day

Comments

For quantitative analysis 40 – 80 ml fluid is needed. Collect into 1 ml saline.

ii. Throat swabs

Indications:

1. To establish microbial cause of pharyngitis
2. Investigation for carriers of *Corynebacterium diphtheriae*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Neisseria meningitidis*.

Collection

- (a) Depress tongue with tongue depressor
- (b) Sample posterior pharynx, tonsils, inflamed areas with sterile swab

Transport and storage

Transport preferably in swab transport medium =<2 hours at room temperature and store =< 24 hours at room temperature

Comments

Throat swab cultures are contraindicated in epiglottitis. Swabs for *Neisseria gonorrhoea* are sent in charcoal containing transport medium and plated =< 12 hours after collection. Transportation in Bio-bags at room temperature is a better option.

3.4.5 Collection, transport & storage of Ear & Eye specimens

A. Ear specimens

- a) Collection of specimens should be done by trained personnel
- b) Collect the aspirate or swab in to a sterile leak proof container
- c) Transfer to laboratory as soon as possible with a duly filled request form
- d) If delay is anticipated send the specimen in anaerobic transport containers (if available) or Stuart's transport medium

B. Eye specimens

- a) Collection of specimens should be done by trained personnel
- b) Use a dry sterile cotton swab for collection and transfer to laboratory immediately with a duly filled request form

Transport media:

For bacterial pathogens – Stuart's transport medium

For viruses – viral transport medium

If *Neisseria gonorrhoeae* is suspected **do not** refrigerate. Ideally streak on a culture plate obtained from the laboratory

If fungal infection is suspected send a kerato/corneal scraping collected by the ophthalmologist.

3.4.6 Collection and transport of specimens from the urogenital tract:

A. Urethral specimens

Indications:

- Urethritis in a male,
- Urethral discharge

Procedure:

- Patient should not have passed urine 2 hours before the specimen is collected.
- Cleans round the urethral opening using a sterile swab moisten with sterile normal saline.
- Gently massage the urethra from above downwards and collect a sample of pus with a sterile bacteriological loop or sterile swab or at least directly onto a clean slide.
- Ideally specimen should be inoculated onto a GC selective medium at the bedside.
- If this is not possible insert the swab into a container of Amies transport medium.

- Break off the swab stick to allow the cap to be replaced tightly.
- Send it to the laboratory immediately with a request form
- Specimen for Chlamydial culture should be sent in chlamydia transport medium.
- Do not refrigerate specimens sent for GC culture
- Make two smear of the discharge on a two separate slides one for Gram stain and another for IF for Chlamydia

B. Cervical specimens:

- Moisten a vaginal speculum with sterile warm water and insert it into the vagina.
- Do not use antiseptics or gynaecological exploration cream.
- Samples for gonococcal and Chlamydial culture should be collected from the endocervix.
- Cervical mucus should be wiped off with a swab moisten with sterile physiological saline.
- Pass a sterile swab into the endocervical canal gently rotate the swab to obtain a specimen.
- Inoculate the specimen at the bedside onto a GC medium or insert the swab into a bottle containing Amies transport medium, close the bottle tightly.
- Make a smear on a slide for Gram staining and another for Chlamydia if facilities available.

C. Vaginal specimens

- For examination of yeasts, *Trichomonas vaginalis* and bacterial vaginosis – A high vaginal swab (HVS) is collected.
- Samples may be collected from the posterior fornix of the vagina using a sterile swab.
- Make a smear on a slide for gram staining.

D. Specimens from genital ulcers

Genital ulcers can be caused by herpes virus, *Treponema pallidum*, *Haemophilus ducreyi*, *Calymmatobacterium granulomatis* and *Chlamydia trachomatis*.

Collection of specimens from a chancre:

- Protective gloves should be worn
- Squeeze the ulcer between two fingers and clean the ulcer surface with saline.
- Remove any crusts if present.
- Wipe away the first few drops of blood
- Collect a sample of serous exudates by touching a clean glass slide to the lesion. Place a clean cover slip
- The specimen may be aspirated from the lesion or the enlarged lymph node using a sterile syringe.
- Examine immediately under a dark-field microscope.
- In case of secondary syphilis specimens may be collected from condylomata lata or mucous patches.

Collection of specimens from chancroid

(Haemophilus ducreyi)

Specimen should be obtained from the base of the ulcer.

When Infection with Chalmvdia suspected:

Make a smear of exudates from bubo, urethra or cervix.

3.4.7 Collection, transport & storage of skin & subcutaneous specimens

General Considerations

- Disinfection of the site is critical. Contamination with normal skin bacteria must be avoided.
- If swabs must be used to collect the specimen, collect at least two swabs for every culture test ordered.
- These sites will be tested for both aerobic and anaerobic bacteria.

Collection container:

Preferred: Collect Aspirate in a sterile screw capped tube or in a capped syringe (with needle removed).

Swabs in a swab transport system can be accepted if necessary.

- Be sure to send at least 2 swabs for every culture test ordered.
- If anaerobes are suspected, a separate sample should be collected and immediately placed into an anaerobic swab transport system.

Storage Requirements:

- Room temperature.

Transport Conditions:

- If delay in transport of more than one (1) hour, transfer a small amount of the sample to an anaerobic transport system and refrigerate.

Lesion-Superficial (Fungal)

Collection container:

- Sterile screw-cap container if delivering to lab or packed in a clean piece of paper

Storage Requirements:

- Room temperature.

Transport Conditions:

- Send promptly to the lab

Using a scalpel blade, scrape the periphery of the lesion border. Samples from scalp lesions should include hair that is selectively collected for examination. If there is nail involvement, obtain scrapings of debris or material beneath the nail plate.

Bacterial Wound-Superficial

Collection container:

- Sterile screw-capped tube, swab transport system

Storage Requirements:

- Room temperature.

Transport Conditions:

- Send promptly to the lab

Syringe aspiration is preferable to swab collection.

- Disinfect the surface of the wound with 70% alcohol and then with 10% povidone-iodine. Or thoroughly clean the wound three times using new sterile saline moistened gauze 4x4. Flush well with sterile normal saline.
- Allow the disinfectant to dry prior to collecting the specimen.
- Using a 3-5 ml syringe with a 22-23 gauge needle, a physician will aspirate the deepest portion of the lesion.
- If the initial aspiration fails to obtain material, inject sterile 0.85% saline under the skin and repeat aspiration.

- Transfer material from syringe into sterile screw-capped tube and deliver promptly to the lab.
- If the specimen will be compromised by transferring it from the syringe, remove the needle and recap the syringe with a sterile cap.
- If swab is used, swab deep areas rather than lesion surface using 2 swabs or swab transport system using a 10 point back and forth motion, rotating the swab throughout the procedure.

Ulcers and Nodules

Collection container:

- Sterile screw-capped tube, swab transport system, or other appropriate transport system

Transport Conditions:

- Send promptly to the lab
- Clean the area with 70% alcohol and then with 10% povidone-iodine. Allow area to dry. Remove overlying debris.
- Curette the base of the ulcer or nodule.
- If exudate is present from ulcer or nodule, collect it with a syringe or sterile swab.
- Transfer material to appropriate transport system (sterile tube for aspirate, swab transport system for bacterial swab culture, viral transport media for viral swab culture.

Punch Skin Biopsies

Collection container:

- Sterile screw-cap tube

Storage Requirements:

- Room temperature.

Transport Conditions:

- Send promptly to the lab

Criteria for Rejection:

- Specimen submitted in formalin

- Disinfect the surface with 70% alcohol and then with a 10% solution of povidone-iodine. Allow area to dry. Collect a 3-4 mm sample with a dermal punch.

Soft Tissue Aspirate

Collection container:

- Sterile screw-cap tube

Storage Requirements:

- Room temperature.

Transport Conditions:

- Send promptly to the lab

Disinfect the surface with 70% alcohol and then with a 10% solution of povidone-iodine. Allow area to dry.

Aspirate the deepest portion of the lesion or sinus tract. Avoid contamination by the wound surface. Transfer material to sterile, screw cap container and deliver promptly to the lab.

Mycological specimens

Superficial sites

a) Hair -

Collection Procedure

- Select infected area. Remove at least 10 hairs. Entire hair shaft is necessary.

Transport Procedure

- Room Temperature
Place hairs between two clean glass slides or in a clean envelope labeled with the patient's data.

b) Nails -

Collection Procedure

- Clean nail with 70% alcohol, scrape away the outer portion and obtain scrapings from the deeper infected areas.

Transport Procedure

- Room Temperature

c) Skin and interspaces -

Collection Procedure

- Clean skin with 70% alcohol. Scrape the entire lesion(s) and both sides of interspaces

Transport Procedure

- Room Temperature

Sub-cutaneous sites

a) Tissue Biopsy –

Collection Procedure

- Collect tissue aseptically from the center and edge of the lesion. Place specimens between moist gauze squares; add a small amount of sterile water or saline to keep tissue from drying out.

Transport Procedure

- Room Temperature

b) Mucus membranes

Collection Procedure

- Two swabs from the lesion to be collected

Transport Procedure

- Room temperature

3.4.8 Collection, Storage & Transport of Cerebrospinal Fluid (CSF)

A. Introduction:

Cerebrospinal Fluid (CSF) is collected from patients with clinically suspected infection of the Central Nervous System (CNS). CSF is used for general laboratory investigation (sugar, protein & cells) & for aetiological investigation to identify the etiological agent.

B. General precautions:

1. CSF samples are obtained by lumbar puncture and less commonly by a ventricular tap. CSF also can be collected from External Ventricular Drain (EVD) & from a CSF shunt (AV or VP).
2. Strict aseptic precautions should be adhered to during the collection of CSF to prevent organisms being introduced into CNS & to prevent contamination of the sample (Reference 1).
3. Adequate disinfection of the skin before the lumbar puncture or ventricular tap with an Iodine containing preparation followed by 70% alcohol (reference 1)
4. CSF for bacteriological investigations should be collected before administration of antibiotics. If antibiotics have been started, information should be entered in the request form (type, dose, duration). CSF for bacterial culture should not be kept or sent in ice.
5. Sample should be sent to laboratory with accompanying request form giving patient identification (name, age, sex, ward, BHT number, hospital) & clinical details (brief history, duration of symptoms)
6. It is difficult to isolate virus from CSF in viral meningitis / viral encephalitis. Therefore, other specimens (faeces, throat swab, and blood) should be collected & sent to the laboratory.

C. Sample collection

- i. **CSF obtained by lumbar puncture / by ventricular tap**
 - a) Clean the skin as mentioned above (6. 2.3)
 - b) CSF is collected into 3 to 4 containers for
 - Sugar (fluoride containing bottle)
 - Proteins, cells & electrolytes
 - Bacterial culture & ABST*
 - Virology *

*should be collected into sterile, screw capped containers as the last two collections

- ii. **CSF obtained from EVD or shunt**
 - Clean the site of puncture with ethanol
 - Using sterile needle & a syringe, aspirate CSF
 - Collect into bottles as described above (B-General Precautions)

- iii. **Additional specimens for bacteriological investigations**
 - Blood culture
 - Skin scrapings from petichiae in the case of Meningococcal meningitis

- iv. **Additional specimens for Biochemical investigations**
 - Blood (3 – 5 ml) in a fluoride containing bottle for blood sugar

- v. **Additional specimens for virological investigations**
 - Throat swab into a dry sterile screw capped container with Virus Transport Medium (VTM)
 - Faeces into a clean dry container (VTM not necessary)
 - Blood into a clean dry container (VTM not necessary)

D. Sample storage & transport

1. CSF specimen should be sent to laboratory immediately (within 2 hours) and processed immediately. Delay in examining CSF leads to disintegration of cells & reduces the chance of isolation of the pathogen.

2. Laboratory staff should be informed before the lumbar puncture (especially if it is done after hours), so that they would be ready to examine the specimen immediately.

3. If there is a delay, specimen for bacterial culture should be kept at room temperature. Do not refrigerate CSF specimens for bacterial culture.

4. **CSF specimen & additional specimens for virological investigations** should be sent to the laboratory **as soon as possible**. It is important to **transport these specimens at 4° C** by using ice (refer to specimen transport for virological investigations).

5. If there is delay in transport, specimen for viral studies, mycobacterial studies and for bacterial antigen detection should be refrigerated.

3.4.9 Blood culture

Collection

Disinfect rubber stopper of culture bottle – apply 70% Isopropyl alcohol to rubber stopper, wait for one minute before injecting the sample

Container and minimum volume

Adult : => 20 ml per set (5-7 ml in 50 ml container)
Infant / Child : 1- 20 ml per set depending on the weight of the patient

Transport time and temperature

=< 2 hours of collection at room temperature

Storage time

=< 2 hours of collection at room temperature or per instructions

Replica limits

3 sets in 24 hours

Comments

Acute febrile episode:

Antibiotics to be started or changed immediately after 2 sets are taken from separate sites within 10 minutes.

Non-acute disease:

Antibiotics will not be started or changed immediately. 2 or 3 sets to be taken from separate sites within 24 hours at intervals no closer than 3 hours (before antibiotics)

Endocarditic:

3 sets from 3 separate sites within 1-2 hours preferably before antibiotics

P.U.O.

2 or 3 sets from separate sites. => apart during a 24 hours period. If negative at 24 – 48 hours obtain 2 or 3 sets.

Collection procedure

Palpate vein before disinfection of venepuncture site.

1. Clean site with Iodine followed by 70% alcohol
 - Swab concentrically, starting at the centre with an Iodine preparation.
 - Allow Iodine to dry. Swab with alcohol
 - Do not palpate vein now without sterile gloves
 - Collect blood

3.4.10 Virus isolation & antigen detection

A. Sample collection for virological investigations

Introduction:

Different methods are available for the diagnosis of viral investigations. The quality & the reliability of

the results depend on the proper sample collected at the correct time.

Methods of diagnosis available are

1. Detection of virus by electron microscopy (EM) / immune electron microscopy (IEM)
2. Detection of viral antigen
3. Detection of viral genome
4. Isolation of the virus
5. Detection of viral specific antibodies (IgM / IgG)

i) General precautions

1. Strict aseptic precautions should be adhered during the collection of specimens to prevent organisms being introduced into the body & to prevent contamination of the sample (Reference 1).
2. Person who collect the specimens should take universal / respiratory precautions to avoid exposure
3. Specimens should be collected into dry, sterile, screw capped containers .(Faeces can be collected into wide mouth dry, clean containers)
4. Each specimen should be labeled with patient name, BHT, ward, date of collection
5. Specimens should be sent to laboratory with accompanying request form giving patient identification (name, age, sex, ward, BHT number, hospital) & clinical details (brief history, duration of symptoms).

6. Specimens for virus isolation, antigen detection & for genome detection should be sent to the laboratory at 4°C with out delay.
7. If there is a delay in transport, specimens for virus isolation, antigen detection & for genome detection should be kept in the refrigerator (at 4°C)

ii) Type of specimen

1. Blood
2. CSF
3. Swabs ((throat swab / nasal swab / swabs from genital lesions / swabs from skin lesions / conjunctival swabs
4. Respiratory secretions (nasopharyngeal aspirates / bronchial secretions / broncho-alveolar lavage
5. Faeces
6. Urine
7. Ante-mortem biopsy * / post mortem biopsy*
8. Corneal impressions / nuchal skin biopsy / CSF / Blood / Post mortem brain biopsy from suspected rabies patients**

*consult the virologist before sending the specimens

** consult the virologist at Rabies division / MRI before taking the specimens

iii) Timing of specimen collection

1. Specimens for antigen detection, genome detection & for virus isolation should be collected within 3 – 5 days of the onset of illness

Note – Faecal specimens from Acute Flaccid Paralysis (AFP) patients are collected on detection at anytime (ideally within 14 days of onset of paralysis)

2. Specimens (blood / CSF) for IgM detection should be collected 5 – 7 days after the onset of illness. If the first sample is negative, second sample should be collected about 7 days later.
3. Two specimens (blood) should be collected for IgG / Total antibody detection. Acute sample is collected within 7 days of onset & convalescent sample is collected 2 – 3 weeks later (Same samples can be used for IgM detection depending on the day of collection)

iv) Collection of specimens

1. Respiratory secretions, swabs & biopsy material should be collected into containers with VTM. Blood, CSF & faeces do not need VTM.

Note – VTM can be collected from MRI / from microbiologists of the respective hospitals

2. Before collecting specimens for genome detection, contact the laboratory to get specific instructions. Specimens may have to be collected into special buffers / into special containers.

v) Collection, storage & transport of blood for virological investigations

1. Blood for virological investigations is collected as per 4.1, 4.2 & 4.3

Note - Blood for Hepatitis B surface antigen is the exception

2. Blood should be collected into dry, sterile, screw capped container with out VTM.

Note – Special instructions for collection of blood for genome detection. Contact the laboratory before collection

3. Blood should be collected aseptically using universal precautions
4. Allow the blood to clot at room temperature
5. Blood collected for antigen detection, genome detection, virus isolation and for IgM detection should be transported to the laboratory without delay at 4 ° C.

Note – Use a reverse cold chain box / regiform container with ice packs / sufficient amount of ice cubes to maintain the temperature

6. Blood collected for IgG / total antibody detection can be transported at room temperature.
7. If there is a delay in transport (for 1 -2 days), keep the blood samples at 4°C and then transport at 4°C.
8. If there is a delay more than 3 days, serum should be separated & it should be stored at – 20°C.

Note – Serum separation of specimens intended for antigen detection, genome detection, virus isolation needs a Bio Safety Cabinet, sterile tips, sterile containers etc.

9. Blood specimens intended for virological investigations should never be kept at 0°C or below (frozen) with out separation of serum / plasma.
10. Blood for HIV serology should be sent to STD / AIDS laboratory

vi) Collection, storage & transport of CSF for virological investigations

1. CSF for genome detection & for virus isolation should be collected within 3 – 5 days of onset of illness. CSF for antibody detection (mainly for IgM)

is collected after 5 – 7 days. If the IgM assay is negative collection of second sample 7 days later should be considered.

2. CSF should be collected into dry, sterile, screw capped container with out VTM.

Note – Special instructions for collection of CSF for genome detection. Contact the laboratory before collection

3. CSF should be collected aseptically using universal precautions
4. CSF collected for genome detection, virus isolation and for IgM detection should be transported to the laboratory without delay at 4°C.

Note – Use a reverse cold chain box / regiform container with ice packs / sufficient amount of ice cubes to maintain the temperature

5. If there is a delay in transport (for 1 -2 days), keep the CSF samples at 4°C & then transport at 4°C.
6. If there is a delay of more than 3 days, it should be stored at – 20°C.

vii) Collection, storage & transport of faeces for virological investigations

1. Faeces for antigen detection, genome detection & for virus isolation should be collected within 3 - 5 days of onset of illness.

Note – Faeces specimens from Acute Flaccid Paralysis (AFP) patients are collected on detection at anytime (ideally within 14 days of onset of paralysis)

2. Faeces should be collected into dry, preferably sterile, screw capped container with out VTM.

Note – Special instructions for collection of faeces for genome detection. Contact the laboratory before collection

3. Patient should pass faeces into a washed bed pan (never use detergent to wash the bed pan). Using a plastic disposable spoon, about 8 g of faeces should be collected into the container.
4. Faeces collected for genome detection and virus isolation should be transport to the laboratory without delay at 4°C.

Note – Use a reverse cold chain box / regiform container with ice packs / sufficient amount of ice cubes to maintain the temperature

5. If there is a delay in transport (for 1 -2 days), keep the samples at 4°C and then transport at 4°C.
6. If there is a delay more than 3 days, it should be stored at – 20°C.

viii) Collection, storage & transport of respiratory specimens for virological investigations

1. Respiratory specimens for antigen detection, genome detection & for virus isolation should be collected within 3 - 5 days of onset of illness.
2. Should be collected aseptically using respiratory precautions
3. Should be collected into dry, sterile, screw capped container with VTM.

Note – Special instructions for collection of specimens for genome detection. Contact the laboratory before collection

4. For methods of collection refer to Laboratory Manual Microbiology (reference 1) section E.1.8

5. Specimens collected should be transported to the laboratory without delay at 4°C.

Note – Use a reverse cold chain box / regiform container with ice packs / sufficient amount of ice cubes to maintain the temperature

6. If there is a delay in transport (for 1 -2 days), keep the samples at 4°C and then transport at 4°C.
7. If there is a delay of more than 3 days, it should be stored at – 20°C.

ix) Collection, storage & transport of swabs & corneal smears for virological investigations

1. Swabs for antigen detection, genome detection & for virus isolation should be collected within 3 - 5 days of onset of illness.

Note - Before collecting corneal smears from suspected Rabies patient, contact Virologist / Department of Rabies, MRI

2. Should be collected aseptically using universal precautions
3. Should be collected into dry, sterile, screw capped container with VTM.

Note – Special instructions for collection of specimens for genome detection. Contact the laboratory before collection

4. For methods of collection refer to Laboratory Manual Microbiology (reference 1) section E.1.8

5. Specimens collected should be transported to the laboratory without delay at 4°C.

Note – Use a reverse cold chain box / regiform container with ice packs / sufficient amount of ice cubes to maintain the temperature

6. If there is a delay in transport (for 1 -2 days), keep the samples at 4°C and then transport at 4°C.
7. If there is a delay more than 3 days, it should be stored at – 20°C.

x) Collection, storage & transport of biopsy material for virological investigations

Note – Before sending the biopsy material, contact the virologist

1. Post mortem biopsy material for antigen detection, genome detection & for virus isolation should be collected as early as possible.
2. Should be collected aseptically using universal precautions

- Should be collected into dry, sterile, screw capped container with VTM. Do not use fixatives / preservatives

Note – Special instructions for collection of specimens for genome detection. Contact the laboratory before collection

- For methods of collection refer to the virologist
- Specimens collected should be transported to the laboratory without delay at 4°C.

Note – Use a reverse cold chain box / regiform container with ice packs / sufficient amount of ice cubes to maintain the temperature

- If there is a delay in transport (for 1 -2 days), keep the samples at 4°C and then transport at 4°C.
- If there is a delay of more than 3 days, it should be stored at – 20°C.

3.4.11 Immunological investigations

Serum Immunoglobulins-

- Serum complement-** Collect 2 ml of venous blood in to a plain bottle Store at room temperature until the specimen is sent to the laboratory. If delay is anticipated in dispatching store at 4°C.

- C- reactive protein-** Collect 2 ml of venous blood in to a plain bottle. Store at 4°C until the specimen is sent to the laboratory.
- Lymphocyte subsets** (by appointment only) - Collect 2 ml of venous blood in to a plain bottle. Store at room temperature until the specimen is sent to the laboratory. If delay is anticipated in dispatching store at 4°C.

NBT test	}	patient has to be sent to the Immunology laboratory (by appointment only)
Lymphocyte function test		

3.4.12 Tissue & Biopsy specimens for microbiological investigations:

- These specimens should be collected under strict aseptic conditions.
- Send to the laboratory in a sterile screw capped container with sterile saline.

Note: do not use formal saline as for histopathology.

Indications:

1. Lymph node biopsy - suspected mycobacterial disease
2. Biopsy of serous membranes for - suspected TB or any other chronic infection.
3. Gastric biopsy – for the detection of *Helicobacter pylori*
4. Brain biopsy – suspected Herpes encephalitis
5. Skin biopsy – leprosy, and other chronic skin ulcers

3.5 References:

1. Laboratory Manual Microbiology by Sri Lanka College of Microbiologists 2001
2. A hand book on Collection and Transport of specimens for Microbiological investigations 2001 by Sirimali Fernando
3. Laboratory diagnosis for viral infections, Practical Medical Microbiology, Mackie & McCartney