Guideline for Collection of Clinical Specimens for Microbiological Testing

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Acronyms

AECB	Acute Exacerbation of Chronic Bronchitis
BAL	Bronchoalveolar Lavage
CDC	Center for Disease Control and Prevention
CID	Citizenship Identity Card
CNS	Central Nervous System
CSF	Cerebrospinal Fluid
DBS	Dry Blood Spot
EDTA	Ethylenediamine Tetra acetic Acid
EPS	Expressed Prostatic Secretion
HVS	High Vaginal Swab
IAI	Intraamniotic Infection
LIMS	Laboratory Information Management System
LRTI	Lower Respiratory Tract Infection
mCB	Modified Cary-Blair
NS	Nasopharyngeal
OP	Oropharyngeal
PCR	Polymerase Chain Rection
PPE	Personal Protective Equipment
RCDC	Royal Centers for Disease Control
SA	Septic Arthritis

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1. Introduction

1.1. Introduction

Specimens play a crucial role in holistic assessment of a patient in building a clinical picture, confirm a diagnosis and make informed decisions in treatment. Inappropriate specimens can lead to inaccurate diagnosis and result in a longer hospital stay due to unnecessary treatment, repeated laboratory testing resulting in delayed diagnosis, and a substantial economic implication, both to the patient and the nation. Collection of specimens for the microbiological study is vital for determining the causative agents and its treatment. Each pathogen requires a unique set of specimen types, collection methods, storage, and handling conditions to optimize diagnostic yield.

It is critical that there is a comprehensive guideline for the proper collection, storage, and transport of specimens according to Good Clinical Laboratory Practices (GCLP) to ensure quality patient laboratory diagnosis and patient care. All laboratory diagnostic results are contingent on the quality of the specimen received. It is also vital that all specimens and requisition form be appropriately labelled. Proper collection of the specimen is essential for the recovery of pathogenic organisms responsible for the disease. A poorly collected, stored or transported specimen may fail to isolate the causative microorganism leading to improper treatment of the patient.

This guideline provides information on general requirements for various clinical specimen collection, storage and transport of different types of specimen collection. General procedures in the annexures are also intended to guide laboratory personnel, nurses and clinicians collect appropriate specimens for microbiological assays.

1.2. Purpose of the guideline

The purpose of this guideline is to guide laboratory professionals, nurses and or clinicians with best practices information regarding specimen collection, storage package and transport in a safe and standardized manner. Adhering with best practice enables the laboratory to produce results that can provide clinically relevant information to all involved in patient care.
Although this guide is not intended to be used as an exhaustive laboratory manual, it covers certain aspects of specimen collection for microbiological testing, standard precaution, biosafety procedures, and other requirements in the collection of specimens.
This guideline is expected to effectively guide laboratory personnel, nurses and clinicians to plan and collect appropriate specimen collection for better diagnosis of bacterial infection, determine its antimicrobial resistance and other diseases where pathogenic microorganisms are implicated. This, in turn, will help clinician provide better patient care, and the data obtained from laboratories will assist public health personnel formulate evidence-based policy for the prevention and control of emerging and re-emerging multidrug-resistant microorganisms.

2. General Consideration for Specimen Collection

2.1. Safety and decontamination

Safety is important part of specimen collection to ensure that both the patient and the specimen collector are safe from potentially contaminated specimen. Decontamination measures should be followed to protect both the specimen collector and the specimen. It is also important for protecting other people and patients from the risk associated with the specimen collection. A person involved in collecting the specimen should treat all clinical specimens infectious and personnel protective equipment (PPE) should be worn while collecting a specimen. Once a specimen is collected, proper packaging methods must be followed for packaging specimens to ensure the safety of the laboratory personnel from the collection site till it the specimen reaches the laboratory.

Decontamination or disinfection is a process of freeing an article from pathogens and harmful substances. Decontamination process is important in the event of specimen leakage, spillage, or accidental exposure with contaminated items such as needles while performing phlebotomy. Chemical disinfection with chlorine-based solutions can be used for disinfecting spillage. Prior to disposal, contaminated or suspected to have contaminated equipment and materials must be disinfected. Combustible materials should be completely burned, which is then buried in a deep pit. Sharps and used glass slides should be discarded directly into a puncture-resistant disposal container, which is then incinerated. Incineration or burning is the preferred method for disposing of contaminated material. However, relevant national agencies such as the National Environment Commission must be consulted, and approval must be sought for the installation of an incineration facility due to environmental concerns.

2.2. Basic safety precautions to be observed by health workers when collecting specimens

1. Use latex or nitrile gloves when taking collecting and handling specimens. Before using it, check for any damage to prevent unintended exposure to infectious disease. Attempt to clean and reuse gloves should be avoided as this may promote the spread of pathogens.
2. Protective clothing (gown, laboratory coat or apron) should be worn when collecting specimens at all times.
3. Contaminated or unused but opened sharp items should be discarded directly into a sharps box and, recapping of needles should be avoided.
4. Work areas and surfaces should be organized and disinfected with 1% household bleach daily before and after each collection procedure. Spillage should be cleaned using 10% bleach and wiped clean with absorbents. Personnel carrying out cleaning or decontamination should wear at least a minimal PPE.
5. Contaminated non-disposable equipment or materials should be sterilized or soaked in 0.5% household bleach for 10 minutes before use. Before using the sterilized items, wash hands thoroughly using soap.
6. Heavily soiled disposable items should be soaked in 10% household bleach before incineration or disposal.

Additional safety equipment, such as respirator masks or goggles, are required to protect eyes, skin and mucous membranes against contact, aspiration, or inhalation of certain pathogens. A respirator mask with a High-Efficiency Particulate Air (HEPA) filter should be used for both known and suspected infection due to highly infectious pathogens.

2.3. General guidelines for specimen collection

Specimens for bacterial assays should be obtained in the acute phase of the disease, preferably prior to administration of antimicrobial drugs whenever possible to effectively yield the infective pathogen. Before the commencement of specimen collection, it is essential to explain the procedure to the patient, attendant or relatives. Risk/benefit ratio of the collection procedure to the patient should also be considered. Specimens should be collected in sturdy, sterile, screwcap, leak-proof containers with lids that do not create an aerosol when opened. An adequate amount of specimens should be collected. Inadequate and/or inappropriately collected specimen culture yield little useful clinical information and may actually produce misleading laboratory and false-negative results and affect the patient's treatment outcome.

When collecting the specimen, a person should avoid contamination and take a sufficient quantity of material as required by the test process. Follow the appropriate precautions for safety during the collection and processing of specimens as outlined in the general consideration for specimen collection. While collecting a specimen, appropriate suitable sterile collection devices must be used, and only sterile equipment should be used. Aseptic techniques to collect specimens must be used to prevent introduction of microorganisms during invasive procedures. Once collected, the transport of specimens to the laboratory should be done as early as possible generally. Refer table 1 for detailed information on collection, storage and transport of different types of specimens in a health microbiology laboratory settings.

2.4. Placing a microbiology test order

Tests are normally generally ordered by a clinician, and occasionally by a nurses occasionally. Orders are normally typically placed using the Laboratory Information Management System (LIMS). However, paper-based test orders are also received. When ordering the test, the test request form should contain as much information as possible. This will help a laboratory personnel to accurately interpret the result accurately. When requesting a laboratory test, minimum information should include the following:

1. Unique patient identification number/Citizen Identity Number (CID)
2. Patient name
3. Patient age and gender
4. Address (current) with contact details
5. Patient ward & bed number (if inpatient), including date of admission
6. List of laboratory test requested
7. Types of specimens
8. Specific anatomic collection site
9. Date and time of specimen collection
10. Details of laboratory test requested
11. Antimicrobials or other drug treatment details (if any)
12. Physician name
13. Name of specimen collector

When appropriate, include clinical information and diagnosis, special microbiological assay request and other relevant patient history histories.
A separate order is needed for each test, including anaerobic cultures. For example, if sputum is collected for AFB, fungus and routine culture, a total of three orders must be entered: one for AFB, one for fungus, and one for routine microbiology. Special requests for culture of unusual isolates (i.e., C. diphtheriae, Leptospira, Actinomyces, Nocardia, Brucella, Hemophilus ducreyi, B. pertussis, etc.) require prior notification of the laboratory in addition to ordering the tests.

2.5. Labelling

The specimen container must be clearly labelled for proper identification and verification to ensure patient safety. Mislabeled or mismatched specimen may lead to delayed and wrong diagnosis. It can also lead to missed and incorrect treatment of the patient. Adequate and accurate labelling should be done while collecting the specimen to enable laboratory staff or microbiologists to adopt appropriate microbiological tests.

Each specimen must have a label firmly attached to the specimen container bearing the following minimum information:

1. Unique patient identification number/CID
2. Date & Time of Collection
3. Patient name
4. Age and sex
5. Specimen type
6. Collection site
7. Initials of the collector

Specimen without request form and label should not be processed. The clinician, nurse or the laboratory staff should be contacted and should be communicated and verified before discarding specimens. If details are not available despite communicating with the relevant hospital staff, the specimen must be rejected and discarded as per the specimen acceptance and rejection criteria.

2.6. Optimal Collection Time for Various Specimens

Optimal collection time for specimens for microbiological testing is mainly based on the type of infectious disease and it is vital that specimens are collected during appropriate timings to obtain reliable microbiology results.

2.7. Quantity of Specimen Collection

Adequate specimens should be collected for culture and other tests. Normally, more than one tests are requested by clinicians and specimens are often inadequate in quantity. Hence it is

crucial for any health staff to consider how much amount of specimen should be collected prior to collecting any specimen. In microbiology laboratory, for instance, swabs specimens are often submitted for multiple cultures and Gram staining. In the laboratory process, swab specimen can become easily dry and get contaminated, affecting the recovery of some of the important pathogenic microorganisms. Quantity ideally required for various types of specimens are given in the table 1.

3. Collection of blood specimen

3.1. Collection of blood specimen for bacterial culture

3.1.1. Specimen collection

Accurate and timely detection of bacteremia and fungemia remains one of the most important functions of clinical microbiology. Contamination should be prevented to avoid repeated venipuncture and delaying of the test process rates be minimized. Blood culture contamination rates can be minimized by strict adherence to aseptic collection technique. However, even with good collection technique, a small proportion of blood cultures are found to be contaminated. Whenever possible, collection of peripheral blood via venipuncture should be performed over other techniques such as via indwelling vascular catheters. The needles should be changed before inoculating a blood specimen into a culture bottle to reduce contamination. For disinfecting the skin surface, an iodophor or tincture of iodine, alone or in combination with isopropyl alcohol should be used. Along with the use of proper disinfecting technique, the use of proper appropriate disinfecting agent plays a critical role in reducing the rate of contamination with normal flora.

3.1.2. Number of cultures

Sufficient amount of blood specimen should be drawn for blood culture. Ideally, blood should be collected 3-4 times for culture to ensure that the pathogen is detected, both during intermittent and continuous bacteraemia. Blood culture for more than four times should be avoided to reduce the resource wastage, even in the event of suspected endocarditis (de Plato et al., 2019; Ombelet et al., 2019). Single specimen for bacterial and fungal culture should be avoided as it will compromise the laboratory's ability to detect sepsis and should encourage physicians to

submit additional specimens. However, culture of Mycobacterium, especially the Mycobacterium avium complex, a single specimen is adequate as first culture yields positive results. Hence second specimen is not recommended although it increases the sensitivity of culture. Also, Mycobacterium is never a contaminant, and the growth is clinically significant.

3.1.3. Volume of blood for culture

Adequate volume of blood also ensures that the proper ratio of blood to broth medium is attained within each bottle. Maintaining a blood-to-broth ratio of between 1:5 and 1:10 enhances microbial recovery in the specimen (Ombelet et al., 2019). Because of the low number of microorganisms present in the blood of adults who are bacteremic or fungemic, the most important variable in recovering bacteria or fungi from adults is the volume of the blood specimen cultured. For adults, the recommended total volume of blood to be drawn for blood culture is 20-30 mL. For infants and small children, the number of organisms in the blood can be higher. Although bacteremia or fungemia can be reliably detected in a small volume of blood in children, collection of 1-5mL of blood is recommended for each culture (University of Iowa, 2014).

3.1.4. Timing of collection

Studies have shown that drawing blood for cultures either simultaneously or over a 24-hour period resulted in similar microbial recovery rates. The blood specimen should be drawn for a set of cultures at the same time and preferably before administration of antibiotics. If a clinician requires to observe for continuous bacteraemia, the specimens should be collected in intervals expanding over 24 hours.

3.2. Collection of blood specimen for other assays

3.2.1. Collection of Acute and convalescent serum specimens

Serum specimens are collected for performing serological tests in microbiology. In general microbiology diagnostic testing, single acute serum specimens can be used. Ideally, during epidemic or public health events, both acute and convalescent sera should be obtained from all patients identified during such events. Unfortunately, collection of both acute and convalescent sera specimens may not be feasible, and these results are not timely enough to guide clinical care.

a. Acute serum specimen : Acute serum specimens should be collected within one week of symptom onset and submitted as soon as possible.
2. Convalescent serum specimen: Convalescent specimens should be collected and submitted at 3-6 weeks after the acute specimen is collected.

3.2.1. Whole blood plasma for PCR

For selected situations, whole blood in EDTA may be obtained for PCR diagnosis of infectious diseases. Whole blood should be collected as soon as possible after illness onset. 2-3mL is the recommended amount for PCR testing.

3.2.1. Dried Blood Spot (DBS)

Dried blood spot are the specimens which are collected on the piece of filter paper in the absence of required transportation facility. This method of collection is mainly used for molecular assays and antigen and antibody detection. Minimum of 100uL of blood specimen is spotted on the filter paper and dried.
Preferably, whole blood specimen is recommended over DBS if there is a refrigerated transport facility during transportation and when there is a higher quantity requirement.

3.2.2. Transport and storage

Blood culture bottles with the specimen inoculated should be transported immediately to the laboratory. If this is not possible, bottles can be kept at room temperature or in an incubator at a temperature of 35°C to 37°C and should be processed within 8 hours. The culture bottles should never be refrigerated. A time gap of 2 hours between specimen collection and incubation into the blood culture system is recommended.

It is recommended that the whole blood and serum specimens for serology and molecular biology assays be stored at 2-80C for up to 7 days if it could not be processed immediately. For long term storage, the specimen should be stored at -200C or -800C and below. For transport of serum specimens for serology and molecular assay, recommended temperature is at 2-80C and -200C respectively.

DBS should be stored and transported at room temperature. Direct sunlight exposure should be avoided at all times.

4. Collection of Cerebrospinal Fluid

4.1. Cerebrospinal fluid specimen

The prompt and accurate diagnosis of bacterial meningitis is among the most critical tasks confronting clinical microbiology laboratories. Clinicians and laboratory staff should carefully coordinate the collection, handling, and processing of the CSF specimens.

4.1.1. Collection

CSF collection should be carried out by clinicians or nurses trained to conduct the procedure. CSF should be collected through a strict aseptic technique to minimize specimen contamination and prevent bacteria from introducing bacteria into the CNS. Prior to collecting the specimen, the tapping site should be adequately disinfected with iodine or chlorohexidine.

4.1.2. Volume

For routine bacterial cultures, a few milliliters of CSF are adequate. In contrast, for fungal and mycobacterial cultures, microbial yield is more proportional to the volume of CSF cultured. Ideally, 10-20 mL should be collected (Teunissen et al., 2009). Amount collected may increase with the increase in the number of tests. The specimen should be divided into 3-4 tubes with 2-4mL and use of glass tubes should be avoided to prevent cell adhesion, which may affect the cell count or differential diagnosis (LabCE, 2020).

4.1.3. Time of collection

There is no specific timing for collecting CSF specimen and should be left to the clinician's discretion depending on the urgency.

4.1.4. Storage and Transport

CSF specimens should be transported immediately to the laboratory. Systematic delays in transport should be identified and eliminated. Laboratories should strive to report the results of initial tests within 30 minutes of receipt of the specimen in the laboratory. From collection through processing, CSF specimens (except aliquots collected for viral cultures) should not be refrigerated until initial processing
is completed. Laboratorians should consider using sequential testing to reduce the number of unnecessary CSF tests. If unavoidable delay is expected, the CSF for microbiological study should be stored and transported in Trans-Isolate (T-I) medium for transport (CDC, 2017).

5. Collection of Respiratory Tract Specimens

5.1. Collection of sputum specimen

Expectorated sputum continues to be the most collected respiratory specimen for bacterial cultures. Expectorated sputum specimens should be screened by Gram staining for contamination with saliva. If a sputum specimen is rejected, another specimen should be collected and screened in the same manner.
Specimens submitted for mycobacterial culture should not be screened with Gram staining, as the results do not reflect the likelihood that mycobacteria will be recovered (Johns Hopkins University, 2021). Similarly, specimens submitted for culture should not be screened on the basis of the relative numbers of neutrophils and alveolar macrophages. If a Gram stain reveals no bacteria or reveals >10 squamous epithelial cells per low-power field, the specimen should be rejected.

5.2. Collection of oropharyngeal and nasopharyngeal specimen

5.2.1. Oropharyngeal (OP) and nasopharyngeal (NP) swabs

Use only sterile dacron or rayon swabs with plastic shafts or if available, flocked swabs. Use of calcium alginate and cotton swabs or swabs with wooden sticks should be avoided as they may contain substances that inactivate some viruses and inhibit some molecular assays.

5.2.2. Nasopharyngeal wash/aspirate

Ideally, nasopharyngeal aspirates should be collected during the early stage of the disease. The specimen should be collected within 3 days of symptom onset and not later than 7 days. Nasopharyngeal aspirates specimen should be collected by instilling 1ml -1.5 ml of non-bacteriostatic saline (pH 7.0) into one nostril and collected using nasal tubing by inserting it into the nostril parallel to the palate (not upwards).

5.3. Collection of other respiratory tract specimens

Lower respiratory tract infection (LRTI) includes acute bronchitis, pneumonia, acute exacerbations of chronic obstructive pulmonary disease/chronic bronchitis (AECB), and acute exacerbation of bronchiectasis. Acute LRTIs (ALRTIs) are one of the common clinical problems in community and hospital settings. Lower respiratory tract specimens include tracheal aspirate, bronchoalveolar lavage (BAL) fluid, pleural fluid, etc. Collection of lower respiratory tract specimens require higher technical skill set and equipment, apart from sputum specimen. Hence, these specimens are mostly collected by trained clinicians or nurses.

Other respiratory tract specimens (e.g., bronchial lavage fluid) should not be rejected on the basis of criteria used for other specimens such as sputum. To obtain optimal result, all types of respiratory specimens should be collected before initiation of antibiotic therapy.

5.4. Transport and storage of respiratory tract specimen

Since most respiratory tract specimens are likely to contain at least a few contaminating microorganisms, specimens should be transported quickly to the laboratory to minimize overgrowth of contaminants (CDC & NCIRD, 2020).

If transportation or processing is delayed, specimens should be refrigerated at 4-8oC for storage up to 1-2 days. Storage for longer duration should be done at -70oC . For fungal and mycobacterial cultures, prompt processing and refrigeration help prevent overgrowth of normal flora in the specimens, which complicates the recovery of pathogens. Repeated freezing and thawing specimens should be avoided. Viability of some pathogens (e.g., respiratory syncytial virus) from specimens that are frozen and then thawed is greatly diminished and may result in false-negative test results (CDC & NCIRD, 2020).

6. Collection of faecal specimens

6.1. Faecal specimen

The laboratory diagnosis of enteric infections is challenging. Problems include the number of potential pathogens; the biologic diversity of these organisms and the emergence of new pathogens. Although faecal specimens are preferred, rectal swabs are convenient way of collecting faecal specimens since it can be collected quickly and without having to pass stool, which, sometimes, the patient feels disgusted while collecting. Specimens from diapers also can be collected for microbiological culture, especially from children.

6.1.1. Collection of fecal specimens

Faecal specimens should be collected within 72 hours (3 days). 4-5g of faeces should be collected in wide mouthed container by the patient. The patient should be advised to avoid contaminating the specimen with urine. Collection of specimens from toilet pot or soiled specimen should be avoided.

Faecal specimens from patient admitted in hospital after 3-4 days of hospitalization does not have benefit and therefore, not recommended for bacterial culture. However, faecal specimens collected from patients who develop diarrhea in the hospital should be tested for the presence of Clostridium difficile.

It is not recommended to test more than two specimens as a part of diagnosis of acute diarrhea. For research purpose, number of specimens collected can be more depending on the purpose of the research.

6.1.2. Collection of rectal swab Specimen

Sterile cotton-tipped swabs may be used to collect specimens. If prepared locally, it is important to ensure that the cotton fits tightly on the stick. The swab should be moistened with sterile non-bacteriostatic fluid or transport medium (not lubricating gel), inserted through the rectal sphincter, rotated, and withdrawn. The swab should be examined for fecal staining. The number of swabs to be collected will depend on the types of investigation required. Swabs taken in this way may also be used for microscopic examination for protozoa, but freshly passed stool is preferred.

6.1.3. Storage and transport

The swab specimen should be processed within 2 hours. If delayed, it should be placed in a sterile empty tube with a cotton plug or screw cap. If it is to be kept for longer than 2 hours, it should be inoculated into transport medium (mCB) and stored refrigerated or at room temperature for up to 7 days.
Stool specimens submitted for culture typically are not stored for any length of time, since most laboratories set up all appropriate cultures at the time of receipt of the
specimen. Rarely it is necessary to retrieve a specimen for additional testing and such specimens should be refrigerated. Specimens should be stored at -70oC for long term storage. Stool specimens submitted for ova and parasite examinations are typically stored at room temperature in a fixative. Specimens stored in 10% neutral-buffered formalin remain stable for many months, even when tested with some enzyme immunoassays for Giardia intestinalis. Specimens should not be stored for investigation of trophozoites as they deteriorate very quickly.

Optimal test results are obtained when microbiological testing is performed on fresh stool specimens. If testing of fresh specimens is not feasible, the specimen should be transported in transport media such as modified Cary-Blair (mCB) medium for culture or fixatives such as polyvinyl alcohol or 10% neutral buffered formalin for microscopy for detection of parasites and ova. For immunological assay for detection of enteric viruses and parasites, the specimen should be transported at -20oC.

7. Collection of wound, abscess, and drainage specimen

In general, before collecting wound specimen, wound surface should be cleaned with normal saline solution. Dead, damaged, or infected tissue should be removed to minimize contamination. Only viable tissues should be collected rather than superficial debris. Preferably, aspirates or biopsy should be taken as specimen, and swab specimen should be the last option. If swab specimens are collected, a hospital should always submit in triplicates (3 swabs per specimen) (CDC, 2020).

7.1. Collection of wound specimens

Wound Specimen should be preferably collected prior to initiation of antibiotic therapy and only from wounds that have clear signs of active infection, are deteriorating, or that fail to heal over a long period. Indiscriminate submission of a wound specimen, especially from a superficial site, may provide useless information that leads to unnecessary antibiotic treatment.

Before collection of specimens, the wound margins and superficial area should be cleaned thoroughly with sterile saline-dipped sponge. Superficial exudates should be all removed and overlying debris should be removed. Collect a biopsy or curette specimen from the base or advancing margin of the lesion. Sufficient tissue specimens (3-4cm) should be collected, however, collection of specimens from necrotic area should be avoided.

For aerobic culture, place tissue in a sterile container with a small amount of non-bacteriostatic saline (just enough to keep the specimen from drying out) and for anaerobic culture place the tissue in an anaerobic transport tube.

7.2. Collection of specimens from closed abscesses

Site of puncture should be disinfected before the specimen collection from abscess. Specimen should be aspirated from the infected material using needle and syringe. If the initial aspiration fails to obtain material, inject sterile non-bacteriostatic saline subcutaneously and repeat the aspiration attempt. The contents should be place in a sterile tube for submission to the laboratory.

7.3. Fine needle aspiration

Fine needle aspiration should be conducted by a trained laboratory staff, clinician, or nurse. Disinfection of puncture sites should be conducted to avoid contamination. Adequate specimens should be obtained. The specimens should be attempted for anaerobic culture.

7.4. Open Wounds

Wound margins and superficial area are thoroughly cleaned with sterile saline, changing sponges with each application. Care should be taken to remove all superficial exudates and overlaying debris with a scalpel and swabs or sponges. Biopsy or curette specimen from the base or advancing margin of the lesion are usually collected in sufficient amount (3-4mm biopsy specimens) and specimen from necrotic areas should not be collected. For aerobic culture, tissue specimen is placed in a sterile container with a small amount of non-bacteriostatic saline (just enough to keep the specimen from drying out). For anaerobic culture, if appropriate, should be attempted from tissue specimens place in an anaerobic transport tube.

7.5. Pus

Pus is thick, opaque, usually yellowish white fluid matter formed due to inflammation caused by the invasion of infective microorganisms (such as bacteria). It is composed of degenerating leukocytes (white blood cells), tissue debris, and living or dead microorganisms. Specimen collection include aspiration of the deepest portion of the lesion or exudate with a syringe and needle and collected in a sterile tube. A biopsy specimen of the advancing margin or base of the infected lesion after excision and drainage may be collected. Aspiration can also be conducted in an infected bite wound, aspirate pus from the drained wound at the bite site. Culture of fresh bite wounds are not recommended as it will harbor resident respiratory flora introduced from the bite, but cultures cannot predict if they will cause infection. Anaerobic culture is not appropriate.

7.6. Additional in formation

Information regarding the type of wound (surgical, traumatic pressure ulcer etc.) and location of the wound is important and should be indicated when a test is ordered. Tissues and aspirates are acceptable specimens for anaerobic culture.

7.7. Storage and transport

The abscess/pus/swab specimen should be transported as soon as possible and processed within 2 hours of collection. If delayed, the swab specimen, add few drops of non-bacteriostatic saline and stored at 2-80C for not more than 24 hours. Do not freeze the pus specimen.

8. Specimen collection from urinary and reproductive organs

Gonorrhea and chlamydia are the two most common bacterial infections which causes urethral discharge both in men and women. Urethral discharge culture or a genital exudate culture testing is a relatively simple but uncomfortable procedure and must be performed by well trained and experienced laboratory personnel. It involves some risks such as fainting due to stimulation of the nerve, bleeding and infection if not collected appropriately. To prepare, patient must be told to refrain from urinating at least 1 hour before the test as urination may wash away some of the germs that the test is trying to capture. If urinated, the test should be done at least 2 hours after urination.

8.1. Collection of urethral discharge specimen

The urethra is the preferred culture site in men, or in women with no cervix. The Laboratory personnel must clean the tip or opening of the genital part with a sterile swab, where the urethra is located. Then, gently insert a sterile cotton swab or sterilized inoculation loop about (1-2 cm for women,2-4 cm for men) and rotate the swab/loop in one direction for minimum of 10 seconds to gather a large enough of discharge specimen. The specimen is then immediately inoculated into a culture media and some portion used in smear preparation for microscopic examination. It must be noted that swabs with wooden shafts should not be used as it possess toxicity affects and therefore Dacron and calcium alginate swabs may all the time be used.

8.1.2. Transportation and storage

It is always recommended that swab for Neisseria gonorrhea culture be processed immediately. Chlamydia are labile bacteria, and viability can be maintained by keeping specimens cold and minimizing the time between specimen collection and processing in the laboratory. For successful culture of chlamydia, swabs, scrapings, and small tissue specimens should be forwarded to the laboratory in a special chlamydial transport medium such as 2SP (0.2 M sucrose-phosphate transport medium containing 10 µg of gentamicin/mL, 25 µg of vancomycin/mL and 25 U of nystatin/mL). Broad-spectrum antibiotics such as tetracyclines, macrolides or penicillin cannot be used in the transport media because they have activity against chlamydiae. Chlamydial specimens should be refrigerated on receipt in the laboratory; if specimens cannot be processed within 24 h after collection, they should be frozen at -70°C

8.2. Collection of High vaginal swab

Vagina discharge is common presentation in practice, potentially indicating the presence of STIs. Abnormal vaginal discharge is characterized by a change of color, consistency, volume, or odor. Investigation and management of vaginal discharge rely on examination of high vaginal swab (HVS). HVS generally tests vaginal discharge for the presence of vaginal thrush, bacterial vaginosis, and trichomonas vaginalis. Using HVS, candida may also be diagnosed with a wet mount microscopy. The specimens are normally collected by medical, female midwifery and nursing staff to maintain the privacy.

The procedure is carried out with good light and in clean conditions. After lubricating the speculum water-based lubricant, it is inserted into the vagina to see the vagina and cervix, whilst also protecting the swab from being contaminated by organisms on the vulva. After inserting the swab to the top of the vagina, it is rotated to obtain a specimen of the discharge and subsequently the speculum is removed and the specimen sent for microscopy, culture, and sensitivity, in charcoal-based transport medium. If the specimen cannot be sent to the laboratory immediately, it may be stored in a fridge.

8.2.1. Storage and Transportation

HVS specimen collected must be attempted to test as immediately as possible. If any delay, obtain the discharge in charcoal-based transport medium and transport to testing laboratory maintaining the temperature at 4°c.

8.3. Prostatic Fluid collection

Prostatic fluid collection which is also known as expressed prostatic secretions (EPS) test a specimen of secretion for sign inflammation of bacterial infection mainly for men. The test is done when one has repeated urinary tract infection, symptoms that would suggest chronic bacterial prostatitis, chronic prostatitis/pelvic pain syndrome, inflammatory, or chronic prostatitis/pelvic pain syndrome, noninflammatory. The test includes microscopy cell count to check inflammation and culture to rule out bacterial infection.

The procedure is normally performed by well-trained medical doctor. Ask patient to avoid ejaculation for 5 days prior to the test. This allows prostrate fluid to build up and prevents increase in the numbers of white blood cells in the prostrate fluid, which could interfere with test result. While the patient bend over or lie on his side or back, the doctor inserts a lubricated ,gloved finger into the rectum and presses each side of the prostate gland 6 or 7 times. The urethra is then gently milked with a gloved finger. The secretions are collected in a tube or swab. Expressed prostatic secretion is also collected by catheter for the purpose of protecting EPS from the contamination of urethral bacteria. The catheter is blind-ended and has several lateral windows and a balloon between the end and the windows. It is inserted into the urethra and balloon blown up. The catheter is extracted during massage of the prostate and finally EPS is aspirated through the internal lumen of the catheter into sterile container.

8.3.1. Storage and Transportation

The specimen is usually stored and shipped at room temperature

8.4. Collection of urine specimen

Urine Specimens are collected in a sterile, screwcap wide mouthed container. A minimum of 10mL is required. Each specimen tube must be properly labeled with the patient's name, medical record number, location and the time and date of specimen collected.

8.4.1. Specimen Storage and Transport

Urine specimens may be transported at 18–25°C. They are stable for 24 hours at this temperature. If it requires shipment to off-site testing centers, shipment must be done within 24 hours. In this case, the specimen may be shipped at 18–25°C. Urine specimens that will not be processed within 24 hours of collection must be stored at 2–8°C. Storage of urine specimens at 18–25°C for more than 24 hours may result in specimen degradation. These specimens should not be used for testing. Specimens stored at 2–8°C must be processed within seven days of collection. Specimens that cannot be processed within seven days may be stored at –20°C or colder and may be stored this way for up to two months.

9. Specimen collection of body fluid

9.1. Collection of pleural fluids

Pleural fluid analysis is a test that examines a specimen of fluid that has collected in pleural space due to various pathological consequences. Specimens are collected by trained Clinician and Nurse to examine the specimens to look for pathogens that cause infection. A procedure called thoracentesis is performed to get a specimen of pleural fluid by inserting needle into pleura cavity to extract pleural fluid.

9.1.1. Volume

It is recommended to extract 10-20ml of pleural fluids. The amount can also be collected while performing thoracentesis for clinical purposes, such as relieving the symptoms.

9.1.2. Storage and Transport

After obtaining the specimens it can be stored at 4°c until processing and subsequent storage (up to 48hrs). The labeled specimen with the request form is transported to the laboratory together. It is recommended that the time between extraction of the Pleural Fluid and freezing at -80ºC is not more than 2 hours.

9.2. Collection of Synovial fluid

Synovial fluid analysis is also known as joint fluid analysis. Each of the joints in the human body contains synovial fluid. This fluid is a thick liquid that lubricates the joint and allows for ease of movement. In joint diseases like arthritis, the synovium of the joint is the main place were inflammation occurs. Synovial fluids help in diagnosis of septic arthritis (SA)

9.2.1. Volume

Synovial fluid should be collected by trained clinician and nurse. Prior to collection of specimens the site of tapping should be adequately disinfected. The recommended synovial fluid specimen volume per container is .5 - 1mL for microbiological testing.

9.2.2. Storage and transport

Specimens collected using a sterile syringe and needle must not be transported with the needle on the syringe. The specimen should be transported and examined promptly or within 8 hours.

9.3. Collection of Ascetic fluid

Ascites (peritoneal fluid) is the pathologic accumulation of fluid within the peritoneal cavity. Because many diseases can cause ascites, in particular cirrhosis, specimens of ascitic fluid are commonly analyzed to develop a differential diagnosis. It is collected by trained clinician and nurse under aseptic condition.

9.3.1. Volume

A peritoneal fluid specimen (50ml) is obtained by inserting a needle into the abdominal cavity. Minimum 50ml is required, if possible more than 500ml or entire volume collected should be submitted to laboratory for diagnosis.

9.3.1. Storage and transport

Refrigerate or keep on wet ice until transported to laboratory.

9.4. Collection of Amniotic fluid

Amniotic fluid are those fluids that fill up the amniotic sac, which is a bag inside a woman's uterus (womb) where an unborn baby develops. Amniotic fluid is a clear, pale straw color and consists mainly of water. The common bacterial infection in the fetal membranes, amniotic fluid and placenta that has ascended from vagina into the uterus is termed chorioamnionitis or intra-amniotic infection (IAI). There are two approved methods of collection: during amniocentesis and during cesarean section surgical procedure.

9.4.1. Volume

Amniotic fluid is collected when health care provider is performing a cesarean section for clinical purposes and fluid (up to 10 ml).

9.4.2. Storage and transport

Store aliquots at refrigerated temperature if it is processed within 7 days. If stored for longer period, a minimum of -20°C for short-term storage (< 30 days), and preferably at - 80°C if delayed further.

9.5. Collection of Pericardial Fluid

Pericardial fluid analysis is used to help diagnose the cause of inflammation of the pericardium (pericarditis) and/or fluid accumulation around the heart (pericardial effusion).
The pericardial fluid collection is done by cleaning the skin of the chest with antibacterial soap or 70% isopropanol alcohol. The clinician then inserts a small needle or catheter into the chest between the ribs and into the pericardium.

9.5.1. Volume

A small amount (2-4ml) of fluid is taken out. Sometimes, the pericardial fluid is obtained during open heart surgery.

9.5.2. Transport and Storage

The pericardial fluid specimen for Laboratory test should be sent to the laboratory as soon as possible in a fresh state or refrigerated at 2-8º C. for microbiology, it can be stored at room temperature (27°C) for 8 hours, refrigerated (2-8°C) for 7 days frozen (below -20°C) for upto month. For long term storage, specimen should be frozen at -80°C.

10. Specimen collection from skin, nails, eyes, ear

10.1. Collection of Skin Scrapping for Fungal Infection

Skin scrapping is a common procedure used to obtain superficial dead layers of skin. Specimens collected are placed in a drop of 10% KOH will dissolve at the greater rate than the fungi within them because fungi have chitinous cell walls. The clearing effect throughout the clinical specimen can be accelerated by gently heating KoH preparation. KOH provides advantageous refractive index to reveal the fungal hyphae.

Single negative KOH wet mount does not rule out the presence of fungal infection. The yield may be reduced by prolonged storage on standing. Therefore, a fresh specimen is preferred. Skin smear for fungal scraping is collected from any sites that is suspected to have fungal infection. The specimen collection sites are cleaned with normal saline swab and allowed to dry. Then using a sterile surgical blade, the skin from infected are scratched and placed on the clean glass slides for microbiology investigation.

10.2. Skin smear for leprosy

Mycobacterium leprae bacilli have large quantities of unsaponifiable wax fraction called “mycolic acid” in its cell wall. In this staining method, application of heat helps the dye (carbolfushsin) to penetrate the bacilli. M. leprae bacilli resist the decolorizing agent (acid alcohol), due to presence of mycolic acid and thus appear as red/pink, while another organism is easily decolorized, take up the counter stain and appear blue. Skin smear are the preferred specimen of choice for Hansen test to diagnose M. leprae (Leprosy) infection.

The skin smears are generally collected from at least 3 sites.

a. Ear lobe.
b. Chin.
c. Forehead.

Occasionally skin smear from lesions, nasal and nodules are taken on the advice of physician. A new sterile scalpel blade in its holder and three clean glass slides. The specimen collection sites are cleaned with 70% alcohol swab and pinched until it becomes pale. Then using a sterile surgical blade, a small cut of 5mm long and 2mm deep is made. If there is any blood drops it is wiped with sterile cotton ball. Turning the blade in right angle to the cut, the tissue fluid is collected with the blunt edge of blade. This tissue fluid is smeared on the clean glass slide. The same procedure is repeated to collected specimen from other sites.

10.3. Collection of Eye swab

Certain conditions that lead to discharge in the eyes can be related to ear pain in some situations. Examples include infections of the ear, eyes, or sinuses. discharge from the eyes that appears to contain pus. There are several indications for taking swabs for the identification of organisms from the conjunctiva and cornea including situations where, Neisseria gonorrhoea infection or chlamydia is suspected, for the identification of herpesvirus infections of cornea or conjunctiva and where fungal infections are suspected. Bacterial swabs should also be taken before the instillation of topical anesthetic, and it should not be used to facilitate swabbing as it inhibits bacterial growth.

Onsite collections:

Conjunctiva:

1. Swab: A moistened swab is used to collect the specimen lower conjunctiva avoiding eyelid border and lashes.
2. If inoculating the specimen immediately at the time of collection, please call the laboratory prior to collection to obtain appropriate media.
3. Scrapings: One or two drops of topical anesthetic instilled, and the lower tarsal conjunctiva is scrapped.
4. Corneal scraping:
5. Procedure performed by ophthalmologist.
6. Contact Microbiology for media for direct inoculation.

Offsite collections:

A moistened swab is used over lower Conjunctiva and placed it in transport medium.

Transport and Storage

Onsite collections: Transport to the laboratory immediately. Directly inoculated plates should be delivered in <15 minutes at room temperature.

Offsite collections: Refrigerate specimen. Specimens must be promptly transported to the

laboratory, and not to exceed 24 hours from the time of collection.

10.4. Collection of ear swab

The ear is a complicated part of the body, made up of several different chambers. Ear infections can strike in any one of these chambers and cause various symptoms. Infections are most common in the middle ear and outer ear. Inner ear infections are less frequent and sometimes a sign of another underlying condition. A swab is not recommended for collecting specimens to diagnose otitis media infections. The specimen of choice is an aspirate from behind the tympanum (eardrum). A small swab may be used only when the eardrum has ruptured, and fluid is collected. Swab any pus or exudates or fluid from external meatus of the ear for investigation of fungal infection, scrapings of material. The swabs for bacterial and fungal culture should then be placed in Amies transport medium with charcoal.

10.4.1. Transport and storage

Transport the specimen as soon as possible. Specimens that cannot be transported or processed immediately should be refrigerated at (2-8°C) if placed in the transport medium.

11. Verification & rejection of specimen

11.1. Information verification

As soon as a specimen is received at the laboratory, a laboratory personnel should verify the specimen label with the information provided on the laboratory request form. if discrepancies are observed, the clinician or the nurse requesting the test should be contacted and information corrected. If the mismatch remains, the laboratory should reject the specimen according to specimen acceptance and rejection criteria below.

11.2. Specimen rejection criteria

Specimen rejection criteria plays vital role in determining specimen quality for the test. Laboratory personnel at testing Laboratory should verify the specimens received to ensure there is no error in the information provided prior to testing. Specimen should be rejected if :

1. The specimen is unlabeled or mislabeled
2. The specimen is collected in a wrong container
3. There is a leakage from container
4. There is indication of specimen deterioration
5. The specimen is determined as prolonged storage at the collection site and delayed shipment
6. The volume is inadequate
7. Required information on specimens and forms are missing
8. Inappropriately packed without following triple packaging system

Table 1: Collection of blood specimen

Specimen	Test	Container	Volume	Minimum Number of specimens	Transport condition	Storage	Remarks
Blood	Blood culture	Blood culture bottle (aerobic and anaerobic)	8-10mL per culture bottle for adults and	2-3	25oC	25oC or 35°C	Avoid delays in processing lysis-
			1-5mL for children				(Aerobic and anaerobic) 1-5 for centrifugation tubes
	Fungal culture	Aerobic blood culture	8-10mL per culture bottle for adults and	2-3	25°C	25oC or 35°C	
		BACTEC HBVFM*	1-5mL for children				
		vials, or lysis centrifugation tube					
	Mycobacterial culture	BACTEC 13A or lysis-centrifu-gation tube	8-10mL per culture bottle	1	25°C	25oC or 35°C	Submit one specimen initially:
							Repeat if negative but
							Mycobacterium is still clinically suspected

Table 2: Collection of respiratory specimens

Specimen	Test	Container	Volume	Minimum Number of specimens	Transport condition	Storage	Remarks
Respiratory tract	Bronchoscopy fluid	Fluid, collected in sterile container	NA	1	25oC		Not for anaerobic culture
	Sputum	Sterile container	NA	1	25oC	4oC	Not for anaerobic culture, check for contamination with saliva.
	Nasopharyngeal swab	Sterile swab	NA	1	Plate it immediately	4oC	Not for anaerobic culture
	sinus	Aspirate materials collected in sterile anaerobic vials	NA	1	25oC	4oC	
	Throat	swab	NA	1	25oC (best result obtained when plated immediately)	4oC	Not for anaerobic culture

Table 3: Collection of fecal specimens

Specimen	Test	Container	Volume	Minimum Number of specimens	Transport condition	Storage	Remarks
Feces	Feces for culture	fecal material collected in wide-mouthed sterile container	2-4g	1	immediately, Trans-port in modified cary blair medium at 4-8oC	4oC, long term storage at -40 to -70oC	Specimens should be collected within 72 hours of diarrhea. Long term stay with diarrhea should be investigated for Clostridium difficile
	Swab for culture	swab	NA	2	immediately, Trans-port in modified cary blair medium at 4-8oC	4oC, long term storage at -40 to -70oC	Specimens should be collected within 72 hours of diarrhea. Long term stay with diarrhea should be investigated for Clostridium difficile
	Feces for parasitology	fecal material collected in a wide mouthed container	2-4g	1	examine immediately or within 30 minutes	4oC	

Table 4: Collection of abscesses, wound, drainage, tissue specimen

Specimen	Test	Container	Volume	Minimum Number of specimen	Transport condition	Storage	Remarks
Hair	Microbial culture	Hair, collected in sterile vile	NA	1	NA	NA	
Deep wound /abscess	Microbial culture	swab/syringe, collected in anaerobic vial	NA	1	25oC	4oC	
Dermatophytes	Microbial culture	Scrap, collected on sterile petri dish	NA	1	25oC	4oC	
Superficial wound	Microbial culture	swab/syringe, collected in anaerobic vial	NA	1	25oC	4oC	Do not collect swabs from wound or ulcers from decubitus and diabetic foot ulcers, margins of nonviable amputation
Eye swabs	Microbial culture	Sterile vials	1	1	25oC	4oC	For bacterial culture
Abscess fluid	Microbial culture	anaerobic vial	1-5	1	25oC	4oC	
Catheter specimen	Urinary catheter	NA	NA	1	25oC	4oC	Only for Gram staining and not recommended for culture
	Vascular catheter	catheter tip in sterile vile	NA	1	25oC	4oC	To detect line sepsis. Venous blood must be obtained for culture
Bone	Aspirate culture	Sterile container	NA	1	25oC	4oC	Infected bone can be cultured. Contamination must be avoided, mainly from sinus tracts and skin

Table 5: Collection of urinary and reproductive specimen

Specimen	Test	Container	Vol-ume	Minimum Number of specimens	Transport condition	Storage	Remarks
Genitourinary tract	swab (candida albicans	Sterile swab	NA	1	25oC	25oC	
	Swab (Chlamydia trachomatis)		NA	1	25oC	4oC	Swab should be immediately transferred into 2- sucrose phosphate
	Swab (Hemophilus)		NA	1	4oC in trans-port media within 48 hours	4oC in trans-port media. Processed immediately or within 2 hours	Processed immediately
	Herpes simplex virus		NA	1	4oC	4oC	
	Swab (Mycoplasma/ Ureaplasma)				25oC		Swab should be immediately transferred into 2- sucrose phosphate
	Swab (Neisseria gonor-rhoeae)	inoculate into the medium immediately	NA	1	25oC	None, incubat-ed immediately	Do not use calcium alginate swab
	Scrap or aspirate from lesion (Treponema pallidum)	scrap or aspirate on the slide	NA	1	Transport immediately	NA	Dark field microscopy should be done immediately
	Swab (Trichomonas vaginalis)	Swab for cul-ture, smear	NA	1	NA	NA	Microscopy should be done immediately
	Smear (Trichomonas vaginalis)	Perform rapid test	NA	1	Na	NA	Microscopy should be done immediately

Table 5: Collection of urinary and reproductive specimen (continued)

Genitourinary	Amniotic fluid	collect fluid in anaerobic vial	10-Jan	1	4oC	4oC	
	Swab (Cervix)		NA	1	NA	NA	Not acceptable for culture
	Endome-trium	Fluid, collected in anaerobic vials	5-Jan	1	25oC	4oC	
	Pelvic fluid	Fluid, collected in anaerobic vials	5-Jan	1	25oC	4oC	
	Prostate	fluid, collected in sterile vial	5-Jan	1	25oC	4oC	Obtain fluid by massaging prostate. Not for anaerobic culture
	Vagina	Swab	NA	1	25oC	4oC	Not for anaerobic culture
Urine	Urine, clean catch	Sterile vial	5-10mL	1	Processed immediately	4-8oC for short duration, -70oC for long duration	Not for anaerobic culture
	indwelling catheter	urine, collected in sterile container	NA	1	NA	NA	Not suitable for culture
	straight catheter	sterile vials	20-Jan	1	Processed immediately	4-8oC for short duration, -70oC for long duration	Not suitable for anaerobic culture
	Suprapubic aspirate	urine, collected in anaerobic vial	20-Jan	1	Processed immediately	4-8oC for short duration, -70oC for long duration	

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Annex 01

General Procedure for Packaging and Transport of Clinical Specimen

Standardized packaging methods and materials ensure safety of personnel and specimen integrity, even if the package is damaged during transport. Laboratory request forms must accompany the labelled specimens. Specimens must be packaged, labelled, and transported in compliance with specific national and international regulations for infectious materials (IATA, 2020).

Address labels on outer packages should display the sender and laboratory name with complete addresses and telephone numbers for both the sender and receiver. Documentation should also contain specimen details (number, type, date of collection), appropriate biohazard labels, and the storage temperature requirements. Copies of letters, forms, permits, airway bills and other identifying/shipping documents for the receiving laboratory should be placed together in a plastic bag and taped onto the outer transport packaging. The transport service must also receive a copy of these documents.

Triple Packaging System

A. Specimen packaging and transportation

1. Prepare the line list of specimens to be shipped in accordance with SOP for line listing specimen information.
2. Arrange documents for specimen accordingly. Documents required for shipment of specimen from site to are given in SOP for documentation for specimen transport and shipment.
3. Follow WHO Triple Packaging System as follows:
    - Primary receptacle holds respiratory specimen container wrapped with parafilm.
    - Secondary container should be durable, watertight, leak-leakproof, several primary receptacles can go into secondary container
    - Outer container should be rigid, durable, and insulated e.g., Styrofoam box.
4. Steps for Packing Specimens
    - Seal specimen tube with parafilm –this is primary receptacle and wrap with tissue paper to absorb the accidental leakage.
    - Place specimen container in watertight zip-lock bag.
    - Place up to 10 single specimens in zip-log bag within another watertight container depending on size of the container (e.g., sturdy plastic container with lid) secondary receptacle.
    - Place absorbent, cushioned material between primary and secondary containers.
    - Put secondary container in a “Wizard Box” or any box (Outer container)

provided for shipment of specimens.

- Place enough ice/cold packs between secondary and outer containers.
- Place test request forms and other relevant documents in a waterproof bag (preferably sealed plastic bag) carefully tapped to the outside of the outer box. Do not place the documents inside the secondary receptacle.
- Mark and label the outer container properly, this should include:
    - Address of the shipper
    - Address of the consignee
    - UN number
    - Category of specimen
    - Biohazard label
    - Orientation
    - Ship the specimen from the nearest Bhutan Post to the reference laboratory.

Figure 1: Triple Packaging System.(Source: biosafetyweb.com)

Annex 02

General Procedure for Blood Specimen Collection for Culture

This guide ensures appropriate collection of blood specimen for quality patient care. All diagnostic information from the microbiology laboratory is contingent on the quality of specimens. Poorly collected blood specimen will lead to failure in isolation of the causative microorganism and recovery of contaminants or normal microorganism, which can lead to improper treatment of the patient. Blood specimen should be collected prior to antibiotic administration.

Volume of blood to collected

1. Adult – 8-10 mL
2. Infant – 1-5mL

Pre-requisites

1. Register
2. Request forms
3. Specimen container (sterile culture bottle)
4. Gloves
5. Marker pen
6. Sterile syringe and needle (5ml and 10ml)
7. 70% isopropanol cotton ball
8. Tourniquet

Collection Procedure

1. Fill up the patient's detail forms
2. Label the container with patients ID (BAR CODE)
3. Use necessary PPE
4. Apply 70% alcohol swab in circular motion and wait for one minute.
5. Withdraw 8-10ml of blood (Adult) and 1-5ml blood from infant.
6. Send blood specimen to the microbiology lab.

Annex 03

General Procedure for Collection of Fungal Scraping

Collection of fungal scraping specimens to ensure quality patient care. All diagnostic information from the microbiology laboratory is contingent on the quality of specimens. Fungal scraping is collected in the lab for etiological investigation of fungal infection of the patients.

Pre-requisites

1. Register
2. Request forms
3. Surgical blade
4. Surgical blade holder
5. Burnson burner
6. Glass slide
7. Cover slip
8. Gloves
9. Marker pen

Collection Procedure

1. Fill up the patient's detail in register.
2. Label the slide with patients ID.
3. Use necessary PPE.
4. Select site for specimen collection.
5. Wash the site thoroughly in case of cream/ointment application.
6. Collect fungal specimen preferably from the margin of the infected sites.
7. With use of surgical blade, scrap the fungal onto to the glass slide.

Annex 04

General Procedure for Collection of Eye Swab

Common mild eye infections include conjunctivitis (conjunctiva. Less common and more severe infections include keratitis (cornea) and endophthalmitis (infection inside the eyeball).

Pre-requisites

1. Register
2. Request forms
3. Sterile swab stick with screw cap.
4. Gloves
5. Marker pen

Collection Procedure

1. Fill up the patient's detail forms
2. Label the primary container with patient's name and lab ID (BAR CODE)
3. Use necessary PPE
4. Collect the eye swab at bed side (Ideally corneal scraping and intra-ocular fluid should be collected by ophthalmologist and should be processed at the patient side).
5. Plate the swab into the agar
6. Transfer to the lab Immediately

Annex 05

General Procedure for Collection of Body Fluid

Collect the body fluid specimen in the acute phase of the infection and before antibiotics are administered for microbiological testing. Specimens must be collected with use of strict aseptic technique from anatomic sites most likely to yield pathogenic microorganism. Very often microbiology laboratories receive specimens collected from sites that are inappropriate for testing.

Cerebrospinal Fluid (CSF)

The prompt, accurate diagnosis of bacterial meningitis is among the most important tasks confronting clinical microbiology laboratories. Clinical personnel and laboratory staff should carefully coordinate the handling of specimens from the time of collection through processing. CSF is precious and handle it carefully and economically. It is not easy to repeat the procedure.

Pre-requisites

1. Register
2. Request forms
3. Necessary PPE
4. Wide mouth container
5. Gloves
6. Marker pen

Collection Procedure

1. To be conducted by Clinician/nurses
2. Use necessary PPE.
3. Collect the body fluid as per the standard procedure (by clinician or trained nurses)
4. Send the specimen to the laboratory along with the request form.

To be conducted by laboratory staff

1. Receive body fluid specimens along with request forms from wards.
2. Verify the patient details with the specimen
3. Assign laboratory specimen identification number

Pleural fluid

Pre-requisites

1. Syringes and/or material required for collecting PF
2. Sterile tube for collecting PF
3. Gloves for protection during handling
4. Sterile 1 milliliter Pasteur pipettes
5. Sterile cryotubes (from 0.5 to 2 milliliters)
6. Cryotube racks

Collection Procedure

1. PF collection is performed in tubes with the anticoagulant chosen by trained clinician or nurse as per the existing SOP.
2. After obtaining the specimen, keep in a refrigerator at 4ºC until processing and sub- sequent storage (up to 48h).
3. The perfectly labeled specimen and the request are transported to the laboratory together with the informed consent, while following the safety guidelines.

Receipt of PF specimen in laboratory

4. Check the information and identification of the tubes and ensure the correct relationship between tubes and patient information.
5. Label and record the specimen according to the specimen management procedure used by a laboratory.

Annex 06

General procedure for collection of genital specimens

Ensure all specimen collected are of good quality. All diagnostic information from the microbiology laboratory is contingent on the quality of specimens. Poorly collected genital specimen include failure to isolate the causative microorganism and recovery of contaminants or normal microorganism, which can lead to improper treatment of the patient. Specimen should be transferred to laboratory immediately after the collection or plate at the bed side. If transport cannot be immediately assured, the specimen should not be refrigerated. The common genital specimen collected are urethral discharge and high vaginal swab.

Pre-requisites

1. Register
2. Request forms
3. Sterile swab stick
4. Gloves
5. Marker pen
6. Nichrome wire loop
7. Glass slide
8. Gas burner/spirit lamp
9. Vaginal speculum
10. Normal saline
11. Match box/ lighter

Collection Procedure

1. Use necessary PPE
2. Fill up the patient details
3. Label the primary container with patient details
4. Sterilize the nichrome wire (urethral discharge)
5. Prepare the glass slide for Gram stain and chocolate agar for culture
6. Collect loopful of specimen and inoculate on the chocolate agar
7. Collect another loopful of specimen for Gram stain
8. Receive HVS specimens along with request forms
9. Verify the patient details with the specimen
10. Assign laboratory specimen identification number

Annex 07

General procedure for collection of fecal specimens

The laboratory diagnosis of enteric infections is challenging. Although faecal specimens are preferred, rectal swabs are convenient way of collecting faecal specimens since it can be collected quickly and without having to pass stool, which, sometimes, the patient feels disgusted while collecting. Specimens from diapers also can be collected for microbiological culture, especially from children.

Pre-requisites

1. Register
2. Request forms
3. Rectal swab stick
4. Gloves
5. Marker pen
6. wide-mouthed container

Collection Procedure

1. Fill in the patient's detail forms
2. Label the container with patients ID (BAR CODE)
3. Use necessary PPE
4. Collect the stool into a clean, wide-mouthed container (2-3g in sterile clinicol) with a tight-fitting and leak-proof lid
5. Transport the specimens immediately to laboratory for processing

Annex 08

General procedure for collection of high vaginal swab

Vaginal collections are taken when a healthcare provider is performing a vaginal or cervical exam (with or without a speculum) or collecting other genital specimens for clinical purposes. Every effort should be taken to acquire specimens without contamination with lubricant. If lubri-can't is present prior to sampling this should be recorded on the Laboratory Requisition forms. Vaginal swabs may also be self-administered.

Pre-requisites

1. Laboratory coats/scrubs
2. Face shield/safety goggles
3. Mask
4. Gloves
5. Sterile swab
6. Speculum
7. 70% Alcohol

Collection Procedure:

1. Before collection of vaginal swabs, make sure all the listed supplies are opened and within easy reach.
2. A speculum may or may not be used for a vaginal swab collection and is at the discretion of the healthcare provider.
3. Insert a lubricated speculum. When sampling, avoid the vaginal area where there has been contact with the speculum.
4. If a speculum is used for cervical exams, partially withdraw the specu-lum then make a full circular swab of the vagina with the swabs.
5. Collect the vaginal midpoint specimen using one sterile swab to specimen an area on the vaginal sidewall about halfway between the introitus and the cervix. Use caution to avoid contamination by the cervical mucus.
6. Gently press the swab into the vaginal sidewall and rotate the swab four times to thoroughly coat the swab.
7. Remove the swab and place it back in the collection tube.

Receipt of HVS specimen in the Laboratory:

1. Verify the specimen received with corresponding requisition form
2. Immediately process for test as per the SOP
3. If the process is delayed it should be placed immediately in a refrigerator at 4- 8°C
4. Swab collected in the cryo-vials for freezing should be frozen at -20°C or -80°C as soon as possible.

Annex 09

General procedure for urethral specimen collection

Swab specimens are collected from the urethra to determine the presence of organisms associated with sexually transmitted infection. *Neisseria gonorrhoea* and *Chlamydia* infection may be characterized by pain, irritation, and discharge. Prior antimicrobial use may result in negative cultures.

Prerequisites:

1. Lab coats/scrubs
2. Closed toed shoes
3. Gloves
4. Mask
5. Sterile swab/loop
6. 70% Alcohol
7. Culture Media
8. Slides
9. Bunsen burner

Specimen collection procedure:

1. Before collection of urethral swabs, make sure all the listed supplies are opened and within easy reach
2. Explain the procedure to the patient
3. Make sure to use sterilized loop or swab
4. Gently insert the loop or swab in the urethra to collect enough discharge
5. Immediately inoculate in the culture media
6. Prepare smear on glass slides with the remaining specimens for microscopy examination

Specimen transport, Receipt, and storage:

Laboratory processing should occur as soon as possible after specimen collection. Specimens should be refrigerated if delays in processing over two hours are unavoidable. Specimens should ideally be stored and transported in sealed plastic bags.

Annex 10

General procedure for prostate fluid collection

Prostatic fluid, which is also known as expressed prostatic secretion (EPS) is collected from men when one has repeated urinary tract infection, to screen for inflammatory/ non-inflammatory pelvic pain syndrome and chronic bacterial infection.

Materials required:

1. Gloves
2. Catheter / swab for collection of the fluid
3. Sterile tube

Specimen collection procedure:

Prostate fluid is collected by a well-trained health care workers following the steps given below:

1. Ask patient to avoid ejaculation for 5 days prior to the test. This allows prostate fluid to build up and prevents increase in the number of WBCs in the prostate fluid, which could interfere with the test results.
2. During collection, ask the patient to bend over, lie on his side or back.
3. Insert a lubricated gloved finger into the rectum and press each side of the prostate gland 6 or 7 times. The urethra is gently milked with a gloved finger. Collect the secretions in the tube or swab.

The prostate fluid may also be collected using a catheter, which is blind ended, has several lateral windows and a balloon between the end and the windows:

4. Insert the catheter into urethra and blow up the balloon.
5. Extract the catheter during massage of the prostate.
6. Aspirate the EPS throughout the internal lumen of the catheter into sterile container.

Storage and transportation

Ship to the laboratory immediately after collection, at room temperature.

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