Integrated Disease Surveillance Programme
State Referral Laboratory Network

Guidelines for collection & transport of clinical specimens during outbreak situation

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Introduction

Outbreaks of communicable diseases cause sufficient morbidity and mortality, affect economic productivity and have the potential for International spread. They must be recognised and controlled rapidly in order to minimise their impact. The effective containment of an outbreak depends on:

- Early detection and reporting of suspect cases
- Rapid epidemiological investigation
- Rapid laboratory confirmation of the diagnosis
- The implementation of effective control measures

Rapid identification of the causative agent and the likely source or mode of transmission is essential. From this perspective, the initial investigation involves two important processes:

- Collection of information on suspect cases, and
- Collection of clinical specimens for laboratory diagnosis.

Successful lab confirmation of a disease depends on:

- Advance planning
- Collection of appropriate specimens
- Correct packaging and rapid transport to an appropriate laboratory
- The ability of the lab to accurately perform the diagnostic test
- Proper biosafety and decontamination procedures to reduce the risk of further spread of the disease

The purpose of this document is to ensure the correct specimens are collected and transported in a safe and standardized manner during a field investigation of an outbreak.

Planning for specimen collection

Once a suspected outbreak has been detected and reported, an epidemiological investigation must be quickly organised. Laboratory investigations wherever applicable, are most useful to confirm the diagnosis but the epidemiological investigations should not be delayed until laboratory results are available. The materials and procedures required for efficient specimen collection and their transport to the laboratory for testing are outlined below.

1. **Define the possible causes of the outbreak**
   An assessment of current clinical and epidemiological information is the starting point for considering the potential aetiology of the outbreak. The historical knowledge of regional, endemic and epidemic diseases, as well as their seasonality, further defines the possible causes. Since a variety of infectious agents can present with a similar clinical picture, the outbreak should be approached in a syndromic manner to obtain the differential diagnosis. One or more specimen types may be required to define the cause of the outbreak.

2. **Decide which clinical specimens are required to confirm the cause of the outbreak**
   After defining the clinical syndrome and suspect pathogen(s), determine the clinical specimens for collection and appropriate laboratory diagnosis. This is best done in consultation with the laboratory(s) which will be performing the diagnostic testing. Review the sampling procedures and the necessary material.

3. **Contact the laboratory for specimen testing**
   Contact the laboratory with appropriate capabilities. Key contact personnel from each referral lab must be nominated in advance who is responsible for:
   - Coordinating the logistical aspects of sample handling and
   - Transmitting information or queries between the field and the laboratory.

   In consultation with the laboratory, organise all aspects of the handling of clinical specimens, from selection of sample type, collection materials, local or on-site processing, transport of specimens, and transmission of results. The laboratory support may be required for supply of special media, rapid kits etc.

4. **Decide who will collect and transport the specimen**
   Decide whether a laboratory specialist/technician should join the team. Otherwise the team must receive training in collection, handling and transport of the required specimens as well as safety and decontamination procedures, the health workers joining the team during the course of investigation must also be offered this training.

5. **Define the procedures necessary for specimen management**
   Consider the logistic requirements for sampling equipment and supplies, specimen handling and transport to the laboratory (timing, route, transit temperature requirements, shipping procedures and documentation) in advance. In addition decide how the specimens would be transported and inform the receiving lab the approximate time at which they can expect the specimen.

6. **Collecting the specimens**
   Specimen collection should commence as early as possible after a suspected outbreak has been notified. Specimens obtained in the acute phase of the disease, preferably prior to administration of antimicrobial drugs, are more likely to yield detectable concentrations of

antibody, antigen or infective pathogen. Before beginning specimen collection, explain the procedure to the patient and relatives. When collecting the specimen avoid contamination and take a sufficient quantity of material (as guided by the laboratory tests). Follow the appropriate precautions for safety during collection and processing of specimens so as to protect the collector and lab personnel.

7. Biosafety

Healthcare workers may be unaware of potential etiologic agents residing in the specimen being transported to the laboratory. To protect the safety of the healthcare worker collecting the sample and the lab personnel, the following precautions must be followed when collecting specimens:

- During specimen collection wear personal protective equipment such as gloves, laboratory coat and where appropriate, a mask and / or goggles.
- Use leak-proof specimen containers and transport bags that have a separate outside compartment for the test requisition form.
- Never transport syringes with needles to the laboratory. Instead, transfer the contents to a sterile tube and place it in a sealable leak proof plastic bag.
- Make sure screw-cap lids are fastened evenly and securely. Ensure that no label material is caught in the threads of the lid.
- Do not transport leaking containers because test results will be compromised and it is a hazard to couriers and laboratory personnel.
- To protect the safety of others, take care not to contaminate the outside of the specimen container or the laboratory requisition form.
- Discard used needles directly into sharps container (puncture proof box) without recapping them
- Use 10% bleach to clean up spills after wiping the surface
- Contaminated non-disposable equipment can be disinfected with 1% household bleach for 5 minutes and heavily soiled equipment can be soaked in 10% household bleach before disposal

8. Labelling and identification of specimens

Each patient should be assigned a unique identification number by the team investigating the outbreak. It is the link between the laboratory results and the patient in the line list, which guides further investigation and response to the outbreak. This unique identification number and the patient name should be present on all specimens, epidemiological data forms and the laboratory request forms and used as a common reference.

Specimens must be labelled and the label must contain the following information:

- Patient name
- Unique identification number
- Specimen type and
- Time and date of collection

9. Case investigation and laboratory request forms

In an outbreak investigation, the relevant information must be filled in the laboratory request forms to be submitted along with the specimen. A case investigation form/epidemiological data form should also be completed for each patient at the time of specimen collection. The originals remain with the investigation team, and should be kept together for analysis and later reference. The epidemiological and clinical data gathered in the investigation can later

be easily tied to the laboratory results for analysis. The laboratory form includes following patient information:

- Age (or date of birth), sex, complete address.
- Clinical information – date of onset of symptoms, clinical and immunisation history, risk factors, antimicrobial taken prior to specimen collection.
- Laboratory information – Acute or convalescent specimen, other specimens from same patient.
- The form records the date and time when specimen is collected, name of the person collecting the specimen and the contact details of the person to whom result is to be communicated.

The receiving lab should record the date and time when specimen was received, name and initials of the person receiving specimens, and record the specimen quality. The investigation team should receive a line list from the laboratory with the unique identification number and laboratory results for each specimen.

10. Storage of specimens

To preserve bacterial or viral viability in specimens for microbiological culture or inoculation, they should be placed in appropriate media and stored at recommended temperatures. These conditions must be preserved throughout the transport of specimen to the laboratory and will vary according to transportation time. They will differ for specimens and pathogens, depending on their sensitivity to desiccation, temperature, nutrient and pH.

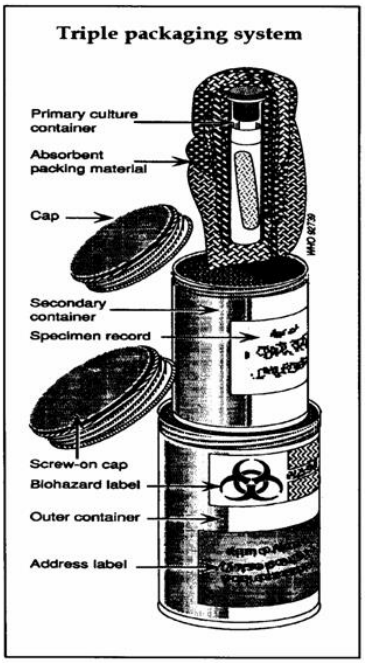
- All specimens must be promptly transported to the laboratory, preferably within 2 hours.
- Specimens for bacterial culture should be kept in appropriate transport media at the recommended temperature. This ensures bacterial viability while minimising overgrowth of other micro-organisms.
- With the exception of urine and sputum, most specimens may be kept at ambient temperature if the specimen will be processed within 24 hours. For longer periods, storage at 4-8°C is advisable with the exception of specimens suspected to yield environmentally sensitive organisms as *Streptococcus pneumoniae, Neisseria sp*. and *Haemophilus influenza, Shigella spp.* (which are sensitive to cold temperatures) which should not be refrigerated. Longer delays are not advisable as the yield of bacteria may fall significantly.
- Many specimens collected for viral isolation are viable for two days if maintained in type specific media at 4-8°C. For longer periods, freeze these specimens as directed by expert advice, as infectivity may be altered.
- Specimens for antigen or antibody detection may be stored at 4-8°C for 24-48 hours, or at –20°C for longer periods.
- Sera for antibody detection may be stored at 4-8°C for up to 10 days.
- Although not ideal, sera stored at room temperature may still be useful for antibody testing even after prolonged periods (weeks). Therefore, do not discard sera which have been collected simply because there are no refrigeration facilities available.

11. Transport of specimens:

- Before transport, the collection team should notify the receiving laboratory of all shipping and specimen details in advance of specimen arrival.

- Specimen containers relating to single case investigation should be placed in a plastic bag with an absorbent material surrounding the specimen so that even if the whole specimen leaks out, it will be absorbed**.**
- The lab report form should be sealed within a separate plastic bag and wrapped round the specimen or attached firmly to box of specimens.
- The material should be packed in an insulated carton / carrier to transport a specimen to the lab.

- All specimens should be considered as potentially pathogenic and accordingly labelled with internationally accepted biohazard label.
- Avoid repeated thawing & freezing of specimens.
- For transportation it should be placed in a *triple container system*:
  - *Primary container* has the specimen and is water tight, leak proof with a screw cap. Label each specimen in a waterproof manner (e.g. tape the label to the container) to prevent it loosening in transit.
  - *Secondary container* made of durable, waterproof material with a screw cap, with an absorbent material (e.g. cotton wool) surrounding the specimen so that even if whole specimen leaks out, it will be absorbed. Alternatively a plastic bag which can be sealed may be used. Specimens from different patients must never be sealed in the same bag.
  - *Tertiary container* is usually made of wood, plastic or cardboard. It should be capable of withstanding the shocks and trauma of transportation. Dry ice can be kept between this and the secondary container along with sufficient absorbents. Multiple secondary containers can be placed in the same tertiary container. Vaccine carriers that have been used for specimen transport must never be used for carrying vaccines. The specimen carriers and ice packs can be reused after disinfection.



- Standardised packaging methods and materials ensure safety of personnel and specimen integrity, even if the package is damaged during transport. Specimens must be packaged, labelled and transported in compliance with specific national and international regulations for infectious materials.
- Address labels on outer packages should display the sender and laboratory name with complete address and telephone numbers for both 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 and other identifying documents for the receiving laboratory should be placed together in aplastic bag and taped onto the outer transport packaging. A copy of these documents should also be given to the transport service.

Maintenance of transit temperature: For 4-8°C, the transport box should be fitted with a minimum of 4 ice packs, or more if room is available, around the secondary container. This will maintain refrigeration for 2-3 days.

SPECIMENS TO BE COLLECTED

Syndrome	Possible agents	Specimen to be collected	Tests that can be done for detection of etiologic agent*
Acute Diarrheal Illness	Viral, Bacterial-Cholera, Shigella, Salmonella, E. coli, Parasitic-Amoeba, Giardia etc.	Faeces	• Stool microscopy • EIA for rotavirus antigen Stool for ova & cyst • Stool culture and antimicrobial sensitivity • Molecular tests for viruses
Acute Jaundice Syndrome	Hepatitis A – E, Leptospirosis	Blood culture (specialised media) Serum	• Serology • Blood for culture for Lepto, virus isolation • Molecular assays
Acute Haemorrhagic Fever syndrome	Dengue, Hantavirus, Malaria	Blood CSF	• Blood smear examination • Virus isolation (Blood, CSF) • Antigen detection, antibody levels (CSF, Serum) • Molecular assays
Acute Respiratory Syndrome	Influenza viruses, RSV, H. influenzae, Streptococcus spp. , Diphtheria, Pneumonic plague, etc.	Throat swab Sputum Serum Blood	• Culture and antimicrobial sensitivity testing (sputum, throat swab, blood) • Virus isolation • Antigen detection • Serology • Molecular assays
Acute Neurological Syndrome	N. meningitides, Streptococcus pneumoniae, Japanese Encephalitis, Enteroviruses, Rabies, polio virus etc	Blood CSF Sera Faeces	• CSF Microscopy • Antigen detection (CSF) • Culture/virus isolation (CSF, Blood) • Stool culture for viruses • Serology • Molecular assays
Acute Febrile Illness	Typhoid, Malaria, Hepatitis, Dengue, Leptospirosis, Anthrax, Brucellosis, Bubonic plague etc.	Blood Sera CSF Urine Bubo aspirate	• Microscopy • Culture (blood, urine, CSF) • Serology • Molecular assays
Acute Dermatological Syndrome	Chicken Pox, Anthrax, Measles, Rubella etc	Sera Sample from lesion	• Microscopy • Serology • Culture • Molecular assays

*Some of tests given in this table may not be available at IDSP SRL s (molecular tests, viral cultures etc), for those tests the samples may have to be referred to other labs when required/possible.

General specimen selection & collection guidelines

- Wash hands before and after the collection.
- Aseptic techniques must be employed during collection to prevent the sample from being contaminated during the process of collection.
- Avoid contamination from indigenous flora, whenever possible, to ensure a sample representative of the infectious process.
- Specimen should be collected before the administration of antimicrobial agents if possible (treatment for severe disease should not be delayed however).
- Collect the specimen at the appropriate stage of disease.
- Ensure that the correct specimen representative of the disease is collected
- Collect adequate volume, as insufficient material may yield false negative results.
- Collect or place the specimen aseptically in a sterile and appropriate screw capped container.
- Ensure that the outside of the specimen container is clean and uncontaminated.
- Close the container tightly so that its contents do not leak during transportation.
- Label the container appropriately and complete the requisition form.
- Arrange for immediate transportation of the specimen to the laboratory.

Faecal specimen collection

- Outbreaks of water borne and food borne diseases can occur throughout the year with a seasonal increase in the monsoon and post monsoon months. Establishing the aetiology of the diarrheal outbreak is an important part of outbreak investigation.
- Stool specimens are most useful for microbiological diagnosis if collected soon after onset of diarrhoea (for viruses < 48 hours and for bacteria < 4 days), and preferably before the initiation of antibiotic therapy. If required, two or three specimens may be collected on separate days, particularly useful when suspecting parasitic infections.
- Stool is the preferred specimen for culture of bacterial, viral and parasitic diarrhoeal pathogens.
- Rectal swabs showing faeces may be collected from infants (where collection of stool sample may not be possible). They are not recommended for the diagnosis of viruses.
- Collect diarrheal stool samples from at least 10 ill persons, if the number of ill persons is less than 10 then collect for all ill persons.

<u>Materials for collection</u>

- Sterile(for culture)/clean (for ova cyst), dry, leak-proof screw cap container and tape
- Appropriate bacterial transport media for transport of stool/ rectal swabs from infants
- Parasitology transport pack: 10% formalin in water

<u>Method of collecting a stool specimen</u>

- Collect freshly passed stool, 5 ml liquid or 5 g solid (pea-size), in the container. (You may first ask the patient to pass the stool in a separate clean wide mouthed container and then transfer enough faeces with a spatula to the specimen container)
- Screw cap the container tightly.
- Label the container and place in a sealed bag and send to laboratory immediately.
- If it is not possible to process the specimen within 2 hours, a small amount of the specimen may be transferred in refrigerated Cary Blair transport medium using two swabs.
- For viral specimens collect a larger volume of stool.

Method of collecting a rectal swab from infants

- Moisten a sterile swab in sterile saline.
- Insert the swab tip just past the anal sphincter and rotate gently.
- Withdraw the swab and examine to ensure that the cotton tip is stained with faeces.
- Place the swab in the sterile tube/container containing the appropriate transport medium.
- Break off the top part of the swab stick without touching the tube and tighten the screw cap firmly.
- Label the specimen tube/container
- Place in a sealed bag and send to laboratory immediately.

Handling and transport

- Fresh stool must be examined within 2 hours of collection
- Cary Blair tubes with stool specimen must be transported to laboratory at 4 - 8°C and must be examined within 48hrs of collection. Bacterial yields may fall significantly if specimens are not processed within 1-2 days of collection. *Shigella spp*. is particularly sensitive to elevated temperatures.
- Specimens to be examined for parasites should be mixed with 10% formalin- 3 parts stool to 1 part preservative and transported at ambient temperature in containers sealed in plastic bags.

How to transfer stool specimen into a tube containing Cary Blair transport medium

- Gloves to be worn at all times when handling the specimen.
- Take a sterile swab. Do not touch the cotton tip of the swab.
- Insert the cotton tip of the swab into the stool specimen. If mucus and shreds of intestinal epithelium are present, these should be sampled with the swab.
- Make sure the cotton tip of the swab is completely coated with the specimen.
- Immediately insert the swab into Cary Blair transport medium. (The transport medium should have been refrigerated for 1 to 2 hours, if possible.)
- The swab should be pushed completely to the bottom of the tube of transport medium.
- Break off the top portion of the stick touching the fingers and discarded so that the cap can be tightly screwed onto the tube.
- Replace the screw cap and tighten firmly, seal the tube with tape to prevent leakage.
- Adhere specimen label to the Cary Blair tube.
- Place the tube in a refrigerator or cold box (4–8°C).
- Safely dispose of all contaminated materials.

Note: Cary Blair is a semi solid medium with long shelf after preparation (about 1 year at 25°C). It must be rejected if solidified, dried, liquefied or if air bubbles or change in color happens

Blood specimen collection

- Blood and separated serum are the most common specimens taken to investigate outbreaks of communicable diseases.
- Venous blood can be used for isolation and identification of the pathogen in culture or separated into serum for the detection of genetic material, specific antibodies (by serology), antigens or toxins (e.g. by immunofluorescence).
- Clustering and sudden increase of acute cases of fever may be due to malaria, typhoid, dengue/chikungunya fever or other viral fevers. Fever with rash may be due to measles or chicken pox.
- In case of fevers suspected to be of viral aetiology, serum sample for detection of antibodies is to be collected. If clinical presentation is compatible with the case definition of typhoid fever, blood culture must also be collected.
- For the processing of most specimens for diagnosis of viral pathogens, serum is preferable to unseparated blood except where otherwise directed.
- When specific antibodies are being assayed, it is often helpful to collect paired sera, i.e. an acute sample at the onset of illness and a convalescent sample one to four weeks later.
- Blood can also be collected by finger prick for the preparation of slides for microscopy or for absorption onto special filter paper discs for analysis.
- Whenever possible, blood specimens for culture should be taken before antibiotics are administered to the patient.
- If suspecting **Measles:** Collect blood between the 3rd and 28th day after onset of rash from at least 5 to 10 suspected cases, for confirmation of an outbreak.
- If suspecting **Viral hemorrhagic fevers:** Collect from the first suspected VHF case. If more than one suspected case present, collect until diagnosis is confirmed.

Venous blood samples

Materials for collection

- Skin disinfection: 70% alcohol (isopropyl alcohol, ethanol) or 10% povidone iodine, swabs, gauze pads, band aid.
- Disposable latex or vinyl gloves
- Tourniquet, Vacutainer or disposable syringes and needles.
- Vacutainer or sterile screw cap tubes (or cryotubes if indicated),
- Blood culture bottles (50ml for adults, 25ml for children) with appropriate media.
- Labels and indelible marker pen.

Method of collection

- Gloves to be worn at all times when handling the specimen.
- Disinfect the tops of blood culture bottles.
- Place a tourniquet above the venepuncture site.
- Palpate and locate the vein. It is critical to disinfect the venepuncture site meticulously with 10% povidone iodine or 70% isopropyl alcohol by swabbing the skin concentrically from the centre of the venipuncture site outwards. Let the disinfectant evaporate. Do not palpate the vein again.
- Perform venipuncture.
- If withdrawing with conventional disposable syringes, withdraw 2-3 ml venous blood and transfer to small blood collection tubes/vials for serological tests.

- For blood culture withdraw 5-10 ml of whole blood from adults, 2-5ml from children and 0.5-2ml for infants. Using aseptic technique, transfer the specimen to relevant culture bottles.
- If withdrawing with vacuum systems, withdraw the desired amount of blood directly into each culture bottle or vacutainer.
- Remove the tourniquet. Apply pressure to site until bleeding stops, and apply bandaid.
- Label the tube, including the unique patient identification number, using permanent marker pen.
- Do not recap used sharps. Destroy the needle using the needle destroyer and discard the remaining syringe directly into the appropriate container for infectious plastic waste.
- Complete the case referral forms.

Handling and transport

- Blood specimen bottles and tubes should be transported upright and secured in a screw cap container or in a rack in a transport box. They should have enough absorbent paper around them to soak up all the liquid in case of spill.
- Blood cultures-If the specimen will reach the laboratory within 24 hour, most pathogens can be recovered from blood cultures transported at ambient temperature. Keep at 4-8°C for longer transit periods, unless one suspects a cold-sensitive bacterial pathogen (eg meningococcus).

How to separate serum from whole blood

- Gloves should be worn at all times when handling the specimen.
- Keep the whole blood at room temperature until there is complete retraction of the clot from the serum.
- If a nearby health facility has a centrifuge, spin the whole blood at 1000 x g for 10 minutes to separate the serum. If centrifuge not available, serum can still be separated carefully.
- Remove the serum using a sterile pipette. Avoid extracting red cells.
- Transfer the serum aseptically to a sterile, screw-capped prelabelled tube.
- Secure cap tightly.
- Safely dispose of all contaminated materials and the remaining clot.
- Keep the tube of serum at 4–8°C.
- If facilities for separation of serum are not available, then the clotted sample should be stored at 4 to 8 °C (NOT FROZEN). Protect such sample from excessive vibration while transporting.

Cerebrospinal fluid (CSF) specimen collection

- Meningitis outbreaks may occur due to bacteria or viruses. A lumber puncture for demonstration and/or isolation of organism from CSF is essential. Commercially available latex agglutination kits may provide diagnosis of some agents of bacterial meningitis.
- The specimen must be taken by a physician or a person experienced in the procedure. CSF is used in the diagnosis of viral, bacterial, parasitic, and fungal meningitis/encephalitis.

Materials for collection
Lumbar puncture tray which includes:
Sterile materials: gloves, cotton wool, towels or drapes.
Local anaesthetic, sterile needle & syringe.
Skin disinfectant: 10% povidone iodine or 70% alcohol.
Two sterile lumbar puncture needles, small bore with stylet.
Small sterile screw-cap tubes and tube rack.

Method of collection
As **only experienced personnel** should be involved in the collection of CSF samples in hospital settings, the method is not described in this document. CSF is collected directly into sterile screw-cap tubes. If the samples will not be promptly transported, separate tubes should be collected for bacterial and viral processing.

Handling and transport
- In general, specimens should be delivered to the laboratory and processed as soon as possible.
- CSF specimens for bacteriology are transported at ambient temperature, generally without transport media. They must never be refrigerated as these pathogens do not survive well at low temperatures.
- CSF specimens for virology do not need transport medium. They may be transported at 4-8°C for up to 48 hours, or at -70°C for longer periods

Respiratory tract specimen collection

- Specimens are collected from the upper or lower respiratory tract, depending on the site of infection. Upper respiratory tract pathogens (viral and bacterial) are found in throat and nasopharyngeal specimens. Lower respiratory tract pathogens are found in sputum specimens. For organisms such as Legionella, culture is difficult, and diagnosis is best based on the detection of antigen excreted in the urine.
- When acute epiglottitis is suspected, no attempt should be made to take throat or pharyngeal specimens since these procedures may precipitate respiratory obstruction. Epiglottitis is generally confirmed by lateral neck X-Ray, but the etiologic agent may be isolated on blood culture.
- For severe hospitalized pneumonia cases, blood culture may be an appropriate test (see above for collection of blood for culture)

Materials for collection
- Transport media – bacterial and viral
- Dacron and cotton swabs
- Tongue depressor
- Flexible wire calcium alginate tipped swab (for nasopharyngeal swab)
- Nasal speculum (for nasopharyngeal swab – not essential)
- Suction apparatus or 20-50 ml syringe
- Sterile screw-cap tubes, and wide-mouthed clean sterile jars (minimum volume 25ml)
- Plastic catheter,
- Sterile normal saline

1. Upper respiratory tract specimens

Method of collecting a throat swab
- Label the specimen containers.
- Hold the tongue down with the depressor.
- Use a strong light source to locate areas of inflammation and exudate in the posterior pharynx and the tonsillar region of the throat behind the uvula.
- Rub the area back and forth with a Dacron or calcium alginate swab. Withdraw the swab without touching cheeks, teeth or gums and insert into a screw cap tube containing transport medium.
- Break off the top part of the stick without touching the tube and tighten the screw cap firmly.
- Complete the case referral form.

Method of collecting nasopharyngeal
- Label the specimen tube.
- Seat the patient comfortably, tilt the head back.
- Insert a flexible calcium alginate/Dacron swab through the nostril to posterior nasopharynx (same distance as from nostrils to external opening of ear) parallel to the floor of nose without pointing upwards.
- Rotate the swab on the nasopharyngeal membrane a few times to obtain infected cells.
- Repeat procedure using other nostril. Collection of specimens from both nostrils increases the amount of material for analysis and the ability to isolate the virus.

- Place the swab(s) in the tube of viral transport medium.

Method of collecting nasal swabs

- Seat the patient comfortably, tilt the head back
- Insert cotton bud end of dry sterile swab into right nostril (approx. 2 cm inside) and rub firmly against the turbinate (to ensure swab contains cells as well as mucus)
- Insert swab into a screw cap tube containing transport medium. Break off the top part of the stick without touching the tube and tighten the screw cap firmly.
- Repeat procedure for left nostril using new sterile swab and insert into same tube of transport medium
- Complete the case referral/lab request form.

Method of collecting nasopharyngeal wash/aspirate

- Label a sterile vial
- Ask the patient to sit with the head tilted back.
- Flush plastic catheter with 2-3 ml VTM/sterile normal saline.
- Instil 1-1.5 ml of VTM/sterile normal saline into one nostril.
- Insert tubing into this nostril parallel to the palate and aspirate secretions.
- Repeat the procedure with the other nostril.
- Collect 1-2 ml in the labelled sterile vial and transport at 2-8 ^{0}C.

2. Lower respiratory tract specimens

Method of collecting sputum

- Label the specimen container.
- Instruct patient to take a deep breath and cough up sputum directly into a wide-mouth sterile container. Avoid saliva or postnasal discharge and avoid soiling the outer walls of the container.
- Good sputum specimen should contain the thick purulent material, and be at least 1 ml in amount.
- Complete the case referral form/laboratory request form.

Handling and transport

- All respiratory specimens except sputum are transported in appropriate bacterial/viral media.
- Transport as quickly as possible to the laboratory to reduce overgrowth by commensal oral flora.
- For transit periods up to 24 hours, transport bacterial specimens at ambient temperature and viruses at 4-8°C in appropriate media.

Collecting specimens of skin lesions

- For most dermatological conditions, diagnosis may be established on the basis of physical examination and clinical history without the collection of diagnostic specimens. Important characteristics to be noted on physical examination include the nature of the skin lesions (erythematous, macular, papular, maculopapular, vesicular, bullous, petechial, purpuric, etc.) and the anatomic distribution of spread (central, peripheral, diffuse, etc.).
- In cases of indeterminate diagnoses, unusual presentations, and some rare conditions, collection of specimens from rashes and/or skin lesions may be necessary.
- In the case of vesicular rashes, specimens for microscopy and culture are taken directly from vesicles. In other exanthemata (macular and/or papular), the diagnosis may be more readily established from alternative specimens (e.g. blood cultures, serology).
- In suspected cutaneous anthrax or bubonic plague, specimens from the skin lesions (eschars and buboes, respectively) and blood cultures may be taken. Regardless of the stage of the lesion, collect 2 separate swabs. Specimens must be collected before initiation of antimicrobial therapy

Materials for collection ·

- Sterile saline
- Sterile swabs and appropriate transport media
- Sterile screw-cap vials
- Sterile lancets or needles (for piercing of vesicles)
- Syringe with wide-bore needle (for aspiration of abscesses/buboes)
- Glass slides and slide boxes

Method of collection Vesicular or vesiculo-pustular rash (for diagnosis of viral infections)

- Pierce roof of fluid-containing vesicle with sterile lancet. ·
- Swab fluid with sterile swab. Try to get a good amount of fluid onto the swab.
- Take a clean labeled microscope slide and make a smear with the swab in the central area of the slide. Make 2 slides if possible. The slides should be left to dry in air.
- Place swab directly into virus transport medium.
- Label the bottles or tubes containing swabs in transport media.
- When glass slides have dried, place carefully into a plastic slide box. Do not refrigerate or freeze the slides during storage or transport. Keep in the closed container at room temperature.

Crusting stage

- Gently ease off crust with a lancet or scalpel and a pair of disposable forceps.
- Take 5-10 crusts; place them in a plastic screw-cap vial. Make sure the lid is tightly closed.
- Label the specimen containers.

- Discard forceps, lancets, and scalpels into sharps disposal container.
- Do not re-use forceps on specimens from another patient.

If cutaneous anthrax is suspected, the vesicular fluid under the eschar is a better diagnostic specimen than a piece of the eschar.

Aspiration of abscesses

- Aspiration of abscesses should only be performed by experienced personnel.
- Disinfect the skin overlying the abscess/bubo with 70% isopropyl alcohol.
- Aspirate the fluid from the abscess with a sterile needle and syringe. Only enough fluid to perform the diagnostic tests is required.
- Transfer the aspirate aseptically into a sterile tube.

Skin biopsy

- Skin biopsies from live patients are generally not appropriate specimens for field outbreak investigations.

Handling and transport

- Specimens for bacteriological analysis should be transported in Amies medium.
- Swabs for suspected viral pathogens should be transported in virus transport medium.
- If processing takes longer than 2 hours, bacteriology specimens can be maintained at ambient temperature for 24 hours. Specimens for virus isolation may be refrigerated at 4-8°C, and transported to the laboratory as rapidly as possible.
- In any outbreak investigation, it should be considered essential to consult the receiving laboratory about the handling of the most likely specimen types before setting out into the field.

Urine specimen collection

Materials for collection

- Sterile plastic cup with screw cap lid (50 ml or more)
- Gauze pads
- Soap and clean water (or normal saline) if possible.

Method of collection

- Give the patient clear instructions to pass urine for a few seconds, and then to hold the cup in the urine stream for a few seconds to catch a midstream urine sample. This should decrease the risk of contamination from organisms living in the urethra.
- To decrease the risk of contamination from skin organisms, the patient should be directed to avoid touching the inside or rim of the plastic cup with the skin of the hands, legs or external genitalia. Tighten the cap firmly when finished.
- For hospitalized or debilitated patients, it may be necessary to wash the external genitalia with soapy water to reduce the risk of contamination. If soap and clean water are not available, the area may be rinsed with normal saline. Dry the area thoroughly with gauze pads before collecting the urine.
- Urine collection bags may be necessary for infants. If used, transfer urine from the urine bag to specimen containers as soon as possible to prevent contamination with skin bacteria. Use a disposable transfer pipette to transfer the urine.
- Label the specimen containers.

Handling and transport

- Transport to the laboratory within 2–3 hours of collection. If this is not possible, do not freeze but keep the specimen refrigerated at 4-8°C. Keeping the specimen refrigerated will decrease the risk of overgrowth of contaminating organisms.
- Ensure that transport containers are leak-proof and tightly sealed.