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Guidelines for the collection of clinical specimens during field investigation of outbreaks

World Health Organization
Department of Communicable Disease Surveillance and Response

Contents

Introduction

Outbreaks of communicable disease cause significant morbidity and mortality, consume scarce national health resources, affect economic productivity, and have the potential for international spread. They must be recognized and controlled rapidly in order to minimize 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 laboratory confirmation of a disease depends on:

- advance planning
- collection of appropriate and adequate specimens
- correct packaging and rapid transport to an appropriate laboratory
- the ability of the laboratory to accurately perform the diagnostic tests
- proper biosafety and decontamination procedures to reduce the risk of further spread of the disease.

The purpose of this manual is to ensure that the correct specimens are collected, packaged, and transported in a safe and standardized manner during a field investigation of an outbreak. It is not intended to be a guide to 'best practice', or an exhaustive manual on laboratory procedures. The safety of field and laboratory investigators, and of patients, has been central to the formulation of these guidelines. Conditions encountered during an outbreak investigation are often difficult, and it may not be feasible to follow the same protocols and procedures which might be employed under optimal conditions in a high technology setting. Essential resources, such as electricity, refrigeration, and clean water, may be absent. The investigation may be conducted in a site remote from the laboratory which will receive and process the specimens. Transport may be difficult and even dangerous. It therefore becomes essential to give careful thought to the diagnostic materials and equipment which are likely to be required before setting out into the field. Advance planning is necessary for all aspects of the flow of diagnostic specimens, from patient through to laboratory. It is hoped that this

manual will assist leaders and members of a multidisciplinary investigative team to plan and guide their specimen collection in the field, leading to rapid diagnostic confirmation and control of the infectious agent causing the outbreak.

Section One: Planning for specimen collection

Once a suspected outbreak has been detected and reported, an epidemiological investigation must be quickly organized. Several key issues must be discussed, and decisions agreed upon, before the team goes to the field. Ultimately these decisions will guide the materials and procedures required for efficient specimen collection and their transport to the laboratory for testing.

1.1 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 now be required to define the cause of the outbreak.

1.2 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 work (see Section 1.3). Review the sampling procedures and the materials necessary for their implementation.

1.3 Select the laboratory for specimen testing

Identify and contact laboratories with appropriate capabilities. Once the receiving laboratory(s) have been identified, 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 should be organized in consultation with them. The laboratory may need to supply special media, equipment, and instructions in advance.

It is essential that key contact personnel be nominated in advance to be responsible for coordinating the logistical aspects of sample handling, and to act to transmit information or queries between the field and the laboratory.

1.4 Decide who will collect, process and transport the specimens

Decide whether a laboratory specialist or technician should join the team. Otherwise, the team must receive training in the collection, handling, and transport of the required specimen, as well as safety and decontamination procedures. Remember to offer this training to persons joining the team during the course of the investigation, e.g. local healthcare workers assisting at a particular site.

1.5 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), and decontamination procedures in advance. In addition arrange transport, accommodation, and protection of the team, and secure lines of communication (satellite phone, etc).

See: Annex 1 for specimens needed for laboratory confirmation of outbreaks
Annexes 2-9 for specific materials and methods of collection for different specimen types

Section Two: Specimen collection and processing

Investigation 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 the 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 samples.

See: Annexes 2-9 for specific materials and methods of collection for different specimen types

2.1 Safety and decontamination procedures

Safety and decontamination measures protect the specimen collector and colleagues, laboratory personnel, and the patient from risks associated with specimen collection. They also reduce the risk of contaminating the samples. Universal safety precautions require that workers should handle all clinical specimens as if they were infectious. Protective equipment (gloves, eye protection, mask) should be worn and safe work practices followed to reduce exposure to potentially infective material. Proper packaging methods also ensure the safety of personnel from collection site to laboratory, even if damage occurs during transit. A first aid kit is essential, and should be readily accessible at the site of specimen collection.

Protective clothing, work premises, equipment, and materials may all become contaminated in the field. Disinfection of work areas and decontamination of spills of blood or infectious body fluids is generally achieved by chemical disinfection with chlorine based solutions (see below and Annex 11). It is generally not practicable to achieve adequate sterilization of contaminated materials in the field. As incompletely 'sterilized' material may expose both the participants in the investigation and the general public to a real risk of infection, the re-use of contaminated equipment or materials such as gloves or clothing is not recommended. Incineration or burning is the preferred method for disposing of contaminated material. Prior to disposal highly infectious equipment and materials must be disinfected. Combustible materials should be completely burned to render sterile ash, which is then buried in a deep pit. If this cannot be accomplished in an institutional-type incinerator, a field incinerator can be improvised from a 200 litre drum. A diagram and instructions for constructing this type of field incinerator are appended in Annex 12. Sharps and soiled glass slides should

be discarded directly into a puncture-resistant disposal container, which is then incinerated.

2.1.1 Basic safety precautions

- Use latex or nitrile gloves when taking and handling specimens. Dispose of gloves between patients and replace with a fresh pair. Do not attempt to clean and reuse gloves as this may promote the spread of pathogens from patient to patient. In addition, unnoticed damage to gloves is common, and places the healthcare worker at risk.
- If possible wear protective clothing (gown, coat or apron) when collecting samples.
- Discard used needles directly into sharps box, without recapping them.
- Work areas and surfaces should be organized and disinfected with 1% household bleach daily or with a change in collection team. Use 10% bleach to clean up spills after wiping the surface clean. Personnel carrying out cleaning or decontamination should wear a protective coat and thick rubber gloves.
- Contaminated non-disposable equipment or materials should be soaked in 1% household bleach for 5 minutes. Before use wash in soapy water and sterilize if necessary.
- Heavily soiled disposable items should be soaked in 10% household bleach before incineration or disposal.

In special circumstances additional safety equipment, such as masks or goggles, are required to protect skin and mucous membranes against contact, aspiration or inhalation of certain pathogens. A respirator mask with a High Efficiency Particulate Air (HEPA) filter is used for highly infective respiratory pathogens.

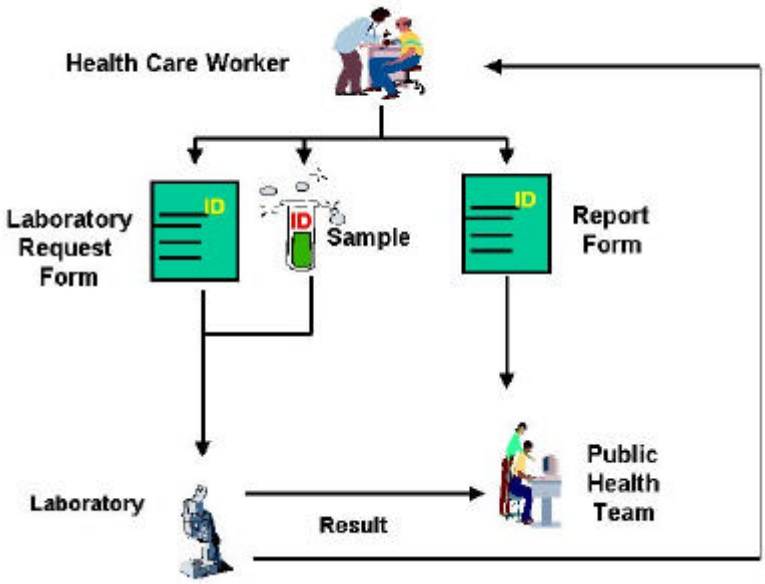
See: Annex 10 for first aid procedures after accidental exposure to infectious material
Annex 11 for chemical disinfectants
Annex 12 for constructing a field incinerator

2.2 Labelling and identification of specimens

In an outbreak investigation the information contained in the case investigation and laboratory request forms is collected along with the specimen. Each patient should be assigned a unique identification number by the collection team. It is the link between the laboratory results on the line listing form, the specimens and the patient, 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 and used as a common reference. Figure 1 illustrates this continuum graphically.

Figure 1:Common elements to link information for outbreak response



2.2.1 Label specimen container/slide

Preprinted labels (at least five) should be used whenever possible. The label should be permanently affixed to the specimen container. It should contain the:

- patient name
- unique identification number
- specimen type and date and place of collection
- name or initials of specimen collector.

Glass slides for microscopy must be labelled individually, and this should not interfere with the staining process. Each slide should bear the patient's name, unique identifier, and date of collection.

2.2.2 Label accompanying forms

A case investigation form should be completed for each patient at the time of collection. The original documents remain with the investigation team, and should be kept together for analysis and later reference. A laboratory request must also be completed for each specimen. For a large number of patients it may be practical to submit the requests to each relevant laboratory as a 'line listing', i.e. a summary request form compiling the necessary data noted in Section 2.2.1. An example of one such line listing is given in Annex 14. The epidemiological and clinical data gathered in the investigation can later be easily tied to the laboratory results for analysis. The laboratory may require other information to select and interpret the necessary tests; this may include:

- Patient information: age (or date of birth), sex, complete address
- Clinical information – date of onset of symptoms, clinical and immunization history, risk factors or contact history where relevant, antimicrobial drugs taken prior to specimen collection
- Laboratory information – acute or convalescent specimen, other specimens from the same patient.

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

See: Annex 14 for examples of case investigation, laboratory request, and line listing forms

Section Three: Storage, packaging, and transport of specimens

3.1 Storage of specimens

To preserve bacterial viability or viral integrity 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 transport 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. In some instances, the outbreak investigation team may bring liquid nitrogen for specimen preservation. If this is the case, follow the instructions of the experienced laboratorian as to appropriate use. If there is any question, check with the laboratory which will ultimately be receiving the specimens. 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.

- Many specimens taken for viral isolation are acceptable for culture after 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. For prolonged storage periods, preservation at –70°C may be indicated.
- Specimens for bacterial culture should be kept in appropriate transport media at the recommended temperature. This ensures bacterial viability while minimizing overgrowth of other microorganisms. With the exceptions 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 on at 4-8°C would be advisable with the exception of particularly cold-sensitive organisms, such as shigella, meningococcus, and pneumococcus. Longer delays are not advisable as the yield of bacteria may fall significantly.
- Specimens for antigen or antibody detection may be stored at 4-8°C for 24-48 hours, or at –20°C for longer periods. Some specimens may require special handling, for example freezing, so specific instructions should always be sought prior to collection. Sera for antibody detection may be stored at 4-8°C for up to 10 days. It is important to avoid unnecessary freeze-thaw cycles, so do not freeze sera unless the facilities are available to keep them frozen until delivery. Although not ideal, sera stored at room temperature may still be useful for antibody testing even after prolonged periods (weeks) if the sample is collected in a sterile container and is not contaminated. Therefore, do not discard sera which have been collected simply because there are no refrigeration facilities available. Valuable information can sometimes be obtained from samples which have not been handled optimally because of resource or logistic limitations, but

for the correct laboratory handling and interpretation of results the samples must be labeled and accompanied by a history of the storage and transport conditions.

3.2 Packaging and labelling of specimens

Detailed packaging, documentation, and handling requirements for the international transport of infectious materials are contained in the regulations of the International Air Transport Association (IATA) and in documentation of the International Health Regulations. Reference to the most recent regulations and guidelines is required, as they are subject to periodic review and modification. In addition to these international requirements, any additional requirements established by national authorities and commercial carriers must also be followed.

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.
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.

See: Annex 13 for the basic triple packaging system and maintenance of transit temperature

3.3 Transport of specimens

Before transport, the collection team should notify the receiving laboratory of all shipping and specimen details in advance of specimen arrival. In many cases, initial surface transportation of specimens from the field site to transport facilities may be required prior to shipment to processing laboratories. If international transport is necessary, authorization to import the specimens should be organized by the laboratory, which should also inform the sender of receipt or non-receipt of the specimens.

3.3.1 Surface transport/courier service

Transport boxes should be securely fastened in place in the transport vehicle. A spill kit containing absorbent materials, chlorine disinfectant, heavy duty reusable gloves, mask,

apron, goggles, and leak proof waste disposal container should be in the vehicle. An adequate amount of refrigerant should be available in case of delays in the travel schedule. Extensive vibration of samples, such as that encountered when travelling for long periods over rough roads, can haemolyse samples, rendering them useless. If possible, serum should be separated from clotted blood samples before transport.

3.3.2 Air transport/postal service

Diagnostic specimens may be sent by mail in conformance with all relevant international, national, and commercial carrier requirements. Contact with the postal authorities should be established prior to the collection of samples to ensure their ability to transport the materials and to verify understanding of the shipping requirements.

For international transport by passenger aircraft, the total quantity of specimens that may be transported in one package is 4 kg or 4 litres (maximum 500 ml per primary receptacle). There are now several commercial organizations specializing in transport of clinical samples and infectious materials. They will provide details of the specimen packaging and documentation requirements, so if it has been decided to employ their services it is advisable to contact them early in the course of an investigation.

Table 1:Diseases and pathogens encountered in outbreak investigations

This table provides a guide to some of the diseases or pathogens which cause outbreaks of the types toward which these guidelines are directed. It is only a guide; many illnesses are restricted geographically, or may present with unexpected clinical features. Some outbreaks may be due to novel or unknown pathogens. When investigating outbreaks, it is important to keep an open mind about possible causes, and ensure that adequate clinical samples are taken to eliminate any uncertainty. The ultimate goals of outbreak investigations are the implementation of successful control measures in the immediate term and prevention measures across the longer term, but these can only be correctly formulated in the light of accurate epidemiological and laboratory data.

Table 1: Diseases and pathogens encountered in outbreak investigations

SYNDROME	DISEASES /PATHOGENS
Acute Diarrhoeal Syndrome	Amoebic Dysentery, Cholera, Cryptosporidiosis, Ebola and other haemorrhagic fevers, *E.coli* (enterotoxigenic and enterohaemorrhagic), Giardiasis, Salmonellosis, Shigellosis,Viral gastroenteritis (Norwalk-like and rotavirus)
Acute Haemorrhagic Fever Syndrome	Crimean-Congo HF, Dengue HF, Ebola HF, Hantaviruses, Lassa fever, Marburg HF, Rift Valley fever, South American arenaviruses, Tick-borne flaviviruses, Yellow fever
Acute Jaundice Syndrome	Hepatitis A, B, E, Leptospirosis, Yellow fever
Acute Neurological Syndrome	Enteroviral meningitis, Japanese encephalitis, Leptospirosis, Malaria, Meningococcal meningitis, Poliomyelitis, Rabies and other lyssaviruses, Tick-borne encephalitis viruses, Trypanosomiasis
Acute Respiratory Syndrome	Anthrax, Diphtheria, Hantavirus Pulmonary Syndrome, Influenza, Mycoplasma, Legionellosis, Pertussis, Pneumonic plague, Respiratory syncitial virus, Scarlet fever
Acute Dermatological Syndrome	Chickenpox*, Cutaneous anthrax, Measles, Monkeypox, Parvovirus B19, Rubella, Typhus
Acute Ophthalmological Syndrome	Epidemic adenoviral keratoconjunctivitis, Haemorrhagic enteroviral conjunctivitis, Trachoma
Acute “Systemic” Syndrome	Anthrax, Arboviral fevers, Brucellosis, Dengue fever, Hantaviral disease, Lassa fever, Leptospirosis, Lyme disease, Plague, Relapsing fever, Rift Valley fever, Typhoid fever, Typhus, Viral hepatitis including Yellow fever

** NOTE: Included only because of need to distinguish from monkeypox in monkeypox-endemic areas*

ANNEXES

Annex 1: Specimens needed for laboratory confirmation of outbreaks

The syndromes are defined according to clinical criteria:

- Acute Diarrhoea Syndrome
- Acute Haemorrhagic Fever Syndrome
- Acute Jaundice Syndrome
- Acute Neurological Syndrome
- Acute Respiratory Syndrome
- Acute Dermatological Syndrome
- Acute Ophthalmological Syndrome
- Acute "Systemic" Syndrome

Glossary of terms

Acute is defined as a period of three weeks or less.
Severe illness may be characterized by:

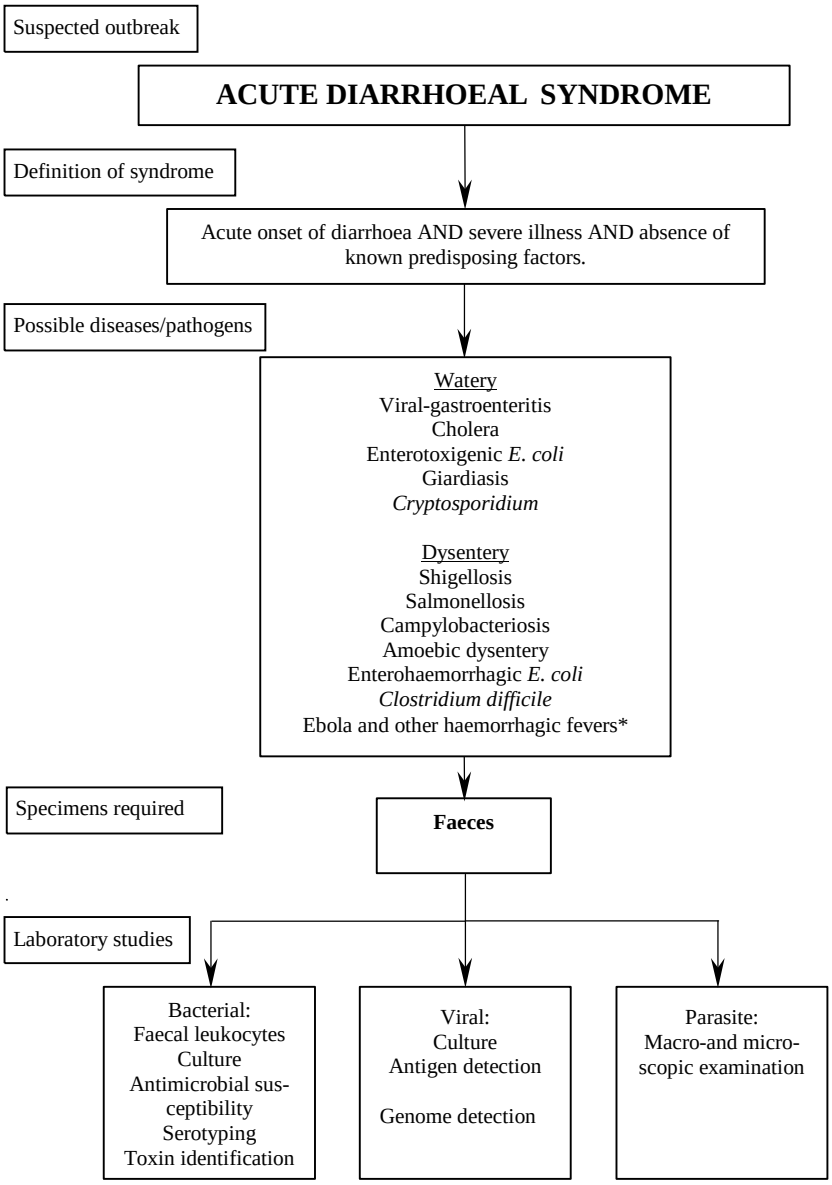
- hospital admission
- major organ failure
- altered state of consciousness
- circulatory collapse
- death

The information presented in this annex is intended for general guidance for appropriate action during the early stages of a field outbreak response. It is not intended to provide guidance for the diagnostic approach to individual patients, nor for the extensive epidemiological and diagnostic aspects of later stages of an outbreak response with a known aetiology.

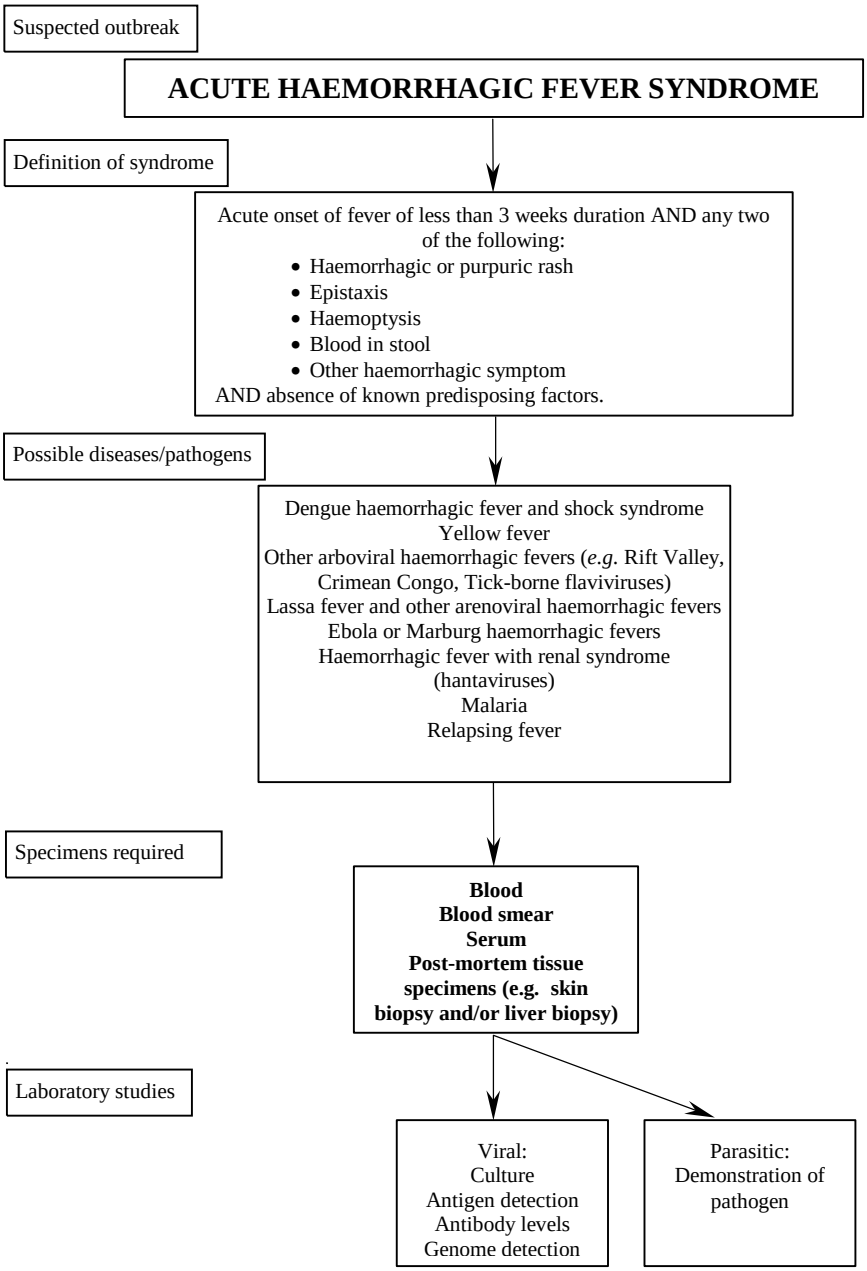
Advice from the receiving laboratory/laboratories prior to departure for the field on appropriate specimens, collection and processing procedures, and transport conditions is essential, as is the preparation of the correct collection and transport materials.

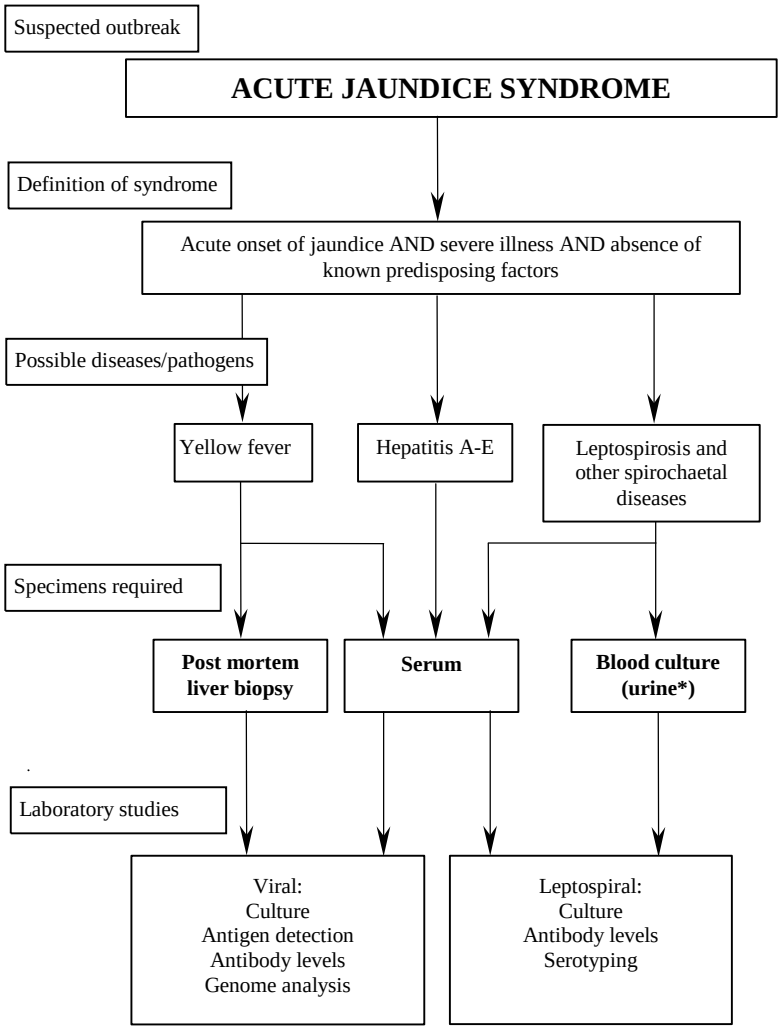
The information is limited to infectious causes of outbreaks of public health priority. No-infectious causes (e.g. heavy metals, industrial products, nutritional deficiencies) are not discussed, but most always be considered in outbreak responses.

See: *Annexes 2-9 for specific materials and methods of collection for different specimen types*
Annex 11 for chemical disinfectants
Annex 12 for construction of a field incinerator
Annex 13 for basic triple packaging system and maintenance of transit temperature

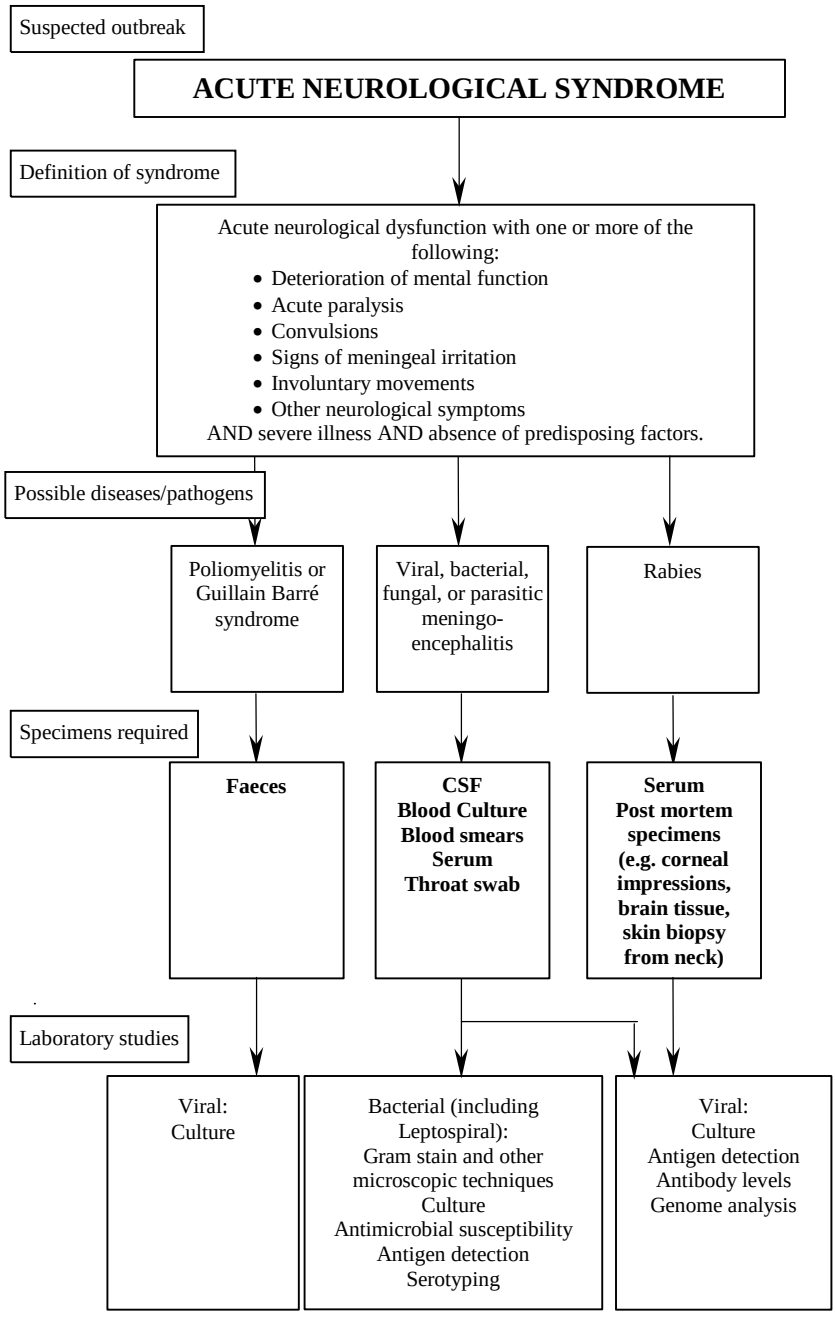


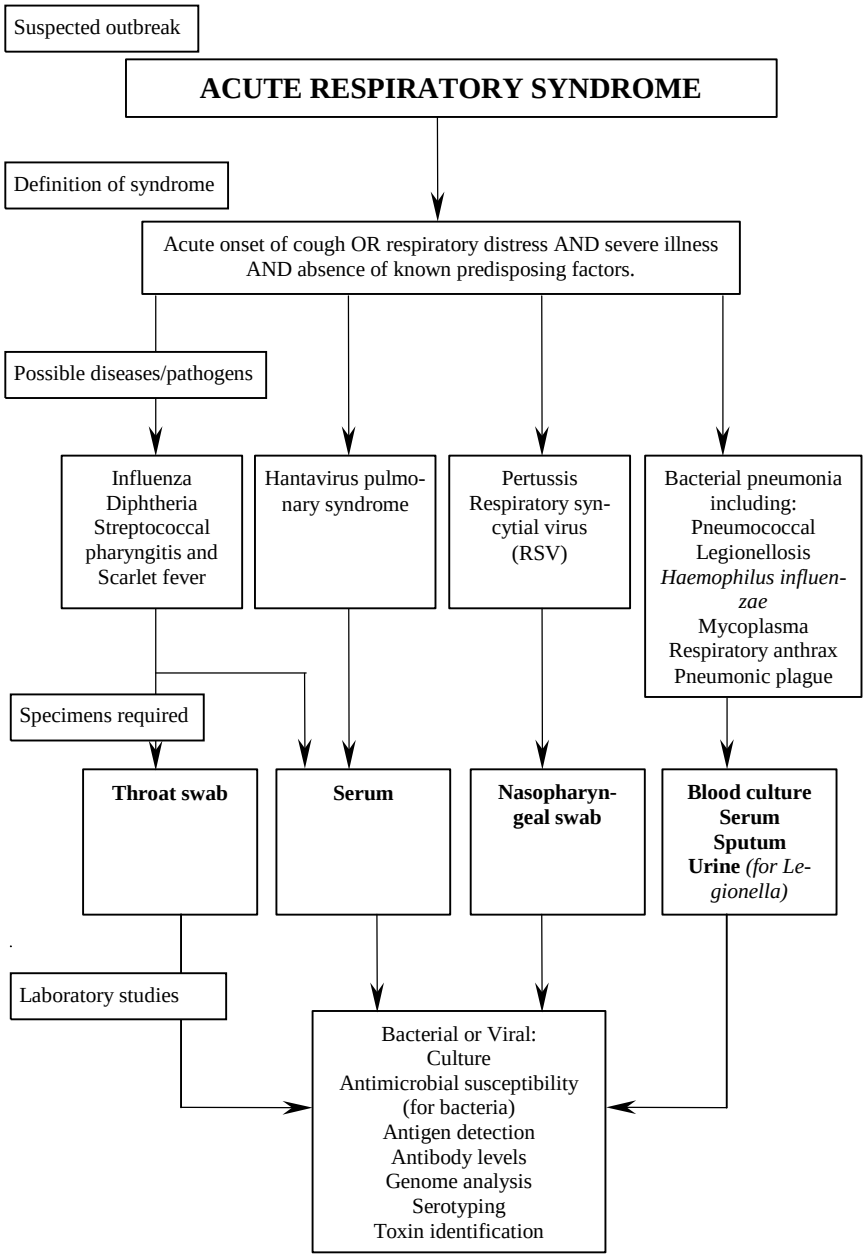
* *NOTE: Ebola and other haemorrhagic fevers may initially present as bloody diarrhoea. If such an aetiology is suspected, refer to "Acute Haemorrhagic Fever Syndrome" for appropriate specimen collection guidelines.*

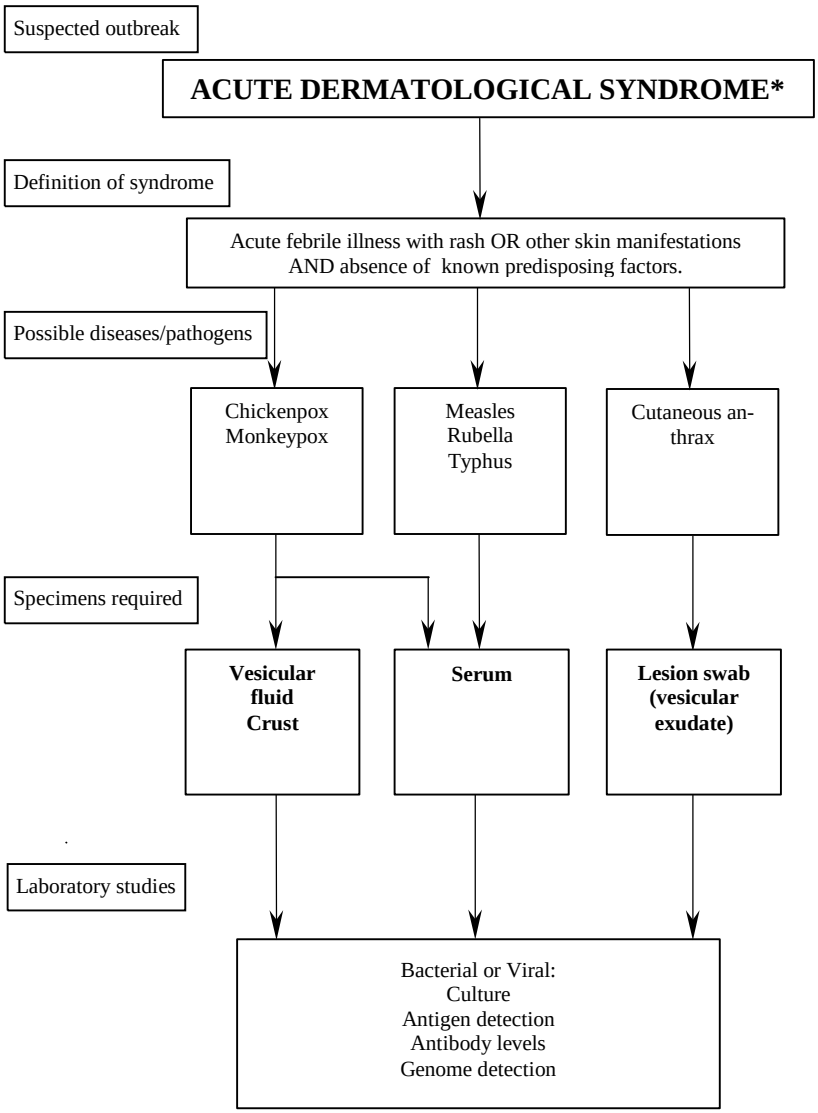

Suspected outbreak
ACUTE HAEMORRHAGIC FEVER SYNDROME
Definition of syndrome
Acute onset of fever of less than 3 weeks duration AND any two of the following:
• Haemorrhagic or purpuric rash
• Epistaxis
• Haemoptysis
• Blood in stool
• Other haemorrhagic symptom
AND absence of known predisposing factors.
Possible diseases/pathogens
Dengue haemorrhagic fever and shock syndrome
Yellow fever
Other arboviral haemorrhagic fevers (e.g. Rift Valley, Crimean Congo, Tick-borne flaviviruses)
Lassa fever and other arenoviral haemorrhagic fevers
Ebola or Marburg haemorrhagic fevers
Haemorrhagic fever with renal syndrome (hantaviruses)
Malaria
Relapsing fever
Specimens required
Blood
Blood smear
Serum
Post-mortem tissue specimens (e.g. skin biopsy and/or liver biopsy)
Laboratory studies
Viral:
Culture
Antigen detection
Antibody levels
Genome detection
Parasitic:
Demonstration of pathogen



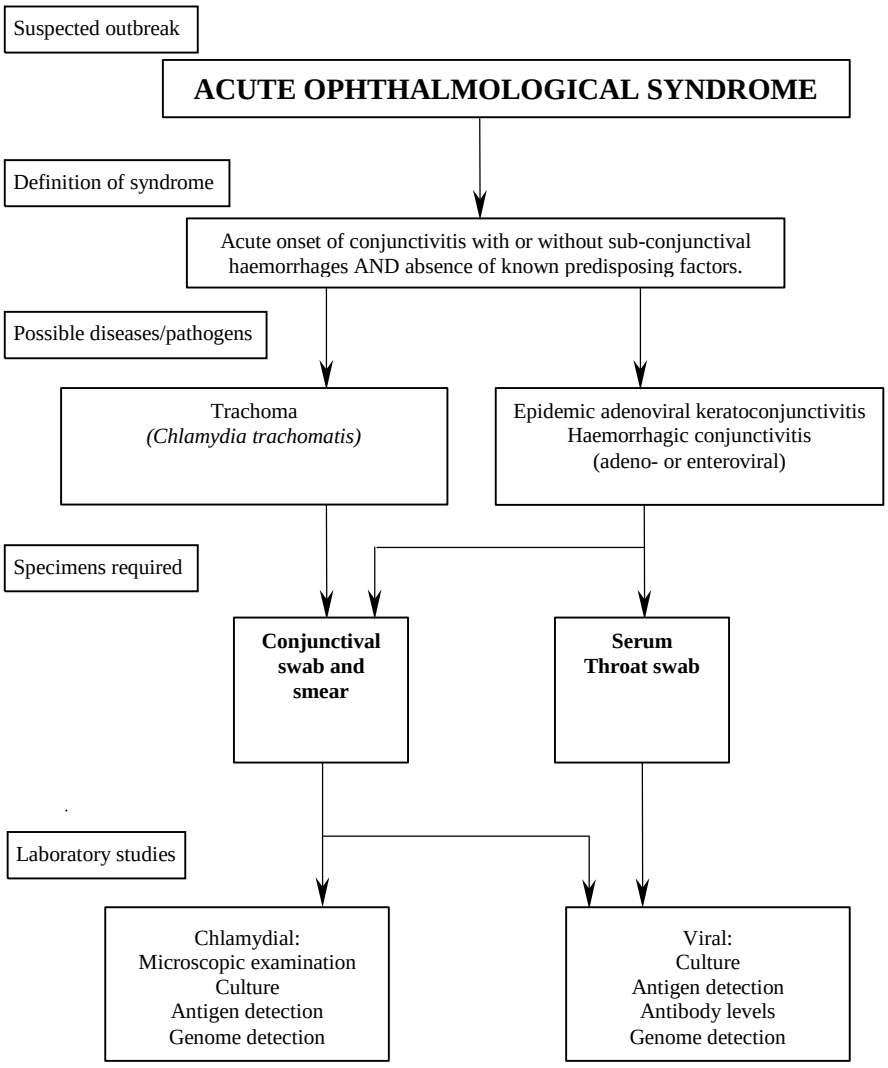
* Requires specialized media and handling procedures

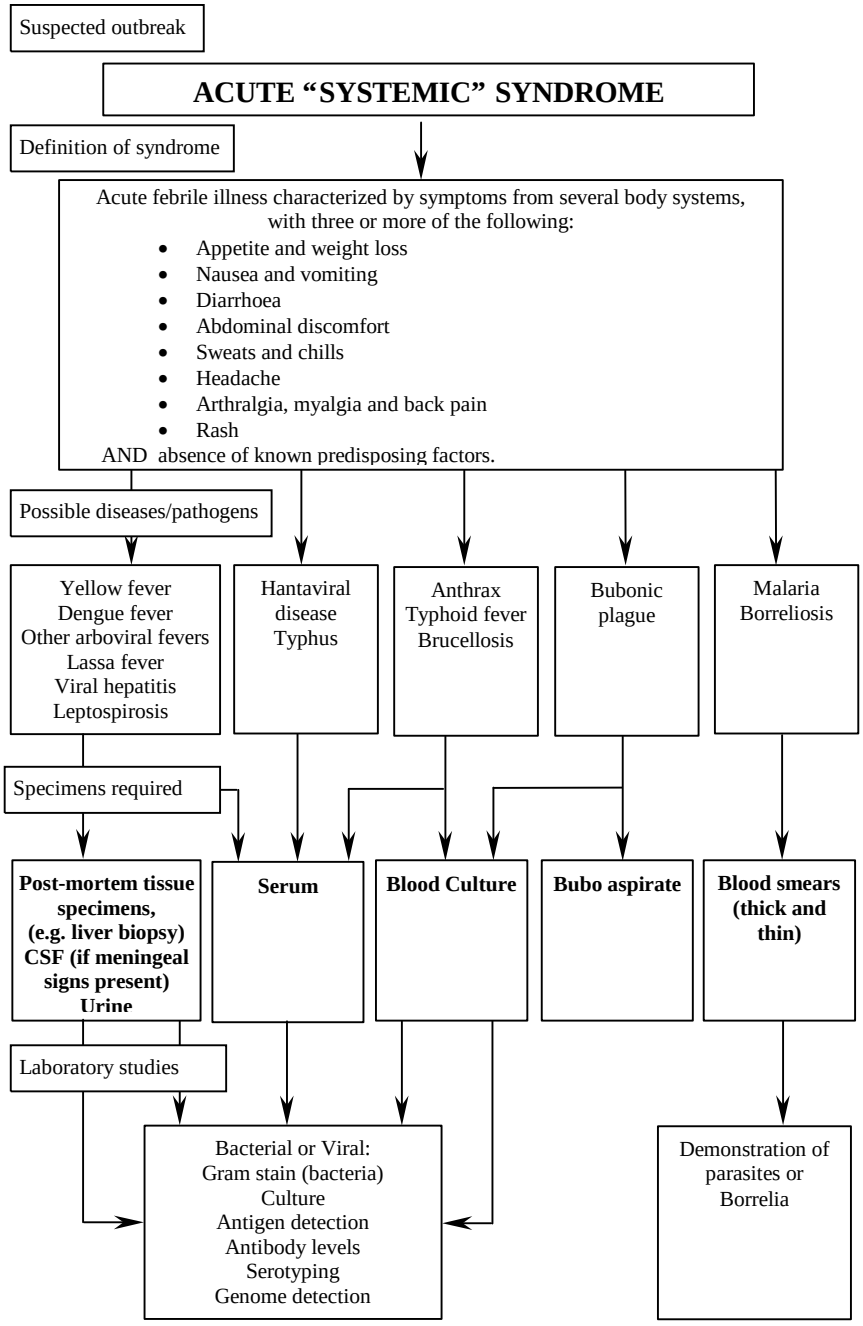

Suspected outbreak
ACUTE NEUROLOGICAL SYNDROME
Definition of syndrome
Acute neurological dysfunction with one or more of the following:
• Deterioration of mental function
• Acute paralysis
• Convulsions
• Signs of meningeal irritation
• Involuntary movements
• Other neurological symptoms
AND severe illness AND absence of predisposing factors.
Possible diseases/pathogens
Poliomyelitis or Guillain Barré syndrome
Viral, bacterial, fungal, or parasitic meningo-encephalitis
Rabies
Specimens required
Faeces
CSF
Blood Culture
Blood smears
Serum
Throat swab
Serum
Post mortem specimens (e.g. corneal impressions, brain tissue, skin biopsy from neck)
Laboratory studies
Viral:
Culture
Bacterial (including Leptospiral):
Gram stain and other microscopic techniques
Culture
Antimicrobial susceptibility
Antigen detection
Serotyping
Viral:
Culture
Antigen detection
Antibody levels
Genome analysis


Suspected outbreak
ACUTE RESPIRATORY SYNDROME
Definition of syndrome
Acute onset of cough OR respiratory distress AND severe illness AND absence of known predisposing factors.
Possible diseases/pathogens
Influenza
Diphtheria
Streptococcal pharyngitis and Scarlet fever
Hantavirus pulmonary syndrome
Pertussis
Respiratory syncytial virus (RSV)
Bacterial pneumonia including:
Pneumococcal
Legionellosis
Haemophilus influenzae
Mycoplasma
Respiratory anthrax
Pneumonic plague
Specimens required
Throat swab
Serum
Nasopharyngeal swab
Blood culture
Serum
Sputum
Urine (for Legionella)
Laboratory studies
Bacterial or Viral:
Culture
Antimicrobial susceptibility (for bacteria)
Antigen detection
Antibody levels
Genome analysis
Serotyping
Toxin identification



* *NOTE: The pathogens listed above reflect only those epidemic-prone conditions in which the principal manifestations may be dermatological. Other common bacterial, fungal, parasitic, and viral conditions are not covered.*


Suspected outbreak
ACUTE OPHTHALMOLOGICAL SYNDROME
Definition of syndrome
Acute onset of conjunctivitis with or without sub-conjunctival haemorrhages AND absence of known predisposing factors.
Possible diseases/pathogens
Trachoma
(Chlamydia trachomatis)
Epidemic adenoviral keratoconjunctivitis
Haemorrhagic conjunctivitis
(adeno- or enteroviral)
Specimens required
Conjunctival swab and smear
Serum
Throat swab
Laboratory studies
Chlamydial:
Microscopic examination
Culture
Antigen detection
Genome detection
Viral:
Culture
Antigen detection
Antibody levels
Genome detection


Suspected outbreak
ACUTE "SYSTEMIC" SYNDROME
Definition of syndrome
Acute febrile illness characterized by symptoms from several body systems, with three or more of the following:
Appetite and weight loss
Nausea and vomiting
Diarrhoea
Abdominal discomfort
Sweats and chills
Headache
Arthralgia, myalgia and back pain
Rash
AND absence of known predisposing factors.
Possible diseases/pathogens
Yellow fever
Dengue fever
Other arboviral fevers
Lassa fever
Viral hepatitis
Leptospirosis
Hantaviral disease
Typhus
Anthrax
Typhoid fever
Brucellosis
Bubonic plague
Malaria
Borreliosis
Specimens required
Post-mortem tissue specimens, (e.g. liver biopsy)
CSF (if meningeal signs present)
Urine
Serum
Blood Culture
Bubo aspirate
Blood smears (thick and thin)
Laboratory studies
Bacterial or Viral:
Gram stain (bacteria)
Culture
Antigen detection
Antibody levels
Serotyping
Genome detection
Demonstration of parasites or Borrelia

Annex 2: Blood specimen collection

Blood and separated serum are the most common specimens taken to investigate outbreaks of communicable disease. Venous blood can be used for isolation and identification of the pathogen in culture and by inoculation, or separated into serum for the detection of genetic material (e.g. using the polymerase chain reaction), specific antibodies, antigens, or toxins (e.g. by ELISA). 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.

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, Monovette, or similar vacuum blood collection devices, 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

- 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 venepuncture site outwards. Let the disinfectant evaporate. Do not repalpate the vein again. Perform venepuncture.
- If withdrawing with conventional disposable syringes, withdraw 5-10 ml of whole blood from adults, 2-5ml from children and 0.5-2ml for infants.
- If withdrawing with vacuum systems, withdraw the desired amount of

blood directly into each transport tube and culture bottle.

- Remove the tourniquet. Apply pressure to site until bleeding stops, and apply sticking plaster (if desired).
- Using aseptic technique, transfer the specimen to relevant cap transport tubes and culture bottles. Secure caps tightly. Be sure to follow manufacturer's instructions on the correct amount and method for inoculation of blood culture bottles.
- Label the tube, including the unique patient identification number, using indelible marker pen.
- Do not recap used sharps. Discard directly into the sharps disposal container.
- Complete the case investigation and the laboratory request forms using the same identification number.

Handling and transport

- Blood culture bottles and blood sample tubes should be transported upright and secured in a screw cap container or in a rack in a transport box. Cushion or suspend bottles during transport over rough terrain to prevent lysis of red cells. They should have enough absorbent paper around them to soak up all the liquid in case of a spill.
- If the specimen will reach the laboratory within 24 hours, most bacterial pathogens can be recovered from blood cultures transported at ambient temperature.

Separation of serum from blood

Additional materials required

- Sterile Pasteur pipettes and bulb, or soft, disposable transfer pipettes (pastettes). The latter are easier to handle and dispose of in the field laboratory.
- Sterile screw-cap tubes - 2 per sample.

Method of separation

- Using the materials and methods described above draw 10 ml of venous blood and transfer to a screw cap tube without anti-coagulant. Alternatively, blood may be collected directly into a proprietary collection and transport tube (e.g., Vacutainer, Monovette, etc.).

- Let the blood specimen clot for 30 minutes at ambient temperature, then place in a cool box to retract at 4 to 8°C for a minimum of 1 to 2 hours (it may be stored at this temperature for 48-72 hours).
- The specimen should be centrifuged at the laboratory at low speed (1000g for 10 minutes) to remove residual blood cells. When serum separation is performed in a field laboratory proper safety precautions should be taken. Ensure that the centrifuge is in good condition and the tubes are properly closed and balanced to avoid breakage and spilling. If a viral haemorrhagic fever is strongly suspected, samples should only be processed in properly equipped, specialized laboratories. Discuss with the laboratory whether a separation gel blood tube (see Note) would be acceptable in this case.
- Separate the serum aseptically from the clot using a sterile Pasteur pipette and bulb or soft, disposable transfer pipette. Transfer equally to 2 plastic screw cap tubes. Secure the caps tightly.
- If a centrifuge is not available and there will be a delay before samples can be transported to a laboratory, serum may still be separated carefully from the retracted clot using a disposable transfer pipette. Allow 4-6 hours to elapse after taking the blood sample to ensure adequate clot retraction. Using the transfer pipette, remove the clear yellow serum whilst taking care to keep the tip as far as possible from the clot, and avoid agitating the blood tube during the removal process. (This may be easier if a separation gel collection tube has been used.) Transfer to plastic screw-cap tubes and secure caps tightly.
- Label the tubes with the same patient details that appear on the blood sample tube.

NOTE: In some instances it may be acceptable to use a special blood tube containing a separation gel, which encourages separation of serum from clot. In this case, the centrifugation step is eliminated. This has advantages for ease and safety of specimen processing under field conditions, but it is important to check with the laboratory in advance to ensure that these devices are appropriate for your particular investigation.

Handling and transport

- If serum will be required for testing, separation from blood should take place as soon as possible, preferably within 24 hours at ambient temperature. If the specimen will not reach a laboratory for processing within 24 hours, serum should, if at all possible, be separated from blood prior to transportation. Sera may be stored at 4-8°C for up to 10 days. If testing is delayed for a long period, serum samples may be frozen.

- If separation on site is not possible, or is inadvisable for safety reasons, the blood sample should be stored at 4-8°C. Protect such unseparated samples from excessive vibration while transporting. Unseparated blood samples should not be frozen.

Capillary blood samples

Materials for collection

- Disposable sterile lancets
- Glass slides, cover slips, slide box
- Filter paper
- Fixatives such as methanol.

Method of collection

- Disinfect finger or thumb for adults, thumb for children, or side of heel for infants by swabbing with 70% isopropyl alcohol, and prick with a sterile lancet. Wipe away the first drop of blood.
- Discard used lancets directly into the sharps disposal container.
- Collect blood directly onto labelled glass microscope slides and/or filter paper.

Method of preparation of blood films

Blood films should be made by trained personnel. If this is not possible, they can be spread from heparinized or EDTA blood specimens sent to the laboratory**.**

Thick films for microscopy:

- Label the slide with patient identification number and name.
- Disinfect and prick site with a lancet as described above.
- Touch one or more large drops of blood onto the centre of the slide making sure that the slide does not touch the skin.
- Spread the blood in a circle about 1 cm in diameter using the corner of another glass slide.
- Air dry the film in a horizontal position. Do not dry the film by heating over a flame or other heat source.

Thin films for microscopy:

- Label the slide with patient identification number and name.
- Touch another drop of blood to the glass slide about 2 cm from one end making sure that the slide does not touch the skin.
- Place the slide horizontally on a flat surface.
- Hold the side of a second clean glass slide (the spreader) on to the center of the specimen slide and move it back until it touches the drop and the blood spreads along its base.
- At an angle of about 45°, move the spreader firmly and steadily across the specimen slide and air dry the film quickly. Do not dry over a flame or other heat source.
- Fix the dried film by dipping the glass slide in methanol or other fixative for a few seconds and air dry.

Handling and transport

- Air dried and/or fixed films are transported at ambient temperature preferably within 24 hours of specimen collection. They must not be refrigerated. Thick and thin films are usually kept in separate slide boxes.

Annex 3: Cerebrospinal fluid (CSF) specimen collection

The specimen must be taken by a physician or a person experienced in the procedure. CSF is used to in the diagnosis of viral, bacterial, parasitic, and fungal meningitis.

Materials for collection

- Lumbar puncture tray which includes:
- Sterile materials: gloves, cotton wool, towels or drapes.
- Local anaesthetic, needle, syringe.
- Skin disinfectant: 10% povidone iodine or 70% alcohol.
- Two lumbar puncture needles, small bore with stylet
- Six small sterile screw-cap tubes and tube rack
- Water manometer.
- Microscope slides and slide boxes.

Method of collection

As **only experienced personnel should be involved in the collection of CSF samples**, the method is not described in this document. CSF is collected directly into the separate 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 many of the relevant 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.

Annex 4: Eye specimen collection

Conjunctival and corneal swabs and smears are the usual specimens collected to diagnose acute bacterial or viral (kerato)conjunctivitis. Label all specimens as conjunctival or corneal and indicate whether the specimen was taken from the left or right eye. Strict aseptic technique is essential when collecting and processing these specimens. All medicines and droppers that have come in contact with patients should be discarded.

While corneal scrapings may occasionally prove useful in improving the utility of corneal specimens for diagnosis of some eye infections, these are not generally infections which are epidemic-prone. C**orneal scrapings must only be collected by an ophthalmologist or other trained person.** For these reasons, instructions for taking corneal scrapings will not be given here.

Materials for collection

- Sterile calcium alginate and/or cotton swabs, sterile saline and sterile transport tubes. (Do not use calcium alginate swabs for virology specimens)
- Sterile gloves
- Glass slides, glass slide marker, slide holder box
- Gloves and protective goggles should be worn if epidemic keratoconjunctivitis is suspected.

Method of collection of conjunctival swabs

- Clean the skin around the eye with a mild antiseptic.
- Moisten a swab in sterile saline and roll over the conjunctiva in a circular manner.
- Insert the swab into a sterile screw-cap tube containing the appropriate transport media for bacteria.
- Break off the top part of the stick without touching the tip of the tube and tighten the screw cap firmly.
- Repeat the procedure with a tube containing the appropriate viral transport medium.
- Label the specimens.

Method of preparation for microscopy smears

- Prepare two smears onto clean glass slides with a fresh conjunctival

swab. This should be done on site if possible. Otherwise, specimens may be sent to the laboratory in appropriate transport media for the preparation of smears. Note that it is not possible to prepare smears from swabs transported in certain media, such as those containing charcoal. For detection of chlamydia, it is essential that smears be prepared on site prior to transport.

- Label the glass slides and put into slide carrier or other appropriate box. Do not refrigerate or freeze the slides.

Handling and transport

- Specimens for detection of bacterial pathogens are transported at ambient temperature in appropriate bacterial transport medium.
- Specimens for viral detection are transported at 4-8°C in virus transport medium. Swabs in viral transport medium may also be frozen in liquid nitrogen.
- Microscopic slides are air dried and transported at ambient temperature in a slide box.

Annex 5: Faecal specimen collection

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. Stool is the preferred specimen for culture of bacterial, viral, and parasitic diarrhoeal pathogens. Rectal swabs showing faeces may also be used from infants. In general, rectal swabs are not recommended for the diagnosis of viruses.

Materials for collection

- Clean, dry, leak-proof screw cap container and tape
- Appropriate bacterial transport media for transport of rectal swabs from infants
- Parasitology transport pack: 10% formalin in water, polyvinyl isopropyl alcohol (PVA).

Method of collecting a stool specimen

- Collect freshly passed stool, 5 ml liquid or 5 g solid (pea-size), in a container.
- Label the container.

Method of collecting a rectal swab from infants

- Moisten a 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 sterile tube/container containing the appropriate bacterial or viral transport medium.
- Break off the top part of the stick without touching the tube and tighten the screw cap firmly.
- Label the specimen tube.

Handling and transport

- Stool specimens should be transported at 4-8°C. Bacterial yields may fall significantly if specimens are not processed within 1-2 days of collection. Shigella are particularly sensitive to elevated temperatures.

- Specimens to be examined for parasites should be mixed with 10% formalin or PVA, 3 parts stool to 1 part preservative. Transport at ambient temperature in containers sealed in plastic bags.

Annex 6: 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 aetiologic agent may be isolated on blood culture.

Materials for collection

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

Upper respiratory tract specimens

Method of collecting a throat swab

- 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.
- Label the specimen containers.
- Complete the laboratory request form.

Method of collecting per-nasal and post-nasal swabs (for suspected pertussis)

- Seat the patient comfortably, tilt the head back and insert the nasal speculum.
- Insert a flexible calcium alginate/Dacron swab through the speculum parallel to the floor of nose without pointing upwards. Alternately, bend the wire and insert it into the throat and move the swab upwards into the nasopharyngeal space.
- Rotate the swab on the nasopharyngeal membrane a few times, remove it carefully and insert it 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.
- Label the specimen tube.

Lower respiratory tract specimens

Method of collecting sputum

- Instruct patient to take a deep breath and cough up sputum directly into a wide-mouth sterile container. Avoid saliva or postnasal discharge. Minimum volume should be about 1 ml.
- Label the specimen containers.
- Complete the 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.

Annex 7: 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.

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)
- Wide-mouth screw-cap containers (for biopsy specimens)
- 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 with transport medium.

Skin biopsy

Skin biopsies from live patients are generally not appropriate specimens for field outbreak investigations. For details of the collection of skin biopsies after death for suspected viral haemorrhagic fevers, see the relevant section in Annex 9, Post-mortem specimen collection.

Handling and transport

Specimens for bacteriological analysis should be transported in Stuart's or Amies medium. Swabs for suspected viral pathogens should be transported in virus transport medium. Other specimens should be handled as described in the relevant section.
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 some instances, the outbreak investigation team may bring liquid nitrogen for specimen preservation. If this is the case, follow the instructions of the experienced laboratorian as to appropriate use. If there is any question, check with the

laboratory which will ultimately be receiving the specimens. 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.

Annex 8: Urine specimen collection

Materials for collection

- Sterile plastic cup with lid (50 ml or more)
- Clean, screw-top specimen transport containers ("universal" containers are often used)
- 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 **mid-stream** 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.

Annex 9: Post-mortem specimen collection

Strict precautions, including respiratory protection from aerosolized particles, must be taken when carrying out post-mortem specimen collection in communicable disease outbreaks. Collect the specimens as soon as possible, since viral titres decline while bacteria multiply rapidly after death. Instructions for taking a skin biopsy from highly contagious cadavers **(**e.g. during suspected Ebola outbreaks**)** are also included in this annex. Thorough post-mortem examinations may only be accomplished by experienced medical personnel. Prior experience and training is also advised even for the minimal collection of specimens from cadavers.

Materials for collection

- Barrier precautions: double gloves, sterile gown, eye goggles, mask
- For collecting blood and other fluids, refer to corresponding annex for materials
- Aseptic surgical and biopsy instruments for collecting tissue specimens
- Fixatives: saline formalin for histology
- Sterile saline, appropriate viral and bacterial transport media
- Sterile containers, sterile screw cap tubes or vials, glass slides and slide box
- Disinfectant such as household bleach 1:10.

Method of collection

- Use a separate sterile instrument for each tissue specimen from affected sites (several fragments with 1-2 grams of each is sufficient). Smaller, but adequate, specimens are taken with a biopsy needle.
- Place different tissues in separate sterile containers containing the relevant medium: fixatives for histopathology; sterile saline for preparation of tissues for immunofluorescence microscopy; and microbiological transport media for the isolation of bacterial and viral pathogens.
- Label all containers and tighten the screw caps firmly.
- Other specimens are collected as per the relevant annex. Blood may be taken from the heart cavities.
- If cerebral malaria is suspected, make several smears from the cerebral cortex on glass slides to detect *Plasmodium falciparum*. Label the slides and transport in a slide box.

Handling and transport

- Fixed specimens can be stored and transported at ambient temperature.
- Tissue specimens for isolation of bacterial pathogens can be transported at ambient temperature in transport media for up to 24 hours.
- Transport tissue specimens for isolation of viral pathogens in viral transport medium or sterile saline at 4-8°C for 24-48 hours. For longer periods, freeze and store at -70°C.
- If rabies is suspected and brain samples are collected, freeze unfixed specimens immediately after collection. Formalin-fixed samples are also useful and may be transported at ambient temperature.

Post-mortem skin biopsy

Materials for collection

- Instruments: Punch-biopsy tool or scissors and forceps
- Screw-cap vial containing sterile saline.

Method of collection

- Take out vial containing the sterile saline. Lay out the instruments.
- If using a punch-biopsy tool, place the open, sharp end on the skin and swivel into the skin, pressing firmly. Remove the tool from the skin and lift the biopsy specimen out with forceps. Cut free with scalpel or scissors. If using scissors to take the biopsy, lift the area of skin to be sampled with forceps. Cut a small piece of skin from the area. Use the forceps to remove the skin specimen.
- Place the specimen in a vial containing the sterile saline, and close the screw cap tightly.
- Label the specimen vial.
- Discard all instruments safely – do not re-use.
- In the diagnosis of rabies, samples from the nape of the neck at the hairline are preferred as concentrations of virus are likely to be high.

Post-mortem skin biopsy for diagnosis of pathogens with high infectious risk (e.g. Ebola haemorrhagic fever)

Materials for collection

All these materials are assembled in one kit. For biosafety reasons, the protective

clothing and gloves are for one-time use only, and should be incinerated after use.

- Disinfectant solution, bucket, soap and water
- Gown, latex gloves, heavy duty rubber gloves, plastic apron
- Masks and, where available, respirators for aerosol protection
- Instruments: punch biopsy tool or scissors, forceps
- Screw-cap vial containing 10% buffered formalin fixative.

Method of collection

- Put on protective clothing beginning with gown followed by latex gloves, rubber gloves, facial mask, and plastic apron.
- Open the vial containing the formalin fixative. Lay out the instruments.
- Gently turn the head of the cadaver to expose the nape of the neck. This area is selected because it is less visible and is highly vascular.
- If using a punch-biopsy tool, place the open, sharp end on the skin and swivel into the skin, pressing firmly. Remove the tool from the skin and lift the biopsy specimen out with forceps. Cut free with scissors. If using scissors to take the biopsy, lift the area of skin to be sampled with forceps. Cut a small piece of skin from the area. Use the forceps to remove the skin specimen.
- Place the specimen in the vial containing the formalin fixative, and close the screw cap tightly. Dip the closed vial in the disinfectant for 1 minute and allow to dry.
- Drop the instruments in the disinfectant. When finished, remove the outer rubber gloves and drop them in the disinfectant. Keep the latex gloves on while removing materials from the disinfectant.
- Dispose of all protective clothing, rubber and latex gloves, and materials in a plastic bag and incinerate everything.
- Wash your hands well and disinfect them with 70% isopropyl alcohol or povidone iodine.
- Label the specimen vial.
- Pack the specimen vial and ship at ambient temperature.

Once the sample has been fixed in formalin, the decontaminated vial may be safely transported to the receiving laboratory.

Annex 10: First aid procedures after accidental exposure to infectious material

Accidental sharps injury

A significant exposure risk is present in any accidental sharps injury, even if no blood is visible and the skin does not appear to be broken.

- Flush the area well in clean running water and wash thoroughly with soap.
- Cover with a dressing if necessary.
- Report the incident to a supervisor or the physician-in-charge immediately.

Accidental contact with infectious material

This includes any unprotected contact between potentially infectious material and broken skin, the mouth, nose or eye.

- Flush the area with soap and clean water. Use water or sterile saline alone for splashes to the eye or mouth.
- Report the incident to a supervisor or the physician-in-charge immediately.

Immediate actions after accidental exposure

Irrespective of the suspected pathogens under investigation, certain procedures must be followed after exposure to potentially infectious material. Patients may be infected with other pathogens unrelated to the outbreak investigation, for example hepatitis B virus or HIV. A baseline blood specimen should be collected immediately from the exposed health care worker and, if feasible, from the source patient. In an outbreak investigation, procedures for possible treatment and for the longer term follow-up of exposed health care workers should be established. Corrective action is required if a procedural cause of the accident is identified.

During a suspected viral haemorrhagic fever outbreak, the general condition and temperature of the health care worker should be monitored twice daily for three weeks.

Annex 11: Chemical disinfectants

Chlorine is the recommended disinfectant for use in field outbreak investigations. An all-purpose disinfectant should have a concentration of 0.05% (= 1 g/litre = 1000 ppm) of available chlorine, with a stronger solution of 0.5% (= 10 g/litre = 10,000 ppm) available chlorine used in situations such as suspected Lassa and Ebola virus outbreaks.

Common methods of describing chlorine concentrations can be very confusing, and often assume that concentrations of chlorine are standard in products which are then diluted to make disinfectants. People and publications frequently refer to routine use of "1%" or "10%" chlorine solutions. What is usually meant by this description is a 1:100 or 1:10 dilution of a liquid product containing 5% available chlorine, which is the concentration in many household bleach preparations. However, chlorine concentrations vary in different products. This information is on the product label, and must be kept in mind when preparing appropriate dilutions. The manufacturer may provide appropriate instructions on how to prepare solutions with concentrations of 0.05% and 0.5% available chlorine. Otherwise, use the guidelines provided below.

An **easy-to-follow Reference Table "Preparation and Use of Chlorine Disinfectants"** is given on page 45. Chlorine solutions gradually lose strength, thus fresh solutions must be prepared daily. Clean water should be used because organic matter reduces the disinfecting properties of the chlorine solution.

Commonly used chlorine-based disinfectants include:

1. Sodium hypochlorite
Commercial liquid bleaches, such as household bleach (e.g. Chlorox, Eau-de-Javel) generally contain 5% (50 g/litre or 50,000 ppm) available chlorine.

To prepare a 0.05 % available chlorine solution, make a 1 in 100 dilution, i.e. 1 part bleach in 99 parts water to give final concentrations of available chlorine of 0.05% (See Reference Table page 45).
Similarly, to make a 0.5% chlorine solution, make a 1 in 10 dilution, i.e. 1 part bleach in 9 parts water to give final concentrations of available chlorine of 0.5% (See Reference Table page 45).

2. Chlorine powder/ Chlorine granules
While the bleach solution described above may satisfy all disinfection needs, calcium hypochlorite powder or chloramine granules 70% may prove convenient for the disinfection of spills of blood and other potentially infectious body fluids.

They may also prove useful under field conditions because of ease of transport. They contain approximately 25% available chlorine. Solutions for disinfection may be made by mixing the powder or granules with clean water (See Reference Table page 45).

In addition to use as a powder on spills, hypochlorite powder or granules may be used to prepare liquid chlorine solutions. The recommended formula is 20 g of powder to 1 litre of clean water to give a solution with 5% available chlorine, equivalent to the concentration in most household bleach preparations. (See Reference Table page 45).

Decontamination of surfaces
Wear an apron, heavy-duty gloves, and other barrier protection if needed, and wipe clean with an absorbent material. Disinfect surface by wiping clean with 1:10 dilution of household bleach, then incinerate all absorbent material in heavy duty garbage bags.

Decontamination of blood or body fluid spills
For spills, hypochlorite granules should be very liberally sprinkled to absorb the spill and left for at least 30 minutes. If chlorine powder is not available, one may use absorbent materials to try to soak up most of the fluid prior to disinfection of the surface with a 1:10 solution of liquid bleach (= 0.5% available chlorine solution, or “10% solution”). These absorbent materials must then be disinfected in bleach prior to disposal.

Sterilization and re-use of instruments and materials
In the field outbreak situation, it is not advisable to consider sterilization and re-use of any instruments or materials. Sterilization techniques are therefore not described in this document.

Disinfection of hands
The principal means for disinfection of hands is thorough washing with soap and water. If available, commercial hand disinfectants such as chlorhexidine or povidone iodine may also be used.

Table 2 (Reference Table): Preparation and Use of Chlorine Disinfectants

Chlorine Product	**To make: 0.5% available chlorine solution for disinfecting:** • **Excreta** • **Cadavers** • **Spills of blood, body fluids**	**To make: 0.05% available chlorine solution for disinfecting:** • **Gloved hands** • **Bare hands and skin** • **Floors** • **Clothing** • **Equipment** • **Bedding**
Household bleach (5% active chlorine)	Add 1 litre of bleach to 9 litres of water (1:10 solution)	Add 100ml of bleach to 9.9 litres of water **Or** Add 1 litre of 1:10 bleach solution to 9 litres of water (yields a 1:100 solution)
Household bleach (30% active chlorine)	Add 16 grams or 1 tablespoon to 1 litre of water	Add 16 grams or 1 tablespoon to 10 litres of water
Calcium hypochlorite powder or chlorine granules 70%	7 grams or ½ tablespoon dissolved in 1 litre of water	7 grams or ½ tablespoon dissolved in 10 litres of water

Annex 12: Constructing a field incinerator

1 Find a 220- liter (55-gallon)drum

2 Cut open the drum. Remove and save the top cutaway piece

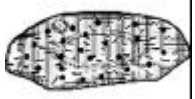
3 Hammer the edges of the drum so they are not sharp

4 Cut 3 half-moon openings just above the top end of the drum

5 Turn the drum upside down. The bottom of the drum now is the top

6 Cut 4 holes on the sides of the drum. Thread 2 metal rods through these holes so that they cross inside the drum

7 Punch holes in the top cutaway piece to make a platform



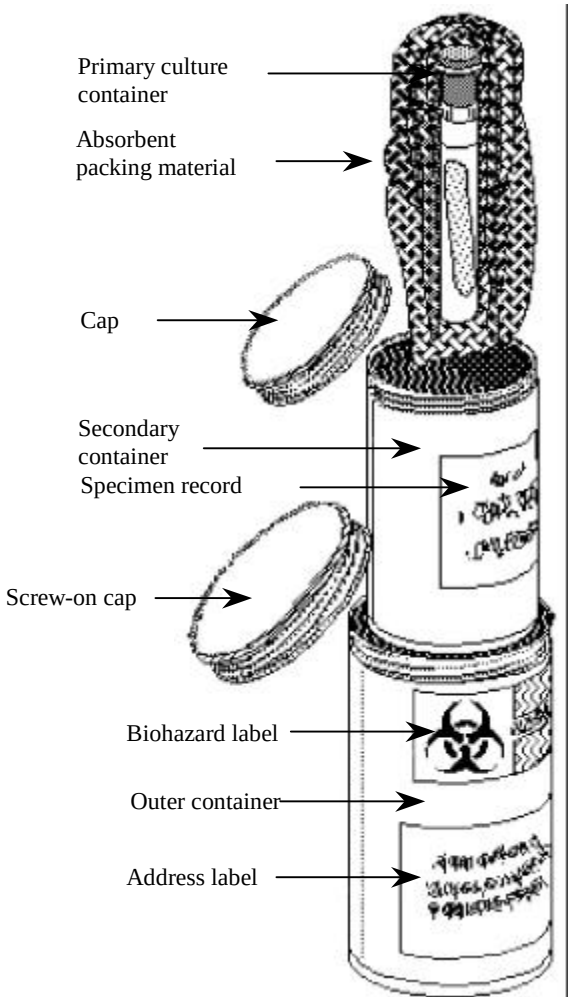
8 Pierce a series of holes on the side of the drum and above the crossed rods to improve the draw of the fire

9 Cut away half of the top. Attach the wire loops to the cutaway half to make a trap door. Attach another loop for a handle to open the trap door.

10 Place the platform inside the drum on top of the rods.

Annex 13: Basic triple packaging system and maintenance of transit temperature

If EPI vaccine carriers or other commercially made containers are not available, make an insulated container as illustrated. Vaccine carriers that have been used for specimen transport must never be reused for carrying vaccines.



Annex 13 (cont'd): Basic triple packaging system and maintenance of transit temperature

- The specimen is in the labeled primary container which must be water-tight, airtight, and wrapped in absorbent material (e.g. cotton wool) in case of leakage. If necessary, tape the label to the container to prevent it loosening in transit.
- Place the primary containers in a single durable, watertight, and leak-proof secondary container or a sealed plastic bag if this is not available. Use additional absorbent material to cushion multiple primary receptacles. A biohazard label and the laboratory request form sealed in a plastic bag should be taped to the outside of this secondary container.
- The outer package protects the contents from physical damage and water while in transit. It should have a resistant, high density external cover (e.g. metal, wood, or fibreboard), shock-absorbent padding on the inside, and a tight-fitting lid. The outer package must be leak-proof and well-insulated, and can contain ice, cold packs or dry ice when required.
- The rigid outer package is placed within an outer carton of double-ply corrugated cardboard or plastic, and a biohazard label is applied.
- The specimen carriers and ice packs can be reused after disinfection (see Annex 11).

Maintenance of transit temperature

- 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. If available, a cold chain monitor should be inserted.
- -20°C:
  Use 2 kg of dry ice within the insulated outer package, which must permit the release of carbon dioxide gas to prevent explosions. This will keep the specimens frozen for 1-2 days.
- -70°C:
  If liquid nitrogen is used for storage and transport, the specimens are placed in special cryotubes.

NOTE: It is advisable that commercially produced, UN-approved packaging systems be included in the materials carried by the investigating team.

Annex 14: Example of case investigation, laboratory request, and line listing

Suspected yellow fever case investigation form

<table>
<tr><td colspan="5">As soon as yellow fever is suspected, contact:
District communicable disease manager:

District EPI programme manager:</td><td colspan="5">Telephone number: _____
Facsimile number: _____
Telephone number: _____
Facsimile number: _____</td></tr>
<tr><td colspan="5">1. Record general information about the patient:</td><td colspan="5">Date reported to district level:</td></tr>
<tr><td colspan="5">Patient’s name and patient record number:</td><td colspan="5">Sex:M [ ]F [ ]

Patient’s occupation:</td></tr>
<tr><td colspan="5">Address:</td><td colspan="5">Village or municipality:</td></tr>
<tr><td colspan="5">District:

State or Province:</td><td colspan="5">Name of head of patient’s household or village chief:</td></tr>
<tr><td colspan="2">Patient’s date of birth</td><td>dd</td><td>mm</td><td>yy</td><td colspan="5">Patient’s age (if date of birth unknown):</td></tr>
<tr><td colspan="5">2. Does suspected case have:</td><td colspan="2">Date of onset:</td><td colspan="3">3. Record travel and yellow fever immunization history.</td></tr>
<tr><td colspan="2">Fever (>38 C or >101 F) that did not respond to antimalarial treatment</td><td>Y</td><td>N</td><td>U</td><td>dd</td><td>mm</td><td>yy</td><td colspan="3" rowspan="2">List names of other areas or districts that patient visited during the last 2 weeks:</td></tr>
<tr><td colspan="5">Jaundice AND at least one of the following:</td></tr>
<tr><td colspan="2">Slow pulse in relation to fever</td><td>Y</td><td>N</td><td>U</td><td>dd</td><td>mm</td><td>yy</td><td colspan="2">Have cases of fever and jaundice been seen or</td><td>Y</td><td>N</td><td>U</td></tr>
<tr><td colspan="2">Bleeding from the nose, gums, skin or gastrointestinal tract</td><td>Y</td><td>N</td><td>U</td><td>dd</td><td>mm</td><td>yy</td><td colspan="2">reported in areas or districts that patient visited during the last 2 weeks?</td><td></td><td></td><td></td></tr>
<tr><td colspan="2">Reduced amount of urine</td><td>Y</td><td>N</td><td>U</td><td>dd</td><td>mm</td><td>yy</td><td colspan="2">Has the patient ever received at least one dose of yellow fever vaccine?</td><td>Y</td><td>N</td><td>U</td></tr>
<tr><td colspan="2">Elevated protein level in urine</td><td>Y</td><td>N</td><td>U</td><td>dd</td><td>mm</td><td>yy</td><td colspan="5">Health facility report by:_____
Contact number for health facility:_____</td></tr>
</table>

Annex 14 (cont'd): Example of a laboratory request form

Laboratory transmittal form for suspected yellow fever case

Specimen Collected *(Circle one)*	Date specimen collected *(dd/mm/yy)*	Date received in laboratory *(dd/mm/yy)*	Type of test	RESULTS				Date results sent to MOH *(dd/mm/yy)*	Date results received at MOH *(dd/mm/yy)*
				Pos	Neg	Not processed	Unkn.		
Blood			IgM						
			IgG (acute)						
			IgG (convalescent)						
Malaria slide			Microscopy						
Other									

1.Were specimens or isolates sent to another laboratory? *(Circle one*) Yes No Unknown	2. If YES, record laboratory's name, address, and telephone number:
3. What is the final classification of the case? (*Circle one)*	Suspected Confirmed Discarded Unknown
4. If case discarded as yellow fever, record diagnosis:	5. What was final outcome for patient? (*Circle one*) Living Dead Unknown
	6. If patient died, record date of death: (dd)__(mm)__(yy)__
Investigator's Name: (*Please print*)	Signature:
Address:	Telephone Number:

Annex 14 (cont'd): Example of a line listing form

Line Listing Form for Suspected Cases of Yellow Fever

District: _____ For Reporting Period:_____ Reported by:_____

CASE ID Nº	Name of patient	Health facility	Received at least 1 dose of YF vaccine? Y/N	Record the patient's:		Record date specimen received in laboratory:				Record results of laboratory testing:				Record date laboratory sent results:				Record:	
				DOB (l)	On set (2)	Acute Blood (3)	Conv Blood (4)	Liver (5)	Other (6)	IgM (7)	VI (7)	IgG (7)	Histo (7)	IgM	VI	IgG	Histo	Final Class (8)	Final Status (9)

(1) Date of birth, if known. If date of birth is unknown, record at least the year, and, if known, the month of birth.
(2) Date of onset of first symptoms
(3) Date first (or acute) blood sample received in laboratory
(4) Date second (or convalescent) blood sample received in laboratory
(5) Date liver sample received in laboratory
(6) Date other sample received laboratory
(7) Results of laboratory testing: Use codes: 1=positive; 2= negative; 3=not done; 4=pending; 5=unknown
VI means "virus isolation". *Histo* means "histopathology"
(8) Final classification of case. Use codes: 1=suspected case; 2=confirmed case; 3=discarded case; 4=suspected case, laboratory results pending; 9=unknown
(9) Final status of patient. Use these codes to record the final status of patient: A = alive; D = deceased; L = lost to follow up