

COMMUNICABLE DISEASE TOOLKIT

IRAQ CRISIS

8. GUIDELINES FOR COLLECTION OF SPECIMENS FOR LABORATORY TESTING

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WORLD HEALTH ORGANIZATION

INTRODUCTION

There is a high risk of communicable disease outbreaks in emergency situations. Outbreaks 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
- implementation of effective control measures.

Rapid identification of the causative agent and the likely source or mode of transmission is essential. 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 carry out the diagnostic tests
- proper biosafety and decontamination procedures to reduce the risk of further spread of the disease.

The purpose of this document is to ensure that the correct specimens are collected, packaged and transported in a safe and standardised manner during a field investigation of an outbreak in Iraq or its neighbouring countries.

This document is adapted for emergency situations from the WHO document *Guidelines for the collection of clinical specimens during field investigation of outbreaks* by WHO/CSR, Geneva.

Section 1: Planning for specimen collection

Once a suspected outbreak has been detected and reported, an epidemiological investigation must be quickly organised. The materials and procedures required for efficient specimen collection and their transport to the laboratory for testing are outlined below.

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 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.

1.3 Laboratory for specimen testing

In the event of an outbreak, WHO will co-ordinate the transport of specimens and follow up on result of laboratory tests.

1.4 Collecting the specimens

For stool samples, the health worker should collect the sample, place in cold box and inform WHO. Transport to the laboratory should be done as soon as possible. For CSF the admitting physician should conduct the lumbar puncture and obtain the sample. Blood samples should be taken by the health worker.

Section 2: 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 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 samples.

2.1 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.

2.2 Labelling specimen container/slide

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.

2.3 Case investigation and laboratory forms

A case investigation form should be completed for each patient at the time of collection. The originals remain with the investigation team, and should be kept together for analysis and later reference. A laboratory form must also be completed for each specimen. The epidemiological and clinical data gathered in the investigation can later be easily tied to the laboratory results for analysis. The form includes 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 received, name of the person collecting the specimen.

Section 3: 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 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.

Many specimens taken for viral isolation are viable for two days if maintained in type specific media at 4-8°C. Freeze these specimens as directed by expert advice, as infectivity may be altered.

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 CSF, 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. 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.

Annex 1: Blood specimen collection

Blood and separated serum are the most common specimens taken in 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. by Polymerase Chain Reaction), specific antibodies (by serology), antigens or toxins (e.g. by immunofluorescence). 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 fingerprick 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 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. Disinfect the tops of blood culture bottles.
- 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 repalpate the vein again. Perform venipuncture.
- 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. Using aseptic technique, transfer the specimen to relevant cap transport tubes and culture bottles. Secure caps tightly.
- 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 bandaid.
- 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 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.
- 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 it is a cold-sensitive bacterial pathogen.

Annex 2: 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/encephalitis.

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 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.

Annex 3: 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. They are not useful 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 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. Transported at ambient temperature in containers sealed in plastic bags.

Annex 4: 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.

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 nasopharyngeal 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, indicating left or right side.
- Complete the laboratory request form.
- Repeat on the other side.

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 5: Urine specimen collection

Material 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

- Labels and indelible marker pen.
- 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 6: Chemical disinfectants

Chlorine is the recommended disinfectant for use in field outbreak investigations. An all-purpose disinfectant should have a concentration of 0.1% (= 1 g/litre = 1000 ppm) of available chlorine, with a stronger solution of 0.5% (= 5 g/litre = 5000 ppm) used in situations such as suspected Lassa and Ebola virus outbreaks.

In preparing appropriate dilutions, one must keep in mind that different products have different concentrations of available chlorine. To prepare solutions with the above concentrations, the manufacturer may provide appropriate instructions. Otherwise, use the guidelines provided below. Chlorine solutions gradually lose strength, thus fresh solutions must be prepared daily. Clear water should be used because organic matter destroys chlorine.

Commonly used chlorine-based disinfectants include:

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 0.1% chlorine solution, make a 1 in 50 dilution, i.e. 1 part bleach in 49 parts water to give final concentrations of available chlorine of 0.1%. (For example, this could entail adding 20 ml of bleach to approximately 1 liter of water.)

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%. (eg add 100 ml of bleach to 900 ml water.)

Chloramine powder

While the above-described bleach solution may satisfy all disinfection needs, chloramine powder may prove convenient for the disinfection of spills of blood and other potentially infectious body fluids. It may also prove useful under field conditions because of ease of transport. It contains approximately 25% available chlorine.

In addition to its use as a powder on spills, chloramine powder may be used to prepare liquid chlorine solutions. The recommended formula is 20 g of chloramine powder to 1 litre of clean water.

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, chloramine granules should be very liberally sprinkled to absorb the spill and left for at least 30 minutes. If chloramine powder is not available, one may use absorbent materials to try to soak up most of the fluid prior to disinfection with 0.5% liquid bleach. These absorbent materials must then be disinfected in bleach prior to disposal.

Check this for accuracy.

Sterilisation and re-use of instruments and materials

In the field outbreak situation, it is not advisable to consider sterilisation and reuse of any instruments or materials. Sterilisation techniques are therefore not required, and are not described in this document

Disinfection of hands

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