17.2: Sample collection

Accurate laboratory results depend on proper collection, processing, and handling of samples. The method of collection, timing, and handling of samples will be determined by the purpose of the trial and specified in the trial protocol. Careful attention must be given to the quantity and quality of samples, aseptic precautions, and prompt transport of samples and their processing and storage in the laboratory. Advances in technology and analytical chemistry have led to the development and use of direct testing in the field, using point of care (POC) diagnostics, and rapid diagnostic tests (RDTs) have been introduced in some areas.

2.1 Types of specimen

The kinds of specimen that are commonly collected in field trials include:

- specimens from humans, including blood, stool, urine, sputum, skin snips, and other tissue biopsies, and swabs or smears collected from skin or mucosal surfaces
- entomological specimens for studies of vectors, and animal or malacological specimens for studies of intermediate hosts
- food, water, and environmental samples.

In this chapter, we discuss only specimens collected from humans, though many of the issues (such as the use of sterile techniques) apply to the other types of specimen.

2.2 Handling specimens

The collection of samples for laboratory studies will usually involve the steps outlined in Box 17.1.
The procedures for collecting and processing samples must be unambiguously specified, including to where they are to be transported and how they will be labelled. Whenever required, the type of shipment must be specified, for example, in dry ice or liquid nitrogen. If samples are to be transported by air, safe shipment of samples is mandatory, and procedures must follow the International Air Transport Association (IATA) guidance for infectious substances and diagnostic specimens, which detail packaging and shipment methods. Each package must contain a primary and a secondary container, and both of these must be leak-proof to avoid accidental spillage during transport. The whole process must be performed only by trained staff, whose competence has been certified. The regulations governing the transport of potentially hazardous samples are designed to ensure that samples reach their destination in good condition and to eliminate exposure of those handling the shipment to any potential hazard. Prior communication with the recipient and tracking information are vital, in order that shipments can be dealt with promptly on arrival. On occasion, this may require staff to receive the specimens outside normal working hours to avoid the specimens sitting around and deteriorating.

Box 17.1 Steps involved in the collection of samples for laboratory studies

1. Collection of specimens from the study participants.
2. Placement in a suitable container.
3. Labelling of the container.
4. Temporary storage at an appropriate temperature.
5. Initial processing (for example, serum separation from whole blood), with appropriate re-labelling.
6. Transport to intermediate or final destination for further processing, testing, and storage.

2.3 Blood

The usual methods by which blood is collected in field surveys are by venepuncture or by finger- or heel-pricks, depending on the nature of the investigations required. If small quantities of blood are required, finger-pricks are usually taken from adults, with heel-pricks more commonly used in infants and young children, whose fingers are very small and whose heels do not yet have calluses. A finger-prick provides an adequate volume of blood for many laboratory tests. Micro-techniques are to be preferred whenever they have acceptable validity, as they either avoid the need for venepuncture altogether or reduce the volume of blood that is needed. Micro-techniques have been, or are being, developed for many assays, and investigations should be conducted before a study starts to find out the latest availability of such techniques (for example, by literature search or contact with those in a central or reference laboratory). It is important to verify that the methods have been adequately validated. Some tests require larger quantities of blood, however, and it will often be necessary to collect blood by venepuncture from at least a sample of the population.

After collection, blood may be separated into several components, including serum, plasma, red cells, and white cells. The separation must be done shortly after the blood has been collected, and it is common for this procedure to be carried out close to where the samples have been collected or in a nearby field laboratory.

A sample of blood taken from a finger-prick may be collected in one of several ways, including:

1. collection into capillary tubes, for example, narrow glass tubes, by capillary action, or microtubes by gentle
squeezing of the finger

2. dropping onto a glass slide for direct examination of a blood smear
3. dropping onto strips or discs of absorbent paper (filter paper).

Fingertips are swabbed with alcohol before pricking, and the first drop is wiped off. Sufficient blood can be obtained for two thick, and two thin, malaria smears to do one or two haemoglobin level measurements (for example, with the Haemocue® system or the older haematocrit tubes), to collect 50–100 microlitres of blood in a microtube or Microtainer® for serum, and to place a drop on filter paper (World Health Organization, 2003). Filter paper samples need to be air-dried, before storing with silica gel. Tubes with plasma or serum can be stored on dry ice, in a freezer, or in liquid nitrogen. The amount of plasma or serum recovered from a finger-prick sample will be sufficient to perform serological tests, such as enzyme-linked immunosorbent assay (ELISA) or Multiplex® assays, and is sufficient for the determination of some micronutrients such as vitamin A or zinc (minimum serum requirements of 25–40 microlitres). Establishing volume requirements for the tests to be conducted is a prerequisite.

If repeated blood sampling is to be undertaken from participants during the course of a study, it is likely to be more appropriate ethically, and easier to maintain the co-operation of most study populations, if finger-prick, rather than venous blood, sampling is used. While filter paper samples are satisfactory in many cases, the larger sample volumes from venous sampling are currently needed for some tests (for example, tests for cell-mediated immunity, human leucocyte antigen (HLA) typing, bacterial cultures). A variety of systems using an evacuated tube, such as Vacutainer® or Vacuette® collection tubes, and blood culture bottles are suitable for this purpose. For repeated sampling, it is also essential to provide feedback to the individuals involved, and to the community if appropriate, about the earlier results (see also Chapter 9).

If multiple types of collection tubes are to be used, the order of draw should be written into the SOP to minimize cross-contamination of tube additives.

Special care in handling and processing samples is needed if any DNA-based work is to be conducted, as the potential for cross-contamination between samples is high. Blood for bacterial cultures is collected by venepuncture and delivered directly into blood culture bottles containing bacterial growth media, before incubation in the laboratory in either a conventional incubator or an automated incubator system such as the BACTEC® series. Blood for immunological and genetic analysis can be collected as whole blood and stored in specialized tubes such as PAXgene® or Tempus™, when only small volumes are available, as spots collected on filter paper for later analysis in a specialist laboratory.

Special precautions should be taken when collecting blood. Disposable gloves should be worn, a sharps box provided, and water and detergent should be available for use by those taking blood. All blood samples should be considered to be potentially infectious, and appropriate handling procedures must be employed to safeguard all those who will come into contact with the specimens during their collection, processing, analysis, or storage (World Health Organization, 2004). Guidelines and drugs should be available for use in the event of a needle-stick injury or blood spillage.

### 2.4 Cerebrospinal fluid

Collection of cerebrospinal fluid (CSF) requires lumbar puncture, which must be performed by a clinically trained member of staff with prior supervised experience. Using aseptic techniques, CSF should be collected into a sterile container for prompt transfer to the laboratory for biochemical and microbiological analysis. An obviously 'bloody' sample
may compromise the laboratory results, especially from biochemical analyses.

### 2.5 Stool and urine

A summary of different methods that may be used for collecting urine and stool samples, with details of different container types, is given in World Health Organization, 2003. The methods considered for use in a particular survey should be discussed with those knowledgeable of local customs and taboos. In some cultures, sensitivity regarding the collection or public display of stool specimens may be greater than that for blood. A container that is technically appropriate may not be acceptable in a particular study community (for example, due to colour, transparency, or resemblance to a cultural design or pattern). In advance of a survey, the proposed stool and urine containers should be shown to the village leaders, and the proposed methods of sample collection discussed. As with all field procedures, it is important to undertake pilot testing to ensure that the procedures planned will be acceptable (both to the investigator and to the study population).

As stool samples can rarely be collected ‘on the spot’, it is usually necessary to leave the container with an individual overnight and to arrange to pick up the specimen on the following day. A potential hazard in doing this is that containers may be exchanged between individuals or, for example, one person may provide a sample for the whole family. It is difficult to rule out this possibility, but it is important for fieldworkers to stress the importance of participants adhering to the correct procedures and to be alert to possible problems.

### 2.6 Sputum

The WHO manual (World Health Organization, 2003) gives a concise description of recommended methods of collecting sputum samples, using different kinds of jars, boxes, and containers, including transport media. Two general points merit special attention:

1. all sputum samples should be considered potentially infectious
2. careful attention should be given to the cold-chain requirements if sputum samples have to be sent to another laboratory for culture.