Annex 9

Procedures and equipment for specimen collection

Clinical specimens

General

Enclose specimens in a secure container and label the container with a waterproof pen. Place this container in a waterproof bag with tissue, towels or other blotting material to absorb any leakage. Put all specimen containers in an insulated box packed with ice or frozen refrigerant packs and deliver them to the laboratory as soon as possible. If sending specimens by post or courier ensure that they are delivered during business hours on a weekday.

Address the package clearly, including the name and telephone number of the receiving laboratory. Write instructions as appropriate, for example “Medical specimens. Call addressee on arrival. Hold refrigerated.”

Faeces

Collect stool specimens as soon as possible, since delay may impede identification of the causative agent.

Ideally, swabs of fresh stool or rectal swabs should be collected for bacteriological examination, large volumes of diarrhoeal stool (at least 30g) for viral examination, and fresh bulk stool (with preservative) for parasite examination.

Bacteria

Collect at least two rectal swabs or swabs of fresh stools (less than one hour old) from each case:

- If possible refrigerate Cary-Blair transport medium in advance, so that the swabs can be placed into a cool medium.
- Insert swab into Cary-Blair medium to moisten it.
- Insert swab 3–5 cm into rectum and rotate gently.
- Remove swab and examine it to ensure that the cotton tip is stained with faeces.
- Insert swab immediately into tube of transport medium.
- Push the swab to the bottom of the tube.
- Repeat procedure with the second swab and place in same tube as the first.
- Break off top parts of sticks, tighten screw-cap firmly.

If specimens will arrive at the laboratory within the 48 hours after collection, they can be refrigerated at 4 °C. Pathogens can still be recovered from refrigerated samples up to 7 days after collection, although the yield decreases after the first 2 days. During transport, refrigeration for up to 36 hours can be achieved by shipping in a well-insulated box with frozen refrigerant packs or wet ice.
If it is impossible for specimens to reach a laboratory within 2 days, they can be frozen at -20 °C (home-type freezer) although freezing at -70 °C (ultra-low freezer) is preferable. Frozen specimens should be shipped with dry ice, observing the following precautions:

- Protect specimens from direct contact with dry ice, as intense cold can crack the glass tubes.
- Protect specimens from carbon dioxide by sealing screw-caps with tape or by sealing tubes in plastic bags.
- Ensure that container is at least one-third full of dry ice.

**Viruses**

Obtain a large quantity (as much as possible but at least 10 ml) of diarrhoeal stool that has not been mixed with urine in a clean, dry, leak-proof container. To permit diagnosis of certain viral agents, specimens must be collected during the first 48 hours of illness. Immediately refrigerate the specimen at 4 °C (do not freeze) and send as soon as possible to the laboratory.

**Parasites**

Obtain fresh bulk-stool that has not been mixed with urine and place in a clean container. Then add preservative solution (10% formalin or 10% polyvinyl alcohol) at a ratio of 1 part stool to 3 parts preservative. If there is a delay in obtaining the preservatives, refrigerate untreated stool specimens at 4 °C (do not freeze) for up to 48 hours. Once preserved, the specimens can be stored and transported at room temperature or refrigerated.

**Vomitus**

If the person is still vomiting at the time of the investigation, collect vomitus. Let the patient vomit directly into a specimen container that has been thoroughly cleaned and boiled in water. Take the specimen directly to the laboratory. If this is not possible refrigerate (but do not freeze) the specimen.

**Serum**

In the investigation of foodborne disease outbreaks, serological examination is sometimes useful to detect the development of antibodies as a result of infection.

Blood should be obtained only by a person legally qualified to undertake the procedure; check appropriate laws. If possible, obtain blood specimens from the same patients from whom stool samples were obtained.

Submit two serum specimens – one acute-phase and one convalescent-phase – for each patient thought to have illness caused by viruses or bacteria. Obtain the acute-phase serum specimen as close to the time of onset of illness as possible (at most, within a week after onset of illness). The convalescent-phase serum specimen should be obtained 3 weeks – or, if a viral agent is suspected, 6 weeks – after the onset of illness.

Collect blood specimens from adults (15 ml) and from children (3 ml) in tubes that do not contain anticoagulants. For antibody studies the specimens need not be refrigerated during the day of the collection (unless the weather is extremely hot) but should be kept out of direct sunlight. Centrifuge the blood and send only the serum for analysis. If no centrifuge is available, store the blood specimens in a refrigerator until a clot has formed; then remove the
serum and pipette it into an empty sterile tube. Refrigerate the tubes of spun or unspun serum and ship them refrigerated.

**Urine**

Clean the area around the urethral orifice with a pad that has been pre-moistened with a 4% tincture of iodine or other appropriate antiseptic. Begin to urinate into the toilet and collect 30ml of midstream urine. The specimen should be refrigerated but not frozen.

**Other clinical specimens (food-handlers)**

**Skin lesions (boils, lesions, abscesses, secretions)**
- Clean skin with normal saline or weak disinfectant to prevent contamination of the specimen with saprophytic organisms.
- Apply pressure to the lesion using sterile gauzes and collect specimen on sterile swab, trying to obtain as much secretion as possible.
- If the lesion is closed, disinfect skin and extract specimen using sterile syringe.
- Transport immediately to laboratory at ambient temperature. If this is not possible, the specimen can be left for up to 24 hours, at which time the swab should be placed in a container of ice.

**Oropharynx and nostrils**
- Collect specimen with a sterile swab and immediately place in transport medium (Stuart’s).
- Transport immediately to laboratory at ambient temperature. If this is not possible, the specimen can be left for up to 24 hours, at which time the swab should be placed in a container of ice.

**Food and environmental specimens**

**Equipment**
- **Sterile sample containers**
  - Disposable plastic bags
  - Wide-mouth jars (100–1000 ml) with screw-caps
  - Bottles for water samples
  - Foil or heavy wrapping paper
  - Metal cans with tightly fitting lids
- **Sterile and wrapped instruments for sample collection**
  - Spoons, scoops, tongue depressors
  - Butcher’s knife
  - Forceps, tongs, spatula
  - Drill bits
Metal tubes (1.25–2.5 cm in diameter, 30–60 cm in length)
Pipettes, scissors
Moore swabs (compact pads of gauze made of 120 x 15 cm strips, tied in the centre with a long, sturdy twin or wire for samples taken from sewers, drains, pipes, etc.)
Sponges

- **Sterilizing agents**
  - 95% ethanol
  - Propane torch

- **Refrigerants**
  - Refrigerant in plastic bags
  - Heavy-duty plastic bags or bottles that can be filled with water and frozen
  - Heavy-duty plastic bags for ice

- **Food temperature measurement**
  - Bayonet-type thermometers (–20 °C to 110 °C), between 13 and 20 cm length
  - Bulb thermometer (–20 °C to 110 °C)

- **General**
  - Marking pen (waterproof)
  - Adhesive tap
  - Cotton
  - Peptone or buffered distilled water (5 ml in screw-capped tubes)
  - Electric drill (if frozen foods to be sampled)
  - Distilled water
  - Insulated chest or polystyrene box

**General**

- Collect samples aseptically. Put them into sterile jars or plastic bags to avoid any cross-contamination.
- If samples are to be examined for organophosphate pesticides or heavy metals, plastic containers should not be used. Chemicals from the plastic may leach into the food and interfere with the analysis.
- Obtain samples of approximately 200 grams or 200 ml.
- Take packaged foods to the laboratory in their original containers. Empty containers can be used to identify micro-leaks, or rinsings from these containers can be used to detect pathogens.
- Check original packages or containers for code numbers that can be used to identify the place and time of processing. Include any unopened packages or cans belonging to the same batch.
- Keep all packages not sent for laboratory examination until the end of the investigation.
- Refrigerate samples of perishable foods at 4 °C until they can be examined. Do not freeze food samples as certain pathogens (e.g. Gram-negative bacteria, vegetative forms of *Clostridium perfringens*) die off rapidly when frozen – **but** foods that were frozen when collected should be kept frozen until examined.
• Enrichment broth and dry materials require no refrigeration.

**Solid foods or mixture of two foods**

• Cut or separate out a portion of food, using a sterile knife or other utensil if necessary. Collect sample aseptically and put into a sterile plastic bag or wide-mouth jar. Collect samples from top centre, and elsewhere, as necessary, refrigerate.

**Liquid food or beverages**

Stir or shake. Collect samples using one of the following methods:

• Using a sterile utensil, transfer approximately 200 ml into a sterile container; refrigerate
• Place a long sterile tube into liquid, cover the opening with finger. Transfer liquid to the sterile container; refrigerate.
• Dip a Moore swab in the liquid or into the pipe so that liquid circulates around it. Leave in place for several hours, if possible. Transfer swab to a jar containing enrichment broth. Refrigeration is not usually necessary.
• If the liquid is not too thick, pour 1 to 2 litres through a membrane filter. Transfer the filter pad aseptically to a jar containing enrichment broth. Refrigeration is not usually necessary.

**Frozen foods**

Keep frozen, using dry ice as necessary. Transport or ship the specimen in an insulated container. Use one of the following methods:

• Send or take small frozen samples to the laboratory, without thawing or opening.
• Break frozen material into pieces using a sterilized hammer and chisel and collect pieces using a sterilized utensil.
• Using a large-diameter sterilized drill, drill from one side at the top of the container diagonally through the centre down to the bottom of the opposite side. Repeat on the other side until sufficient material has been collected.

**Raw meat or poultry**

Use one of the following methods:

• Using a sterile utensil or sterile glove, place poultry carcass or large piece of meat in a large sterile plastic bag. Add 100–300 ml enrichment broth. Remove sample and seal the bag.
• Wipe a sterile sponge over a large section of the carcass or piece of meat. Place swab in a jar containing enrichment broth.
• Moisten a swab in buffered distilled water or 0.1% peptone water. Wipe the swab over a large section of the carcass or piece of meat. Place swab in enrichment broth.
• Using a sterile glove wipe the carcass or the piece of meat with sterile gauze pads and place the pads in a jar containing enrichment broth.
• Aseptically cut a piece of meat or skin from different parts of the carcass or large piece of meat, or remove part of the carcass. Place at least 200 g of sample in a sterile plastic bag or glass jar; refrigerate.

**Dried foods**

• Insert a sterile hollow tube near one edge at the top of the container diagonally through the centre down to the bottom of the opposite side.

• Keep the top part of the sample and transfer to sterile container.

• Repeat the procedure on the other side of the container until a sufficiently large sample has been collected.

• Alternatively, use sterile spoon, spatula, tongue depressor or similar utensil to collect sample. Transfer to sterile jar.

• Keep in water- and airtight container.

**Scrapings from food equipment, pipes, filters etc.**

• Cut or collect sufficient amount of material with a sterile tongue depressor, spatula, spoon or similar utensil and place in sterile bags or wide-mouth jars.

• Refrigerate as required (depending on material, see above).

**Environmental swabs**

• Moisten swab with 0.1% peptone water or buffered distilled water and wipe over contact surfaces of equipment or environmental surfaces. Place in enrichment broth.

• **Air:** Touch plate or liquid with the device for sampling air, or let airborne particles settle on broth or agar plates obtained from microbiology laboratory. Seal with insulation tape. Refrigerate liquid samples.

• **Water:** Collect water from suspected areas, including from bottles in refrigerators, ice cubes, basins, etc. When taking water from a tap, let the water run for 10 seconds before collecting the sample. To sample water that has not been standing in proximal pipes, let water run for 5 minutes. Place sterile jar under running water and let it fill to 2.5 cm from the top. Collect 1–5 litres. Alternatively, membrane filters can be used. Moore swabs may be used to collect water samples from streams or plumbing; they should be left in place for up to 48 hours and then transferred to sterile jars containing enrichment broth.

**Specimen collection for suspected chemical toxicants**

• Avoid contamination at all cost.

• Refrigerate or freeze specimens as rapidly as possible.

• Used only screened collection material if possible. This material has been tested for extraneous contaminants, and is specially washed and packaged. If unscreened material is used, randomly select at least three of each of the containers being used (collection cup, vacutainer, etc), seal them in a clean bag and submit them with the other samples to the

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laboratory. This may allow evaluation of possible extraneous contaminants from the collection material at hand.

- Urine is the preferred specimen if the suspected toxicant is an inorganic chemical (e.g. lead, arsenic, mercury). Urine should also be collected if the toxicant is unknown. Freeze promptly.

<table>
<thead>
<tr>
<th>Suspected toxicant</th>
<th>Preferred specimen (in decreasing order)</th>
<th>Adults and children &gt;10 years (children &lt;10 years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic</td>
<td>Serum</td>
<td>Two (one) 10-ml silicon-free vacutainers; freeze</td>
</tr>
<tr>
<td></td>
<td>Urine</td>
<td>50–100 ml (25–50 ml) in prescreened collection cup; store in Wheaton glass bottle, freeze</td>
</tr>
<tr>
<td></td>
<td>Whole blood (usually heparinised)</td>
<td>One–two (one) 10-ml tubes; refrigerate</td>
</tr>
<tr>
<td>Inorganic</td>
<td>Urine</td>
<td>50–100 ml (25–50 ml) in prescreened collection cup; (no preservative if frozen promptly)</td>
</tr>
<tr>
<td></td>
<td>Whole blood (usually with EDTA)</td>
<td>One 2–3-ml prescreened container; refrigerate</td>
</tr>
<tr>
<td></td>
<td>Serum</td>
<td>One 7-ml trace elements vacutainer; freeze</td>
</tr>
<tr>
<td>Unknown</td>
<td>Serum</td>
<td>Three (one) 10-ml silicon-free vacutainers; freeze</td>
</tr>
<tr>
<td></td>
<td>Urine</td>
<td>50–100 ml (25–50 ml) in prescreened collection cup; store in Wheaton glass bottle, freeze</td>
</tr>
<tr>
<td></td>
<td>Whole blood (EDTA)</td>
<td>One 2–3-ml prescreened container; refrigerate</td>
</tr>
<tr>
<td></td>
<td>Whole blood (heparin)</td>
<td>One 7–10-ml (5–ml) heparin vacutainer; refrigerate</td>
</tr>
<tr>
<td></td>
<td>Tissues, stomach contents</td>
<td>10–50 g, no preservatives; seal in small zip-lock bag, freeze</td>
</tr>
<tr>
<td></td>
<td>Food</td>
<td>As much as possible, place in large ziplock bag, freeze</td>
</tr>
</tbody>
</table>