Sampling Protocol for Foodborne Illness Outbreak Field Investigations

The environmental health specialist (EHS) is an important part of the Epi Team at the local health department. There are times when the EHS will be expected to collect clinical samples from food service workers. For information on this process, please refer to the NC Communicable Disease Control Manual. The manual can be found at http://www.epi.state.nc.us/epi/gcdc/manual/toc.html. The EHS may also help with the development of the questionnaire, obtaining food histories, etc. For further information, consult the Epi Team website which may be accessed at www.epi.state.us/epi/gcdc.html.

If the outbreak is connected with a church supper, reunion, homecoming, etc, then getting food samples and determining who prepared what dish, how it was prepared and how it was stored prior to service may be difficult but well worth the effort. If food from the event is not available for sampling, then food from the same lot may be sampled. If there is no food to be sampled, determining who prepared the food and what steps they took in preparation is an important method of learning where there was a breakdown that could have caused the problem. This information can then be used to educate the manager and food service workers.

Preparing for Sampling

The NC State Laboratory of Public Health (SLPH) will not process food samples unless there are at least two people ill. It is important to remember that your Regional Environmental Health Specialist in the Food Protection Branch can be of great help to you. It is recommended to make them aware of the outbreak as soon as possible.

Before proceeding with the investigation, the EHS must call the SLPH at (919) 733-7367 and alert them to the possibility of incoming food samples. The SLPH can be a great resource for the EHS. Sterile sample containers, mailing packages, etc., are available through the SLPH. If the pathogen is known through clinical samples, the SLPH may be able to offer guidance for collection of specific food items with a higher probability of containing the pathogen than others. The SLPH may not process the samples you submit, but they will be able to assist you in finding out where to send them. More information on the resources available and services provided by SLPH can be found at:
http://slph.ncpublichealth.com/doc/administration/SCOPE_07.pdf

You will also need access to refrigeration for storage of the samples if it is necessary. Therefore, EHSs must create and maintain a list of weekend/after hours contacts to gain access to parts of the building where refrigeration is available.

Epi Kits

An Epi Kit is a collection of all the supplies you will need to do a field investigation. It should be stored in a place that is readily accessible to all personnel who may need to use it. This includes access to the kit on the weekends and in the evening. A checklist should be used prior to using the epi kit to ensure the necessary items are included. It is also a good idea to do this after the investigation is over to see what needs to be replaced. The following Epi Kit materials list can be used as a checklist. The minimal Epi Kit will contain the following items:

- A large cooler
- Ice packs
- Sterile containers (e.g., WhirlPak bags)
- Sterile sampling utensils
- Non-cotton swabs (resin in the cotton swab, it may affect the growth of microbes) or swab test kits.
- At least 15 sterile bags.
- At least 15 sterile spoons.
- Six sterile specimen collection containers or devices (e.g. WhirlPak bags).
- Non-sterile self-sealing plastic bags (e.g. unused Zip-Lock® bags)
- Properly calibrated temperature-measuring devices.
- Sterilizing equipment.
- One of each item listed under supporting equipment.

Supporting Equipment

- DHHS form #1814 (Copies can be printed off the SLPH Website.)
- Ballpoint pen with waterproof ink
- Fine point waterproof permanent marker such as a Sharpie
- Roll of adhesive or masking tape
- Labels and waterproof tags with eyelet and wire ties
- Tamper-evident seals*
- Matches
- Buffered distilled water or 0.1% peptone water (5 ml in screw capped tubes)
- Test tube rack
- Investigational forms

*Tamper-evident seals and labels can be obtained from laboratory supply companies or by searching for "tamper-evident seals (or labels)" online.

In addition to the items specified above, the following supplies may also be necessary to include in the Epi Kit.

Miscellaneous
- Sterile tubes

Sterilizing & Disinfecting Agents
- Ethyl alcohol 95% solution
- Propane torch
- Sodium or calcium hypochlorite

Refrigerants
- Ice
- Blue ice packs
- Rubber or plastic bags which can be filled with water and frozen
- Heavy-duty plastic bags for ice

Media
- Tubes of transport media
- Pre-enrichment or enrichment broth as appropriate

Substitute Sample Containers
There are times when the EHS is not prepared to take food samples (e.g. out of the office or the Epi Kit may be inaccessible or the supplies depleted). The following is a list of articles that may be used instead of the items typically found in the Epi kit. It is important to call the SLPH to confirm that the sampling technique is adequate.

1. The SLPH has on its website copies of the collection form you can print out and is available at: http://slph.ncpublichealth.com/Forms/DHHS-1814.pdf . You will need DHHS form #1814 (use one form for each sample collected).

2. For sample containers, sterile urine collection cups from a local hospital lab or from the health department make excellent substitutes. In the event these are not available, use an unopened box of plastic storage bags such as Ziploc® gallon baggies.

3. The use of the ladle or spoon in the product for sampling can be substituted for using a sterile collection tool. A sanitized spoon or knife, sanitized in accordance with 15A NCAC 18A .2600, the *Rules Governing the Sanitation of Food Service Establishments*, is also acceptable.

4. On each sample collected label the primary container and outside bag with the product's name to match the form used when sampling.

5. For shipping, if ice packs are not available, pack collected samples in an insulated cooler/ box with bags of ice surrounding the zip locked bags.

Sample Collection
Proper food sample collection and handling techniques are very important to ensure accurate laboratory results. The following general guidelines should be kept in mind when sampling food:

1) Collect representative samples of all foods prior to sampling because non-uniform distribution of microorganisms can occur in any food item.
2) Use proper aseptic technique during sample collection.
3) Clearly label the primary container and seal it with tamper-evident tape.
4) Deliver or ship all samples to the SLPH as quickly as possible. Unless the food being sampled is already frozen, **do not freeze** food samples because certain foodborne bacteria (such as gram-negative bacteria and *Clostridium perfringens)* die off rapidly during frozen storage. All samples that are not frozen should be stored and shipped at 40ºF. If possible, rapidly lower the temperature of the sample to 40ºF before storing it prior to shipment. The procedure for proper collection and handling of a food sample depends on the physical state and packaging of the food item being sampled. The specific labeling and sampling procedures are described in the following sections.

Specimen Identification
Each sample should be clearly labeled. If sampling food, different batches should be individually identified. Required information for the label is as follows:

- Type of food,
- date and time sampled,
- name of sampler,
- name of county in which the investigation is being conducted, and
- sample source and location taken.

The source of environmental samples should be identified on the label. This should be specific, e.g. swab from prep surface A in main kitchen. Use a separate DHHS form #1814 for each food item. Multiple samples of the same food item may be listed on a single form. When this form is completed, it should be placed in a waterproof bag and placed in the secondary container with the sample. When submitting multiple samples, at least one form should be completed with all requested information. When including forms with samples, make sure the forms are enclosed in separate plastic bags to protect them.

Solid Food or Mixture of Two or More Food Items
This category includes all solid foods, such as turkeys or roasts. In addition, it includes mixtures of two or more food items such as casseroles (e.g. lasagna), meat in gravy, etc.

1) Cut or separate portions of food with a sterile knife or other sterile implement. For a solid uniform food (e.g. roast), whenever possible collect at least four samples of 0.875 ounces (for a total of 3.5 ounces) each from the center and other representative locations throughout the food item.

2) For food mixtures, collect at least four samples of 0.875 oz (25 grams) for a total of 3.5 oz (100 grams) each from the center and other representative locations throughout the food item.
3) Transfer the sample to a sterile primary container.
4) Label the primary container, taking care to identify food from different batches.
5) Seal the primary container with tamper-evident tape.
6) Place the primary container in a secondary self-closing plastic bag such as an unused Ziploc® bag.
7) Pack the secondary container in an insulated container (e.g. a cooler) with cold packs or other refrigerant around the sample containers. Do not freeze the sample or use dry ice in the cooler.
8) Take or ship all samples to the SLPH as quickly as possible.

Liquid Food or Beverages
This category includes all beverages and liquid food such as gravy, soup, sauce, etc.

Stir or thoroughly shake the item to be sampled and collect the sample in one of the following ways:

1) Pour or ladle, with sterile utensil, at least 3.3 oz (100 ml) of the liquid into a sterile primary container; **OR**
Put a long sterile tube into the liquid and then cover the top with a gloved finger, transferring a 3.3 oz (100 ml) sample to a sterile primary container.
2) Label the primary container.
3) Seal the primary container with tamper-evident tape.
4) Place the primary container in a secondary self-closing plastic bag such as an unused Ziploc® bag.
5) Pack in an insulated container with cold packs or refrigerant around the sample container. Do not freeze or use dry ice.
6) Take or ship all samples to the SLPH as quickly as possible.

Raw/Cooked Meat or Poultry
There are several methods of collecting these samples depending on the type of sample being taken. Use **ONE** of the following methods:

1) Using a sterile utensil or sterile gloved hand, put at least 3.5 ounces (100 grams) of the chicken, poultry part or large cut of meat into a large sterile primary container; **OR**
For large cuts of meat, a sterile sponge should be wiped over a large area of the meat. Then put the sponge into a primary sterile container; **OR**
Using a sterile utensil(s), cut four 0.875 oz (25 gram) portions of meat or skin from different areas of the carcass or cut of meat and put it into a sterile container.
2) Label the primary container.
3) Seal the primary container with tamper-evident tape.
4) Place the primary container in a secondary self-closing plastic bag such as an unused Ziploc® bag.

5) Pack in an insulated container with cold packs or refrigerant around the sample container. Do not freeze or use dry ice.
6) Take or ship all samples to the laboratory as quickly as possible.

Frozen Foods

Use one of the following methods:

1) Place unopened packaged frozen food items in a sterile plastic bag (primary container). Call the SLPH for instructions if the sample is greater than one pound; **OR**
   Use sterile utensils to chip unwrapped frozen material and transfer at least 0.875 oz (25 grams) of chips taken from each of four different locations for a total of 3.5 oz (100 grams) of the frozen food item or from each food item (e.g., all foods in a frozen dinner) into a sterile primary container.
2) Label the primary container.
3) Seal the primary container with tamper-evident tape.
4) Place the primary container in a secondary self-closing plastic bag such as an unused Ziploc® bag.
5) Pack in an insulated container with cold packs or refrigerant around the sample container and ship or store on dry ice to maintain frozen state.
6) Take or ship all samples to the SLPH as quickly as possible.

Reduced Oxygen Packaging

One organism of concern when dealing with foods in reduced oxygen packaging (ROP) is *Clostridium botulinum*. This is the bacteria that cause botulism. As this is a rare occurrence in the United States, it takes only one case to be considered an outbreak. Testing for *C. botulinum* must be conducted at the Centers for Disease Control laboratories. This must be set up through the SLPH. The second organism of concern is *Listeria monocytogenes*. The SLPH does not test food samples for *Listeria monocytogenes*. For foods that have been packaged prior to preparation for service in ROP use the following method:

1) Obtain a sample of 3.5 oz (100 grams) of the suspected food from the same ROP.
2) If possible, submit an unopened package that was processed in the same lot as the suspect food.
3) Label the primary containers.
4) Seal the primary containers with tamper-evident tape.
5) Place the primary containers in secondary self-closing plastic bags such as an unused Ziploc® bag.
6) If testing for C. botulinum, package to transport to the CDC Lab as instructed by the SLPH.
7) Pack in an insulated container with cold packs or refrigerant around the sample container. Do not freeze or use dry ice.
8) Take or ship all samples to the SLPH as quickly as possible. If the sample is being tested for C. botulinum, ship to the CDC lab as instructed by the SLPH.

Environmental or Equipment Surface Samples

NOTE: Remember to contact the SLPH or your Regional Environmental Health Specialist before taking any environmental samples.

1) If available, use a commercial swab collection/transport system. Consult with SLPH for appropriate transport media if a commercial swab collection kit is unavailable. (A sterile non-cotton swab moistened with sterile 0.1% peptone water or buffered distilled water may be used.)
2) Swab the food contact surfaces of the equipment or environmental surfaces. Put swab in a sterile container with enrichment broth.
3) Label the primary container (container with swab) with the following:
   - date and time sampled
   - equipment or sample source and location sampled,
   - name of sampler, and
   - name of county in which the investigation is being conducted.
4) Seal the primary container with tamper-evident tape
5) Complete a DHHS Form #1814 and place with the primary container in a secondary self-closing plastic bag such as an unused Ziploc® bag.
6) Pack in an insulated container with cold packs or refrigerant around the sample container. Do not freeze or use dry ice.
7) Take or ship all samples to the SLPH as quickly as possible.