Washington State Department of Health

Foodborne Disease and the Public Health Labs:

A Foodborne Pathogen Quick-Reference Guide for Food Sanitarians

PUBLIC HEALTH
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Disclaimer

This document is intended only as a basic guide for general information about the organisms food sanitarians may encounter. It is not intended to be an exhaustive reference. The information it contains, including organism information, laboratory sampling requirements, and tests offered, are subject to change at any time. Food sanitarians should always consult the most recent Public Health Laboratories Directory of Services for the most current information. The current PHL Directory of Services and most current version of this document are both available at www.doh.wa.gov/EHSPHL/PHL/default.htm under "Publications."

Outbreak Contacts

When local health jurisdictions (LHJs) suspect a foodborne illness outbreak, they must notify the Washington State Department of Health (DOH) Communicable Disease Epidemiology (CD Epi) section immediately at 206-418-5500 or 1-877-539-4344. They should also contact the DOH Food Safety Program at 360-236-3385. Both CD Epi and the Food Safety Program will assist LHJs during an outbreak investigation. CD Epi will determine when it is appropriate to collect food or patient specimens to send to the DOH Public Health Laboratories (PHL) for testing. **Do not send ANY samples to the lab without consulting with CD Epi first – samples that arrive without notification will be rejected.** In general, it is better to collect samples early on. It is easier to discard excess samples than it is to locate appropriate samples later. Always refer to the current DOH Directory of Services, available online at http://www.doh.wa.gov/EHSPHL/PHL/Forms/directory_of_services.pdf, for the most current information on how and where to send the samples. If you have any questions, please call CD Epi at 206-418-5500 or the PHL Food Lab at 206-418-5442.

Helpful Phone Numbers

CD Epidemiology: 1-877-539-4344 or 206-418-5500
Kathryn MacDonald, Food Epidemiologist: 206-418-5432
Food Safety Program: 360-236-3385
Public Health Laboratories Front Desk: 206-418-5400
PHL Food Lab: 206-418-5442
PHL Enteric Bacteriology Lab: 206-418-5456
PHL Mail Room: 206-418-5579
PHL Parasitology Lab: 206-418-5469
PHL Special Bacteriological Pathogens Lab: 206-418-5452
PHL Molecular Lab: 206-418-5462

Aseptic Technique for Food Collection

Purpose: Food samples must be handled with aseptic technique to prevent them from being contaminated by the collector or the environment. Using aseptic technique will prevent the introduction of organisms into the specimen. The diagnosis of pathogens in a specimen is only as valid as the method used to collect and ship the specimen. In addition, aseptic technique will protect the sampler from potential pathogens in the samples.

Procedure: Wash hands before collecting samples and wear clean gloves during collection. Use sterile equipment to collect samples. **DO NOT** touch specimens with hands, gloves, non-sterile equipment, or other contaminated items. Place specimens in sterile containers or whirl-pak bags that seal completely. When feasible, send food specimens in the original packaging or storage containers. Seal these in zip-close or other sealable bags for transport. Extra sealable bags may be used to protect specimens in any type of container. When shipping on ice, use frozen gel packs, **NOT** wet ice, to prevent contamination. Protect the samples from direct contact with gel packs.

Safety: Make sure that samples will not leak in transit. Ship in hard-sided containers such as coolers or cardboard boxes in good condition. Follow specimen storage guidelines for the suspected pathogen. Ship specimen to the PHL as quickly as possible. Remember that shipping regulations for human specimens are much more stringent than for food samples (see Packaging and Shipping on page 4). Wash hands thoroughly with soap and water when finished.

Documentation: Clearly label all samples and fill out all laboratory forms as completely as possible. Fill out the appropriate form for the lab that the sample will go to, i.e. PHL Food Lab, PHL Enteric Bacteriology Lab, PHL Parasitology Lab, etc. Be sure to fill out a separate form for each sample sent and ensure that samples can be matched to the correct forms. Do not send unlabeled specimens, specimens without forms, or multiple specimens on the same form. Protect all forms from moisture.

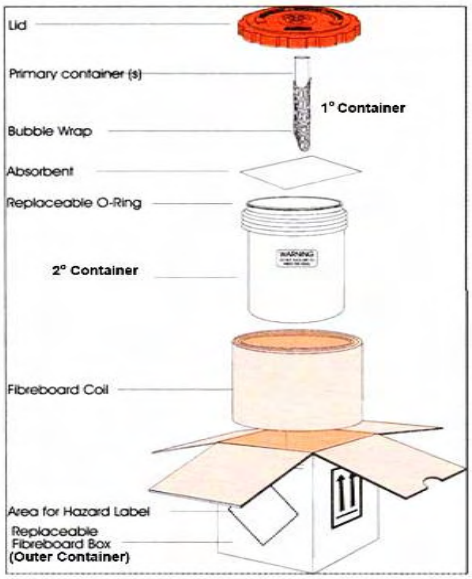
Packaging & Shipping

Federal laws govern the shipping of potentially infectious substances derived from humans, such as stool, blood, or vomitus. **It is the shipper's responsibility to ensure that packages being shipped meet current regulations.** Both the institution sending the package and the individual person responsible for packaging and shipping the package may be held liable for violations. For current regulations please visit the Code of Federal Regulations website: http://www.gpoaccess.gov/cfr/index.html.

DO NOT PACKAGE OR SHIP CLINICAL SPECIMENS UNLESS YOU HAVE BEEN SPECIFICALLY TRAINED TO DO SO ACCORDING TO FEDERAL REGULATIONS!

This page is only a "cheat sheet" reminder for personnel that have already had specific training. The PHL Training Program offers a training course concerning the shipping of infectious materials. For more information please visit their website: http://www.doh.wa.gov/EHSPHL/PHL/Training.

Specimens derived from humans, such as blood, stool or vomitus, are known as clinical specimens. Clinical specimens are classified as "Category B Substances" for shipping purposes. Federal regulations for shipping Category B substances must be followed using the triple packaging method described below. Only packaging systems certified for use with Category B Substances may be used.



Federal Department of Transportation definition of Category B Substances:
"An **infectious substance** that is not in a form generally capable of causing permanent disability or life-threatening or fatal disease in otherwise healthy humans or animals when exposure to it occurs."

Triple Packaging Using a Certified System for Category B Substances:

Primary receptacle - contains the specimen and must be leakproof or sift proof.

Secondary packaging - contains absorbent material, may contain multiple primary receptacles, and has a biohazard label and list of contents.

Outer packaging - outer dimensions must be a minimum 4" x 4", made of rigid material, and have appropriate labels and identification.

Specimens must be clearly and completely identified. Include a specimen submission form for the appropriate PHL laboratory with **EVERY** sample. Forms for all PHL labs may be obtained by calling the PHL Mailroom at (206) 418-5579. Include as much of the following information as possible on the form:

Name and address of submitting organization
Collector's name
Date, place, time of collection
The reason for testing and organism to be tested for
Specimen description
Specimen condition and other notes
Name and address of product manufacturer or patient
Lot number and dealer or distributor information
Patient history including symptoms and travel

Food samples are not covered by federal shipping regulations, however appropriate care should be taken when packaging them to prevent any leakage or contamination. The PHL will reject any leaking containers. In general, leave the food sample in the original container, if it is available. Give each sample a securely attached label and a sample submission form. Keep frozen samples frozen. Keep samples cold if the sample is already refrigerated or if the sample is at risk of spoiling during shipment, otherwise ship at ambient temperature.

The CD Epi staff screens all complaints of foodborne illness before food samples are tested in the laboratory. **Food samples should not be submitted to the PHL without the approval of CD Epi.** Call 206-418-5500 (24-hour telephone number) if you would like to discuss food testing with CD Epi staff.

Bacillus cereus

Organism Profile	*Bacillus cereus* is a sporeforming bacterium. It is Gram positive and aerobic, with large, rod-shaped cells. Its spores are highly resistant to environmental stressors such as heat and desiccation. It is commonly found in soil and the environment.
Associated Illness	*B. cereus* causes foodborne intoxication due to two distinct toxins produced by the bacteria. These toxins cause two recognized types of illness. Diarrheal illness is associated with a heat-labile protein. Emetic (vomiting) illness is associated with a heat-stable protein. Diarrheal type food poisoning caused by *B. cereus* is similar to that caused by *Clostridium perfringens*. The onset of symptoms occurs 6 to 24 hours after consumption of contaminated food. Symptoms may include watery diarrhea, abdominal cramps, and pain. Nausea may accompany diarrhea, but vomiting is rare. Symptoms are self-limiting and rarely last more than 24 hours. Emetic type food poisoning caused by *B. cereus* is similar to *Staphylococcus aureus* intoxication. The onset of symptoms occurs 0.5 to 6 hours after consumption. Nausea and vomiting are characteristic of this illness, although occasionally abdominal cramps and/or diarrhea may occur. Symptoms are self-limiting and usually last less than 24 hours. Complications have generally not been associated with the illnesses caused by the diarrheal or emetic toxins produced by *B. cereus*, but the bacteria may cause other forms of disease. *B. cereus* has been associated with gangrene, meningitis, septicemia, and other infections.
At-Risk Populations	All people are believed to be susceptible to *B. cereus* food poisoning.
Implicated Foods	Diarrheal illness has been associated with a wide variety of foods, including meats, milk, vegetables, fish, and shellfish. Emetic illness outbreaks have often been associated with rice products, although other starchy foods such as potato, pasta, and cheese products have been implicated as well. Food mixtures such as sauces, puddings, soups, casseroles, pastries, and salads have also been incriminated in food poisoning outbreaks.
Preventative Measures	*B. cereus* spores may not be killed by conventional cooking methods, so food should not remain at ambient temperature after cooking to prevent proliferation of the bacteria and toxin production. Hot food should be reheated quickly and kept at greater than 60° C. Food should be cooled quickly in shallow containers to less than 10° C and covered if it will be stored for more than 2 hours.
Sample Collection	Food and stool specimens should be collected for testing and both should be sent to the PHL Food Lab. Vomitus may also be collected. A PHL Food Lab form should be filled out for each sample sent and included with the sample. Send a minimum of 25 grams of each specimen, preferably more. If possible, send samples of both patient stool/vomitus and the suspect food they ate so the results can be matched to each other. Collect and package each specimen with care to avoid contamination of other specimens. Food samples should be stored under refrigeration and shipped between 0° C and 6° C. Do not freeze. If the samples are already frozen at collection, do not thaw. Keep them frozen during shipping. Stool specimens should be unpreserved and as fresh as possible, ideally less than 24 hours old. Stool should not be sent in any kind of enrichment media, preservative, or other diluent. Vomitus should be collected immediately and neutralized with baking soda until it stops bubbling. Store and ship specimens between 0° C and 6° C. Do not freeze. Stool and vomit specimens are clinical specimens and must adhere to all applicable shipping regulations (see Packaging and Shipping on page 4). An accurate count of organisms in a sample may not be obtained if the samples are temperature abused, including freezing and heating above 6° C. If samples are suspected of being temperature abused, note it on the lab form to alert the lab of the potential influence on the results.
Laboratory Testing and Results	Since *B. cereus* spores are commonly found in soil and the environment as well as in raw, dried, and unprocessed foods, isolating it in low numbers from food or stools is not unusual and does not indicate it as the cause of illness. However, the presence of large numbers of *B. cereus* (greater than 10^6 colony forming organisms per gram) in a food is indicative of active growth and proliferation

	of the organism and is consistent with a potential hazard to health. Confirmation of *B. cereus* as the etiologic agent in a foodborne illness outbreak requires either isolation of the same *B. cereus* serotype from a suspect food and the feces or vomitus of the patient, or isolation of greater than 10^6 colony forming organisms per gram of *B. cereus* from a suspect food or from the feces or vomitus of the patient. The PHL Food Lab uses quantitative tests and biochemical confirmations to detect *B. cereus* in food and stool samples. Results are generally available within 14 working days. Isolates will be genetically typed using pulse-field gel electrophoresis (PFGE) in the PHL PFGE Lab to match them to other outbreak samples.

Campylobacter jejuni

Organism Profile	*Campylobacter jejuni* is a microaerophilic bacteria, which means it requires low levels of oxygen to grow, but cannot survive without oxygen or in a high concentration of oxygen. It is a Gram negative, slender, curved, motile, rod-shaped organism. It is relatively fragile and sensitive to environmental stresses such as oxygen concentration, drying, heating, disinfectants, and acidic conditions. Because of its microaerophilic characteristics, the organism requires 3% to 5% oxygen and 2% to 10% carbon dioxide for optimal growth conditions.
Associated Illness	*C. jejuni* causes an infection known as campylobacteriosis. The incubation period is 1 to 10 days, although the onset of symptoms is usually between 2 and 5 days. Illness is often over within 2 to 5 days, and usually lasts no more than 10 days. The major symptom is diarrhea that may be watery or sticky and may contain mucus, blood, and fecal leukocytes (white blood cells). Other symptoms may include fever, abdominal pain, nausea, headache, and muscle pain. Infections are usually self-limiting, but relapses may occur. Antibiotic therapy may shorten the duration of symptoms and the length of time that infected individuals shed the bacteria. Complications of campylobacteriosis include Guillian-Barre syndrome, reactive arthritis, and meningitis. Some cases mimic acute appendicitis and may lead to unnecessary surgery.
At-Risk Populations	Children under 5 and young adults are the groups that most commonly develop campylobacteriosis.
Implicated Foods	*C. jejuni* frequently contaminates raw chicken and other poultry. Surveys have found 20 to 100% of analyzed retail chickens to be contaminated. *C. jejuni* is a normal flora of the intestinal tract of many healthy birds and contamination of the meat occurs during slaughter. Raw milk and non-chlorinated water have been additional sources of infection. The bacteria may be carried by healthy cattle and even flies on farms. Contact with infected puppies or kittens has also been linked to infection.
Preventative Measures	The infective dose of *C. jejuni* is small, so the bacteria need to be completely eliminated from foods before consumption. Properly cooking poultry, pasteurizing milk, and chlorinating or boiling drinking water will kill the bacteria. Cross-contamination should be avoided during the preparation of foods, particularly the reuse of cutting boards and knives. Hands should be thoroughly washed after contact with any animal.
Sample Collection	Send stool swab specimens to the PHL Enteric Bacteriology Lab in Cary-Blair media at ambient temperature. Follow all applicable shipping regulations for clinical samples (see Packaging and Shipping on page 4) and include a completed PHL Enteric Bacteriology Lab form with every sample. The PHL Food Lab is not yet equipped to test food for *C. jejuni*, although it is in the process of developing the capability. At this time, food samples will not be accepted.
Laboratory Testing and Results	*C. jejuni* is usually present in high numbers in the diarrheal stools of infected individuals, but enrichment and isolation requires special antibiotic-containing media and a microaerophilic atmosphere of 5% oxygen. Results should be available from the PHL Enteric Bacteriology Lab in 6 working days.

Clostridium botulinum

Organism Profile	*Clostridium botulinum* is a sporeforming, Gram positive, rod-shaped bacterium that produces a powerful neurotoxin. It is anaerobic, so it only grows in the absence of oxygen. Its spores are very resistant to heat and other environmental stressors and are widely distributed in nature. There are seven recognized types of *C. botulinum* toxin, A, B, C, D, E, F, and G, although not all of them are regularly associated with human disease.
Associated Illness	*C. botulinum* causes an intoxication known as botulism. Three types of botulism are recognized: foodborne, intestinal (also known as infant), and wound. Foodborne botulism is caused by consumption of foods containing the neurotoxin produced by *C. botulinum*. Intestinal botulism occurs when a person, usually an infant, ingests *C. botulinum* spores that then colonize its intestine and begin to produce the toxin inside the body. Wound botulism occurs when *C. botulinum* infects a wound that has anaerobic conditions and begins producing toxin. It has similar symptoms to foodborne botulism, but is not related to food. Foodborne botulism is a severe type of foodborne intoxication that occurs when a person ingests botulinal neurotoxin formed during the growth of the organism. Just a few nanograms of toxin may cause illness. The toxin is heat labile and can be destroyed by boiling, although spores may survive to germinate and produce more toxin, if the food is mishandled. The onset of symptoms is usually 18 to 36 hours after ingestion of toxin, although it may range from 4 hours to eight days. The speed of onset is often related to the dose of toxin ingested. Neurological symptoms are progressive. Early signs are constipation, weakness, vertigo, and double vision, advancing to paralysis of the eyes, face, and throat, difficulty speaking and swallowing, then difficulty breathing and respiratory arrest. It can be rapidly fatal without medical intervention. Intestinal botulism occurs when *C. botulinum* spores colonize the intestine of a patient and begin producing toxin. Infants less than 12 months old are most at risk, since their intestinal flora is not fully developed, but adults with altered gastrointestinal anatomy or microflora are also at risk. The incubation period between ingestion of spores and onset of symptoms is not known. In infants, progressive neurological symptoms often start with constipation, then lethargy and listlessness, poor feeding, and an altered or wailing cry, finally extending to loss of head control, generalized weakness ("floppy baby"), and potential respiratory insufficiency or arrest. Intestinal botulism may be the cause of some cases of sudden infant death syndrome (SIDS). In adults, symptoms are similar to those of foodborne botulism. The condition may be misdiagnosed, but the number of cases confirmed has increased due to greater awareness of health officials. The incidence of botulism is low, but is regarded with concern due to its high mortality rate if not treated quickly and properly. Death in botulism patients is caused by inhibited respiration leading to asphyxia. Medical treatment generally involves intensive supportive therapy, including mechanical respiratory assistance if necessary, and equine botulism antitoxin or human botulism immune globulin.
At-Risk Populations	All people are believed to be susceptible to foodborne intoxication with *C. botulinum* toxin. Infants less than 12 months old and adults with altered gastrointestinal anatomy or microflora are at risk for intestinal botulism.
Implicated Foods	The types of foods involved in botulism vary according to food preservation and eating habits in different regions. Most outbreaks in the United States are associated with inadequately processed home-canned foods, but commercial products are sometimes involved. Meat products, seafood products, and canned vegetables have been the most frequently implicated items. Any food with low acidity (pH above 4.6) and an anaerobic environment is conducive to the growth of *C. botulinum* and the production of toxin. Botulism may occur if the initial processing and preservation of the food allows the survival of spores and the final product is not heated sufficiently before consumption. Honey is often associated with intestinal botulism as a potential source of spores in an infant's diet. Toxin type isolated from infants usually coincides with the type found locally in the soil and environment.
Preventative Measures	*C. botulinum* spores are widely distributed in nature, including in soil, sediment, and the intestinal tract of mammals, fish, and shellfish. Anaerobically packaged food must either be treated to destroy spores, or be acidic enough to prevent growth. Botulism toxin is heat labile and can be destroyed if heated to 80° C (176° F) for 10 minutes or longer.

	A single case of botulism is enough to trigger a foodborne illness outbreak investigation. Due to the severity of the disease, the FDA initiates a Class I recall of any food associated with a botulism risk.
Sample Collection	Food, food remnants or containers, stool, blood serum, or other clinical samples should all be sent to the PHL Special Bacteriological Pathogens lab. 10g to 50g of specimen is preferred for most types of samples. Specimens should be shipped between 0° C and 6° C according to all applicable shipping regulations (see Packaging and Shipping on page 4) and should not be frozen. All specimens must be accompanied by a completed PHL Reference Bacteriology Laboratory Request form. If the patient has received any drugs or treatment, it should be noted on the lab form.
Laboratory Testing and Results	The PHL Special Bacteriological Pathogens lab will test specimens for the presence and type of botulinal toxin and will culture the *C. botulinum* organism as well. Confirmation and typing of toxin takes 4 days. Culture will take 7 to 14 days.

Clostridium perfringens

Organism Profile	*Clostridium perfringens* is an anaerobic, Gram positive, sporeforming, rod-shaped bacterium. It is widely distributed in the environment and frequently occurs in the intestines of humans and many domestic and wild animals. Spores of the organism persist in soil, sediments, and areas subject to human or animal fecal pollution.
Associated Illness	*C. perfringens* food poisoning is one of the most common foodborne intoxications in the United States. It is caused by an enterotoxin produced by the bacteria in the alkaline environment of the intestine during the sporulation process. Usually, it causes a mild illness, but certain strains may produce a severe disease called enteritis necroticans, also known as pig-bel disease. *C. perfringens* is also a causative agent of gas gangrene and septicemia related to wound infections and abscesses. The incubation period for common perfringens food poisoning is from 6 to 30 hours, with the onset of symptoms usually occurring between 7 and 12 hours after the ingestion of contaminated food. Symptoms are similar to diarrheal illness caused by *Bacillus cereus* and include intense crampy abdominal pain, diarrhea, and foamy, foul-smelling stools. Nausea or vomiting may occur in up to one third of patients. Fever is absent. Patients are usually fully recovered in two or three days, however, mild symptoms may persist for up to two weeks, and dehydration or other complications are concerns in the elderly and immunocompromised individuals. Pig-bel disease is often fatal. It occurs when *C. perfringens* proliferates and causes infection and necrosis of the intestine. The organism may be present as part of the normal intestinal flora or be ingested with contaminated pork or other meat. The condition is associated with overeating, poor nutrition, and eating foods rich in trypsin inhibitors, such as the sweet potatoes eaten at pig feasts. The disease is seen mostly in children and is very rare in the United States.
At-Risk Populations	Most people are susceptible to perfringens food poisoning. The elderly and immunocompromised individuals are most at risk for a longer or more serious illness. Children are most at risk for pig-bel disease.
Implicated Foods	Meats, meat products, stews, and gravy are the foods most frequently implicated. Institutional settings, such as cafeterias in schools, hospitals, nursing homes, and prisons where large quantities of food are prepared hours before serving, are the most common situations where perfringens poisoning occurs.
Preventative Measures	In most instances, the actual cause of poisoning by *C. perfringens* is temperature abuse of prepared foods, particularly meat products. Symptoms are caused by ingesting large numbers of vegetative cells. Small numbers of spores often survive proper cooking processes and can multiply to illness-causing levels during improper cooling and storage of prepared foods. The organisms are allowed to survive by incomplete reheating. To avoid the proliferation of the organism to infective-dose levels, foods should be served hot from the initial cooking. If this is not possible, food must be cooled quickly in shallow pans, and thoroughly reheated to an internal temperature of 75° C or higher.
Sample Collection	Food and stool specimens should be collected for *C. perfringens* testing and both should be sent to the PHL Food Lab. Send at least 50g of stool and 100g of food. If possible, send specimens of both patient stool and the suspect food the patient ate so that the results can be matched to each other.

	The specimens should be kept cool, but above 10° C, since a rapid die-off of vegetative cells occurs at temperatures below 10° C. Do not freeze or refrigerate. If the samples are frozen at collection, do not thaw and keep them frozen during shipping. Food and stool should be collected in sterile containers. Stool should be as fresh as possible, ideally less than 24 hours old, and should not be sent in any kind of enrichment media, preservative, or other diluent. A completed PHL Food Lab form should accompany each specimen, and specimens should be sent according to all applicable shipping regulations (see Packaging and Shipping on page 4). An accurate count of organisms in a sample can not be obtained if the samples are temperature abused, including freezing or chilling below 10° C. If samples are suspected of being temperature abused, note it on the lab form.
Laboratory Testing and Results	The PHL Food Lab uses standard bacteriological culturing methods to isolate and confirm *C. perfringens* in food and stool related to a suspected outbreak. *C. perfringens* spores are commonly present in the environment, in food and in the human digestive tract. Therefore, the presence of *C. perfringens* in a suspect food or patient stool specimen does not necessarily indicate that it is the causative agent of illness. At the lab, the bacterial load is calculated in colony forming units per gram (cfu/g) of sample. A bacterial count of 10^5 cfu/g in food or 10^6 cfu/g in stool is required for *C. perfringens* to be considered the cause of an outbreak. Alternatively, lower numbers may be considered causative if the food and stool tested contain *C. perfringens* of the same type using PFGE genetic typing performed by the PHL PFGE Lab.

Escherichia coli: Coliforms and Diarrheogenic *E. coli*

Organism Profile	*Escherichia coli* is a Gram negative, aerobic, rod-shaped bacterium. It is universally found in the digestive tract of humans and animals. It does not survive in the environment for extended periods of time. Because of these traits, *E. coli* is a prime indicator of fecal contamination in food and water sources. Generally, *E. coli* is not pathogenic, although it may cause infections such as bacteremia, urinary tract infections, or wound infections. There are currently six recognized classes of *E. coli* strains known to cause diarrhea: enterohemorrhagic *E. coli* (EHEC) (also referred to as Shiga toxin-producing *E. coli* [STEC]), enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAggEC), and diffusely adherent *E. coli* (DAEC).
Associated Illness	Fecal coliform testing does not search for disease-causing *E. coli*, however, the presence of fecal coliforms in food or water indicates an increased risk for the presence of disease-causing organisms, such as diarrheogenic *E. coli*, *Salmonella* species, or Norovirus. Foodborne illness caused by diarrheogenic *E. coli* is due to intestinal infection with the organism, not a foodborne intoxication. The type and severity of the illness varies with the class of *E. coli* infecting the patient. EHEC is the *E. coli* class most commonly associated with serious disease in the United States. This class contains *E. coli* 0157:H7, the most widely recognized strain of diarrheogenic *E. coli*. EHEC produce Shiga toxins identical to those produced by *Shigella*. The incubation period lasts from 2 to 8 days, although 3 to 4 days is most common. Diarrhea may range from mild to severe and bloody. Abdominal cramps may occur and fever is usually absent. A particularly dangerous complication of EHEC infection is the development of hemolytic uremic syndrome (HUS), which can cause anemia, kidney failure and death. HUS occurs in approximately 6% of EHEC patients, with children under 5 years old being most at risk. The reservoir for *E. coli* 0157:H7 is cattle and possibly deer. The infection is easily spread due to the low infectious dose. ETEC is an important cause of infant diarrhea in developing countries. Consequently, adults in these areas generally have some immunity. Fecal-oral contamination of food or water can lead to diarrhea in non-immune adults. This is a common cause of traveler's diarrhea. Humans are the main reservoir for this class of *E. coli*. There is a 10 to 72 hour incubation period and the illness usually lasts less than 5 days, though it may be prolonged. It causes non life-threatening diarrhea in healthy adults, but is a leading cause of infant mortality in developing countries. Symptoms include watery diarrhea, abdominal cramps, occasionally nausea and headache, but rarely vomiting

	or fever. EPEC is another common cause of infant diarrhea in developing countries. It almost always occurs in infants less than 1 year old. It causes a watery diarrhea that contains mucus and may be severe, but not bloody. Vomiting and fever may also occur. Infection may cause chronic diarrhea leading to malnutrition and weight loss or growth retardation. It has a high fatality rate where medical assistance is unavailable or inadequate. It is uncommon in exclusively breast-fed infants and may be transmitted by contaminated formula and weaning foods. EIEC infection causes an inflammatory disease of the gut mucosa as the bacteria invade the cells of the colon, similar to *Shigella*. It has a 10 to 18 hour incubation period. It causes diarrhea that is generally watery, but may be bloody. Contaminated food or water are the most likely sources of infection. The two other classes of diarrheogenic *E. coli*, EAggEC and DAEC, are uncommon and information about them is scarce. They are associated with illness in infants and young children.
At-Risk Populations	Infants and young children are most at risk from all types of diarrheogenic *E. coli*. Children, the elderly, and immunocompromised individuals are those most at risk of serious illness due to EHEC infection in the United States, with children under 5 years old being most at risk of developing HUS. Travelers from areas with adequate sanitation to areas without adequate sanitation are at risk of illness from ETEC.
Implicated Foods	*E. coli* 0157:H7 and other EHEC strains are commonly carried by cattle, leading to the most commonly implicated food, ground beef. Raw milk, unchlorinated water, unpasteurized apple cider, and raw vegetables have also caused EHEC outbreaks. Infected food handlers may contaminate prepared food with any class of *E. coli*. Food or water contaminated with sewage or other sources of fecal coliforms may also contain any class of *E. coli*.
Preventative Measures	Clean water, basic sanitation, and strict food handling standards, including pasteurization and minimum cooking temperatures, are the most effective preventative measures for all types of *E. coli*.
Sample Collection	Food samples should be sent to the PHL Food Lab. 100g of specimen is preferred and a completed PHL Food Lab form must be included. Perishable food samples, such as produce, should be stored and shipped between 0° C and 6° C. Non-perishable items, such as dry mixes, may be sent at ambient temperature. Do not freeze. If samples are already frozen at collection, do not thaw. Keep them frozen during shipping. Stool swab specimens should be sent to the PHL Enteric Bacteriology Lab in Cary-Blair media at ambient temperature with a completed PHL Enteric Bacteriology form. All applicable shipping regulations must be followed (see Packaging and Shipping on page 4).
Laboratory Testing and Results	The PHL Food Lab can test food and water for fecal coliforms and *E. coli* 0157:H7. Coliform testing is a presence/absence test that does not require isolation of the organisms. Coliforms, fecal coliforms, and *E. coli* levels are quantified. Results are usually available in 5 to 10 business days. Coliform testing is only performed on specimens reasonably associated with coliform contamination, such as well water or shellfish. In both the PHL Food Lab and PHL Enteric Bacteriology Lab, *E. coli* 0157:H7 is isolated, confirmed using biochemical tests, serotyped, and genetically typed using PFGE at the PHL PFGE Lab. The PHL Enteric Bacteriology Lab can also test for Shiga toxin production and other diarrheogenic *E. coli* strains upon request. Results should be available in 7 to 14 business days.

Listeria monocytogenes

Organism Profile	*Listeria monocytogenes* is a short, Gram positive, rod-shaped, motile, aerobic bacterium. It is non-sporeforming, but is hardy and resistant to the effects of freezing, drying, and heat. It can grow slowly at common refrigeration temperatures, as low as 3° C. It is widely found in the environment, including in soil, water, sewage, animal feed, animal products, and asymptomatic human carriers.
Associated Illness	*L. monocytogenes* causes an infection known as listeriosis. Listeriosis includes a variety of potential symptoms, including diarrhea, septicemia, and meningoencephalitis. The incubation time is not definitively known, but is suspected to be from 3 to 70 days, with a median incubation time of about 3 weeks. Listeriosis poses the greatest risk to pregnant women and their babies. Infection may cause high fevers and spontaneous abortion or premature birth in pregnant women. The bacteria can travel transplacentally, even in asymptomatic mothers. Infants may be stillborn, born with listeriosis, or develop listeriosis as a newborn. Listeriosis may cause septicemia or meningitis in the infant, with fatality rates as high as 80%. The mother usually makes a full recovery. *L. monocytogenes* has the ability to migrate through the gut wall into the bloodstream, causing particularly serious disease in immunocompromised patients and the elderly. Consuming food containing high numbers of bacteria may cause gastrointestinal illness in healthy adults.
At-Risk Populations	Pregnant women, newborn infants of infected women, the immunocompromised, and the elderly are at risk for serious illness from listeriosis. Healthy adults may also develop gastrointestinal symptoms after consuming foods highly contaminated with *L. monocytogenes*. There is some evidence that gastric acidity and antacid use may also be risk factors for developing listeriosis.
Implicated Foods	*L. monocytogenes* has been associated with raw or insufficiently pasteurized milk and milk products, soft cheeses (particularly homemade, Mexican-style cheeses), ready-to-eat (RTE) meats such as lunch meat, ice cream, raw vegetables, and raw meats and fish. The common presence of *L. monocytogenes* in the environment and its ability to grow at refrigeration temperatures means that all raw and RTE foods are at risk of *L. monocytogenes* contamination.
Preventative Measures	Pregnant women and other at-risk individuals should avoid consuming soft cheeses, raw milk, and RTE meats such as lunchmeat and hotdogs. Raw vegetables should be washed thoroughly. Leftovers should be heated until steaming hot. Meats should be thoroughly cooked.
Sample Collection	Food samples to be tested for *L. monocytogenes* should be sent to the PHL Food Lab with completed lab forms. Send at least 50g of food. Store and ship samples between 0° C and 6° C. Do not freeze. If samples are already frozen at collection, do not thaw. Keep them frozen during shipping. Clinical specimens are not routinely tested for *L. monocytogenes* at the PHL. Requests will be handled on a case-by-base basis in consultation with CD Epi and the PHL Enteric Bacteriology Lab.
Laboratory Testing and Results	*L. monocytogenes* is a slow-growing organism and may be present in a contaminated food in low numbers. The PHL Food Lab will culture the bacteria and isolates will by typed at the PHL PFGE Lab. Testing may take up to 30 working days.

Salmonella Species

Organism Profile	*Salmonella* species are Gram negative, rod-shaped, aerobic, usually motile, non-sporeforming bacteria. There are only two species of *Salmonella*, *S. enterica* and *S. bongori*, but more than 2400 distinct serotypes within these species. *Salmonella* strains pathogenic to humans are generally *S. enterica* species, subtype I serotypes. *Salmonella* bacteria are commonly found in a wide variety of animals, particularly birds and reptiles. They may also be found in mammals, water, soil, insects, and seafood, and are easily spread around a kitchen through cross-contamination of utensils and surfaces.
Associated Illness	*Salmonella* species are known to cause two types of foodborne illness, a gastrointestinal infection known as salmonellosis and a systemic infection known as typhoid or paratyphoid fever. Salmonellosis may be caused by many *S. enterica* subtype I serotypes and is usually spread by inadequately cooked or cross-contaminated food. Typhoid fever is caused by *S. enterica* serotype Typhi (*S.* Typhi) and paratyphoid fever is caused by *S.* Paratyphi. Typhoid fever is most commonly spread by infected food workers through the fecal-oral route. Salmonellosis has an incubation period ranging from 6 to 72 hours, but illness most commonly begins 12 to 36 hours after consumption of contaminated food. The onset of symptoms is sudden and usually includes fever, headache, diarrhea, abdominal pain, nausea, and occasionally vomiting. Symptoms, particularly diarrhea and anorexia, may last a week or longer. The bacteria may be shed in the stool for months. Dehydration may be severe and is a particular risk for infants and the elderly. The bacteria may penetrate the gut wall and cause complicating infections, including abscesses, endocarditis, meningitis, septic arthritis, and septicemia. Death is uncommon, usually occurring in infants and young children, the elderly, and the immunocompromised. However, salmonellosis has a high cost in terms of illness and missed work and school days for healthy adults and older children. Typhoid fever has an incubation time from 3 days to 1 month, usually from 8 to 14 days. Symptoms may include fever, severe headache, malaise, anorexia, constipation or diarrhea, splenomegaly, and cough. Severe cases may develop intestinal ulcers or perforations and cerebral involvement, including mild deafness and mental dullness. Untreated, the fatality rate may be as high as 20%. Proper treatment drops the fatality rate to less than 1%. Paratyphoid fever is similar, but tends to be milder. The main reservoir is humans and an asymptomatic, permanent carrier state is possible (Typhoid Mary). In endemic areas with poor sanitation, typhoid fever is a common childhood illness.
At-Risk Populations	All people are believed to be susceptible to salmonellosis, although infants, the elderly, and immunocompromised individuals have a greater risk of severe disease and complications. All people not previously exposed to typhoid fever are believed to be susceptible to infection. Some specific immunity is conferred to the patient after recovery from the illness.
Implicated Foods	Salmonellosis is most commonly associated with raw or inadequately cooked poultry and egg products. The bacteria may be deposited inside an egg by an infected hen during shell formation, meaning that disinfection of the exterior of an egg will not prevent contamination. Other implicated foods include milk and dairy products, raw meat, fish, shellfish, produce, cream fillings, cake mix, sauces, salads, salad dressings, and non-chlorinated water supplies. Cross-contamination is a common source of salmonellosis linked to ready-to-eat items. Typhoid fever is most commonly associated with inadequate hygiene of infected food workers. It may be transmitted by flies traveling from infected human waste to food that will support multiplication of the bacteria. Shellfish and non-chlorinated water contaminated with human sewage have also been implicated.
Preventative Measures	Proper handwashing technique should be routinely performed by food workers and caregivers. Cross-contamination should be avoided. Raw meats and eggs should be thoroughly cooked. Fruits and vegetables should be washed before cutting or consuming. Pooled eggs should be pasteurized. Adequate sanitation systems should be maintained.
Sample Collection	Food samples should be sent to the PHL Food Lab. 100g of specimen is preferred and a completed PHL Food Lab form must be included. Perishable food samples, such as meat, should be stored and shipped between 0° C and 6° C. Non-perishable items, such as dry mixes, may be sent at ambient temperature. Do not freeze. If samples are already frozen at collection, do not thaw. Keep them

	frozen during shipping. Stool swab specimens should be sent to the PHL Enteric Bacteriology Lab in Cary-Blair media at ambient temperature with a completed PHL Enteric Bacteriology form. All applicable shipping regulations must be followed (see Packaging and Shipping on page 4).
Laboratory Testing and Results	Both the PHL Food Lab and the PHL Enteric Bacteriology Lab will culture the specimen, isolate the *Salmonella*, serotype it, and have the PHL PFGE Lab genetically type it. Results will report either the name of the serotype, i.e. *Salmonella* Wenatchee, or the type itself, i.e. *Salmonella* group E, depending on the extent of testing performed. Testing will take 7 to 14 business days.

Shigella Species

Organism Profile	*Shigella* species are Gram negative, nonmotile, non-sporeforming, aerobic, rod-shaped bacteria. There are four species of *Shigella*: *S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei*, with some species having a number of serotypes. All four species may cause illness, although the severity differs between them. *Shigella* is very closely related to *E. coli* and some strains may cross-react during serotyping. Humans are the main reservoir for *Shigella*, although it may also be found in large primates.
Associated Illness	Infection with *Shigella* is known as shigellosis or bacillary dysentery. The incubation period is 12 hours to 1 week, usually 1 to 3 days. Symptoms include diarrhea, which may be watery or contain blood, mucus, and/or pus (dysentery), fever, abdominal cramps, nausea, vomiting, and tenesmus (straining to defecate). Some strains of *Shigella* produce Shiga toxins identical to enterohemorrhagic *E. coli*. Complications of shigellosis may include hemolytic-uremic syndrome (HUS), toxic megacolon, and Reiter chronic arthritis syndrome. Illness usually lasts a week or less and is most severe in children, although the elderly and the immunocompromised are also at risk for severe illness. Healthy adults may have mild or asymptomatic illness. The human *Shigella* reservoir makes fecal-oral transmission the most important route of infection. The infectious dose may be as low as 10 organisms, making thorough handwashing by foodworkers and caregivers very important.
At-Risk Populations	All humans are believed to be susceptible to shigellosis, although infants and children, the elderly, and the immunocompromised are at greater risk for severe illness and complications. In particular, children under 5 years old are at risk of developing HUS.
Implicated Foods	Foods that require a lot of manipulation of the ingredients, such as potato, tuna, or macaroni salads, have been implicated as causing shigellosis after being contaminated by infected food workers. Fecally contaminated water is another common source. Outbreaks have also been linked to raw vegetables, poultry, milk, and dairy products.
Preventative Measures	Since the fecal-oral route is the most common way *Shigella* is spread, strict hygiene practices including handwashing, chlorinated water, and good sanitation are the most effective preventative measures.
Sample Collection	Food samples should be sent to the PHL Food Lab. 100g of specimen is preferred and a completed PHL Food Lab form must be included. Perishable food samples, such as produce, should be stored and shipped between 0° C and 6° C. Non-perishable items, such as dry mixes, may be sent at ambient temperature. Do not freeze. If samples are frozen at collection, do not thaw and keep frozen during shipping. Stool swab specimens should be sent to the PHL Enteric Bacteriology Lab in Cary-Blair media at ambient temperature with a completed PHL Enteric Bacteriology form. All applicable shipping regulations must be followed (see Packaging and Shipping on page 4).
Laboratory Testing and Results	Both the PHL Food Lab and the PHL Enteric Bacteriology Lab will culture the specimen, isolate the *Shigella*, serotype it, and have the PHL PFGE Lab genetically type it. Testing will take 7 to 14 business days.

Staphylococcus aureus

Organism Profile	*S. aureus* is a Gram positive, nonmotile, non-sporeforming, aerobic, spherical bacterium (coccus) that is commonly seen in grape-like clusters under the microscope. Approximately 25% of the human population is colonized with *S. aureus*, carrying the bacteria on their skin or mucous membranes, making human carriers the primary reservoir. The bacteria may also be found in the environment and in domestic animals such as dogs and cattle, in which it may cause udder infections.
Associated Illness	Illness caused by *S. aureus* is an acute foodborne intoxication similar to emetic illness caused by *Bacillus cereus*. Some strains of the bacteria produce a heat-stable enterotoxin during multiplication in foods. Cooking contaminated foods will not destroy the toxin and will not prevent illness, although it may kill the bacteria. Food should never be left at room temperature for more than 2 hours to prevent bacterial proliferation and toxin production. The onset of symptoms in *S. aureus* intoxication is rapid, sudden, and occasionally violent. Onset may occur in as little as 30 minutes after consumption of contaminated food, although 2 to 4 hours is most common and symptoms may take up to 8 hours to appear. Symptoms may include nausea, vomiting, abdominal cramps, diarrhea, and sometimes headache, muscle cramps, and lowered temperature and blood pressure. Severity of symptoms, duration, and onset time depend on the amount of toxin ingested and the health of the patient. Illness is usually self-limiting, with recovery taking two days or more, but severe cases may require hospitalization related to dehydration. *S. aureus* is responsible for many other opportunistic infections, including boils, eye infections, pericarditis, pneumonia, and wound infections. The bacteria may spread from these infections to food during preparation. Persons with hand, eye, or skin infections, including acne, should not prepare food.
At-Risk Populations	Everyone is believed to be at risk of illness from staphylococcal food poisoning, but infants, the elderly, and severely debilitated persons are most at risk of more severe illness, dehydration, and complications.
Implicated Foods	Food handlers colonized by the bacteria are the primary source of *S. aureus* contamination in food due to bare hand contact. The most frequently incriminated foods in staphylococcal food poisoning are those that require a lot of manipulation, are stored at room temperature, and provide a good growing medium, such as egg, tuna, chicken, or potato salads and cream-filled bakery goods. Meat, poultry, eggs, mayonnaise, milk, and dairy products have also been implicated.
Preventative Measures	Strict handwashing measures and glove usage by food handlers will reduce the staphylococcal contamination of food. However, since *S. aureus* may survive in the environment, on food equipment or preparation surfaces, and may be present in the foods themselves, care should be taken not to temperature-abuse foods. Food should never be left at room temperature for more than two hours. Hot food should be kept above 60° C, and cold food should be kept below 7.0° C.
Sample Collection	Food and stool specimens should be collected for testing and both should be sent to the PHL Food Lab. Vomitus may also be collected. A PHL Food Lab form should be completed for each sample sent and included with the sample. Send a minimum of 50 grams of each specimen. If possible, send samples of both patient stool or vomitus and the suspect food they ate so the results can be matched to each other. Food samples should be stored under refrigeration and shipped between 0° C and 6° C. Do not freeze. If the samples are frozen at collection, do not thaw. Keep them frozen during shipping. Stool specimens should be unpreserved and as fresh as possible, ideally less than 24 hours old. Stool should not be sent in any kind of enrichment media, preservative, or other diluent. Vomitus should be collected immediately and neutralized with baking soda until it stops bubbling. Store and ship them between 0° C and 6° C. Do not freeze. Stool and vomit specimens are clinical specimens and must adhere to all applicable shipping regulations (see Packaging and Shipping on page 4). An accurate count of organisms in a sample can not be obtained if the samples are temperature abused, including freezing and heating above 6° C. If samples are suspected of being temperature abused, note it on the lab form to alert the lab of the potential influence on the results.
Laboratory	The PHL Food Lab quantifies *S. aureus* in food, stool, or vomitus and calculates the bacterial load in

Testing and Results	cfu/g of specimen. Isolates are confirmed with biochemical tests and genetically typed by the PHL PFGE Lab. The PHL Food Lab does not routinely test for the presence of staphylococcal toxin, although testing may be performed on a case-by-case basis in consultation with CD Epi and the PHL Food Lab. Since *S. aureus* is common in the human environment but sensitive to cooking temperatures and the toxin may cause illness even the absence of viable organisms, there is no "magic number" of cfu/g that designates *S. aureus* as the causative agent of illness. The presence or absence of *S. aureus* in food and patient specimens and the symptoms of the patients must all be considered by CD Epi to make a determination. PFGE matching is particularly useful in linking *S. aureus* in food to *S. aureus* from patient samples. Testing will take 7 to 14 business days.

Vibrio Species

Organism Profile	*Vibrio* species are Gram negative, motile, non-sporeforming, straight or curved, aerobic, rod-shaped bacteria with a single flagella at one end that usually prefer or require salt for growth. Three species, *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus*, make up the main human pathogens, although other *Vibrio* species are occasional opportunistic human pathogens. *Vibrio* species are universally found in marine and aquatic environments. They may grow in association with copepods and other zooplankton. Humans are a known reservoir for *V. cholerae*. *Vibrio* bacteria are extremely fast-growing. *V. parahaemolyticus* is the quickest-multiplying organism known, with the capability of doubling its numbers every nine minutes in ideal conditions.
Associated Illness	Most *Vibrio* species have the potential to cause gastroenteritis, wound infections, or septicemia. *V. vulnificus* in particular is known for causing rapidly fatal septicemia from wound infections. The severity of gastrointestinal symptoms depends on the *Vibrio* species and strain, the health of the patient, and the number of organisms consumed. *V. cholerae* is the causative agent of cholera, a severe gastrointestinal illness that may be rapidly fatal. The bacteria attach to the small intestine and produce a toxin that causes diarrhea. It has the potential to cause large, devastating outbreaks, usually spread through contaminated drinking water. The most striking feature of cholera is a severe dehydrating diarrhea known as cholera gravis, although mild or asymptomatic infections are common. The incubation period ranges from six hours to five days, with two to three days being common. The illness has a sudden onset and symptoms include profuse, watery diarrhea progressing to characteristic "rice-water" stools usually containing mucus but not blood. Nausea and vomiting may occur, particularly in the early stages. Severe fluid and electrolyte loss may lead to shock, acidosis, hypoglycemia, circulatory collapse, and death. Death may occur within hours of onset of symptoms. The fatality rate may exceed 50% in untreated cholera cases. Proper treatment, including aggressive oral or intravenous rehydration, may drop the fatality rate to less than 1%. In mild cases and in patients given supportive therapy, the disease is self-limiting. The *V. cholerae* serotype infecting the patient may have an impact on the severity of the illness. Serotyping *V. cholerae*, especially differentiating serogroups O1, O139, and non O1, provides important information for epidemiology. *V. parahaemolyticus* and *V. vulnificus* cause a gastroenteritis known as vibriosis, which is not as severe as cholera. Symptoms include watery diarrhea that may contain blood or mucus, abdominal cramps, nausea, vomiting, fever, and headache. Illness is usually self-limiting and lasts less than a week. In cases of vibriosis due to *V. vulnificus*, serious complications and death may occur in patients with liver disease.
At-Risk Populations	All people are believed to be susceptible to infection with *Vibrio* species. Children, the elderly, malnourished individuals, and the immunocompromised are more likely to develop severe symptoms or complications and have a more difficult time preventing dehydration, particularly in cases of cholera. Reduced gastric acidity may allow more *Vibrio* bacteria to survive to reach the intestine, lowering the infectious dose and potentially increasing the severity of illness. Healthy adults in cholera-endemic areas may develop some resistance to prevalent local serotypes of *V. cholerae*. Individuals with liver disease are particularly at risk of complications of infection with *V. vulnificus*.
Implicated Foods	Cholera epidemics are generally due to poor sanitation and untreated, contaminated water supplies. Cholera may also be spread in food through the fecal-oral route if infected food handlers have poor

	handwashing practices. In the U.S., sporadic cholera cases may be associated with shellfish consumption due to the natural presence of *V. cholerae* in the marine environment. In the United States, raw oysters and other shellfish are the primary vehicle for vibriosis. *V. parahaemolyticus* is commonly found in shellfish in Washington State, particularly during warm months. *V. vulnificus* is more commonly associated with shellfish from the Gulf Coast, although both organisms may be found in marine waters throughout the world.
Preventative Measures	Drinking water must be treated and food should be cooked thoroughly. Proper sanitation, particularly sewage treatment, prevents the spread of *V. cholerae*. Handwashing, particularly for food handlers, helps prevent transmission through the fecal-oral route. In the United States, oysters are monitored for the presence of *V. parahaemolyticus* or *V. vulnificus*, depending on the prevalent organism in the growing region.
Sample Collection	Send 100g of food to be tested for *V. parahaemolyticus* to the PHL Food Lab with a completed form. Store and ship between 0° C and 10° C. Do not freeze. If samples are frozen at collection, do not thaw and keep frozen during shipping. If shellfish are collected, be sure to get the tracking tag and all related information so that the shellfish can be traced back to their growing area. Do not allow food or shellfish samples to directly contact ice packs during shipping. Food is not regularly tested for *V. cholerae* or *V. vulnificus*, but outbreaks of illness will be handled on a case-by-case basis in consultation with CD Epi and the PHL Food Lab. Water is not routinely tested for any *Vibrio*, but may also be tested by the PHL Food Lab on a case-by-case basis in consultation with CD Epi and the PHL Food Lab. For all three *Vibrio* species, send stool swab specimens to the PHL Special Bacteriological Pathogens Lab in Cary Blair media at ambient temperature. Include a completed PHL Special Bacteriological Pathogens Lab form and follow all applicable shipping regulations (see Packaging and Shipping on page 4). On the lab form, specify which *Vibrio* species is suspected. Specimens will not automatically be tested for all three species.
Laboratory Testing and Results	Both the PHL Food Lab and PHL Special Bacteriological Pathogens Lab will culture, isolate, identify, and serotype *Vibrio* organisms using biochemical tests in an outbreak situation. *V. cholerae* will be serotyped, particularly to identify O1, O139, and non-O1 *V. cholerae*. PFGE will be run on isolates to genetically match outbreak specimens or food to patient specimens. The Food Lab also has a PCR test for *V. parahaemolyticus* that may be utilized, which tests both for the presence of the bacteria and the presence of a toxin-producing gene that some *V. parahaemolyticus* strains carry. The PCR test does not require an isolate. Testing will take 7 to 14 business days. The PHL Food Lab performs routine monitoring of locally harvested oysters for *V. parahaemolyticus* for the DOH Shellfish Program during warm summer months.

Yersinia species

Organism Profile	*Yersinia* species are Gram negative or Gram variable, non-sporeforming, rod-shaped or rounded bacteria that may or may not be motile and can grow at low temperatures and in microaerophilic conditions. Three species are known human pathogens, *Yersinia pestis*, which causes plague, *Y. enterocolitica*, and *Y. pseudotuberculosis*, both of which may cause gastrointestinal illness. Pathogenic *Yersinia* species are found in many domestic and wild animals, including rodents, pigs, fowl, cats, beavers, and squirrels. They are not considered normal flora of humans and are not usually found free-living in the environment, although they may survive in fecally polluted soil or water for an extended period of time.
Associated Illness	Intestinal infection with a *Yersinia* species is known as yersiniosis. The incubation period is less than 10 days, usually 3 to 7 days. Symptoms include watery diarrhea that may be bloody, fever, vomiting, abdominal pain, and acute mesenteric lymphadenitis which may mimic appendicitis. Complications include postinfectious arthritis, septicemia, and unnecessary appendectomies resulting from incorrect diagnosis. None of the *Yersinia* species are common sources of gastrointestinal illness in the United States, although food and waterborne outbreaks of *Y. enterocolitica* and *Y. pseudotuberculosis* have

	occurred in the United States, Europe, and Japan. *Y. pestis* is usually transmitted through routes other than food, causing bubonic, pneumonic, or septicemic plague, although it has the potential to cause gastrointestinal yersiniosis. Outbreaks of foodborne *Y. pestis* are extremely unusual, but possible. All *Yersinia* species, including common environmental isolates, may be opportunistic pathogens causing wound infections, urinary tract infections, septicemia, and other infections.
At-Risk Populations	Children, the elderly, and the immunocompromised are most at risk of developing serious yersiniosis and complications. Older children and teens are most at risk for developing acute mesenteric lymphadenitis mimicking appendicitis, and therefore are most at risk for unnecessary appendectomies. Patients with the blood antigen HLA-B27 and related antigens may be most susceptible to developing postinfectious arthritis.
Implicated Foods	*Y. enterocolitica* is commonly found in the nasopharyngeal tract of pigs, and improper slaughtering of pork increases the risk of contamination. Foods implicated in outbreaks of yersiniosis include meat and meat products such as chitterlings (chitlins), fish, oysters and other shellfish, raw milk, dairy products, tofu, non-chlorinated water, and contaminated milk containers.
Preventative Measures	Since the organisms may be found in the environment, proper sanitation including chlorinated water and strict food handling regulations must be enforced. Handwashing, disinfection of food containers and other contact surfaces, proper slaughter of pigs, proper cooking of meat, pasteurization of milk and dairy, and proper storage of hot and cold foods should prevent contamination and illness. Outbreaks are usually caused by a breakdown of food processing techniques.
Sample Collection	Send stool swab specimens to the PHL Special Bacteriological Pathogens Lab in Cary-Blair media. Include a completed PHL Special Bacteriological Pathogens Lab form specifying the test and follow all applicable shipping regulations (see Packaging and Shipping on page 4). Food related to normal yersiniosis is not routinely tested at the PHL. Foods linked to a suspected *Y. pestis* BT event will be handled on a case-by-case basis in conjunction with CD Epi, the PHL BT Team, and the PHL Food Lab. At least 100g of suspect food should be collected.
Laboratory Testing and Results	The PHL Special Bacteriological Pathogens Lab will culture specimens, isolate, and identify *Yersinia* species. Special procedures such as cold enrichment may be necessary, causing confirmation to take up to three weeks.

Norovirus

Organism Profile	Noroviruses are a group of related viruses known to cause viral “stomach flu” in humans, although they have no relation to the influenza virus. They are small, rounded, non-enveloped, single-stranded RNA viruses in the family *Caliciviridae*. They have been previously referred to as Norwalk viruses or Norwalk-like viruses. Norovirus cannot multiply outside the human body, but can survive for extended periods of time on contaminated surfaces, known as fomites, such as doorknobs, railings, bathroom fixtures, kitchen equipment, and food.
Associated Illness	Norovirus infection causes acute gastroenteritis, also known as viral gastroenteritis or non-bacterial gastroenteritis. The illness has an incubation period of 12 to 48 hours, usually between 33 to 36 hours. Symptoms have a sudden onset and include vomiting, watery diarrhea, abdominal cramps, nausea, and low grade fever. Complications arise from dehydration. Symptoms are generally self-limiting and last 24 to 60 hours with no long-term consequences. The CDC estimates that as many as half of all cases of gastroenteritis are likely caused by Norovirus infection. The infectious dose may be as low as 10 virions.
At-Risk Populations	Everyone is believed to be at risk for Norovirus gastroenteritis. Infection confers some amount of immunity, but there are many strains of the virus, making total immunity unlikely and reinfection possible. Children and the elderly are more at risk of complications from dehydration.
Implicated Foods	Even though it cannot multiply outside the human body, the extremely low infectious dose for Norovirus makes it very easy to spread through contamination of food through the fecal-oral route, contaminated fomites including food contact surfaces and tools, and sewage-contaminated well and recreational water. Salad dressing, frosting, raspberries, salads, sandwiches, bakery products, and shellfish have all been implicated in outbreaks. Sewage dumped by cruise ships over shellfish beds

	have also been linked to Norovirus outbreaks.
Preventative Measures	Proper sanitation including clean water, thorough handwashing, decontamination of fomites, and sewage treatment, washing produce, eating only shellfish obtained from approved sources, and regulation of cruise ship waste will all help prevent Norovirus gastroenteritis.
Sample Collection	Stool specimens may be sent to the PHL Molecular Lab for Norovirus testing. Stool should be unpreserved and as fresh as possible. Ship between 0°C and 6°C, include a completed PHL Molecular Lab form, and follow all applicable shipping regulations (see Packaging and Shipping on page 4). Food and environmental swabs are **NOT** tested for Norovirus at the PHL because the virus is not present at a high enough concentration to be detectable.
Laboratory Testing and Results	The PHL Molecular Lab performs PCR on human specimens to test for the presence or absence of Norovirus. PFGE cannot be performed on viruses due to their tiny amount of nucleic acid. Results should be available in 3 to 7 business days.

Trichinella Species

Organism Profile	*Trichinella* species are a type of nematode found in a variety of carnivorous and omnivorous animal tissue. In addition to the most common species, *T. spiralis*, which is found worldwide, several other species of *Trichinella* are now recognized, including *T. pseudospiralis*, found in mammals and birds worldwide, *T. nativa*, found in cougars and Arctic bears, *T. nelsoni*, found in African carnivores, and *T. britovi*, found in European and western Asian carnivores. The reservoir for most species of *Trichinella* are rodents, so any carnivore or omnivore (including domestic pigs) with access to rodents as part of its diet are likely to be infected with *Trichinella*.
Associated Illness	Infection with *Trichinella* larvae causes trichinosis. *Trichinella* larvae form cysts in the muscle tissue of an animal. When meat infected with cysts containing viable, infective larvae is ingested, the stomach acid dissolves the cysts and the larvae hatch. They invade the intestinal mucosa, mature, and mate by the second day. By the sixth day, females begin to deposit motile larvae in the blood stream which are carried throughout the body. An adult female may produce as many as 1500 larvae in a month. These larvae invade the tissues of the body, usually ending up in highly oxygenated striated muscle tissue such as the diaphragm, tongue, and jaw muscles. There, the larvae encyst themselves to begin the cycle again. Symptoms of trichinosis may occur within 24 hours of ingesting infected meat. These early symptoms may include diarrhea, nausea, cramps, and malaise. The diarrhea may be prolonged, lasting up to 14 weeks. Subsequently, during muscle invasion by the larvae, symptoms may include facial edema, muscle pain, joint pain, swelling and weakness.
At-Risk Populations	All people are believed to be susceptible to trichinosis, but the individuals most at risk to contract it are people who consume raw or undercooked carnivore or omnivore meat, particularly wild game meat.
Implicated Foods	Any meat taken from a carnivorous or omnivorous animal may potentially carry *Trichinella*. This is especially true for wild or feral animals. Pork, bear, walrus, and cougar meat have all been found to contain infectious *Trichinella* larvae.
Preventative Measures	Ensure that all carnivore, omnivore, or wild game meat is fully cooked to 77°C (170°F). Freezing, drying, smoking, curing, or microwaving meat do not reliably kill infective larvae and should not be relied upon as preventative measures.
Sample Collection	*Trichinella* is most commonly isolated from certain highly oxygenated muscle groups, such as the jaw muscle, diaphragm muscle, and tongue. Send at least 100g of these tissues, if available, to the PHL Parasitology Lab. If these specimens are not available, send 100g of intact muscle tissue such as roasts or steaks and 100g of whatever ground tissue is available. Include a PHL Parasitology Lab form with each tissue specimen. Human tissue specimens are not routinely tested at the PHL, but may be examined on a case-by-case basis in consultation with CD Epi and the PHL Parasitology Lab.

Laboratory Testing and Results	The PHL Parasitology Lab performs a digestive sedimentation procedure on tissue samples to search for *Trichinella* larvae. Once the tissue is digested away, the remnant is examined for larvae using direct microscopy. The larvae are counted and the number of organisms per gram of tissue is calculated.

Enteric Parasites

Organism Profile	Many types of parasites may be associated with contaminated food or water. Single-celled protozoans such as amoebae, *Cryptosporidium*, or *Giardia* and helminths such as tapeworms, roundworms, and flukes may all parasitize human hosts.
Associated Illness	Enteric parasites cause many symptoms. Diarrhea is common and may be severe and watery, as in amoebic dysentery. *Giardia* may cause alternating diarrhea and constipation. Helminths may cause a variety of problems. Tapeworms feed on nutrients in the gut ingested by their host and may cause or exacerbate malnutrition. Hookworms attach to the gut wall and feed on blood, causing anemia. Flukes and roundworms such as *Ascaris* may cause wider systemic problems as they burrow through the gut wall and invade the blood vessels, liver, lungs, bladder, or other organs. Cysticercosis is one of the most dangerous conditions caused by an enteric parasite. It is caused by the pork tapeworm, *Taenia solium*. Usually, humans develop adult *Taenia solium* in their gut after eating infective larvae in undercooked pork. These adults produce eggs that are shed in the host's feces. If a human ingests these *Taenia solium* eggs, they will hatch and release larvae that travel throughout the body and create cysts known as cysticerci in tissues. Symptoms will depend on the tissues invaded and may range from mild soreness from cysticerci in muscles to blurred vision from cysticerci in eyes, to headaches, seizures, and even death from cysticerci in the brain.
At-Risk Populations	Anyone exposed to unsanitary or contaminated water or food is at risk for infection with enteric parasites. In the U.S., immunocompromised individuals are the most at risk of serious illness caused by protozoan parasites, particularly *Cryptosporidium*. Enteric parasites may cause more persistent and difficult to treat infections in the immunocompromised than in those with healthy immune systems. In areas with poor sanitation, children are most at risk of complications from malnourishment and anemia resulting from heavy parasite loads.
Implicated Foods	All food and water is susceptible to contamination with parasites, but particular risks are undercooked meat, fish, and shellfish, untreated surface or well water, unwashed produce, and underchlorinated recreational water.
Preventative Measures	Adequate sanitation will prevent most parasites from entering the food supply. Untreated surface water should never be used for drinking. Produce should be washed thoroughly with sanitary water before eating. Meat, particularly pork and wild game, should be cooked thoroughly. Recreational water should be sufficiently chlorinated.
Sample Collection	The PHL Mailroom provides Meridian ParaPak ULTRA EcoFix kits and collection instructions for PHL Parasitology Lab stool specimens. Stool should be passed on clean paper or plastic and should not come into contact with water, soil, or other contaminants. Using the scoop attached to the lid, the EcoFix kit should be completely filled to the red fill line, but not overfilled, and thoroughly stirred to allow the fixative to evenly and completely penetrate the sample. The tube must be labeled and a PHL Parasitology Lab form **must** be completed including all patient and submitter information. EcoFix kits and paperwork should be shipped to the PHL Parasitology Lab at ambient temperature as soon as possible in accordance with applicable shipping regulations (see Packaging and Shipping on page 4). Food and water are not regularly examined for parasites due to low numbers and difficult screening methods. However, outbreak samples will be handled on a case-by-case basis in consultation with the PHL Parasitology Lab and CD Epi staff.
Laboratory Testing and Results	Laboratory examination of parasitology specimens involves making and staining slides, then directly examining the specimen under the microscope, scanning for parasites and diagnostic morphological features. Results are available in 2 to 5 days.

pH and Aw

pH and water activity (Aw) are two physical properties of a food or other substance that affect microorganism growth. pH is the measure of acidity or alkalinity. Aw is the measure of free water available in a substance. These properties may be manipulated in foods to inhibit the growth of microorganisms.

pH is, specifically, the measure of the difference in concentration between free hydrogen ions (H+) and free hydroxyl ions (OH-). Pure water has a pH of 7.0, which is perfectly neutral and has an equal concentration of H+ and OH-. Substances with a pH higher than 7.0 are basic (alkaline), meaning the OH- concentration is greater than the H+ concentration. Substances with a pH lower than 7.0 are acidic, with the H+ concentration greater than OH-. Acids tend to taste sour while bases tend to taste bitter. Most foods are somewhat acidic, including dairy products, and acid may be added to food to make it less hospitable to microbes. The acetic acid in vinegar is often added to foods, such as ketchup, to make them acidic enough to be safely stored at room temperature. Most bacteria will only grow at pH from 5.5 to 8.0. Other organisms, such as yeasts and molds, have a wider range and may grow from a pH of 2 to 11. Extremophile bacteria may grow in conditions even more acidic or basic, but are rarely human pathogens.

Aw is the measure of free water available in a substance. It is defined as the ratio of the water vapor pressure of the substance to the water vapor pressure of pure water. Therefore, the Aw of pure water is 1.0. It is different from moisture content in that Aw measures only the water available for microbial use whereas moisture content includes free and bound water in a substance. Many methods of preserving food, including salting, drying, freezing, curing, concentrating, and adding sugar all reduce Aw. Most bacteria need an Aw of at least 0.91 to grow, while fungi can survive in foods with an Aw as low as 0.70. When the Aw is too low, solutes in the substance interfere with the cell membrane and processes of the microorganism, reducing or inhibiting growth.

The PHL Food Lab will test the pH and Aw of suspicious foods to ensure that they are within safe ranges. Call the PHL Food Lab for consultation at 206-418-5442 before shipping any samples. Samples that arrive without notice will be discarded. Approval from CD Epi is not required for pH and Aw testing. Send a minimum of 50 grams of sample. Results should be available in 3 business days.

Heterotrophic Plate Count

A heterotrophic plate count (HPC) is a way to calculate the total colony forming units (CFU) of bacteria in a sample. The media supports the growth of aerobic organisms with simple nutritional requirements ("heterotrophic" means "requiring organic carbon and nitrogen compounds for nourishment"). It does not differentiate pathogens and non-pathogens, nor does it measure anaerobic bacteria. Most foods contain many harmless bacteria, even when properly cooked. Fresh produce, in particular, contains many bacteria from the soil it is grown in. If a specific pathogen is suspected, the food should be cultured for that pathogen and not have an HPC performed on it. However, HPCs may be useful for determining the effectiveness of sterilization, pasteurization, or cooking methods. CD Epi and the PHL Food Lab will assist in selecting the appropriate test to perform. Results generated from an HPC are reported out as CFU per gram of food. CFUs are an approximation of the number of live bacteria present.

Tips to Remember!

* When in doubt, collect a sample.
* Always use aseptic sampling technique.
* Contact CD Epi at 1-877-539-4344 as soon as possible and **ALWAYS** before sending a specimen.
* Call the Food Safety Program at 360-236-3385 for guidance and advice.
* Consult the PHL Directory of Services for the most up-to-date sampling and testing information.
* Store and ship specimens according to the recommendations for the suspected organism and **ALL** applicable shipping regulations.
* **DO NOT** ship a clinical specimen without specific Packaging and Shipping training!
* Always send a completed form for the appropriate lab with every sample.
* If you have a question, please call us and ask! The PHL main line is 206-418-5400. We are here to help you.

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