VOLUME 4, ISSUE 2

FOCUS on Field Epidemiology

Collecting Specimens in Outbreak Investigations

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The North Carolina Center for Public Health Preparedness is funded by Grant/Cooperative Agreement Number U90/CCU424255 from the Centers for Disease Control and Prevention. The contents of this publication are solely the responsibility of the authors and do not necessarily represent the views of the CDC.

As part of an outbreak investigation team, you and your colleagues are going to collect specimens from a school for laboratory testing. It's like junior year all over again: what to wear? Will your laboratory notebook look too geeky?

Maybe you should bring an entourage of coworkers to collect samples at the snap of your fingers while you sit back looking like you don't care.

What if you have to collect something that makes you squirm? Maybe you should wear a space suit and bring tongs.

It really won't matter whether your clothes are in style, but your specimen collection practices have to be A+ material. Specimen collection is one of the first and most important steps in an outbreak investigation. Laboratory results are extremely valuable, as they can help identify the causative agent, provide information about the mode of transmission and identify possible outbreak sources.

Clinical, demographic and risk factor information from an outbreak investigation can also provide important clues about the cause(s) of an outbreak (and steps to control it). Since time can be lost waiting for laboratory results, collection and processing of outbreak-associated specimens usually occur simultaneously with other outbreak investigation steps, such as epidemiologic data collection and hypothesis generation.

Using examples from real outbreak investigations, this issue of FOCUS discusses some of the different types of specimen collection that may be required during an outbreak. While we review each type of specimen separately for the sake of clarity, more than one type of specimen is often collected in a real outbreak investigation. We will also discuss some of the practical issues of clinical specimen collection, including packaging, shipping, and other logistical issues.

Outbreaks Involving Clinical Specimens

Many outbreak investigations involve the collection of human clinical specimens from outbreak-associated case-patients. Human clinical specimens include blood, serum, urine, saliva, hair, feces, etc. The type of specimen collected depends on the nature of the outbreak. For example, stool specimens may be collected during an outbreak of diarrheal illness, while blood may be collected during an outbreak of fever and rash suggestive of rubella.

Similar specimens can also be taken from animals when investigators suspect that they may be involved in an outbreak.

- An interesting outbreak investigation that relied on the collection of human clinical specimens was conducted a few years ago in the Midwestern United States (1). More than 70 individuals experi-

enced a febrile rash thought to be caused by the monkeypox virus. Monkeypox is a rare zoonotic disease that usually occurs in African rain forests. Cases were laboratory confirmed using blood, skin, lymph node or pharyngeal specimens. All laboratory-confirmed human cases in this outbreak were associated with the purchase of prairie dogs as pets. Samples taken from prairie dogs confirmed the infection. The prairie dogs were thought to have been infected at an animal distribution facility, where they were housed or transported with exotic rodents imported from Africa. As this example demonstrates, clinical specimens from animals are often collected in conjunction with human clinical specimens in outbreaks of zoonotic diseases.

- An historic example involving animal specimens occurred in 1993 in the southwestern United States (2). An outbreak of a fatal unexplained pulmonary illness was found to be associated with a previously unknown type of hantavirus, which is a zoonotic virus. Rodents were known to transmit hantavirus to humans, so rodents found near the homes of the case-patients were trapped and tested to find out if they were infected with the same virus type as the case-patients. Within several months, the same strain of hantavirus was cultured from the tissue of a deer mouse captured near the home of a case-patient who had died from the hantavirus strain. In addition to the laboratory-based link found between humans and rodents, the results of a case-control study were consistent with the hypothesis that the fatal pulmonary disease observed in case-patients was associated with proximity to infected deer mice. Like the outbreak of monkeypox, this is an example of dual collection of animal and human clinical specimens.

Outbreaks Involving Environmental Specimens

Environmental specimens such as food, water, or fomites (see glossary on page 5) may be collected during disease outbreak investigations to confirm a source. Food and/or water samples are often collected in food or waterborne outbreaks. Environmental samples are frequently collected in conjunction with human clinical samples; when outbreak-associated clinical and environmental specimens yield the same results, the hypothesis that the outbreak source is the same as the environmental specimen source is supported.

- In 1991, an individual in Maryland tested positive for cholera while hospitalized for diarrhea and dehydration (3). This incident was particularly unusual because the case-patient had not reported any of the common risk factors for cholera, such as recent consumption of raw shellfish, travel to a foreign country, or cholera vaccination. An epidemiologic investigation revealed that she had attended a party 2 days prior to hospitalization, and that several other attendees also experienced diarrhea and had laboratory evidence of cholera. All case-patients reported eating a homemade Thai-style rice pudding prepared with frozen coconut milk imported from Thailand. Although there was no food left from the party to test for cholera, laboratory professionals cultured unopened packages of frozen coconut milk of the same brand used to prepare the rice pudding. These specimens tested

Sampling Onboard Ship: The Vessel Sanitation Program

The Vessel Sanitation Program (VSP) was jointly established by the Centers for Disease Control and Prevention (CDC) and the cruise ship industry in the 1970s in response to several disease outbreaks on cruise ships. The CDC inspects ships, performs surveillance of diarrheal illness, conducts outbreak investigations and offers sanitation training seminars to ship staff members.

The epidemiological aspect of an outbreak investigation involves interviewing all crew members and passengers who are ill and then collecting information (using a standardized questionnaire) from all ship crew and passengers. The laboratory aspect of the investigation is performed to confirm clinical illness and may involve the collection of stool, blood and vomitus from ill persons (as well as non-ill persons for purposes of comparison). Finally, environmental health is assessed by examining and sampling potential outbreak sources such as on-board potable water, ice and food.

Many diarrheal illness outbreaks on cruise ships have been investigated by the VSP since its inception. For example, VSP investigated 21 acute gastroenteritis outbreaks in 2001. Stool samples revealed that 9 outbreaks were caused by noroviruses and 3 were caused by bacteria, while 9 were of unknown etiology. Laboratory results on these human clinical specimens shaped recommendations developed by the CDC to stop the outbreaks and to prevent future outbreaks from occurring on cruise ships.

Source: Centers for Disease Control and Prevention. Vessel Sanitation Program. Available at: http://www.cdc.gov/nceh/vsp/default.htm. Accessed December 12, 2006.

positive for several types of *Vibrio cholerae,* as well as *Aeromonas* and *Salmonella* species.

- Another interesting outbreak investigation using food specimens involved consumption of fresh cheese curds (4). In 1998, more than 50 cases of *Escherichia coli* 0157:H7 were laboratory confirmed in Wisconsin. A case-control study was conducted, and consumption of fresh cheese curds produced by a particular cheese factory was found to be highly associated with illness. Cheese samples taken from an opened package of curds that had been served at a party attended by several case-patients tested positive for *E. coli* 0157:H7. Furthermore, pulsed-field gel electrophoresis (see glossary on page 5) demonstrated that 42 of the 44 case-patient isolates were indistinguishable from the curd isolates and from each other. In this outbreak, a batch of unpasteurized cheddar cheese was inadvertently used to make fresh cheese curds, and was incorrectly sold as pasteurized cheese curds.
- In the spring of 1993, a widespread outbreak of acute watery diarrhea occurred among an estimated 403,000 residents of Milwaukee, WI (5). Suspecting drinking water as the culprit, investigators performed laboratory tests for enteric pathogens and examined ice made during the time of the outbreak for the presence of cryptosporidium oocysts. Additionally, investigators surveyed residents with confirmed or probable cryptosporidium infections. Through a combination of efforts, including both clinical and environmental sampling, this massive outbreak was determined to have been caused by cryptosporidium oocysts that passed through the filtration system of one of the city's water-treatment plants.
- An unforgettable case illustrating the importance of fomite sampling occurred during the anthrax investigation in 2001 (6). Sampling of envelopes containing white powder confirmed the suspicion of an anthrax attack. Results from sampling of envelopes, postal facilities, clothing, news media offices, residences, and other sites were used to evaluate the presence and extent of anthrax contamination and to guide the decontamination process.

Challenges to Detecting Infectious Agents in the Environment

Despite the many successes described in these examples, infectious pathogens can be difficult to isolate and identify. Infectious enteric agents in water can be particularly difficult to detect and quantify because their concentration is more diluted than in clinical specimens. For example, only 13% of the causative organisms of U.S. waterborne infectious disease outbreaks in 1991 and 1992 were identified (7).

Furthermore, some methods, such as those used for *Cryptosporidium* oocysts and *Giardia* cysts, cannot determine the viability or infectivity of the organisms (7).

Identification of infectious agents in food can also be challenging because they are often present in small quantities (8).

Moreover, detection of infectious agents in both food and water may require costly specialized methods that many laboratories lack. For example, in a 2004 survey of 56 state and territorial public health laboratories, only 18% of the responding laboratories reported testing food specimens for viral pathogens, despite the fact that viruses are responsible for approximately 50% of foodborne illness in this country (9).

A final challenge in detecting infectious agents in the environment has to do with the time sensitivity of specimen collection. To ensure sample integrity and proper diagnosis and treatment, specimens need to be collected

PulseNet and DNA "Fingerprinting"

PulseNet is a national network of public health and food regulatory agency laboratories coordinated by the CDC. PulseNet consists of state and local health departments and federal agencies such as the CDC, USDA/FSIS, and FDA.

PulseNet members perform standardized molecular subtyping (or "fingerprinting") of foodborne disease-causing bacteria using a technique referred to as pulsed-field gel electrophoresis (PFGE). (We will cover PFGE in more detail in the next issue of *FOCUS*.)

PFGE allows laboratory professionals to distinguish strains of infectious organisms such as *Escherichia coli O157:H7, Salmonella*, *Shigella*, *Listeria*, or *Campylobacter* at the DNA level. DNA "fingerprints," or patterns, are then submitted electronically to a comprehensive database at the CDC.

These databases are made available on demand to participants to enable rapid comparison of patterns from state to state, thus permitting early identification of common source outbreaks.

Source: Centers for Disease Control and Prevention. PulseNet. Available at: http://www.cdc.gov/pulsenet/index.htm. Accessed on December 7, 2006.

as quickly as possible once an outbreak is suspected. For instance, in a foodborne disease outbreak, the implicated food may be discarded or consumed in a matter of days.

Logistics of Human Clinical Specimen Collection and Transportation

Typically, most specimen collection that occurs during a disease outbreak involves human clinical specimens. Here we will touch upon some important issues in planning, collecting, processing, labeling, storing, and transporting human clinical specimens.

Laboratory confirmation of an etiologic agent is a critical component of a successful outbreak investigation. For this reason, it is important to remember that the ability of a laboratory to successfully identify a pathogen depends on appropriate specimen collection and transportation (10).

Planning for Human Clinical Specimen Collection

When a suspected outbreak is first reported, the clinical and epidemiological data collected should be used to narrow the range of possible causative agents. Once this is done, the clinical specimens needed to make a laboratory-confirmed diagnosis should be determined.

Next, a laboratory must be selected to perform the testing and analysis. This may be determined in part by the test(s) needed. Routine laboratory tests, such as those used for the detection of *Salmonella* species, can be performed by most clinical laboratories. However, the laboratory capability to test for agents such as *Clostridium botulinum* toxin is more limited.

Because each laboratory has its own specific guidelines for specimen collection, all aspects of the specimen collection process (including the sample type, materials needed, local or on-site processing, transportation, and communication of results) should be discussed with the laboratory *before* specimen collection begins.

Transportation details such as the timing and delivery of the collected samples, required transport media, transit route, shipping requirements, temperature requirements, and documentation (i.e., chain of custody forms—see *FOCUS* Volume 2, Issue 6 for more information) should also be discussed. Packaging and transportation requirements must comply with national regulations for transporting infectious material and should also be reviewed with the transport service.

Collecting Human Clinical Specimens

Specimens should be collected as soon as possible once an outbreak has been identified. Human specimens obtained early, particularly before antimicrobials are given to the patient, are more likely to yield the pathogen.

In certain situations, however, specimen collection after a person recovers from illness may be equally important, because the presence of antibodies in serum samples after recovery can confirm whether or not an individual's illness or infection was in fact related to the outbreak in question.

Before obtaining human clinical specimens, it is important to explain the purpose and procedure to the case-patient. The investigator must obtain an adequate amount of the specimen and handle it with care, because this may be the only opportunity to obtain a specimen during the outbreak. A sample must be collected properly in order to ensure that the pathogen or infectious agent can be recovered in a viable form.

Again, it is important to stress that communication with the laboratory *before* specimen collection is critical to ensure an appropriate collection technique, maintain the sample, and allow for proper diagnosis and treatment decisions. For example, it is not advisable to collect most fungal cultures with swabs because the swab fibers can interfere with interpretation of the results (10).

Appropriate specimen collection technique is also important because the laboratory may otherwise reject the

Resources:

World Health Organization. Guidelines for the collection of clinical specimens during field investigations of outbreaks, 2000. http://www.who.int/csr/resources/publications/surveillance/WHO_CDS_CSR_EDC_2000_4/en/.

CDC. Guidelines for specimen collection. http://www.cdc.gov/foodborneoutbreaks/guide_sc.htm.

State health department websites often have information on specimen collection; searching the appropriate state health department website for this information may be useful.

specimen. For example, if there is an insufficient sample quantity or contamination from other body fluids, the laboratory may not be able to process the specimen (10).

Labeling and Identification of Human Clinical Specimens

Over 70% of the information used by a clinician to diagnose and treat a patient is derived from laboratory testing. Thus, ensuring that specimens are accurately labeled at collection time is essential. Misidentification of a specimen leads to misidentification of a patient, which can result in improper diagnosis and treatment (11).

While different laboratories may have different requirements, in general, labels should be affixed to the specimen container and should include the patient's name (first and last to prevent confusion); a unique identification number; date, hour, and place of collection; type of sample; specific anatomic culture site (to validate the specimen and to help select the appropriate medium); and the name of the specimen collector. Although laboratories handle all specimens as potentially infectious, specimens that are known to contain a particularly dangerous pathogen should be clearly marked as such.

A case investigation form with matching information should be completed for each specimen at the time of specimen collection and retained by the investigation team for reference. And, of course, all of the above information should be printed legibly.

Storage and Transport of Human Clinical Specimens

Specimens must be stored appropriately before and during transportation in order to preserve the integrity of the specimen. Since microorganisms are living beings, environmental conditions can affect their maintenance and survival.

If they multiply or die during collection, transport, or storage, they no longer accurately represent the disease process in the person from whom the specimen was taken (10). For this reason, storage in an appropriate medium and maintenance of the proper temperature is critical.

Requirements depend on the type of specimen and sample and should be determined in consultation with the laboratory *before* specimen collection begins.

Most specimens (with the exception of feces) need to be transported in sterile containers (10). Specimens transported in incorrect containers (i.e., a non-sterile container when sterility is required) may be rejected by the laboratory.

All specimen containers should be closed tightly. Laboratories may reject a specimen if it shows signs of leakage or seepage, since this could potentially expose laboratory personnel to the contents (10).

Packaging of clinical specimens must comply with postal and commercial regulations for transport of infectious materials. These regulations depend on the type of transport (i.e., ground or air delivery) and should be determined in consultation with the laboratory and carrier *prior* to specimen collection.

Finally, the receiving laboratory should be notified of the pending shipment before transport.

Summary

This issue has touched upon the ways in which clinical and environmental specimens can provide valuable information to an outbreak investigation, and the importance of appropriate and timely specimen collection.

The next few issues of *FOCUS* will discuss in more detail what happens after a specimen is collected and sent to the laboratory and what types of laboratory diagnostics may be used to help identify an agent suspected in an outbreak.

Glossary:

Fomites: Articles that convey infection to others because they have been contaminated by pathogenic organisms (12). An example of a fomite is an environmental surface such as a desktop or counter, which may be swabbed or wiped to detect the presence of an infectious agent.

Pulse-field gel electrophoresis: Electrophoresis is the separation of substances achieved by applying an electrical field to samples in a solution. The pulse-field gel technique is used with large molecules and is performed in a gel that slows the migration of molecules depending on molecular size (13).

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