VOLUME 4, ISSUE 3

FOCUS on Field Epidemiology

Laboratory Diagnosis: An Overview

CONTRIBUTORS

Authors:

Amy Nelson, PhD, MPH

Lauren N. Bradley, MHS

FOCUS Workgroup*

Reviewers:

FOCUS Workgroup*

Production Editors:

Tara P. Rybka, MPH

Lorraine Alexander, DrPH

Rachel A. Wilfert, MD, MPH

Editor in chief:

Pia D.M. MacDonald, PhD, MPH

*** All members of the FOCUS Workgroup are named on the last page of this issue.**

UNC SCHOOL OF PUBLIC HEALTH

NORTH CAROLINA CENTER FOR PUBLIC HEALTH PREPAREDNESS

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.

You are in the middle of investigating an outbreak of gastrointestinal illness in your county. You collect the proper clinical specimens and promptly ship them to the laboratory for testing. Then at the laboratory, someone with wiry hair in a long white coat waves a wand over the samples and chants until the true identity of the organism appears in a puff of smoke. Right?

Well, not exactly. Instead, we rely on the efforts of public health microbiologists. And given the importance of laboratory results in outbreak investigations and other public health arenas, it is helpful to have a basic understanding of public health laboratories and how they work. This issue of *FOCUS* provides an overview of the pathogens tested in public health laboratories and describes some commonly used lab tests.

A Review of Specimens

Once a specimen has been properly collected and shipped to the laboratory, as discussed in the last issue of *FOCUS*, the laboratory staff analyze the specimen to determine the presence or absence of suspected pathogens. Specimens can tell us whether different individuals are infected with the same pathogen and whether a particular source is causing an outbreak. To determine the source of infection, an investigator might take a potentially contaminated environmental sample for testing. Environmental samples include:

- food (items suspected in a foodborne outbreak),
- water (from a lake, water supply, or drinking fountain), and
- surfaces (medical equipment, countertops, etc., for example, the post office and letter sorting machines sampled in the 2001 anthrax outbreaks).

A good specimen is needed for laboratory testing, so proper specimen collection is important (see *FOCUS* Volume 4, Issue 2). Not only must the right sample be collected—such as feces in a diarrhea outbreak, or throat cultures in a strep throat outbreak—but the sample must be collected in the proper medium for survival and transported within a time frame and at a temperature that ensures the organism will still be in good condition when it arrives at the laboratory. And the sample must be accompanied by enough information that the laboratory knows what kind of test to conduct when it arrives. In an investigation, there is no such thing as having too much information!

Microorganisms

Understanding how the microbiology laboratory identifies the agent responsible for an outbreak requires knowing what the microorganisms causing the outbreak could be.

Bacteria are single-celled organisms. Bacteria that commonly cause illness in humans include *Salmonella*, *Streptococcus* ("strep"), *Staphylococcus* ("staph"), and *Escherichia Coli* (*E. coli*).

Viruses do not have a cell-like structure. They are composed of DNA (or

RNA) surrounded by a protective coat made of proteins. Viruses that infect humans include Influenza, HIV, West Nile, Noroviruses (a.k.a. Norwalk-like viruses), and common cold viruses such as Coronavirus and Rhinovirus.

Other pathogens that can infect humans and may cause outbreaks include toxins produced by bacteria, parasites, fungi, and chemicals.

Why is Laboratory Diagnosis Necessary?

Laboratory identification of the agent causing an outbreak is crucial. Diagnosis generally should not be based on clinical symptoms alone, because many agents can cause the same or similar symptoms in humans. For example, various agents that infect the gastrointestinal tract might all result in symptoms of abdominal cramping and diarrhea. Clinical symptoms may also be unclear or too general to definitively identify the pathogen. In addition, physicians recording symptoms might not recognize a rare disease that they have never encountered and then could misdiagnose a patient. Proper laboratory diagnosis is therefore important not only to connect individual cases that could be involved in an outbreak, but also to ensure proper medical treatment for the patient.

- For example, Norovirus and *Shigella* infections both cause diarrhea, cramping, and related gastrointestinal symptoms, but Norovirus can only be treated by providing symptomatic relief, while *Shigella* can be treated with an antibiotic.

Gross identification of the organism responsible for an outbreak is often just the first step in a laboratory investigation. In some cases, it is necessary to conduct further laboratory studies to determine the specific strain, or serotype, of a virus or bacterium responsible for the disease. This process is known as subtyping and is often an important part of an outbreak investigation.

- For example, there are dozens of potential strains of Noroviruses, including Hawaii virus, Snow Mountain virus, Desert Shield virus, and Toronto virus. Several people could be infected with a Norovirus, but if they all have different strains, the infections are not likely to have been acquired from the same source and they are therefore unrelated.

Determining that patients are infected with the same strain of a virus or bacterium can help identify outbreaks that occur across state lines. This might happen when a food item is contaminated at the processing plant and subsequently distributed to a large geographic region.

- For example, in the fall of 2006, CDC officials were notified of several small clusters of *E. coli* 0157:H7 infections in the states of Wisconsin and Oregon (1). Fresh spinach was implicated as the probable source of these infections. The same day, New Mexico epidemiologists contacted Wisconsin and Oregon epidemiologists regarding a similar cluster of *E. coli* 0157:H7 infections in their state, also believed to be associated with fresh spinach consumption. A few days later, CDC's PulseNet (described in *FOCUS* Volume 4, Issue 2) was able to confirm through laboratory testing that the *E. coli* 0157:H7 strains obtained from infected patients in Wisconsin had the same pulsed-field gel electrophoresis (PFGE) pattern and also identified that pattern in patient isolates from several other states.

Pathogen Identification and Typing

There are many methods of identifying the agent causing an outbreak. The method used depends on the type of organism (e.g., virus, bacteria, fungus). Some methods are well established for particular organisms, and guidelines exist for identifying the organism.

Table 1 lists various methods of detecting and identifying pathogens, specific types of tests performed with each method, and some of the advantages and disadvantages of the different approaches. Several of these laboratory methods and techniques are discussed in more detail later in this issue.

Laboratory Diagnosis and Surveillance Programs

Local and state diagnostic labs participate in national disease surveillance programs of the Centers for Disease Control and Prevention (CDC). The Council of State and Territorial Epidemiologists (CSTE), with input from the CDC, recommends surveillance for a long list of pathogens. Based on the CSTE recommendations, each state decides which pathogens state law will require healthcare providers and laboratories to report.

If a reportable disease-causing organism is identified in a participating lab, the lab reports this to the state health department through a disease reporting system. Guidelines specify which identification methods are to be used to report certain organisms, to ensure that only confirmed cases are reported. For example, only culture-confirmed cases of *Salmonella* are reported.

The state laboratory is responsible for identification when local labs do not have the necessary expertise, and the state lab has final responsibility for reporting these cases to the state health department. In some cases, identification of an organism may not be possible at the state level, and the CDC may be asked to help.

Microscopy

A microbiologist may be able to examine a clinical specimen directly under the microscope. This is useful for larger organisms such as bacteria or fungi. Typically, with a standard optical or light microscope, a small part of the specimen is smeared onto a glass slide and stains may be applied that function as a dye to help identify cells and substances within a specimen.

- For example, when using the Gram stain, a specific series of stains/reagents is applied to a bacterial specimen. Bacteria that are "Gram-positive" have a cell wall that will stain purple while "Gram-negative" bacteria stain as red.

Along with staining, the shape of a microorganism gives a clue to its identity. Two common bacterial shapes are round (cocci), and rod-shaped (bacilli). Furthermore, bac-

Table 1. Methods of pathogen identification and typing and examples of laboratory tests using each method

Identification method	Tests	Pros (+) and cons (-)
Microscopy Examination of organisms under magnification	• After preparation with various stains and reagents, specimen samples are put onto glass slides and examined with a light microscope • Smaller microorganisms (viruses) may require use of an electron microscope	+ Relatively quick and may provide immediate answers - Clinical specimen may not contain sufficient numbers of microorganisms for visualization without culture
Culture Propagation of microorganisms in a growth medium	• Organism is grown in a nutrient medium (culture plates, stab culture, slab culture, or liquid culture) OR • Organism is grown in live cells or tissue (cell culture or tissue culture)	+ Is the "gold standard": growth of the organism provides a definitive diagnosis - Limited by the quality of the specimen from which the organism is grown - Not all pathogens can be cultured - Does not detect past infection
Antigen detection Uses antibodies to detect antigens	• Latex agglutination (LA), complement fixation (CF), enzyme-linked immuno-assay (EIA), fluorescent antibody (FA) assay	+ Results often discernable by eye (no microscope needed) - Does not detect past infection - Not possible for all pathogens
Serology Detects any past immunological response to pathogen	• Latex agglutination (LA), complement fixation (CF), enzyme-linked immuno-assay (EIA), fluorescent antibody (FA) assay	+ Safe, because it does not require further growth of the pathogen + Routine methods of measurement available + Detects past infection - Not all pathogens create an immune response - May require sequential specimens
Typing method	**Tests**	**Pros (+) and cons (-)**
Phage typing Uses viruses (phages) that infect specific bacteria	• Tests using lambda phage, gamma phage, T4 phage, T7 phage, leviviruses, microviruses	+ Very useful for particular strains (*Staphylococcus*) - Many organisms are not typeable by this method - Not standardized for many organisms
Identification and typing method	**Tests**	**Pros (+) and cons (-)**
Molecular techniques Uses nucleic acid identification methods	• Pulsed field gel electrophoresis (PFGE), random fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), ribotyping	+ Relatively quick + High sensitivity - Often initially expensive (high start-up costs)

From: Herwaldt, et al. Microbial Molecular Techniques. In: *Epidemiologic Methods for the Study of Infectious Diseases*, JC Thomas, DJ Weber, eds. Oxford University Press, 2001: 163-191.

teria can cluster in pairs, chains, or other arrangements that help in their identification.

- For example, *E. coli* is a Gram-negative rod, while *S. pneumoniae* or pneumococcus is a Gram-positive diplococcus, a round bacterium that clusters in pairs.

Shapes and growth patterns can also be used to help identify fungi and fungal spores.

Viruses can also be viewed under a microscope. However, viruses are much smaller than bacteria or fungi and require a very high degree of magnification, so an electron microscope is typically used. This microscope shoots electrons at the virus to take its picture, much like a camera flash shoots light at an object to capture the image. Many viruses have a characteristic shape and can be fairly accurately identified from a microscope image. (See the Additional Resources on page 7 for sample pictures.)

Culture

Another method of identification is "culture." The laboratorian provides the right temperature, moisture, and nutrients for a pathogen to thrive and replicate, introduces a sample, and waits to see what, if anything, grows. The pathogen grown in this controlled laboratory environment can then be identified. In some outbreak situations, the case definition may require a definite case to be 'culture confirmed.'

Culturing a Clinical Specimen

Typically a clinical specimen is cultured for the types of microorganisms that are known to thrive in the particular environment and are associated with certain clinical symptoms. For example:

- Fecal samples in diarrheal illnesses are cultured for the presence of enteric pathogenic bacteria, including *Salmonella* serotypes (*typhi, enteritidis, typhimurium,* etc.), *Shigella*, *Campylobacter*, *Yersinia*, *Escherichia coli* 0157:H7, and *Vibrio,* for identification and/or serotyping.
- Respiratory samples are cultured for pathogens such as *Streptococcus pneumoniae, Bordetella pertussis, Haemophilus influenzae,* Influenza, *Legionella,* mycobacterium, or other organisms, depending on the clinical circumstances.
- Cervical, vaginal or penile specimens may be cultured for *Neisseria gonorrhoeae,* herpes, or other organisms known to cause genital infections.
- For example, in an outbreak of *E. coli* 0157:H7 infections among Colorado residents in June 2002, part of the case definition was that specimens taken from patients were culture-positive for *E. coli*. Contaminated beef was implicated in this outbreak and over 350,000 pounds of beef sold in retail stores were recalled (2).

Culture can also be used to increase the amount of the organism available to perform other types of tests, such as antigen detection assays or nucleic acid-based tests (discussed later in this issue).

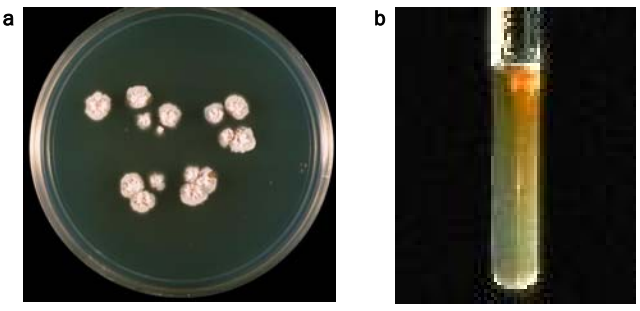
Different microorganisms require different growth environments. Bacteria are often grown on a Petri dish or plate containing a growth medium (often a gelatin-like substance called agar, as well as nutrients and other materials that make a bacterium feel comfortable). If the bacteria thrive, they pile up on each other to form distinctive-looking colonies that a laboratorian can identify just by looking at them (Figure 1a). Some bacteria are happier when grown inside the culture nutrients instead of on top of them. For these bacteria, a test tube is filled with the agar and nutrients, and a sterile wire is dipped into the sample of bacteria to be grown and stabbed into the agar-filled test tube. This is called a stab culture (Figure 1b). There are also other methods of growing different kinds of bacteria.

Viruses can also be grown in culture, but because viruses need living cells to reproduce themselves, they are often grown in tissue culture, which, as the name suggests, is derived from growing cells or tissues. Again, the idea is to grow enough virus from a clinical specimen to identify or

Figure 1.

a) Culture of *Nocardia asteroids*, a mycobacterium commonly found in soils. It causes illness in people with defects in cellular immunity.

b) Stab culture of *Legionella pnuemophila*, the agent that causes Legionnaire's disease. It is found in aqueous environments.



Photos courtesy of the CDC image library: http://phil.cdc.gov

confirm the identification of the disease-causing agent. After culture, viruses may be tested by nucleic acid-based methods or viewed under an electron microscope.

- For example, in June of 2003, there was a multistate monkeypox outbreak. The monkeypox virus was isolated from multiple patients and cultured. All case patients were found to have links to prairie dogs. The virus from the patients was grown in cell culture and confirmed using electron microscopy (3).

While culture is a very useful tool, different organisms require different conditions, and not all organisms that cause disease can be grown in culture. Other methods must be used for these organisms. There are other limitations to culture, such as the requirement of a considerable amount of time to grow certain organisms, which can have a negative impact on the pace of an outbreak investigation. For example, pulmonary blastomycosis, a fungal infection that causes severe respiratory symptoms, can require up to 5 weeks in culture before confirmatory diagnostic tests can be done (4).

Serology

In some cases, a person's immune response can be used to determine if the person has recently been infected by a particular pathogen. This is known as serology. From examining a blood sample, a laboratorian can detect an immune response to a recent infection by a specific pathogen. A previous infection can also be detected using the same method. Thus, one way of determining whether a person has fought off an infection by a particular pathogen is to have blood samples taken at the time of exposure (or shortly thereafter) and then several weeks later. If the person has a recent infection at the first blood sample and then evidence of an old infection by the second blood sample, you can conclude that the person was recently infected with that pathogen. Whether the infection is recent or old is determined by looking at antibodies, or immunoglobulins, which are part of the immune system's pathogen-fighting artillery. If the antibodies designed to fight off a pathogen are not present at the first blood sample, or are present in a very early form, and the fully mature antibodies designed to fight that pathogen are present at the second blood draw, it is safe to conclude that this person has been recently exposed to that particular pathogen. (See Table 2 on page 6 for descriptions of the different types of human antibodies.)

- One example of serology testing is the syphillis rapid plasma reagin or RPR test. This test determines whether a person has been exposed to syphilis by detecting the presence of antibodies against syphilis in a blood sample.

As you might imagine, this method of identification is not useful for a rapid intervention. It is often difficult to obtain a sample of blood from a patient even once, let alone twice. However, in some cases this method of detection may be useful, especially when the pathogen is not easily detected in other types of samples or the source of exposure has been eliminated with no remaining sample to test. Serology is also useful for research purposes.

Antigen detection

In clinical samples, we can also find small parts of a viral or bacterial pathogen, called antigens. In antigen detection, a laboratory processes the clinical or environmental sample to separate antigens from all the other material in the sample, and then performs a test using antibodies designed to find a particular antigen. If the test comes out positive, the antibodies have attached to the target antigen and the pathogen has been identified. If the test is negative (that is, the antibodies do not find anything to attach

Glossary:

Agar— a gelatinous colloidal extractive of a red alga (as of the genera *Gelidium, Gracilaria,* and *Eucheuma*) used especially in culture media or as a gelling and stabilizing agent in foods

Assay—laboratory test

Bacteria—round, spiral, or rod-shaped single-celled microorganisms that are often aggregated into colonies that live in water, soil, organic matter, or the bodies of plants and animals

DNA—any of various nucleic acids that are usually the molecular basis of heredity; constructed of a double helix held together by hydrogen bonds

DNA fingerprinting—a method of identification by determining the sequence of base pairs in the DNA of a person (or other creature).

Nucleic Acid—acids that are composed of nucleotide chains

Ribosome—RNA-rich structure in the cell that is the site of making proteins

RNA—any of various nucleic acids that contrain ribose and uracil as components; involved with the control of cellular chemical activities.

Virus—any of a large group of sub-microscopic infective agents composed of a protein coat that surrounds an RNA or DNA core; capable of growth and multiplication only in living cells

Table 2. Human antibodies used in laboratory testing

Antibody/ immunoglobulin:	General characteristics:	High levels may indicate:	Low levels may indicate:
IgA	• Located mainly in the nose, breathing passages, digestive tract, ears, eyes, and vagina; also in saliva and tears • Protect body surfaces exposed to outside organisms, bacteria, etc. • Comprise 10-15% of antibodies present in the human body • A small percentage of humans do not manufacture IgA antibodies	• IgA multiple myeloma • Autoimmune disease • Liver disease	• Some types of leukemia • Kidney damage • Enteropathy • Ataxia-telangiectasia
IgG	• Located in all types of body fluids • Comprise 75-80% of antibodies present in the human body • Considered to be most important in the fight against viral and bacterial infections • Only type of antibody capable of permeating placenta, are thus important during pregnancy	• Long-term chronic infection (e.g., AIDS) • IgG multiple myeloma • Long-term hepatitis • Multiple sclerosis • Certain autoimmune conditions	• Macroglobulinemia • Some types of leukemia • Kidney damage
IgM	• First antibody produced in response to infection • Located in blood and lymph fluid • Stimulate other immune system cells to generate compounds capable of eliminating foreign cells • Comprise 5-10% of antibodies present in the human body	• Macroglobulinemia • Early viral hepatitis • Mononucleosis • Rheumatoid arthritis • Kidney damage • Parasitic infection	• Multiple myeloma • Some types of leukemia • Some inherited types of immune diseases
IgD	• Located in the tissues lining the abdominal or chest cavity • Function not well understood, but may play a role in allergic reactions related to certain substances (e.g., milk, poisons, certain medications)	• IgD multiple myeloma	
IgE	• Located in the lungs, mucous membranes, skin • Cause body to react when in contact with foreign substances like pollen, mold, pet dander	• Parasitic infection • Allergic reaction (e.g., asthma, atopic dermatitis) • Some types of cancer • Certain autoimmune conditions • IgE multiple myeloma (rare)	• Ataxia-telangiectasia

Source: WebMD. Immunoglobulins. Available at: http://www.webmd.com/hw/lab_tests/hw41342.asp. Accessed January 3, 2007.

to), we still do not know which organism is causing the infection. There are many ways that antigens can be separated from the other matter in a specimen, and many ways the test for the antigen can be performed. (See the Additional Resources below for further information on this subject.)

Phage typing

A phage, short for "bacteriophage," is a virus that infects bacteria (5). There are many different types of phages, and each type only attacks a particular type of bacteria. Thus, phage typing is used to identify specific strains of bacteria (6). Phage typing is most often used to identify strains of *Staphylococcus aureus*, and the methods have been standardized for this organism.

When trying to identify an unknown bacterium taken from a clinical or environmental sample, microbiologists use a phage known to infect a specific strain of bacteria. The mixture of the "known" phage and the "unknown" bacterium is poured onto an agar plate. The plate is then left in temperature and humidity conditions that the bacteria should like, and the bacteria are allowed to grow. If the bacteria are the strain that the phage likes to attack, there will be a clear lack of growth of bacteria (called a plaque) wherever there is a phage, like a lawn with holes in it (Figure 2). If the plaques appear, the bacteria can be identified based on the phage that was used. If there are no plaques—the bacteria grow well and there are no holes–then the phage did not attack the bacteria, and that strain of bacteria can be eliminated as the possible pathogen.

Figure 2. Phage typing.

A "gamma phage" is used to identify *Bacillus anthracis* growing on a nutrient agar plate. The lawn of bacteria is interrupted where the gamma phage has attacked the bacteria, causing a "plaque," or hole in the bacterial growth.

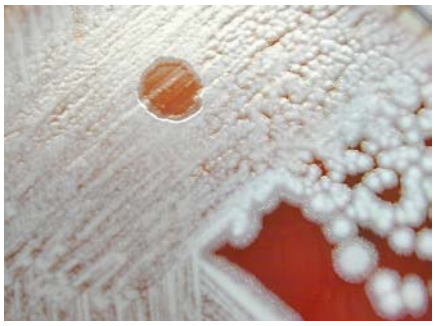


Photo courtesy CDC image library: http://phil.cdc.gov

Molecular techniques

We can also identify a pathogen by using nucleic acid (DNA, RNA) methods. Since every pathogen has either DNA or RNA, or both, microbiologists can look at genetic material from bacteria and viruses to find specific patterns. Each organism has a unique DNA fingerprint , so we often test a clinical or environmental sample for the presence of a bacteria or virus by looking for the tell-tale DNA . If the DNA of a particular pathogen is present in many of the cases in an outbreak, then you may have identified the cause of the outbreak. Identification techniques that rely on DNA and RNA are often referred to as molecular methods for typing organisms.

Molecular techniques are also very useful for distinguishing between the strains of an organism. For example, these methods can be employed when trying to distinguish between the strains of *E. coli* normally found in the human gut and a pathogenic strain that is causing disease during an outbreak. Identifying the exact strain of *E. coli* is important for finding the source of an outbreak.

This overview of diagnostic techniques can give you a better sense of what happens once you send that specimen off to the laboratory. In a future issue of *FOCUS*, we will delve further into more advanced laboratory techniques, such as molecular identification and typing.

Additional Resources:

To see examples of microorganisms that can often be identified with a Gram stain, go to http://www.uphs.upenn.edu/bugdrug/antibiotic_manual/gram.htm and click on "Typical Gram stains."

To see electron micrographs of viruses, go to http://www.ncbi.nlm.nih.gov/ICTVdb/Images/index.htm.

To find information on the diseases most often tested at public health labs, visit the North Carolina State Laboratory of Public Health Microbiology Web site: http://204.211.171.13/Microbiology/default.asp.

To find infectious disease information from the National Center for Infectious Diseases, go to http://www.cdc.gov/ncidod/diseases/index.htm.

To use the American Society for Microbiology Microbe Library, visit http://www.microbelibrary.org.

CONTACT US:

The North Carolina Center for Public Health Preparedness
The University of North Carolina at Chapel Hill
Campus Box 8165
Chapel Hill, NC 27599-8165

Phone: 919-843-5561
Fax: 919-843-5563
Email: nccphp@unc.edu

REFERENCES:

1. Centers for Disease Control and Prevention. Ongoing multistate outbreak of *Escherichia coli* serotype O157:H7 infections associated with consumption of fresh spinach --- United States, September 2006. *MMWR Morb Mort Wkly Rep*. 2006; 55(Dispatch):1-2. Available at: http://www.cdc.gov/mmwr/preview/mmwrhtml/mm55d926a1.htm. Accessed December 8, 2006.
2. Centers for Disease Control and Prevention. Multistate outbreak of *Escherichia coli* O157:H7 infections associated with eating ground beef --- United States, June--July 2002. *MMWR Morb Mort Wkly Rep*. 2002;51:637—639. Available at: http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5129a1.htm. Accessed November 30, 2006.
3. Centers for Disease Control and Prevention. Multistate outbreak of monkeypox—Illinois, Indiana, and Wisconsin, 2003. *MMWR Morb Mort Wkly Rep.* 2003;52:537-540. Available at: http://www.cdc.gov/mmwr/PDF/wk/mm5223.pdf. Accessed November 30, 2006.
4. Martynowicz MA, Prakash, UBS. Pulmonary blastomycosis: An appraisal of diagnostic techniques. *Chest.* 2002;121:768-773.
5. Mayer G. Bacteriology Chapter 7: Bacteriophage. In: University of South Carolina School of Medicine. Microbiology and Immunology On-line [Internet]. September 11, 2003. Available at: http://www.med.sc.edu:85/mayer/phage.htm. Accessed November 30, 2006.
6. Herwaldt, et al. Microbial Molecular Techniques. In: *Epidemiologic Methods for the Study of Infectious Diseases*, JC Thomas, DJ Weber, eds. Oxford University Press, 2001: 163-191.

If you would like to receive electronic copies of FOCUS on Field Epidemiology, please fill out the form below:

- NAME: _____
- DEGREE (S): _____
- AFFILIATION: _____
- E-MAIL ADDRESS: _____
- May we e-mail any of your colleagues? If so, please include their e-mail addresses here:

  _____

  _____

  _____

Please fax to: (919) 919-843-5563

or mail to: North Carolina Center for Public Health Preparedness
The University of North Carolina at Chapel Hill
Campus Box 8165
Chapel Hill, NC 27599-8165

Or go online: http://www.sph.unc.edu/nccphp/focus/

UPCOMING TOPICS!

- **Laboratory Diagnosis: Molecular Techniques**
- **Laboratory Diagnosis in Outbreak Investigations**
- **Contact Tracing**

We are on the web!
http://www.sph.unc.edu/nccphp