Chapter 34

LABORATORY SUPPORT FOR INFECTIOUS DISEASE INVESTIGATIONS IN THE FIELD

EDWARD T. CLAYSON, PhD AND CARL J. MASON, MD, MPH

E.T. CLAYSON; Lieutenant Colonel, MS, US Army; Product Manager, Joint Vaccine Acquisition Program, 64 Thomas Jefferson Dr., Frederick, MD 21702

C.J. MASON; Colonel, Medical Corps, US Army; Commander, USAMC-AFRIMS, APO AP 96546-5000

INTRODUCTION

Infectious disease surveillance and outbreak investigations in a field environment differ significantly from those conducted in a hospital, clinic, or laboratory and require special planning and coordination. Microbiology laboratory support may be hundreds or even thousands of miles away from a theater of military operations. Methods of specimen collection, storage, and transportation routinely used in a clinical setting are often not appropriate in a field setting. This chapter describes some of the special laboratory considerations needed to conduct infectious disease surveillance and outbreak investigations in a field environment. It was written for the military specimen collector and is not meant to be a laboratory guide for microbiologists. Considerations are discussed below for planning investigations, such as study purpose, study design, and laboratory support. Brief descriptions of common microbiological diagnostic methods are provided to aid in the selection of proper specimens for examination. Special considerations for specimen collection, handling, and storage are described. And finally, issues relating to specimen shipping are discussed. This information provides the medical investigator with the knowledge necessary to ensure that an investigation yields results that are useful to both the health care provider and the military commander.

STUDY CONSIDERATIONS

The usefulness of the information to be gained from a field study depends greatly on careful planning and coordination before the study begins. Issues such as study purpose and design must be addressed before the start of the study (see Chapter 32, Outbreak Investigation). Laboratory support is also an issue that needs to be addressed early. Preventive medicine personnel need to understand the available laboratory resources; this will aid in coordination and facilitate testing and reporting. Deployable military medical facilities are not usually equipped with microbiology laboratory capabilities, so organic microbiology laboratory capabilities are often lacking in the theater. However, during some large-scale operations, the Theater Area Medical Laboratory or the Navy Forward Laboratory may be deployed to provide some diagnostic capabilities to the theater of operations.[1] These laboratories are staffed with selected personnel and equipped with relevant but limited diagnostic capabilities. Often permanent (nondeployable) laboratories must be solicited for support. Army and Navy infectious disease research laboratories can serve as both reference and support centers (Exhibit 34-1). Because these laboratories have been given a formal mission of surveillance for emerging infectious diseases and provided with a budget to conduct surveillance and outbreak investigations, they can be considered for laboratory support for an infectious disease outbreak or surveillance investigation. If support from the military medical system is not available for the disease of interest, support from an international reference laboratory may be obtained. A partial list of international laboratories is shown in Exhibit 34-2.

LABORATORY DIAGNOSIS OF INFECTION

A principal role of a clinical microbiology laboratory is to provide accurate information about the presence or absence of microorganisms that may be involved in a patient's disease process. In a military environment, the identification of causative organisms is important for two reasons: first, therapeutic intervention is now possible for many infectious diseases, thus allowing for earlier return to duty of infected personnel, and second, combat commanders need to be informed of the medical threats facing their units.

A fundamental step in any diagnosis is the choice of an appropriate specimen, which ultimately depends on an understanding of the pathogenesis of infectious diseases. Awareness of the types of diagnostic tests available and their complexity will facilitate choosing the appropriate specimen (Table 34-1). Microbiological tests fall into four main categories: (1) identification of microorganisms by microscopic examination, (2) identification of microorganisms by isolation and culture, (3) detection of a specific microbial component or product, and (4) detection of specific antibodies to a microorganism.[2] General principles and applicability of various diagnostic assays in each of these four categories will be discussed below and are shown in Table 34-2. The information provided is not meant to be a "cookbook" description of each assay for use by a microbiologist. Rather, information is provided to aid medical investigators in the field in identifying and collecting appropriate specimens for transport to supporting laboratories.

EXHIBIT 34-1

LIST OF US DEPARTMENT OF DEFENSE MEDIAL RESEARCH LABORATORIES FOR INFECTIOUS DISEASES*

Army CONUS Laboratories

Walter Reed Army Institute of Research
503 Robert Grant Avenue
Silver Spring, MD 20910-7500
Phone: (301) 319-9100
Fax: (301) 319-9227

US Army Medical Research Institute of Infectious Diseases
1425 Porter Street
Fort Detrick, MD 21702-5011
Phone: (301) 619-2833
Fax: (301) 619-4625

Army OCONUS Laboratories

Armed Forces Research Institute of Medical Sciences
US Army Medical Component—Thailand
APO AP 96546
Phone: 66-2-644-4888
Fax: 66-2-247-6030

US Army Medical Research Unit—Kenya
Unit 64109 Box 401
APO AE 09831-4109
Phone: 254-2-729-303
Fax: 254-2-714-592

Navy CONUS Laboratory

Naval Medical Research Command
503 Robert Grant Ave
Silver Spring, MD 20910-7500
Phone: (301) 319-9208
Fax: (301) 319-7410

Navy OCONUS Laboratories

Naval Medical Research Institute Detachment (Peru)
American Embassy, Unit 3800
APO AA 34031
Phone: 51-1-561-2733
Fax: 51-1-561-3042

US Naval Medical Research Unit No. 2 (Indonesia)
Box 3, Unit 8132
APO AP 96520-8132
Phone: 62-21-421-4457
Fax: 62-21-424-4507

US Naval Medical Research Unit No. 3 (Egypt)
PSC 452, Box 5000
FPO AE 09835-0007
Phone: 20-2-684-1375
Fax: 20-2-684-7139

*Telephone and fax numbers may have changed.
CONUS: continental United States
OCONUS: outside the continental United States

Identification of Microorganisms by Microscopic Examination

Direct microscopic inspection of clinical specimens is usually the fastest and most economical method available for the detection of microorganisms. Some infectious agents can be reliably identified with only a few simple stains and a basic microscope—without the need for time-consuming and expensive cultivation techniques. In addition, microscopic methods sometimes permit diagnosis of infections when microorganisms cannot be cultivated and in specimens where the microorganism is no longer viable. Numerous methods for microscopic examination exist, including bright field, dark field, phase-contrast, fluorescence, and electron microscopy. The best method to use in a given situation depends on the equipment and reagents available in the support laboratory. A description of the principles and applicability of the various microscopy methods is beyond the scope of this chapter but can be found in common microbiological laboratory texts.[3–5]

Identification of Microorganisms by Isolation and Culture

In general, rapid diagnostic methods, which today exist for a broad range of microorganisms, are preferred over the time-consuming and labor-intensive methods of isolation and cultivation. Cultivation of microorganisms requires a day to several weeks, but many of today's rapid diagnostic methods require only a few minutes to a few hours. Nevertheless, cultivation is still the gold standard for diagnosis of infectious diseases, and it is likely to remain one

EXHIBIT 34-2

PARTIAL LIST OF INTERNATIONAL REFERENCE LABORATORIES FOR COMMUNICABLE DISEASES*

Center for Applied Microbiology Research
Porton Down
Salisbury Wiltshire SP4 0JG
United Kingdom
Phone: 44-1980-612100
Fax: 44-1980-611096
www.camr.org.uk

Centers for Disease Control and Prevention
National Center for Infectious Diseases
1600 Clifton Road, N.E.
Atlanta, Georgia 30333
USA
Phone: 1-800-311-3435
Fax: 1-404-639-2334
www.cdc.gov

Epicentre
8 Rue St Sabin
75011 Paris
France
Phone: 33-1-40-212850
Fax: 33-1-40-212803

Institut de Medicine Tropicale Prince Leopold
Nationalestraat 155
B-2000 Antwerp
Belgium
Phone: 32-3-247-6666
Fax: 32-3-216-1431
www.itg.be

National Institute of Infectious Disease, Japan
1-23-1 Toyama
Tokyo 162-8640
Japan
Phone: 81-3-5285-1111
Fax: 81-3-5282-1150
www.nih.go.jp

Public Health Laboratory Service
61 Colindale Ave
Colindale, London NW9 5HT
United Kingdom
Phone: 44-181-2004400
Fax: 44-181-2007874
www.phls.co.uk

South African Medical Research Council
PO Box 9070
7505 Tygerberg
Republic of South Africa
Phone: 27-21-9380911
Fax: 27-21-9380200
www.mrc.ac.za

Statens Seruminstitut
Artillerivej 5
2300 Kobenhavn S
Denmark
Phone: 45-32-683268
Fax: 45-32-683868
www.serum.dk

*Specimens should be forwarded to these or other laboratories only after consultation. Proper shipping containers must be used, and all regulations for shipping hazardous, high-risk specimens must be observed. Telephone and fax numbers may have changed.

of the more important microbiological methods in the laboratory for some time. Cultivation of microorganisms isolated from patient specimens is often necessary before laboratory technicians can carry out the multitude of assays needed to identify or differentiate the agent from among various disease-causing agents or determine the susceptibility of isolated microorganisms to various antimicrobial agents.

Unfortunately, there is no universal method for the cultivation of all microorganisms. Each microorganism has its own optimum conditions for growth, which vary widely. The determination of the optimum cultivation conditions for various microorganisms has taken years of experimentation, and the optimum conditions for many microorganisms remain unknown. However, the various methods available for the cultivation of a broad range of microorganisms can be divided into three main categories: cultivation using artificial media, cultivation using cell culture, and cultivation using experimental animals. Descriptions of these methods can be found in common microbiological laboratory texts.[2,6]

Detection of Microbial Components or Products

Clinical microbiologists now have many new techniques to aid them in the laboratory diagnosis of infectious diseases. These new methods may involve the detection of structural components of the microorganism, detection of microbial products (eg, toxins), or

TABLE 34-1

LABORATORY SPECIMENS REQUIRED FOR TESTS FOR PARTICULAR CAUSATIVE AGENTS

Suspected Agent or Disease	Specimen	Test
Arbovirus	Blood or brain (-70°C)	Isolation
	Blood or serum (4°C)	Serology
Cholera	Rectal swabs or stool specimens in transport medium, as recommended by the laboratory	Culture
Gastroenteritis	Stool	Culture (bacterial,viral), electron microscopy, ELISA
	Blood or serum (4°C)	Serology
Hepatitis	Serum (4°C)	ELISA
Legionella	Sputum in enrichment broth, blood	Culture; FA
Malaria	Blood (thick and thin smears)	Staining
Meningococcal meningitis	Spinal fluid, blood, pharyngeal swabs (all on transport media)	Culture, counter-immunoelectrophoresis
Plague	Bubo fluid, blood (in broth or on blood agar slants)	Culture, FA
Rabies	Brain (-70°C)	FA and isolation
Salmonella typhi	Blood (early in disease) in enrichment broth	Culture
Shigella	Rectal swabs in enrichment broth or fecal specimens	Culture
Typhus	Blood	Inoculation
	Serum (4°C)	Serology
Varicella and Suspected Smallpox	Lesion fluid, crusts	Electron microscopy, cell culture

ELISA: Enzyme-linked immunosorbent assay
FA: Fluorescent antibody test
Source: Reprinted with permission from Brès P. *Public Health Action in Emergencies Caused by Epidemics.* Geneva: World Health Organization; 1986: 238.

detection of specific gene sequences. Since these methods do not depend on the growth and multiplication of microorganisms, they are potentially more rapid than culture methods. In addition, these methods permit diagnosis of uncultivatable agents and are more specific than direct microscopic examination. They are potentially applicable to all microorganisms, as long as specific antibodies or probes are available. A brief explanation of the principles of several of the more common techniques is provided below.

Detection of Microbial Antigens or Products

Methods for the detection of microbial antigens or products rely on the ability of microorganism-specific antibodies to bind microbial antigens or products. Monoclonal antibodies, which are increasingly being used in microbiology, are highly specific and allow for distinction among different species and among strains of the same species. As more antigen-specific monoclonal antibodies become commercially available, the detection of microorganisms will be accomplished with a higher degree of sensitivity and specificity than was ever possible before. However, these methods are limited by the availability of specific antibody probes. Detection of the antibody–antigen reaction is facilitated by the use of labeled antibodies. Antibodies can be labeled with fluorescent markers, particles (eg, latex), enzymes that permit colorimetric analysis, or radioisotopes. Available

TABLE 34-2

USE OF VISUALIZATION AND OTHER TECHNIQUES FOR LABORATORY EXAMINATIONS

	Techniques					
	Direct Microscopy					
Agent	Gram Stain	Geimsa Stain	IF	EIA and ELISA	Histopathological and Cytological	Biochemical
Parasitic		+	+	+	+	+
Mycotic		+	+	+	+	+
Bacterial	+	+	+	+	+	+
Mycoplasmal		+	+	+		+
Rickettsial		+	+	+	+	+
Chlamydial		+	+	+	+	+
Viral			+	+	+*	+

*Electron microscopy in certain diseases
IF: immunofluorescence
EIA: enzyme immunoassay
ELISA: enzyme-linked immunosorbent assay
Source: Reprinted with permission from Brès P. *Public Health Action in Emergencies Caused by Epidemics.* Geneva: World Health Organization; 1986: 101.

detection methods include immunofluorescence, latex agglutination, enzyme-linked immunosorbent assay (ELISA), and radioimmunoassay.

Detection of Microbial Genes

An alternative approach to using antibodies to detect microbial antigens is to use specific nucleic acid probes to detect microbial gene sequences. Nucleic acid–based detection methods were first described in the mid 1970s, but they have only recently been accepted in the clinical laboratory. The early procedures were not widely adopted in clinical laboratories because they were more expensive, more labor-intensive, more time-consuming, less sensitive, and potentially more hazardous than prevailing antibody-based methods. Recent improvements in nucleic acid amplification and nonradioactive detection systems, however, have greatly improved the sensitivity and specificity of nucleic acid–based detection systems and have made these methods more acceptable for the clinical laboratory. In addition, these methods are useful in detecting nonviable microorganisms or those that are difficult or dangerous to cultivate.

The key to nucleic acid–based methods is the gene probe, a nucleic acid molecule (usually DNA) with a sequence complementary to the gene to be detected. Polynucleotide probes can be synthesized from scratch if the genomic sequence of the microorganism is known or can be obtained from naturally occurring DNA by cloning DNA fragments into appropriate vectors and isolating the cloned DNA. Nucleic acid probes can be labeled with a radioisotope (usually phosphorus-32P [^{32}P]) with biotin, a specific enzyme or a fluorescent marker, or with digoxigenin, a chemiluminescent marker. Biotinylated probes using streptavidin detection systems have been used in some laboratories, but these methods are not as sensitive as radioactive methods. A commonly used label today is the chemiluminescent marker because of the long shelf-life of the assay components, the marker's sensitivity, which approaches that of radioactivity, and the elimination of hazards associated with the use of radioactivity. Increasingly, nucleic acid probes for a wide variety of microorganisms are becoming commercially available.

Gene detection methods rely on the ability of one or more nucleic acid probes to hybridize with the genome of the microorganism. In some methods, the nucleic acid of a tissue or of a culture of a patient specimen is first extracted and then hybridized with one or more microbe-specific probes. Alternatively, hybridization can occur in situ in patient tissues or laboratory cultures. Specific infected cells contain-

ing intracellular parasites (eg, viruses, rickettsia, chlamydia) may be identified by the in situ technique. Nucleic acid–based detection methods include blot assay, in situ hybridization, and polymerase chain reaction. Descriptions of these methods can be found elsewhere.[7–10]

Detection of Antibodies to Microorganisms

During the early stages of an infection, the immune system is exposed to a rise in the amount of microbial antigen as the infecting microorganism replicates. The initial host response is nonspecific, directed at infectious processes in general, until the host can generate a more efficient, specific immune response. For assay purposes, the specific immune response is divided into cellular and humoral response mechanisms. These two response mechanisms are closely related and interdependent. The cellular mechanism is more difficult to measure and is not used as the basis of any routinely performed assays.

Early in infection, there is a surge in available antigen (the microorganism itself or one of its products) in patient serum. Culture, antigen detection, and genomic detection techniques are most likely to be useful during this period. A transient rise in specific IgM antibodies and a subsequent, more persistent rise in specific IgG antibodies follow the surge in antigen. For microorganisms that invade mucosal surfaces, there will also be an accompanying rise in specific IgA antibodies at the mucosal surface. The rise in IgA antibodies may not be detectable in serum or plasma.

Detection of antibodies specific for the infecting microorganism will be most successful following the rise in IgM antibodies. Evidence for infection by a given microorganism may be provided by detection of high levels of specific IgM antibodies or by detection of a 4-fold or greater rise in specific antibodies in paired serum or plasma samples (Table 34-3). The finding of a high titer of agent-specific IgG in a single specimen is not considered indicative of recent infection, but a rise in agent-specific IgG as detected in paired specimens suggests recent infection. In addition, a serum with high levels of agent-specific IgM strongly suggests recent infection. Ideally, the paired samples should consist of one sample collected at the onset of infection (when antibody production is minimal) and a second sample collected at least 7 to 14 days later, when antibody is maximal (or longer for some rickettsial infections). This provides strong evidence of recent infection with the given microorganism. Unfortunately, the initial acute sample is not always collected. For US military personnel, a predeployment sample from the Department of Defense Serum Repository in Rockville, Md, may sometimes be substituted for the initial acute specimen.

In parallel to the rapid developments in nucleic acid techniques, advances have been made in the detection of antibodies specific for microorganisms. Two of these techniques, ELISA and agglutination, have been widely adapted for use in assays in both clinical and research settings and serve as the basis of most commercially available antibody assays. Three other techniques—complement fixation, hemagglutination, and precipitation—although important in the past are used less frequently today.

Diagnostic and Reporting Issues

Understanding the various diagnostic assays described above is not enough. There are other issues that must be understood to interpret test results properly. The sensitivity and specificity of the diagnostic test must be known to determine the reliabil-

TABLE 34-3

SEROLOGICAL EVIDENCE OF RECENT INFECTION

Specimen	Result
Single Serum	Presence of IgM specific for the suspected agent (non-specific reactions must be eliminated)
	High titer of specific undifferentiated (IgM + IgG) antibody, if higher than the long-lasting immunity level for the disease concerned
Paired Sera*	4-fold increase in titer (IgM + IgG) with specific antigen

*The first sample should be collected soon after disease onset, the second ideally 10 to 14 days later for most diseases.
Adapted with permission from Brès P. *Public Health Action in Emergencies Caused by Epidemics.* Geneva: World Health Organization; 1986: 103.

ity of test results. An understanding of the incidence of the specific disease among the population examined is also required to determine the predictive value of the test.

Sensitivity and Specificity

Sensitivity and specificity are terms used to describe the performance of a given test or assay compared with one or more widely accepted standards. A widely accepted standard is often referred to as the gold standard. Sensitivity is a measure of the capability of the test or assay to detect all true positive cases in a given population. It is usually expressed as a percent and given by this formula:

$$\begin{aligned}\text{Sensitivity (\%)} &= 100\ (\text{Assay True Positives})/[(\text{Assay True Positives}) + (\text{Assay False Negatives})] \\ &= 100\ (\text{Assay True Positives})/(\text{True Positives})\end{aligned}$$

For example, a test with a stated sensitivity of 99% is expected to detect 99 of 100 positive cases when correctly performed (and interpreted) on properly collected specimens from a population similar to that used originally to determine the sensitivity.

Specificity is a measure of the capability of the test or assay to detect all true negative cases. It is also usually expressed as a percent and given by the formula:

$$\begin{aligned}\text{Specificity (\%)} &= 100\ (\text{Assay True Negatives})/[(\text{Assay True Negatives})+(\text{Assay False Positives})] \\ &= 100\ (\text{Assay True Positives})/(\text{True Positives})\end{aligned}$$

For example, a test with a stated specificity of 99% is expected to detect 99 of 100 negative cases when correctly performed (and interpreted) on properly collected specimens from a population similar to that originally used to determine the sensitivity.

Positive Predictive Value

The positive predictive value is a measure of the meaning of a positive test or assay result. It depends on the prevalence of the disease or condition in the population from which the specimens being tested were collected. The positive predictive value is usually expressed as a percent and given by the formula:

$$\text{Positive Predictive Value (\%)} = 100\ (\text{Assay True Positives})/[\text{Assay Positives (True + False)}]$$

For example, given a population with a prevalence of disease markers of 20% and an assay with a stated sensitivity and specificity of 99%, the positive predictive value of the assay will be 96%. This implies that a positive result on this assay in this population will predict the presence of disease markers 96% of the time. Given a different population with a prevalence of disease markers of 1% and the same assay with a stated sensitivity and specificity of 99%, the positive predictive value of the assay will be 50%, which implies that a positive result in this population will correctly predict the presence of disease markers only 50% of the time. Therefore, the use of tests (even highly sensitive and specific tests) to screen for diseases of low prevalence is problematic.

The positive predictive value can be improved by combining two or more tests in series. For example, a positive test result on an ELISA test for human immunodeficiency virus-1 (HIV-1) is usually followed by a Western blot assay for HIV-1; this improves the positive predictive value of the screening program in the United States, where the prevalence of antibodies to HIV-1 is extremely low.

Interpretation

Laboratory results should always be interpreted in combination with epidemiologic data, historical information, and other clinical and laboratory findings. Several reasons for test error are included in Table 34-4. Inappropriate sample collection, sample storage, and sample shipping are major sources of laboratory error. Steps to minimize these problems are covered elsewhere in this chapter.

The selection of appropriate laboratory tests and assays must be a coordinated effort with the laboratory to ensure that the results obtained are meaningful and useful. Many of the assays that are approved by the Food and Drug Administration (FDA) and are commercially available have been standardized on Western European or North American populations. They can thus be expected to perform as stated when applied to specimens collected from US military personnel or civilians in the United States. But FDA-approved tests and well-characterized assays are not available for many of the agents from areas of the world that are of potential interest and importance to deploying US forces.

Within the United States, unapproved medical devices (including diagnostic assays) may not be used in clinical care settings without an FDA Investigational Device Exemption (IDE). By custom, the US Department of Defense attempts to comply

TABLE 34-4

CAUSES OF ERROR IN THE INTERPRETATION OF LABORATORY TESTS

False Positive Results	False Negative Results
Microscopic Methods	
Saprophyte present	Inappropriate sampling
Nonspecific staining	Inappropriate dye
	Need for electron microscopy
	Inappropriate specimen
	Inexperience of microscopist
Isolation Methods	
Concurrent agent in specimen is easier to detect than causative agent or withstands storage conditions better	Inappropriate sampling: specimen inadequate or taken at wrong time
Concurrent chronic infections (eg, malaria, schistosomiasis)	Damage to agent by shipping or storage conditions
Concurrent pathogen in an outbreak primarily caused by a toxic agent	Inappropriate laboratory techniques
Contamination of specimens or reagents	"New" agent requiring unusual conditions for isolation
	Presence of immune complexes
	Bacterial or contaminant overgrowth
Antibody Detection Methods	
Presence of antibodies (IgG) to endemic disease	Sample taken at wrong time
Cross reaction of antibodies among antigenically-related agents	Damage to specimen during shipping and storage
Nonspecific reactions	Inappropriate laboratory techniques
Previous immunization or skin test	Inappropriate antigen battery
Presence of excess IgG or rheumatoid factors when detecting IgM	Presence of immune complexes
	Immunosuppression

Adapted with permission from Brès P. *Public Health Action in Emergencies Caused by Epidemics.* Geneva: World Health Organization. 1986: 104.

with FDA requirements in the diagnosis and treatment of US military personnel and dependents overseas, but procedures exist for emergency use of an unapproved medical device for medical care. These procedures require the user to notify the Institutional Review Board within 5 days of use of the device or assay and to submit an application for an IDE if future use is likely. If clinical use of an unapproved diagnostic assay is being considered, every effort should be made to seek guidance. For the US Army, guidance may be obtained from the Human Subjects Protection Division at the US Army Medical Research and Materiel Command, Fort Detrick, Md.

SPECIMEN COLLECTION, HANDLING, AND STORAGE

All too often, the importance of collecting the proper specimens and transporting them to the laboratory is neglected or not emphasized. Perhaps the most common reason for failure to establish a diagnosis (or suggesting a wrong one) is the collection of an inappropriate specimen. To obtain a test result that correctly identifies the etiology of the infection, it is important to collect an appropriate specimen, use appropriate transport, and deliver specimens to the laboratory rapidly. No matter how sophisticated the laboratory or how expert its personnel, if the specimen is inappropriately chosen,

TABLE 34-5

METHODS TO BE EMPLOYED IN COLLECTING RESPIRATORY TRACT SPECIMENS

Purpose	Procedure
Bacteriology, mycology or parasitology	Direct examination of sputum: thin smear on a slide for Gram staining
	Cultivation: make a cough swab. Fragile bacteria require special media and particular precautions (ask laboratory for guidance)
Virology	Direct examination by immunofluorescence: cough swab transported in Hanks' medium at 4ºC, or preferably nasopharyngeal aspirate obtained with a suction apparatus
	Cultivation: same specimens. Fragile viruses require special media and particular precautions (ask laboratory for guidance)

Source: Reprinted with permission from Brès P. *Public Health Action in Emergencies Caused by Epidemics.* Geneva: World Health Organization; 1986: 241.

collected, or transported, results will be less than optimal and will often be misleading. Early communication between the investigator and the laboratory is extremely important. Laboratory personnel can advise the investigator on the collection of optimum specimens and will be better able to guide the processing of these specimens if potential problems have been discussed. Some specific methods are listed in Table 34-5, Table 34-6, Table 34-7, and Table 34-8. This section discusses some general issues regarding specimen collection in the field and provides specific guidance in the collection of specimens for microscopic examination, isolation and culture, detection of microbial components or products, and detection of antibodies to infectious agents.

Practical Issues

All clinical specimens (including serum samples for antibody detection) should be handled as if they are infectious. Universal precautions as outlined by the Occupational Safety and Health Act should be followed during collection and transport to the laboratory.[11] In particular, appropriate barriers should be used during specimen collection to prevent exposure of skin and mucous membranes to specimens. Gloves must be worn at all times, and masks, goggles, and gowns or aprons must be worn in situations in which there is a risk of splashes or droplet formation. After specimen containers are sealed, they should not be opened until they reach the laboratory, where they can be opened safely in

TABLE 34-6

METHODS TO BE EMPLOYED IN COLLECTING SPECIMENS OF FECES

Purpose	Procedure
All examinations	3 mL (or equivalent in solid) in screw-cap "bijou" bottle (capacity 7 mL). Store at 4ºC or normal refrigerator temperature
Parasitology	3 parts of 10% formaldehyde solution are added to 1 part of stool
Bacteriology	Use special transport medium for cholera, other vibrios, *Salmonella*, *Shigella*, etc; store at room temperature in shade, not in refrigerator. If medium not available, consult laboratory
Virology	A suitable virus transport medium may be provided by the laboratory

Source: Reprinted with permission from Brès P. *Public Health Action in Emergencies Caused by Epidemics.* Geneva: World Health Organization; 1986: 241.

TABLE 34-7

METHODS OF COLLECTING SPECIMENS OF FOOD AND OTHER MATERIALS

Type of Specimen	Method
Liquid food	Shake, pour 200 mL into sterile container, refrigerate but do not freeze
Solid or mixed food	Separate portions with sterile knife, transfer to a sterile glass jar (eg, jam jar); take samples from periphery to central laboratory; refrigerate
Meat and poultry	Cut portion of meat or skin aseptically from different parts of carcass; alternatively, wipe large portions of carcass with sterile gauze squares or swabs; place in transport medium
Water	See Table 34-8
Other	Collect any fabric (eg, sheets or towels) known or suspected to contain poison, vomit, urine, or feces

Source: Reprinted with permission from Brès P. *Public Health Action in Emergencies Caused by Epidemics.* Geneva: World Health Organization; 1986: 245.

a biological safety cabinet.[12]

Whenever possible, specimens should be collected before antimicrobial agents have been given. Collection of specimens after initiation of antimicrobial therapy may drastically affect recovery of bacteria and parasites and lead to misdiagnosis. Likewise, fecal specimens to be examined for parasites should be collected before barium is used for radiological examination.[13] Specimens should be obtained from the site of infection with minimal contamination from adjacent tissues and organ secretions. Knowledge of the stage of the infection (eg, acute vs. chronic, time of onset) is helpful in the selection of appropriate specimens. The type of specimen and stage of infection when a culture is taken may determine whether or not the etiological agent will be isolated. Specimens should be large enough to allow thorough examination. Many microbiological techniques are relatively insensitive, so it is important to collect large enough samples and to maintain viable organisms in the samples while they are in transit to the laboratory. However, due to regulations regarding the shipment of clinical specimens, 50 mL per specimen should be considered the upper limit.[14] Specimens should be collected in appropriate, safe, and sterile containers with tight-fitting lids. In general, the quicker the specimen is delivered to the laboratory, the better the laboratory results will be. Quick delivery, though, is not usually possible in a field setting.

Each specimen should be labeled with the name or

TABLE 34-8

COLLECTION OF WATER SAMPLES*

Type of Water	Method of Collection
Tap water	1. Disinfect the mouth of the tap with a burning cotton wool swab soaked in alcohol 2. Let the water flow for 2 m 3. Fill the bottle
Well-water	Weight a bottle with a sterile stone attached with sterile string and dip into well
Open water†	Plunge the bottle neck down into the water and then turn it upwards with the mouth facing the current

*Water should be collected in sterile bottles (1–5 L)
†Water from springs, streams, rivers, and lakes
Source: Reprinted with permission from Brès P. *Public Health Action in Emergencies Caused by Epidemics.* Geneva: World Health Organization; 1986: 249.

identification number of the person from whom the specimen was collected, the source or type of specimen, and the date and time of collection. Labeling of specimens is especially important in surveillance and outbreak investigations. These investigations often generate hundreds or thousands of specimens that can easily be misidentified if improperly labeled. Unfortunately, errors in specimen labeling are too common and often lead to faulty results. Maintaining correct specimen identification during the specimen's passage through the laboratory is critical.

Sufficient clinical information should be provided to guide the microbiologist in proper, optimal processing of the specimen. A list of the tests desired by the investigator is helpful. Age and sex of the patient are important with respect to some infections, and the patient's clinical features must be included. Relevant geographic and travel details are also important to indicate possible exposure to pathogens in endemic areas. Finally, the date and time of specimen collection and of arrival in the laboratory indicate how much time has elapsed since the specimen was collected.

Specimens for Microscopic Examination

Microscopy plays a fundamental role in microbiology and is an important first step in the examination of all diagnostic specimens, especially when a parasite is suspected as the etiological agent. Preferably, slides for microscopy are prepared with fresh specimens, but in a field setting this is not always possible. If blood is to be examined for parasites, then it should be collected using an anticoagulant ([EDTA] is preferred).[13] Thick or thin films for parasite identification may be prepared on slides in the field and sent to the laboratory; alternately, tubes of blood containing an anticoagulant may be sent to the laboratory and slides prepared there. If examination of a sputum specimen is to be delayed for any reason, then a portion of the sputum should be fixed in 5% or 10% formalin so that helminth eggs or larvae may be preserved. The remainder of the specimen should be saved for bacterial and viral cultivation. Wet mount slides may be prepared in the field (preferably) or in the laboratory. If examination of a fecal specimen is to be delayed (more than 30 minutes for liquid specimens, 1 hour for soft specimens, and 1 day for formed specimens), then the specimen should be fixed in one of several available fixatives. A series of at least three specimens collected 48 hours apart is preferred for the diagnosis of intestinal parasites. Slides of fecal material should be prepared in the laboratory.[13] Other aspects of collecting fecal samples are listed in Table 34-6.

Slides and specimens prepared for microscopic examination can be stored at room temperature or in a refrigerator (4°C). Care should be taken to prevent overheating specimens intended for microscopic examination (Table 34-9).

Specimens for Culture

A fluid specimen (including exudate and excreta) collected early in the acute phase of illness is the most desirable specimen for isolation and culture. In a typical clinical setting, a sterile swab is commonly used to collect fluid specimens.[2] In a field environment, however, swab collection usually fails to provide suitable specimens for analysis. Swabs absorb only a small quantity of sample and provide an arid, unfriendly environment for transport to the laboratory. Therefore actual fluid should be collected whenever possible to ensure that a large

TABLE 34-9

GENERAL GUIDELINES FOR STORAGE AND SHIPPING TEMPERATURES OF BIOLOGICAL SPECIMENS

Type of Specimen	Storage Conditions	Shipping Conditions
Specimens for microscopic examination	Room temperature or refrigerator temperature (4°C)	Ambient temperature or wet ice
Specimens for culture and isolation	Refrigerator temperature (4°C) if for less than 24 h; < –70°C otherwise	Dry ice or liquid nitrogen
Specimens for detection of microbial components or products	Refrigerator temperature (4°C) if for less than 24 h; < -70°C otherwise	Dry ice or liquid nitrogen
Specimens for detection of antibodies	Freezer temperature (-20°C)	Wet ice

enough volume of sample is collected.

Maintaining viable organisms in the sample until the specimen reaches the laboratory is critical. Specimens for culture should be collected in sterile containers and should not be put into fixatives or preservatives, which are lethal to microorganisms. In cases when a virus is suspected as the etiological agent, placing the specimen in a viral transport medium is preferred. A virus transport medium should preserve the virus in the specimen, prevent loss of the specimen due to bacterial or fungal contamination, be nontoxic to cell cultures, and not interfere with direct tests to detect and identify virus antigens (eg, immunofluorescence, enzyme immunoassays). Several transport media are commercially available. Antibiotics can be added to specimens for the isolation of viruses, fungi, and algae to reduce bacterial contamination, but specimens containing antibiotics cannot be used for the isolation of bacteria.

Specimens for culture may be stored in a refrigerator or on ice (0°C–4°C) if less than a 5-day delay is expected. Specimens expected to be stored or in transit longer than 5 days should be frozen at or below -70°C initially and shipped on dry ice or liquid nitrogen. Specimens for culture should not be stored or transported at room temperature, in normal kitchen freezers (-20°C), or in incubators (30°C–38°C) because many microorganisms degrade quickly at these temperatures. Care should be taken to prevent thawing of specimens, as multiple freezing and thawing of specimens is known to reduce recovery of microorganisms.

Specimens for Detection of Microbial Components or Products

Specimens suitable for culture are also suitable for the detection of microbial components or products, but the detection of microbial components or products does not depend on the presence of living organisms. Therefore, samples containing fixatives, preservatives, or sometimes antibiotics are acceptable. However, minimizing contamination by extraneous organisms is very important, so sterile containers should be used. Specimens should be stored and shipped at the coldest temperature achievable under field conditions or at temperatures equal to or lower than -70°C in the laboratory.

Specimens for Detection of Antibodies

Samples of sera and sometimes cerebrospinal fluid are used to detect antibody responses. Specimens should be collected in a sterile manner. Paired sera, collected in the acute and convalescent phases of the disease (ideally 10 to 14 days or more apart), should be tested at the same time. Whenever possible, whole blood should not be sent to the laboratory. Serum should be separated from the cells in the blood and the serum sent to the laboratory. Serum samples can be stored at normal scientific freezer temperatures (-20°C to -25°C) for months or years without loss of antibody titer. The thermal cycling associated with residential-type frost-free freezers can, however, reduce antibody levels.

SPECIMEN SHIPPING

The job of the field investigator is not over when the specimens have been collected. The specimens must be sent to the laboratory for examination. Care should be taken to get the specimens to the laboratory as soon as possible and in a condition useful for examination. In addition, certain regulatory guidelines must be followed so that persons handling potentially infectious material are not exposed to it.

Timely Delivery

Specimens should be transported to the laboratory as quickly as possible. By their very nature, many clinical specimens provide a good medium for the growth of bacteria and fungi. Microorganisms may multiply during the time between collection and cultivation in the laboratory. With time, the hardy species may overgrow the fastidious, giving a false impression of the balance between species; in some cases, this will make it impossible to isolate and identify the less-hardy species. Bacteria or fungi may render specimens for the detection of viruses useless if allowed to grow unchecked. The addition of antibiotics in viral transport media may be necessary to preserve these specimens. Some specimens are naturally prone to drying (eg, specimens collected with swabs), which will complicate or make impossible the isolation and cultivation of microorganisms. Rapid transport to the laboratory will help prevent many of these problems.

Regulatory Issues

Packages of clinical or laboratory specimens prepared for mailing or shipping must conform to US postal regulations and those of the US Department of Transportation and the US Public Health Service.[14–17] Packages shipped between overseas locations must conform to these regulations as well as the Dangerous Goods Regulations of the Interna-

tional Air Transport Association (Figure 34-1). The regulations, which govern package containers, volume limitations, and labeling requirements, were designed to protect all who may come in contact with the package. Compliance with these regulations is the responsibility of the sender, and large financial penalties may be levied on senders of improperly packaged and labeled shipments.

Containers

In general, diagnostic specimens or infectious substances must be mailed in double containers. Each specimen is contained in a primary container (eg, tube, vial). The cap of the primary container should be sealed with waterproof tape. One or more primary containers may be packed together in a secondary container filled with cotton or other absorbent material that is capable of absorbing the entire contents of all the primary containers. The secondary container must be sealable and break-resistant, and its lid should be taped to prevent loss of contents during shipment. The secondary container should be placed in a shipping container. Wet or dry ice to be included with the shipment, if any, should be placed between the secondary container and the shipping container.

Microscope slides containing specimens do not require double containers for shipment. They may be packed in boxes, cardboard slide holders, or any other suitable container that will prevent damage or breakage. Slides should be completely dry before packing and should be individually wrapped in soft tissue. If more than one slide is to be shipped or mailed, the slides can be wrapped together in tissue as follows: a slide is placed on the tissue and wrapped several times, and then the next slide is placed on top of the first and both are wrapped several times. The series of slides wrapped this way will be padded and can be easily unwrapped on arrival. Slides packed in flat cardboard containers need additional protection. Plastic slide containers with snap tops work well for shipping slides.[13]

Volume Limitations

Various regulations limit the amount or volume of specimen that may be shipped in any one package.[14–16] The limit depends on whether the specimen is categorized as a diagnostic specimen or an infectious substance. In general, a diagnostic specimen is defined as any human or animal material, including but not limited to excreta, secreta, blood and its components, tissue, and tissue fluids, being shipped for purposes of diagnosis. It is not known at the time of shipment whether a diagnostic specimen contains microorganisms or toxins that may cause human disease. For diagnostic specimens, primary containers must not exceed 1L. The maximum volume that may be shipped in a secondary container is 4 L. Likewise, the maximum volume that may be shipped in a shipping container is 4 L. An infectious substance is defined as any substance that is known to contain a viable microorganism or its toxin that is known or suspected to cause disease in animals or humans. The etiological agent is known at the time of shipment. The maximum volume of infectious substance that may be shipped in a shipping container is 50 mL.

Temperature

Specimens should be shipped at the same or similar temperatures as that recommended for storage. In general, specimens prepared for microscopic examination or for detection of antibodies should be shipped on wet ice but not be frozen; they may be shipped at ambient temperature (< 24°C) if necessary. Prepared slides may also be shipped at ambient temperature. Specimens for culture or for detection of microbial components should be shipped on dry ice or liquid nitrogen (see Table 34-9). Properly collected sera for antibody testing can usually be transported, if necessary, at ambient temperature for a day or so without substantial loss of antibody.

Finding dry ice or liquid nitrogen in developing countries can be challenging. In some developing countries, ice cream makers and distributors produce dry ice and may be willing to sell sufficient quantities to support the temporary storage and shipment of clinical specimens. In some countries where cattle breeding is conducted, local veterinary centers often produce liquid nitrogen for the storage of bull sperm. These centers may be willing to sell excess liquid nitrogen for the right price. In large urban areas in developing countries, larger medical centers may have access to either dry ice or liquid nitrogen. However, many developing countries do not have the capability to produce either dry ice or liquid nitrogen locally, so dry ice or liquid nitrogen must be imported. Arrangements can be made with a supporting laboratory to ship sufficient quantities of dry ice or liquid nitrogen to support specimen storage and return shipment.

Labeling

All packages prepared for shipping should contain complete information sheets about the specimens. The information sheets should be placed between the sec-

a

SHIPPER'S DECLARATION FOR DANGEROUS GOODS (Provide at least two copies to the airline)

Shipper	Air Waybill No. Page of Pages Shipper's Reference Number *(optional)*
Consignes	

Two completed and signed copies of this Declaration must be handed to the operator

TRANSPORT DETAILS

This shipment is within the limitations prescribed for: *(delete non-applicable)*

PASSENGER AND CARGO AIRCRAFT | **CARGO AIRCRAFT ONLY**

Airport of Departure

Airport of Destination:

WARNING

Failure to comply in all respects with the applicable Dangerous Goods Regulations may be in breach of the applicable law, subject to legal penalties. This Declaration must not, in any circumstances, be completed and/or signed by a consolidator, a forwarder of an IATA cargo agent.

Shipment type: *(delete non-applicable)*

NON-RADIOACTIVE | RADIOACTIVE

NATURE AND QUANTITY OF DANGEROUS GOODS

Dangerous Goods identification					Quantity and Type of packing	Packing inst.	Authorization
Proper Shipping Name	Class or Division	UN or ID No.	Packing Group	Subsidiary Risk			

Additional Handling Information

24 hr. Emergency Contact Tel. No.

I hereby declare that the contents of this consignment are fully and accurately described above by the proper shipping name and are classified, packaged, marked and labelled/placarded, and are in all respects in proper condition for transport according to applicable international and national governmental regulations.

Name/Title of Signatory

Place and Dete

Signature *(see warning above)*

(**Fig 34-1** continues)

b

Fig. 34-1. The form (**a**) and label (**b**) that must be fixed to packages containing infectious substances before they are mailed. These are required by US and international postal regulations.

ondary and shipping containers. Labels should have names, addresses, and telephone numbers of both the sender and the recipient and should be inside the package on the secondary container, as well as on the outside of the shipping container. If the package contains known infectious substances (not likely in the context of this chapter), then an "Etiological Agents" label must be displayed on the outside of the shipping container.[14–17] Likewise, if the shipment contains dry ice (officially declared a hazardous material), then the shipping container must be marked "Dry Ice, Frozen Medical Specimens."[17]

Inventory and Clinical Information

If more than one specimen is contained in a shipment, then a complete inventory should accompany the shipment. Sufficient clinical information should also be provided to the laboratory to guide the microbiologist in the selection of suitable diagnostic assays. The inventory and clinical information should be placed between the secondary and shipping containers. When the package is opened, the recipient should reconcile the specimens with the inventory list. A notice should be sent back to the sender that the shipment was received, along with a report of any discrepancies between the inventory list and the specimens received.

SUMMARY

Surveillance and outbreak investigations in a field environment require additional planning and coordination beyond that required for similar investigations in a hospital or clinical setting. In particular, laboratory support must be coordinated before the investigation begins. Plans for specimen collection, handling, storage, and shipment must be made early and potential problems addressed. Successful laboratory results depend on the selection of a proper specimen that will survive storage and transport. A general knowledge of the types of diagnostic assays available for infectious diseases will aid the investigator in the proper selection of patient specimens. For purposes of specimen storage and transport, specimens may be divided into four categories, and storage and transport conditions vary between these specimen categories. In addition, federal regulations must be observed when shipping biological specimens, even between two locations outside the United States. When done with the proper coordination, field medical investigations can be an important tool for the commander to identify medical threats facing US forces.

REFERENCES

1. US Dept of the Army. *Planning for Health Service Support*. Washington, DC: DA; 1994. Field Manual 8-55.

2. Mims CA, Playfair JHL, Roitt IM, Wakelin D, Williams R. *Medical Microbiology.* Hong Kong: Mosby-Year Book Europe; 1993: Chap 17, 18.

3. Hoeprich PD, Jordan MC, Ronald AR, eds. *Infectious Diseases.* Philadelphia: J.B. Lippincott; 1994.

4. Lennette EH, ed. *Laboratory Diagnosis of Viral Infections.* 2nd ed. New York: Marcel Dekker; 1992.

5. Galasso GJ, Whitley RJ, Merigan TC, eds. *Practical Diagnosis of Viral Infections.* New York: Ravin Press; 1993.

6. Baron EJ, Peterson LR, Finegold SM. *Bailey & Scott's Diagnostic Microbiology.* 9th ed. St. Louis: Mosby-Year Book; 1994.

7. Southern EM. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J Mol Biol.* 1975;98:503–517.

8. Alwine JC, Kemp DJ, Stark GR. Method for detection of specific RNAs in agarose gels by transfer to diazobenzyloxymethyl-paper and hybridization with DNA probes. *Proc Natl Acad Sci USA.* 1977;74:5350–5354.

9. Kafatos FC, Jones CW, Efstratiadis A. Determination of nucleic acid sequence homologies and relative concentrations by a dot hybridization procedure. *Nucleic Acids Res.* 1979;7:1541–1552.

10. Saiki RK, Scharf S, Faloona F, et al. Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science*. 1985;230:1350–1354.

11. US Dept of Labor, Occupational Safety and Health Administration. Occupational exposure to bloodborne pathogens: final rule. *Fed Regist*. 1991;56:64175–64182. 29 CFR Part 1910.1030.

12. Woods GL, Gutierrez Y. *Diagnostic Pathology of Infectious Diseases.* Philadelphia: Lea & Febiger; 1993: Chap 54.

13. Garcia LS, Bruckner DA. *Diagnostic Medical Parasitology*. 2nd ed. Washington, DC: American Society for Microbiology; 1993: Chap 25, 29, 30.

14. Public Health Service. Interstate shipment of etiologic agents. *Fed Regist.* 1980;45:28–29. 42 CFR part 72.

15. US Postal Service. Nonmailability of etiologic agents. *Fed Regist.* 1988;53:23775–23776. 39 CFR part II.

16. US Postal Service. Mailability of etiologic agents. *Fed Regist.* 1989;54:11970–11972. 39 CFR part III.

17. US Dept of Transportation. Hazardous materials regulations. *Fed Regist*. 1998;63:52843–52851. 49 CFR part 173.