



Microbiology

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Referral of Cultures to Division of Laboratory Services

Directors of hospital and private laboratories across Tennessee are required by state law to submit the following cultures to the TDH Division of Laboratory Services. These cultures form the basis for surveillance of infectious diseases in Tennessee by the TDH Communicable and Environmental Disease Services Program and play a vital part in determining the accurate state of infectious diseases in Tennessee. Under the Tennessee Medical Laboratory Act (T.C.A. 53-4105) the rules for referral of cultures to the TDH Division of Laboratory Services are as follows:

1200-6-3-.12 REFERRAL OF CULTURES TO THE DEPARTMENT OF HEALTH.

(1) It shall be the responsibility of the director of a medical laboratory to submit cultures of those microorganisms designated by the Board to the Department of Health, Laboratory Services for confirmation, typing and/or antibiotic sensitivity, including, but not limited to:

- (a) *Salmonella* species (including *S. typhi*)
 - (b) *Shigella* species
 - (c) *Corynebacterium diphtheriae*
 - (d) *Francisella tularensis*
 - (e) *Brucella* species
 - (f) *Mycobacterium* species
 - (g) *Legionella* species
 - (h) *Plasmodium* species
 - (i) *Vibrio* species
 - (j) *Clostridium tetani*
 - (k) *Listeria* species
 - (l) *Listeria monocytogenes*, isolated from sterile sites
 - (m) *Francisella* species
 - (n) *Yersinia pestis*
 - (o) *Escherichia coli* O157
 - (p) Shiga-like toxin producing *Escherichia Coli* non-O157 (STEC)
 - (q) Shiga-like toxin positive stools and/or EIA positive broth for shiga-like toxin
 - (r) *Clostridium botulinum*
 - (s) *Haemophilus influenzae*, isolated from sterile sites
 - (t) *Neisseria meningitidis*, isolated from sterile sites
 - (u) *Streptococcus pneumoniae*, isolated from sterile sites
 - (v) *Streptococcus*, Group A, isolated from necrotizing fasciitis wound cultures or normally sterile sites *
 - (w) *Bacillus anthracis*
 - (x) *Burkholderia mallei*
 - (y) *Burkholderia pseudomallei*
 - (z) Vancomycin resistant *Staphylococcus aureus* (VRSA)
 - (aa) Vancomycin intermediate *Staphylococcus aureus* (VISA)
- (2) All cultures shall be accompanied by the following information:
- (a) Patient's full name, address (including county), age and sex.
 - (b) Physician's (submitters) name and address.
 - (c) Anatomic source of culture and specimen collection date.

Authority: T.C.A. §§4-5-202, 4-5-204, 68-29-105 and 68-29-107. **Administrative History:** Original rule filed October 26, 1979; effective December 10, 1979. Repeal filed May 3, 1995; effective July 17, 1995. New rule filed January 7, 1997; effective March 23, 1997. Repeal and new rule filed June 18, 2002; effective September 1, 2002. Amendment filed September 17, 2003; effective December 1, 2003. Amendment filed February 15, 2006; effective May 1, 2006. Amendment filed July 10, 2006; effective September 23, 2006. \

*A normally "sterile site" is defined as: Blood, CSF, Pleural fluid (includes chest fluid, thoracentesis fluid), Peritoneal fluid (includes abdominal fluid, ascites), Pericardial fluid, Bone (includes bone marrow), Joint (includes synovial fluid; fluid, needle aspirate or culture of any specific joint: knee, ankle, elbow, hip, wrist), Internal body sites (specimen obtained from surgery or aspirate from one of the following: lymph node, brain, heart, liver, spleen, vitreous fluid, kidney, pancreas or ovary).

***Yersinia enterocolitica* is currently undergoing the process to be added to the list of referral cultures.**



TENNESSEE DEPARTMENT OF HEALTH LIST AND REPORTING OF NOTIFIABLE DISEASES

For reporting forms and the most up-to-date list go to:
<http://health.state.tn.us/CEDS/index.htm>

The diseases and conditions listed below are declared to be communicable and/or dangerous to the public and are to be reported to the local health department by all hospitals, physicians, laboratories and other person knowing of or suspecting a case in accordance with the provision of the statutes and regulations governing the control of communicable diseases in Tennessee.

The Notifiable Disease Report Form (PH-1600) is available as a Microsoft® Word document that can be saved to your computer and emailed to your Regional Office. It is also available as an Adobe® Acrobat® PDF file that can be printed.

Category 1: Immediate telephonic reporting to 615-741-7247 is required followed with a written report using PH-1600

Anthrax (2)
Botulism
 1. Foodborne (5)
 2. Wound (4)
Diphtheria (11)
Disease Outbreaks
 1. Foodborne
 2. Waterborne
 3. All Other
Encephalitis, Arboviral
 1. California/LaCrosse serogroups
 2. Eastern Equine (122)
 3. St. Louis (123)
 4. Western Equine (124)
Group A Strep Invasive Disease (53)
Group B Strep Invasive Disease (47)
Haemophilus influenzae Invasive Disease (54)
Hantavirus Disease (23)
Hepatitis – Type A acute (16)

Listeriosis (94)
Measles (96 Imported, 26 indigenous)
Meningococcal Disease (95)
Meningitis – Other Bacterial (102)
Mumps (31)
Pertussis (32)
Plague (33)
Poliomyelitis (34 Paralytic, 35 Nonpara)
Prion Disease
 1. Creutzfeldt-Jakob Disease (118)
 2. variant Creutzfeldt-Jakob Disease
Rabies – Human (37)
Rubella & Congenital Rubella Syndrome
Severe Acute Respiratory Syndrome (SARS)
Staph aureus Vancomycin nonsen-~~iforms~~
Tuberculosis – all forms
Typhoid Fever (41)
West Nile Infections
 1. West Nile Encephalitis (125)
 2. West Nile Fever (126)

Possible Bioterrorism Indicators

Anthrax (2)
Plague (33)
Venezuelan Equine Encephalitis (108)
Smallpox (107)
Botulism (5)
Q Fever (109)
Staph enterotoxin B pulmonary poisoning (110)
Viral Hemorrhagic Fever (111)
Brucellosis (6)
Ricin poisoning (112)
Tularemia (113)

Category 2: Only written report using form PH-1600 required

Botulism – infant (3)
Brucellosis (6)
Invasive (130)
Campylobacteriosis (7)
Chancroid (69)
Chlamydia trachomatis (55 Gen, 56 PID, 57 other)
Cholera (9)
Cyclospora (106)
Cryptosporidiosis (1)
Ehrlichiosis (61 HME, 116 HGA, 117 Other)
Escherichia coli 0157:H7 (52)
Giardiasis (acute) (15)
Gonorrhea (60 Gen, 61 Oral, 62 Rectal, 63 PID, 64 Other)
Guillain-Barre Syndrome (133)
Hemolytic Uremic Syndrome (58)

Hepatitis, Viral
 1. Type B acute (17)
 2. HBsAg positive pregnant female (48)
 3. HBsAg positive infant (480)
 4. Type C acute (18)
Influenza – weekly casecount (20)
Legionellosis (21)
Leprosy (Hansen Disease) (22)
Lyme Disease
Malaria (25)
Psittacosis (36)
Rabies – Animal (105)
Rocky Mountain Spotted Fever (39)
Salmonellosis – other than S. typhi (42)
Shiga-like Toxin positive stool (115)

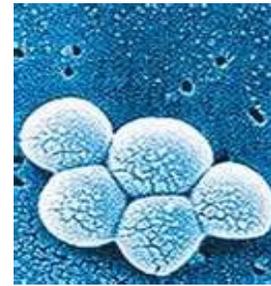
Shigellosis (43)
Staph aureus Methicillin Resistant –

Strep pneumoniae Invasive Disease
 1. Penicillin resistant (50)
 2. Penicillin sensitive (49)
Syphilis (70-78)
Tetanus (44)
Toxic Shock Syndrome
 1. Staphylococcal (45)
 2. Streptococcal (97)
Trichinosis (46)
Vancomycin Resistant Enterococci Invasive
Varicella Deaths (114)
Vibrio infections (104)
Yellow Fever (98)
Yersiniosis (103)

Category 3: Requires special confidential reporting to designated health department personnel
Acquired Immunodeficiency Syndrome (AIDS) Human Immunodeficiency Virus (HIV)

Category 4: Laboratories required to report all blood lead test results (#79)
Physicians required to report all blood lead test results \geq $\mu\text{g/dl}$

Aerobic Bacteriology



Methicillin Resistant
Staph aureus (MRSA)

Introduction

The Aerobic Bacteriology Section serves primarily as a referral laboratory for bacteria that are unusual or difficult to identify. In this context, aerobic bacteriology refers to the examination of a wide variety of microorganisms. Reference cultures will be accepted from public and private I. Pure culture isolates are required for serotyping and identification of reference specimens. Clinical specimens are accepted for the isolation and identification of a specific pathogen if this testing is unavailable at the sending facility. Refer to Chart II - 1 AEROBIC SPECIMENS REQUIRING SPECIAL HANDLING.

Refer to sections on *Bordetella* (whooping cough), *Legionella*, Gonorrhea and *Streptococcus* Group A for specific information on these organisms.

Services available in the Aerobic Section include, but are not limited to, the following:

- Serotyping of *Neisseria meningitidis* from sterile sites.
- Culture of nasopharyngeal specimens for *Bordetella pertussis*.
- Biochemical identification of non-fermentative gram-negative bacilli and fermentative gram-negative bacilli not included in the family *Enterobacteriaceae*, *Vibrionaceae* or *Aeromonadaceae*.
- Culture and direct fluorescent antibody staining for *Legionella*.
- Grouping of beta hemolytic streptococci and biochemical identification of clinically significant strains of other gram-positive cocci on referred isolates only.
- Biochemical identification or confirmation of any bacterial isolate (other than anaerobes, enterics or mycobacterium) that is unidentifiable at the local level, due to unusual or special requirements of the organisms. These may include growth requirements, aberrant biochemical results, additional test requirements, as well as the rarity or hazardous nature of the suspected organism.

The Aerobic Bacteriology Section does not perform antimicrobial susceptibility testing for patient treatment.

The Tennessee Medical Laboratory Act requires that laboratory directors send isolates of *Brucella* species, *Corynebacterium diphtheriae*, *Francisella* species, *Francisella tularensis*, **Haemophilus influenzae*, *Legionella* species, *Listeria* species, **Neisseria meningitidis*, **Streptococcus* Group A, **Streptococcus pneumoniae* and *Yersinia pestis* to the Tennessee Department of Health (TDH) Laboratory for surveillance purposes. Whatever the source, cultures of *L. monocytogenes* from all sites are requested.

*Sterile sites only

All specimens forwarded to the CDC must be accompanied by a clinical history documenting the need for special testing. Services available at the Centers for Disease Control and Prevention (CDC), through the Aerobic Section, include:

- Serotyping of certain bacteria under specific circumstances.
- Toxigenicity testing of *Corynebacterium diphtheriae*.
- Direct fluorescent antibody staining for *Leptospira*.
- Antimicrobial susceptibility testing under special circumstances.

Aerobic Bacteriology (Continued)

**Chart II - 1
 Aerobic Specimens Requiring Special Handling**

Organism or Disease	Collection Instructions	Shipping Requirements	Special Requirements
<i>Bacillus anthracis</i> anthrax	Aseptically collect specimens from lesion, contaminated hair products or sputum. Subculture isolates to blood or nutrient agar slants. Use extreme caution.	Blood culture bottles for blood and spinal fluid, TB plastic tube for sputum. Sterile containers for other specimens.	USE BIOLOGICAL SAFETY HOOD. Do not create aerosols. *Notify Bioterrorism (BT) laboratory before shipping.
<i>Bordetella pertussis</i> - whooping cough. Refer to BORDETELLA SECTION.			
¹ <i>Brucella</i> species brucellosis or undulant fever	Aseptically collect multiple blood samples, infected tissues, abscess material, bone marrow or liver biopsies. Subculture isolates to sheep blood, nutrient or Brucella agar slants. Use extreme caution.	Blood culture bottles, vented and incubated under 5 to 10% CO ₂ . Use sterile container. .	USE BIOLOGICAL SAFETY HOOD. Refrigerated clinical specimen if delay is anticipated. *Notify BT laboratory before shipping.
<i>Burkholderia mallei</i> <i>B. pseudomallei</i> melioidosis	Aseptically collect blood, sputum or pus. Subculture isolates to nutrient or infusion agar slant.	Blood culture bottles for blood, TB plastic tube or sputum, sterile container for pus.	*Notify BT laboratory before shipping.
<i>Clostridium botulinum</i> <i>Clostridium tetani</i>	Collect stool for culture or toxin assay. Contact TDH Lab. Aspirate obtained by needle and syringe	Sterile container, no transport medium. Ship cold, not frozen. Anaerobic transport vial	
¹ <i>Corynebacterium diphtheriae</i> diphtheria	Collect throat or skin lesion swabs. Subculture isolates to Pai or Loeffler's medium. Collect specimens aseptically.	Sterile container or place on Loeffler's, Pai, cystine blood tellurite or infusion medium.	Toxigenicity testing Performed at CDC.
¹ <i>Francisella tularensis</i> tularemia or rabbit fever or <i>Francisella</i> species	Collect specimens aseptically. Specimens include material from lesions, lymph nodes, sputum, gastric aspirates, nasopharyngeal washings and blood cultures. Use extreme caution.	Sterile container, no transport medium. Ship frozen on dry ice. Package to prevent leakage.	DO NOT ATTEMPT ISOLATION *Notify BT laboratory before shipping.

* Federal regulations require that these specimens be shipped in Certified 6.2 mailing systems as UN 3373 Category B Biological Substances (training of shippers is required) or as higher Category A Infectious Substances (training and certification is required), depending upon critical judgment of the shipper..

¹ Tennessee law requires laboratory directors to submit isolates of these organisms to the TDH Laboratory for surveillance purposes.

² Tennessee law requires laboratory directors to submit isolates of these organisms when recovered from a sterile site to the TDH Laboratory for surveillance purposes.

Aerobic Bacteriology (Continued)

**Chart II - 1
 Aerobic Specimens Requiring Special Handling**

Organism or Disease	Collection Instructions	Shipping Requirements	Special Requirements
<i>Haemophilus ducreyi</i> chancroid	Collect specimens from lesions or inguinal bubo and inoculate onto enriched chocolate agar and incubate at 35 to 37°C in 5 to 10% CO ₂ .	Heavy growth of 24 to 48 hour culture scraped with sterile swab, transport as subsurface stabs in chocolate agar.	Primary culture must be done at the local level.
² <i>Haemophilus influenzae</i> - Isolates from sterile sites required for surveillance purposes.			
¹ <i>Legionella</i> species- <i>legionellosis</i> - Refer to LEGIONELLA SECTION.			
² <i>Listeria monocytogenes</i> - Isolates from sterile sites required for surveillance purposes. Isolates from all sites are requested.			
Listeria species	Collect specimens aseptically. Obtain from normally sterile sites, except in outbreak situations.	Isolated organisms on non-glucose containing media such as heart infusion agar or tryptic soy agar.	
<i>Neisseria gonorrhoeae</i> - gonorrhea - Refer to GONORRHEA AND CHLAMYDIA, DNA PROBE TECHNOLOGY and GONORRHEA, CULTURE METHOD, SECTIONS.			
² <i>Neisseria meningitidis</i> - Isolates from sterile sites are required for surveillance purposes.			
<i>Staphylococcus aureus</i>	Isolates from documented outbreak. Only coagulase positive staphylococci accepted.	Isolated organisms on nutrient or infusion agar slants.	Documentation must accompany specimens. *Notify Section before shipping. Pulsed Field Gel Electrophoresis (PFGE) is performed at TDH Laboratory Services in Nashville.
² <i>Streptococcus pneumoniae</i> – Isolates from sterile sites are required for surveillance purposes.			
² <i>Streptococcus</i> Group A – Isolates from sterile sites are required for surveillance purposes..			
Miscellaneous Bacteria	Use blood, chocolate or TSA slant or Cary-Blair transport.		
(VRSA) Vancomycin Resistant <i>Staphylococcus aureus</i>	Coagulase positive MRSA/VIRSA. Contact Communicable and Environmental Disease Services	Isolated organism on heart infusion agar or tryptic soy agar	
(VISA) Vancomycin Intermediate <i>Staphylococcus aureus</i>	Coagulase positive MRSA/VIRSA. Contact Communicable and Environmental Disease Services	Isolated organism on heart infusion agar or tryptic soy agar	

* Federal regulations require that these specimens be shipped in Certified 6.2 mailing systems as UN 3373 Category B Biological Substances (training of shippers is required) or as higher Category A Infectious Substances (training and certification is required), depending upon critical judgment of the shipper..

¹ Tennessee law requires laboratory directors to submit isolates of these organisms to the TDH Laboratory for surveillance purposes.

² Tennessee law requires laboratory directors to submit isolates of these organisms when recovered from a sterile site to the TDH Laboratory for surveillance purposes.

Aerobic Bacteriology (Continued)

Specimen Collection

Aseptically collect specimens from sites such as autopsy material, surgically obtained tissue, urine and the respiratory and urogenital tract using appropriate techniques for the individual type of specimen. Aseptically collect blood samples and inoculate directly into appropriate commercial blood culture bottles. Preferably, all specimens should be cultured at the local laboratory using recommended isolation procedures. (For exceptions refer to Chart II - 1 AEROBIC SPECIMENS REQUIRING SPECIAL HANDLING.) To ensure purity, isolates should be subcultured onto appropriate media before transportation to the TDH Laboratory.

Isolated organisms should be submitted on non-carbohydrate-containing agar slants such as infusion, nutrient, Trypticase soy, blood or chocolate. Do not mail broth or plate cultures. A single block of agar from plating media with good growth can be cut out and placed in a sterile tube.

Telephone the Aerobic Bacteriology Section at (615) 262-6362 to make special arrangements in urgent situations or unusual circumstances. **Always** telephone in advance when submitting large numbers of isolates, as in an outbreak situation or when the organism being submitted is classified as a biologically hazardous organism. For specimens requiring special handling refer to Chart II - 1 AEROBIC SPECIMENS REQUIRING SPECIAL HANDLING.

Specimen Identification – The Specimen Request Form (Some Basic Information is Required by the STARLiMS Electronic Reporting System)

1. Include the last name, first name, date of birth, sex, county, date of collection, source, submitting organization, and the test requested.

Specimen Identification – The Primary Container (Tube, Bottle, etc.)

1. Complete all the provider and patient information areas on the Miscellaneous Exam Form PH-1573. Include pertinent clinical information with each specimen.
2. Using indelible ink, label each specimen with the date of collection and the patient's first and last name. Attach the control number on the tear strip to the specimen and secure it with transparent tape. Unlabeled specimens or specimens where the patient identifier on the specimen does not exactly match the identifier on the form **will not be tested**.

Shipment of Specimens

1. Packing and shipping specimens to the state public health laboratory requires personnel trained in current regulations. Follow the shipping guidelines for Category B substances and any additional guidelines from your current carrier or method of shipment. See Section 7, page 5 for instructions.
2. Ship to the Tennessee Department of Health Laboratory in **Nashville**.
3. Use first-class postage on US mail.
4. If *Burkholderia mallei*, *Burkholderia pseudomallei*, *Bacillus anthracis*, *Brucella* species or *Francisella tularensis* is suspected, **telephone the Bioterrorism Laboratory (BT) Section @ 615-262-6359 before shipping**.

Aerobic Bacteriology (Continued)

Reporting Procedures and Interpretation of Results

Reporting of Results
Organisms are reported by genus and species.

Organisms are identified to a genus and species level only when culture, morphology and biochemical test results support the species identification. Genus and species designations are those consistent with designations in the American Society for Microbiology's *Manual of Clinical Microbiology* or according to the *International Code of Nomenclature of Bacteria*. Some organisms encountered in aerobic bacteriology can be identified accurately only to the genus level and are reported as such. Organisms normally encountered as contaminants or those believed to lack clinical significance may be reported only to the genus level, especially if the culture was not accompanied by clinical information to the contrary.

Organisms reported as "unidentified" are those which do not fit the description of recognized genera and/or species. These organisms are routinely forwarded to the CDC for further study when the nature of the isolate, source of isolation and/or the clinical history of the patient warrant further identification efforts or a special request is made to forward the isolate (such a request requires justifying information from the submitter).

The results of all specimen requests are reported to the health care provider who submitted the specimen. In addition, the TDH Communicable and Environmental Disease Services and the health department in the county (including out of state) where the patient lives are sent reports on the following organisms:

<i>Bacillus anthracis</i> , BT	<i>Haemophilus influenzae</i> (sterile body sites)
<i>Bordetella pertussis</i>	<i>Listeria monocytogenes</i> (sterile body sites)
<i>Brucella</i> species, BT	<i>Listeria</i> species
<i>Burkholderia mallei</i> , BT	<i>Legionella</i> species
<i>Burkholderia pseudomallei</i> , BT	<i>Neisseria meningitidis</i> (sterile body site)
<i>Clostridium botulinum</i> , BT	<i>Streptococcus pneumoniae</i> (sterile body sites)
<i>Clostridium tetani</i>	<i>Streptococcus</i> Group A (sterile body sites and necrotizing fasciitis)
<i>Corynebacterium diphtheriae</i>	Vancomycin resistant <i>Staphylococcus aureus</i> (VRSA)
<i>Francisella</i> species, BT	Vancomycin intermediate <i>Staphylococcus aureus</i> (VISA)
<i>Francisella tularensis</i> , TB	

In addition organisms that appear on the National Select Agent Registry (NSAR) will be reported immediately on Form 4 (*Report of the Identification of a Select Agent or Toxin*) to the CDC/APHIS Select Agent Program. The provider of the specimen will be advised about the need to complete and submit a Form 4 to NSAR.

The TDH Sexually Transmitted Disease (STD) Control Division, the regional STD representative and the health department in the county where the patient lives are sent reports on *Haemophilus ducreyi*.

Aerobic Bacteriology (Continued)

Criteria for Unacceptable Specimens

All specimens

1. The specimen was not properly identified with the patient's name/or and the tear strip control number.
2. The patient identifier on the specimen did not exactly match the identifier on the form.
3. The specimen was broken in transit.

Clinical specimens

1. The type of specimen was improper for the test requested.

Reference specimens

1. The specimen was non-viable.
2. A mixed specimen was submitted.

**Miscellaneous Exam Form PH-1573
 FRONT OF FORM**

SOCIAL SECURITY NO.			TENN CARE NO.			MCO			MISCELLANEOUS EXAM			A418027											
MEDICARE NO.			RECORD FOLDER NO.			DATE REPORTED			DATE/TIME RECEIVED			LAB NO. ▼											
PATIENTS NAME - LAST, FIRST, MIDDLE						SPOUSE - FIRST NAME						COLLECTION DATE			DATE OF ONSET								
STREET AND NUMBER												SOURCE: <input type="checkbox"/> BLOOD <input type="checkbox"/> URINE <input type="checkbox"/> FECES <input type="checkbox"/> THROAT <input type="checkbox"/> OTHER			EXAMINATION RESULTS								
TOWN			STATE			ZIP			PATHOGEN/DISEASE SUSPECTED/SYMPTOMS														
DATE OF BIRTH			RACE			ETHNICITY			SEX			PHONE NO.			EXAMINATION REQUESTED								
COUNTY NO.			COUNTY NAME			SITE NO.			RECENT TRAVEL 1 <input type="checkbox"/> YES 2 <input type="checkbox"/> NO STATE/COUNTRY						EXAMINATION RESULTS								
NAME												<input type="checkbox"/> SUBMITTED TO REFERENCE LABORATORY FOR EXAMINATION. RESULTS FORTHCOMING.											
ADDRESS																							
CITY			STATE			ZIP CODE																	
PH-1573 REV. 11/06															TENNESSEE DEPT. OF HEALTH LABORATORY SERVICES			DAVID L. SMALLEY, PH.D, MSS, BCLD, DIRECTOR			LABORATORY PERFORMING EXAMINATION		

**TIGHTEN CAPS SECURELY
 SUBMIT IN DOUBLE MAILING CONTAINER**

STOOL CULTURES FOR SALMONELLA, SHIGELLA AND CAMPYLOBACTER
 Place two (2) cotton tipped swabs dipped into feces or other specimens and insert into Amies, Stuarts, or Cary-Blair transport medium. Submit the transport medium refrigerated within two (2) days of collection.

INTESTINAL PARASITES: Place amount of feces equal to volume of formalin in container designated for intestinal parasites (5% Formalin).

CULTURES FOR IDENTIFICATION - Submit pure cultures on non-selective media such as trypticase soy agar slants or enriched slants (Blood or Chocolate) when required.

ANAEROBIC ORGANISMS - Submit in semi-solid media such as thioglycollate, overlaid with sterile vaspar to prevent exposing culture to oxygen.

TESTING LABORATORY LOCATION CODES
 J = JACKSON BRANCH LAB, 295 SUMMAR DRIVE, JACKSON, TN - DR. ORISTYNE E. WALKER, PhD, MSS
 K = KNOXVILLE BRANCH LAB, 1522 CHEROKEE TRAIL, KNOXVILLE, TN - DR. DAVID L. SMALLEY, PhD, MSS, BCLD
 N = NASHVILLE REFERENCE LAB, 630 HART LANE, NASHVILLE, TN - DR. DAVID L. SMALLEY, PhD, MSS, BCLD

BACK OF FORM PH-1573



***Bordetella* species**

Includes *B. pertussis* (whooping cough)

Introduction

Specimens for isolation of *Bordetella pertussis* in suspected cases of whooping cough are accepted from public and private health care providers. Only **symptomatic** contacts of diagnosed cases of pertussis will be accepted for examination, since a carrier state in asymptomatic contacts has not been demonstrated as an important source of transmission. Reference cultures are accepted for confirmation of *Bordetella pertussis*, *B. parapertussis* and *B. bronchiseptica*. *B. pertussis* and *B. parapertussis* are identified by culture, Polymerase Chain Reaction (PCR) and confirmed by Direct Fluorescent Antibody testing. *B. bronchiseptica* is identified by culture techniques.

Specimen Collection for Testing for Whooping Cough

Collect nasopharyngeal swabs as soon as possible after the onset of symptoms and before antibiotic treatment is started. There is a greater likelihood of positive cultures in the first two weeks of symptomatic infection than during the later weeks of illness. There is a 95% likelihood of positive isolation during the first week of disease, with a subsequent drop in likelihood of isolation to 50% by the fourth week of disease.

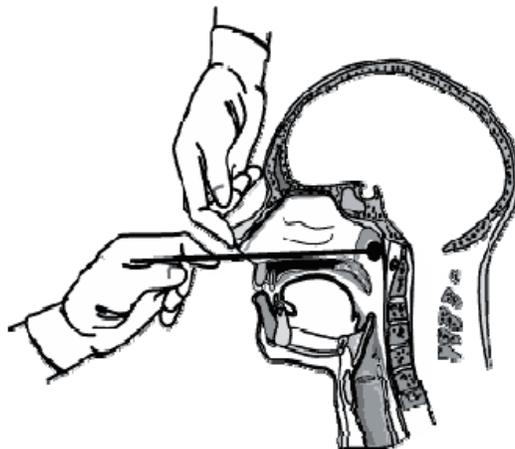
A kit containing the materials and instructions necessary for collecting a nasopharyngeal specimen is available from the Tennessee Department of Health (TDH) Laboratory. Do not order more kits than are needed. They contain culture medium with a short shelf life. Store refrigerated and allow to come to room temperature for 15 minutes prior to use.

The kit contains:

- Instructions for collection of specimens
- 2 Dacron nasopharyngeal swabs (one for PCR, one for culture)
- 2 tubes of Regan-Lowe transport medium
- 1 Form PH-1573
- 1 Mailing label

Collection Procedure

1. To obtain the specimen, immobilize the patient's head and pass the swab through the nostril completely to the back of the **nasopharynx** and leave it in place for at least 5 seconds before withdrawing. If entry cannot be gained through either nostril, enter through the mouth; however, the **throat area provides an inferior method for culture**.



2. Repeat step 1 to collect a second swab.

Bordetella (Continued)

After collecting the samples:

1. Place one swab into a labeled Regan-Lowe transport medium for PCR. Replace the cap with the swab still in the tube. Keep cold at 4°C.
2. After collecting the second specimen, immediately place the swab in the second Regan-Lowe transport tube. Replace the cap with the swab still in the tube. Keep cold at 4°C.
3. Label the Regan-Lowe transport medium tubes with the patient's name.
4. Complete the Miscellaneous Form PH-1573. No culture confirmations or PCR will be performed without a completed form accompanying each specimen. Return the completed form PH-1573 to the mailing container and ship to the TDH Division of Laboratory Services in Nashville following the instructions for submitting a Category B Biological Substance.

Keep the Regan-Lowe transport medium, containing the swab, at 4°C while held at the clinic and during transport. If specimens cannot be sent or transported to laboratory immediately, **ship on cold packs.** Do not hold longer than 24 hours; specimens must reach the laboratory as soon as possible after collection.

Specimen Preparation - Reference Cultures

Subculture isolated organisms for identification or confirmation to appropriate media slants and incubate until growth is apparent. Do not mail reference cultures on plates. If only plated media is available, cut out a chunk of the plating media with good growth and place it in a sterile tube. Plates are acceptable only if a courier delivers them directly to the laboratory.

Specimen Identification

1. Complete **all** the provider and patient information areas on the Miscellaneous Exam Form PH-1573. Submit the following information: nature of symptoms, date of onset of symptoms, immunization history and contact with other cases of whooping cough, any antibiotic therapy before specimen collection and other pertinent information. Include biochemical information with reference cultures.
2. Using indelible ink, label each specimen (transport medium) with the date of collection and the patient's first and last name. Attach the control number on the tear strip to the transport medium specimen and secure it with transparent tape. Unlabeled specimens or specimens where the patient identifier on the specimen does not exactly match the identifier on the form, **will not be tested.**

Shipment of Specimens

1. Wrap the transport medium in absorbent material. Pack the specimens in the pertussis mailing container provided by the TDH Laboratory. Packing and shipping specimens to the state public health laboratory requires personnel trained in current regulations.
2. Affix the mailing label (PH-0838), return address and infectious substance (etiologic agent) or clinical (diagnostic) specimen label to the outer mailing container.
3. Ship the specimens on cold packs to the Tennessee Department of Health Laboratory in Nashville.
4. Use first-class postage on US mail.
5. Contact the Aerobic Bacteriology Section if an outbreak is suspected and specimens will be submitted.

Bordetella (Continued)

Reporting Procedures and Interpretation of Results

Bordetella pertussis – Generally, a preliminary report with PCR results is mailed within 1 to 4 days of sample receipt. Final reports are generated when both the PCR and culture results are complete. Typically this is within 7 to 10 working days after the specimen is received. Positive PCR and culture results for *B. pertussis* are reported by telephone to the Tennessee Department of Health Immunization Program.

PCR results are reported as:
Negative for <i>Bordetella pertussis</i> and <i>parapertussis</i> by real-time PCR.
Positive for <i>Bordetella pertussis</i> by real-time PCR.

Cultures are reported as:
No <i>Bordetella pertussis</i> isolated.
<i>Bordetella pertussis</i> isolated.

Culture examinations may fail to detect *B. pertussis*. As the disease process may continue for weeks or months after viable organisms no longer remain in the nasopharynx, a negative culture report does not rule out infection, especially if the specimens were collected late in the course of the illness. Organisms present in low numbers may be difficult to detect. Prior antibiotic therapy or overgrowth of the transport medium with contaminants may result in a negative culture.

The PCR methodology is inhibited by certain collection devices such as cotton swabs and wooden swab shafts. A negative PCR result with a positive culture is usually the result of a PCR-inhibitory sample. One of the benefits of PCR is greater sensitivity over culture. A positive PCR result with a negative culture is usually the result of a viability or sensitivity difference. A positive PCR result indicates the presence of the target DNA, not necessarily an active infection. PCR methodology can not differentiate between viable and non-viable organisms. Conscientious interpretation of all test results and correlation with patient signs and symptoms is the sole responsibility of the ordering physician.

Other *Bordetella* - Cultures for *B. parapertussis* and *B. bronchiseptica* are reported 7 to 10 working days after receipt of the specimen. The real-time PCR assay will detect *B. parapertussis* and this will be reflected in the report.

Reporting of results
Organisms are reported by genus and species.

Bordetella (Continued)

The results of all specimen requests are reported to the health care provider who submitted the specimen. In addition, the Tennessee Department of Health Communicable and Environmental Disease Services and the health department in the county where the patient lives are sent reports on *Bordetella pertussis*.

Criteria for Unacceptable Specimens

All specimens

1. The specimen was not properly identified with the patient's name and/or the tear strip control number.
2. The patient identifier on the specimen did not exactly match the identifier on the form.
3. The specimen or slide was broken in transit.

Clinical

1. Out-dated media or dehydrated media was used.

Reference

1. The specimen was not viable.
2. A mixed specimen was submitted.

**Miscellaneous Exam Form PH-1573
 FRONT**

SOCIAL SECURITY NO.		TENN CARE NO.		MCO		MISCELLANEOUS EXAM		A418027		
MEDICARE NO.			RECORD FOLDER NO.			DATE REPORTED	DATE/TIME RECEIVED	LAB NO. ▼		
PATIENTS NAME - LAST, FIRST, MIDDLE				SPOUSE - FIRST NAME				COLLECTION DATE	DATE OF ONSET	
STREET AND NUMBER										
TOWN			STATE			ZIP				
DATE OF BIRTH	RACE	ETHNICITY	SEX	PHONE NO.						
COUNTY NO.	COUNTY NAME				SITE NO.					
SOURCE: <input type="checkbox"/> BLOOD <input type="checkbox"/> URINE <input type="checkbox"/> FECES <input type="checkbox"/> THROAT <input type="checkbox"/> OTHER _____										
PATHOGEN/DISEASE SUSPECTED/SYMPTOMS										
EXAMINATION REQUESTED										
RECENT TRAVEL 1 <input type="checkbox"/> YES 2 <input type="checkbox"/> NO STATE/COUNTRY										
EXAMINATION RESULTS										
<input type="checkbox"/> SUBMITTED TO REFERENCE LABORATORY FOR EXAMINATION. RESULTS FORTHCOMING.										
PH-1573 REV. 11/06		TENNESSEE DEPT. OF HEALTH LABORATORY SERVICES DAVID L. SMALLEY, PhD, MSS, BCLD, DIRECTOR				<input type="checkbox"/> K <input type="checkbox"/> J <input type="checkbox"/> N		EXAMINED BY:		RD-1160

**TIGHTEN CAPS SECURELY
 SUBMIT IN DOUBLE MAILING CONTAINER**

STOOL CULTURES FOR SALMONELLA, SHIGELLA AND CAMPYLOBACTER
 Place two (2) cotton tipped swabs dipped into feces or other specimens and insert into Amies, Stuarts, or Cary-Blair transport medium. Submit the transport medium refrigerated within two (2) days of collection.

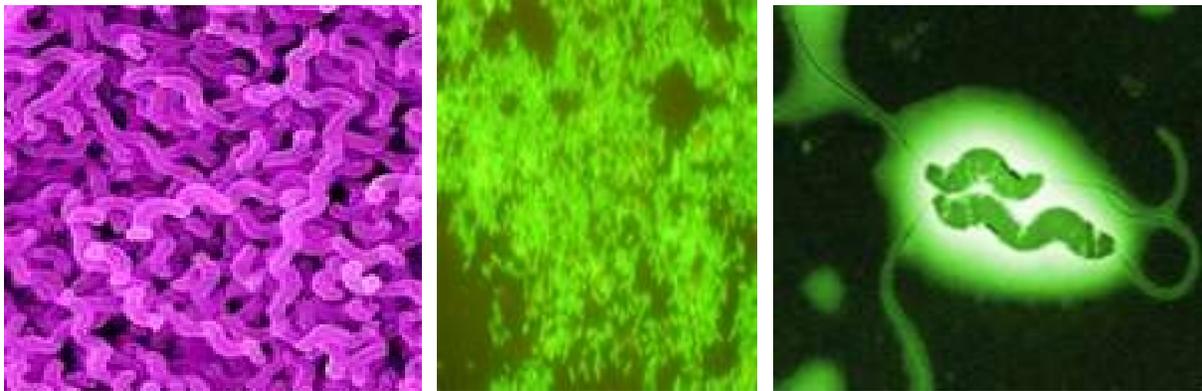
INTESTINAL PARASITES: Place amount of feces equal to volume of formalin in container designated for intestinal parasites (5% Formalin).

CULTURES FOR IDENTIFICATION - Submit pure cultures on non-selective media such as trypticase soy agar slants or enriched slants (Blood or Chocolate) when required.

ANAEROBIC ORGANISMS - Submit in semi-solid media such as thioglycollate, overlaid with sterile vaspar to prevent exposing culture to oxygen.

TESTING LABORATORY LOCATION CODES
 J = JACKSON BRANCH LAB, 295 SUMMAR DRIVE, JACKSON, TN - DR. ORISTYNE E. WALKER, PhD, MSS
 K = KNOXVILLE BRANCH LAB, 1522 CHEROKEE TRAIL, KNOXVILLE, TN - DR. DAVID L. SMALLEY, PhD, MSS, BCLD
 N = NASHVILLE REFERENCE LAB, 630 HART LANE, NASHVILLE, TN - DR. DAVID L. SMALLEY, PhD, MSS, BCLD

BACK



Campylobacter

Introduction

Campylobacter are microaerophilic organisms that are identified by morphological and biochemical characteristics. They are small, curved or spiral gram-negative rods with a corkscrew-like motility. *Campylobacter* organisms may be isolated from fecal specimens or blood cultures. Antimicrobial susceptibilities are not performed on these isolates.

Stool specimens for suspected *Campylobacter* infections are tested by the Tennessee Department of Health (TDH) Enteric Bacteriology Section. Refer to ENTERIC BACTERIOLOGY, Section II.

***Campylobacter* illness is a notifiable disease. Complete a Miscellaneous Exam Form PH-1573 with the patient's name and address and send the form to the Regional Health Office. It is not necessary to send a culture.**

Specimen Collection

Use thioglycollate medium as a transport medium. Pick a single colony to a tube of thioglycollate. Incubate it for 24 to 48 hours until good growth is observed. Overlay with 3/4 inch of sterile Vaspar * to maintain proper atmospheric conditions. Seal the cap with parafilm to prevent leakage. An alternative method involves harvesting the pure growth from a plate with a swab. Inoculate a Cary-Blair and leave the swab in the medium. Send the inoculated Cary-Blair on cold packs.

* Vaspar -- melt together equal portions (w/w) Vaseline and paraffin. Dispense in 3 ml amounts and autoclave at 121°C for 30 minutes. Store at room temperature. Melt the Vaspar for use, as needed.

Specimen Identification

1. Complete **all** the provider and patient information areas on the Miscellaneous Exam Form PH-1537. Include pertinent clinical and biochemical information with each specimen.
2. Using indelible ink, label each specimen with the date of collection and the patient's first and last name. Attach the control number on the tear strip to the specimen and secure it with transparent tape. Unlabeled specimens or specimens where the patient identifier on the specimen does not exactly match the identifier on the form **will not be tested**.

Shipment of Specimens

1. Thioglycollate Transport Medium

Pack the thioglycollate specimen with absorbent material to prevent breakage and to absorb the fluid if breakage or leakage should occur.

Campylobacter (Continued)

Packing and shipping specimens to the state public health laboratory requires personnel trained in current regulations. Use cold packs to keep the organisms cold. Follow the shipping guidelines for Category B substances and any additional guidelines from your current carrier or method of shipment. See Section 7, page 5 for instructions.

Cary-Blair Transport Medium

Packing and shipping specimens to the state public health laboratory requires personnel trained in current regulations. Follow the shipping guidelines for Category B substances and any additional guidelines from your current carrier or method of shipment. Use cold packs to keep the organisms cold. See Section 7, page 5 for instructions.

3. Ship cultures to the TDH Laboratory in Nashville.
4. Use first-class postage on US mail.

Specimens submitted on plates are acceptable only if they are properly closed in a *Campylobacter* transport bag and delivered by courier to the laboratory.

Reporting Procedures and Interpretation of Results

Most *Campylobacter* cultures are reported within 5 to 7 working days after receipt in the laboratory.

Reporting of Results
Organisms are reported by genus and species.

Organisms are identified to the genus and species level only when culture, morphology and biochemical test results support the species identification. *Campylobacter* species designations are consistent with the American Society for Microbiology's *Manual of Clinical Microbiology* or according to the *International Code of Nomenclature of Bacteria*.

The results of all specimens are reported to the health care provider who submitted the specimen. In addition, the TDH Communicable and Environmental Disease Services and the health department in the county where the patient lives are sent reports on *Campylobacter* isolates.

Criteria for Unacceptable Specimens

1. The specimen was not properly identified with the patient's name and/or the tear strip control number.
2. The patient identifier on the specimen does not exactly match that on the form.
3. The specimen was broken or leaked in transit.
4. The specimen was non-viable.
5. The specimen was submitted under improper atmospheric conditions.
6. A mixed specimen was submitted.

Miscellaneous Exam Form PH-1537

FRONT

SOCIAL SECURITY NO.		TENNCARE NO.		MCO		MISCELLANEOUS EXAM		A418027	
MEDICARE NO.			RECORD FOLDER NO.			DATE REPORTED		DATE/TIME RECEIVED	
PATIENTS NAME - LAST, FIRST, MIDDLE				SPOUSE - FIRST NAME				LAB NO. ▼	
STREET AND NUMBER						COLLECTION DATE		DATE OF ONSET	
TOWN		STATE		ZIP		SOURCE: <input type="checkbox"/> BLOOD <input type="checkbox"/> URINE <input type="checkbox"/> FECES <input type="checkbox"/> THROAT <input type="checkbox"/> OTHER			
DATE OF BIRTH		RACE		ETHNICITY		SEX		PHONE NO.	
COUNTY NO.		COUNTY NAME		SITE NO.		PATHOGEN/DISEASE SUSPECTED/SYMPTOMS			
NAME		ADDRESS		CITY		STATE		ZIP CODE	
PH-1573 REV. 11/06		TENNESSEE DEPT. OF HEALTH LABORATORY SERVICES		DAVID L. SMALLEY, PH.D, MSS, BCLD, DIRECTOR		<input type="checkbox"/> K <input type="checkbox"/> J <input type="checkbox"/> N		LABORATORY PERFORMING EXAMINATION	
EXAMINATION REQUESTED		RECENT TRAVEL 1 <input type="checkbox"/> YES 2 <input type="checkbox"/> NO		STATE/COUNTRY		EXAMINATION RESULTS			
SUBMITTED TO REFERENCE LABORATORY FOR EXAMINATION. RESULTS FORTHCOMING.		EXAMINED BY:		RDA-1160					

**TIGHTEN CAPS SECURELY
 SUBMIT IN DOUBLE MAILING CONTAINER**

STOOL CULTURES FOR SALMONELLA, SHIGELLA AND CAMPYLOBACTER
 Place two (2) cotton tipped swabs dipped into feces or other specimens and insert into Amies, Stuarts, or Cary-Blair transport medium. Submit the transport medium refrigerated within two (2) days of collection.

INTESTINAL PARASITES: Place amount of feces equal to volume of formalin in container designated for intestinal parasites (5% Formalin).

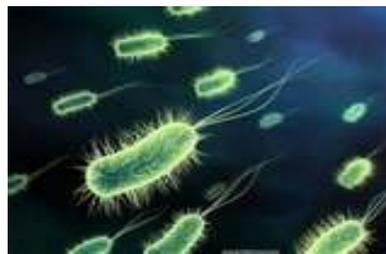
CULTURES FOR IDENTIFICATION - Submit pure cultures on non-selective media such as trypticase soy agar slants or enriched slants (Blood or Chocolate) when required.

ANAEROBIC ORGANISMS - Submit in semi-solid media such as thioglycollate, overlaid with sterile vaspar to prevent exposing culture to oxygen.

TESTING LABORATORY LOCATION CODES
 J = JACKSON BRANCH LAB, 295 SUMMAR DRIVE, JACKSON, TN - DR. ORISTYNE E. WALKER, PhD, MSS
 K = KNOXVILLE BRANCH LAB, 1522 CHEROKEE TRAIL, KNOXVILLE, TN - DR. DAVID L. SMALLEY, PhD, MSS, BCLD
 N = NASHVILLE REFERENCE LAB, 630 HART LANE, NASHVILLE, TN - DR. DAVID L. SMALLEY, PhD, MSS, BCLD

BACK

Enteric Bacteriology



Introduction

Feces and other specimens are tested for the presence of enteric pathogens, in particular *Salmonella* Typhi, other *Salmonella* serotypes, *Shigella* species, *Campylobacter jejuni* and *Escherichia coli* 0157:H7. Testing of clinical specimens for *Yersinia* and *Vibrio* is performed only upon request. Reference specimens are accepted from public and private health care providers for identification and confirmation of members of families of *Enterobacteriaceae* and *Vibrionaceae*. The Tennessee Department of Health (TDH) Laboratory is the serotyping center for *Salmonella* and *Shigella* isolates for the state and participates in the national surveillance programs of the Centers for Disease Control and Prevention (CDC). Isolation and identification techniques include culture procedures, morphological and biochemical characterization and complete serogrouping and serotyping. Refer to Chart II - 3 ENTERIC TESTING AVAILABLE AT THE TDH LABORATORIES.

Antimicrobial susceptibility testing for patient treatment is not performed in this laboratory.

Feces and other specimens associated with foodborne illness are screened for foodborne disease agents. Refer to FOODBORNE ILLNESS in Section II.

The Tennessee Medical Laboratory Act requires laboratory directors to submit pure cultures of the following organisms to the TDH Laboratory: *Escherichia coli* 0157:H7, *Salmonella* species, *Shigella* species, *Vibrio* species and * *Yersinia pestis*.

Patient information only is requested on *Campylobacter* species. You may complete a Miscellaneous Exam Form PH-1573 with the patient's name and address and send the form to the Bacteriology Section of the TDH Laboratory in Nashville.

Specimen Collection

Specimens for Routine Screening of Enteric Pathogens: Specimens should be collected early in the course of enteric disease and before antimicrobial therapy is begun. These specimens are routinely cultured for *Salmonella*, *Shigella*, *Campylobacter jejuni* and *Escherichia coli* 0157:H7. Please indicate if the patient has bloody diarrhea or a suspected *E. coli* 0157:H7 infection. The physician must specifically request *Yersinia* or *Vibrio* for the specimen to be tested for these pathogens.

Collect at least two rectal swabs or swabs of fresh stools from the patient and place the swabs in refrigerated (chilled 1 to 2 hours before use) Cary-Blair transport medium. When obtaining swabs from a patient, first moisten each rectal swab in the holding medium, insert the moistened swab into the rectum 1 to 1 inches, rotate the swab gently and then return the swab to the same tube of holding medium. Try to ensure that visible fecal material is present on each swab. After obtaining the two fecal swabs, insert both into the same tube of medium and push them to the bottom of the tube. CUT off PLASTIC SHAFT and discard the excess top portion of the swab.

IMPORTANT: Refrigerate or freeze tubes after specimens are placed in them. If the specimens will be tested within 48 hours after collection, they can be refrigerated; however, if the specimens must be held longer than 48 hours, freeze them as soon as possible after they are collected. Although storage in an ultra-low freezer (-70°C) is preferable, storage in a home-type freezer (if it is properly set at -20°C) is acceptable for short periods of time.

* *Yersinia pestis* is a select agent regulated by the National Select Agent Registry. Isolation of *Y. pestis* requires the laboratory that makes such an identification to make an immediate report

Enteric Bacteriology (Continued)

Chart II - 3

Enteric Testing Available at the TDH Laboratories

	Isolation & Preliminary Identification	Identification	Typing/ Grouping	Toxin Testing	Other
<i>Aeromonas/ Plesiomonas</i>		N			
<i>Campylobacter jejuni</i>	A	N			
<i>Escherichia coli</i> 0157:H7	A	N	N		
<i>E. coli</i> , other		N	¹ CDC		
<i>Salmonella</i>	A	J, N, K	N		
Shiga-toxin producing <i>E. coli</i>	A	N			
<i>Shigella</i> species	A	J, N, K	J, N, K		
<i>Vibrio cholera</i> & noncholera vibrios	² A	N	N	¹ CDC	
<i>Yersinia pestis</i> (plague)	² A	A			***
<i>Yersinia</i> species (other than <i>Y. pestis</i>)	² A	N			
Other <i>Enterobacteriaceae</i>		N			
Noroviruses (outbreaks only)	A	N			⁺ PCR

A Test are performed at the TDH Laboratories in Jackson, Knoxville and Nashville.

N Tests are performed at the TDH Laboratory in Nashville.

¹ CDC Centers for Disease Control and Prevention, Atlanta, Georgia.

² Testing of clinical specimens for *Yersinia* and *Vibrio* is performed only upon request.

*** Prior consultation with the laboratory is required before sending the specimen. Send by registered mail. This is so that packages can be tracked and undelivered packages may be located quickly. Before shipping, telephone the Enteric Bacteriology Section.

+ PCR Prior consultation with Communicable and Environmental Disease Services required.

Enteric Bacteriology (Continued)

Reference Cultures: Reference cultures for further identification should meet the criteria for inclusion in the families of *Enterobacteriaceae* or *Vibrionaceae*. Grow a pure culture of the isolated organism on a carbohydrate-free agar slant, such as nutrient agar or trypticase soy agar, for 24 hours.

Specimens for Isolation of *Yersinia pestis* (Plague): Inoculate blood (as least two samples) and aspirated fluids from lymph nodes or bubo into blood culture bottles. Use a sterile container for other specimens, such as sputum, tracheal, bronchial washings or throat swabs.

Specimens for Norovirus: During an outbreak, stools or feces from a representative sample of minimally 3 persons should be submitted in a Para-paks. Para-paks may be obtained from regional health departments only during outbreaks. Prior consultation CEDS is required.

Specimen Identification

1. Complete **all** the provider and patient information areas on the Miscellaneous Exam Form PH-1573. Include pertinent clinical and epidemiological information for all specimens and biochemical information on isolates. Indicate the organism suspected.
2. Using indelible ink, label each specimen with the date of collection and the patient's first and last name. Attach the control number on the tear strip to the specimen and secure it with transparent tape. Unlabeled specimens or specimens where the patient identifier on the specimen does not exactly match the identifier on the form **will not be tested**.
3. Clinical specimens will not be tested for *Yersinia* or *Vibrio* unless this request is stated on the form and/or the laboratory has been contacted. Contact the laboratory as soon as you know you will submit a specimen for *Yersinia* or *Vibrio* so that appropriate media can be prepared and testing can begin as soon as the specimen is received.

Shipment of Specimens

Ship clinical specimens for routine screening of enteric pathogens on cold packs to the Tennessee Department of Health Laboratory in Jackson, Knoxville or Nashville. Ship specimens for isolation of *Yersinia pestis* and all reference cultures to the TDH Laboratory in Nashville.

1. Routine screening specimen – Wrap the specimen in absorbent material to prevent breakage and to absorb the fluid if breakage or leakage should occur. Place it in a leak-proof insulated container and pack with wet ice or cold packs. Place the form in a plastic bag to prevent wetting or contamination. Follow the shipping guidelines of your current carrier or method of shipment. The specimen must be received in the laboratory within 48 hours. If the specimen cannot reach the laboratory within this time, freeze it at -20°C. (Storage at a temperature of -20°C is acceptable for 3 days.) Ship the specimen frozen with dry ice. It must arrive at laboratory in a frozen state.
2. Reference isolates – Pack the specimen with absorbent material to prevent breakage and to absorb the fluid if breakage or leakage should occur. Place it in a double-walled shipping container or the equivalent. Place the form in the outer container. Place the cap on securely. Cold packs are not required.
3. *Yersinia pestis* specimens – Notify the laboratory before shipping the specimen. Place the specimen in a double-walled shipping container or the equivalent. Pack it with absorbent material to prevent breakage and to absorb the fluid if breakage or leakage should occur. Place the form in the outer container. **Contact the Enteric Bacteriology Section before shipping.**

Enteric Bacteriology (Continued)

4. Follow the instructions in Section 7, page 5 for shipping Category B Specimens to the TDH Laboratory in Nashville, using the mailing label PH-0838. Use first-class postage on US mail.
5. Category A Infectious Substances Affecting Humans must be packaged and shipped by certified personnel following Department of Transportation (DOT) or International Air Transport Association regulations.
6. When an unusually large number of specimens are anticipated (as in outbreaks), telephone the laboratory before mailing the specimen so that necessary preparations may be made. Notify the laboratory by telephone when one or more specimens for *Yersinia pestis* or *Vibrio cholerae* are being submitted.

Note: **Do not mail specimens on plates.** Specimens submitted on plates are acceptable only if a courier delivers them to the laboratory.

Reporting Procedures and Interpretation of Results

Results from routine screening specimens and from reference specimens are usually reported within 3 to 6 working days after receipt of the specimen. Isolates that are submitted to the Centers for Disease Control and Prevention (CDC) for further testing might require several weeks for identification.

Reporting negative routine screening specimens
No <i>Salmonella</i> , <i>Shigella</i> , <i>Campylobacter jejuni</i> or <i>Escherichia coli</i> O157 isolated. All specimens are tested for Shiga-toxins to detect other Shiga-toxin producing E. coli.
Reporting enteric pathogens from screening and reference specimens
<p style="text-align: center;"><i>Salmonella</i></p> <p style="text-align: center;"><i>Salmonella</i> serotype _____.</p> <p style="text-align: center;">There are over 2000 known serotypes of <i>Salmonella</i>. <i>Salmonella</i> Typhi is the organism that causes typhoid fever.</p>
<p style="text-align: center;"><i>Shigella</i></p> <p style="text-align: center;"><i>Shigella dysenteriae</i> <i>Shigella flexneri</i> <i>Shigella boydii</i> <i>Shigella sonnei</i></p>

Enteric Bacteriology (Continued)

Reporting enteric pathogens from screening and reference specimens (continued)

Escherichia coli 0157:H7

Sorbitol negative isolates that agglutinate in 0157 latex reagent are identified biochemically and tested for the H7 antigen.

Campylobacter jejuni

Campylobacter jejuni is identified by culture, morphology and Biochemical means.

Reporting of other isolates

Organisms are reported by genus and species.

Follow-up of cases of salmonellosis and shigellosis and their contacts is recommended following the procedures outlined in the 18th Edition of *Control of Communicable Diseases in Man*. Questions concerning follow-up should be addressed to the epidemiologists in the TDH Communicable and Environmental Disease Section at (615) 532-8515.

Organisms are identified to genus, species and subspecies level when appropriate and only if culture, morphology and biochemical test results support the identification. Genus, species and subspecies designations are consistent with designations in the American Society for Microbiology's *Manual of Clinical Microbiology* or according to the *International Code of Nomenclature of Bacteria*.

The results of all diagnostic tests are reported to the health care provider who submitted the specimen. In addition, the TDH Communicable and Environmental Disease Services and the health department in the county where the patient lives are sent reports on the following organisms:

Campylobacter jejuni
Escherichia coli 0157:H7
Salmonella Typhi
Salmonella species
Shigella species
Vibrio species
Other Shiga-toxin producing *E. coli*
Yersinia enterocolitica

Enteric Bacteriology (Continued)

Criteria for Unacceptable Specimens

All specimens

1. The specimen was not properly identified with the patient's name and/or the tear strip control number.
2. The patient identifier on the specimen does not exactly match the identifier on the form.
3. The specimen was broken or leaked in transit.

Clinical specimens

A provider who submits an unsatisfactory specimen is notified by phone.

If *Shigella* or *Campylobacter* is not isolated, a second specimen must be submitted. (*Shigella* and *Campylobacter* are more dependent on proper transportation conditions for survival than *Salmonella* and *E. coli*.)

1. No preservative was used for transport. If there is a delay of more than 2 to 3 hours after collection, a transport medium is necessary. Cary-Blair transport is available from your local county health department.
2. The specimen was submitted in 5 to 10% formalin.
3. The specimen was not transported under refrigerated (2 to 8°C) or frozen (-20°C) conditions.
4. An unfrozen specimen was received 48 hours after collection.
5. The specimen was resting on top of Cary-Blair transport media. The swab was not preserved by stabbing the Cary-Blair.
6. The specimen contained too much inoculum. A small amount of feces, on a cotton-tipped applicator, should be inserted into the preservative.
7. The Cary-Blair was not inoculated.
8. The Cary-Blair medium had expired.
9. No growth was obtained from the specimen. There was no apparent inoculum.

Reference specimens

1. The specimen was not viable.
2. A mixed specimen was submitted.

Enteric Bacteriology (Continued)

Miscellaneous Exam Form PH-1573

FRONT

SOCIAL SECURITY NO.		TENN CARE NO.		MCO		MISCELLANEOUS EXAM		A418027		LAB NO. ▼	
MEDICARE NO.		RECORD FOLDER NO.									
PATIENT'S NAME - LAST, FIRST, MIDDLE					SPOUSE - FIRST NAME			DATE REPORTED		DATE/TIME RECEIVED	
STREET AND NUMBER						COLLECTION DATE		DATE OF ONSET			
TOWN			STATE		ZIP		SOURCE: <input type="checkbox"/> BLOOD <input type="checkbox"/> URINE <input type="checkbox"/> FECES <input type="checkbox"/> THROAT <input type="checkbox"/> OTHER				
DATE OF BIRTH		RACE	ETHNICITY	SEX	PHONE NO.		PATHOGEN/DISEASE SUSPECTED/SYMPTOMS				
COUNTY NO.		COUNTY NAME			SITE NO.		EXAMINATION REQUESTED				
NAME		RECENT TRAVEL 1 <input type="checkbox"/> YES 2 <input type="checkbox"/> NO STATE/COUNTRY									
ADDRESS		EXAMINATION RESULTS									
CITY		STATE		ZIP CODE		<input type="checkbox"/> SUBMITTED TO REFERENCE LABORATORY FOR EXAMINATION. RESULTS FORTHCOMING.					
PH-1573 REV. 11/06		TENNESSEE DEPT. OF HEALTH LABORATORY SERVICES			<input type="checkbox"/> K <input type="checkbox"/> J <input type="checkbox"/> N		EXAMINED BY:		RDA-1160		
DAVID L. SMALLEY, PhD, MSc, BCID, DIRECTOR		LABORATORY PERFORMING EXAMINATION									

PLEASE FILL OUT SHADDED AREA COMPLETELY
 TN. DEPT. OF HEALTH DIV. OF LAB SERVICES
 PRINTED BY STAN WARD ON 08/08/10 10:58 AM

DETACH THIS STRIP

A418027

Gonorrhea (*Neisseria gonorrhoeae*) and *Chlamydia trachomatis* Nucleic Acid Amplification Technology

Introduction

The label in the box on the left is placed correctly.



Screening for the presence of *Neisseria gonorrhoeae* (Gonorrhea, Gonococcus, GNDC, GC) is performed state-wide as part of a state and federally supported program to control sexually transmitted diseases. Gonorrhea affects males and females with symptoms from purulent discharge in males a few days after exposure to very mild symptoms in females. Symptoms may pass unnoticed with the consequence that an asymptomatic carrier state is formed. These carriers contribute significantly to the public health problem of gonorrhea. Chlamydiae are bacteria that are obligate intracellular parasites of eukaryotic cells. Today *Chlamydia trachomatis* is considered the most common sexually transmitted disease (STD) in the United States. The infections it causes are among the most damaging of the STD diseases. Epididymitis is the major complication of chlamydial urethritis in men. *C. trachomatis* has been implicated in salpingitis and pelvic inflammatory disease in women.

The Tennessee Department of Health (TDH) Laboratories in Jackson, Knoxville and Nashville accept female urine, vaginal and endocervical specimens and male urethral and urine specimens from public health clinics. Nashville will accept oropharyngeal and rectal swabs from designated STD clinics. These specimens are tested for gonorrhea and *C. trachomatis*. *N. gonorrhoeae* and *C. trachomatis* are identified directly from the clinical specimens by nucleic acid amplification technology (NAAT) using the Gen-Probe Aptima System in Jackson and Memphis and TIGRIS in Nashville and Knoxville.

The TDH Laboratory Services will continue to perform culture tests for *N. gonorrhoeae* on non-traditional sites, in the case of treatment failure and under special circumstances. Antimicrobial tests are not performed in the TDH Laboratories, but can be forwarded to the Centers for Disease Control and Prevention (CDC) in special circumstances. The TDH Laboratory does not perform culture tests for Chlamydia.

ONLY SWABS SUPPLIED WITH THE NAAT SPECIMEN COLLECTION SYSTEMS CAN BE USED FOR SPECIMEN COLLECTION. The specimen must be tested within 60 days of collection, urine specimens must be tested within 30 days of collection.

Specimen Collection

Female specimen types: oropharyngeal, rectal (designated STD clinics), vaginal and urine.

Female – Endocervical

1. Insert a speculum into the vagina using only water as a lubricant.
2. Clean the cervical os with a swab from the kit to remove excess mucus. Discard the cleaning swab.
3. Insert a second swab from collection kit 1-1 1/2 cm into the endocervical canal and rotate it for 30 seconds.
4. Withdraw the swab without touching the vaginal surface and place it in transport medium.
5. Insert the blue shaft swab into the unisex Transport Tube.
6. Break off or cut the swab's shaft to fit the tube and cap the tube leaving the swab in the tube.

Gonorrhea and Chlamydia by NAA (Continued)

Female - Urethra

1. If vaginal discharge is present at urethral orifice, remove it with cotton swab before proceeding.
2. Insert a second swab 1 cm into the urethra, rotate it, remove it and place in transport medium.
3. Insert the blue shaft swab into the unisex Transport Tube.
4. Break off or cut the swab's shaft to fit the tube and cap the tube leaving the swab in the tube.

Male Urethra

1. The patient should not have urinated for at least one hour before sample collection.
2. Collect the urethral exudate or insert the blue shaft swab from urethral collection kit 2 to 4 cm into the urethra using a rotating motion to facilitate insertion.
3. Once inserted, rotate the swab gently, using sufficient pressure to ensure the swab comes into contact with all urethral surfaces. Allow the swab to remain inserted for 2 to 3 seconds.
4. Withdraw the swab.
5. Insert the swab into the unisex Transport Tube.
6. Break off or cut the swab's shaft to fit the tube and cap the tube leaving the swab in the tube.

Specimen Identification

1. Use the established electronic information system or complete **all** the provider and patient information areas on the Chlamydia/Gonorrhea Detection Form PH-3179. Include pertinent clinical information with each specimen.
2. Indicate the source on the tube for endocervical, urethral, throat and rectal specimens being sent.
3. Using indelible ink, label each specimen with the date of collection and the patient's first and last name. Attach the control number on the tear strip to the specimen and secure it with transparent tape. Unlabeled specimens or specimens where the patient identifier on the specimen does not exactly match the identifier on the form will not be tested.

Shipment of Specimens

1. Packing and shipping specimens to the state public health laboratory requires personnel trained in current regulations. Follow the shipping guidelines of your current carrier or method of shipment.
2. Affix the mailing label (PH-0838), return address and infectious substance (etiologic agent) or clinical (diagnostic) specimen label to the outer container.
3. Ship the specimen to the nearest Tennessee Department of Health Laboratory in Jackson, Knoxville or Nashville.
4. Use first-class postage if shipping by US mail.

Gonorrhea and Chlamydia by NAA (Continued)

Reporting and Interpretation of Results

Specimens are reported within 2 to 4 working days after receipt in the laboratory.

Gonorrhea results are reported
Negative for <i>Neisseria gonorrhoeae</i> .
Positive for <i>Neisseria gonorrhoeae</i> .
Indeterminate for <i>Neisseria gonorrhoeae</i> . (Submit another specimen.)

Chlamydia results are reported
Negative for <i>Chlamydia trachomatis</i> .
Positive for <i>Chlamydia trachomatis</i> .
Indeterminate for <i>Chlamydia trachomatis</i> . (Submit another specimen.)

In the NAAT System a relative light unit (RLU) value is printed out for each specimen. Select positive specimens undergo additional testing. Reports indicate that the specimen was positive, indeterminate (submit another specimen) or negative for *C. trachomatis* and *N. gonorrhoeae*.

The results of all specimens are reported to the provider who submitted the specimen. In addition, the TDH Sexually Transmitted Disease (STD) Control, the regional STD control representative and the health department in the county where the patient lives are sent reports on positive gonorrhea and *Chlamydia* specimens.

Gonorrhea and Chlamydia by NAA (Continued)

Criteria for Unacceptable Specimens

1. The specimen was not properly identified with the patient's name, the tear strip control number or other appropriate identifier.
2. The patient identifier on the specimen did not exactly match the identifier on the form.
3. The specimen was collected by use of swabs and/or tubes (collection kit) other than by the NAAT System kit.
4. The specimen was collected from a site other than endocervical, rectal, oropharyngeal, urethral, or urine.
5. The specimen was too old for testing. All specimens should be tested within 60 days after collection (30 days for urine.)
6. The specimen had no collection swab in the transport tube upon receipt in the laboratory.
7. The specimen had two collection swabs in the transport tube.
8. The specimen was received in an out-of-date collection kit.
9. The media had leaked in transport or something has been added to the tube for (example the tube was too full or was a strange color.)

Chlamydia/Gonorrhea Detection Form PH-3179

FRONT

SOCIAL SECURITY NO.			TENNCARE NO.			MCO			CHLAMYDIA/GONORRHEA DETECTION D710605																																															
MEDICARE NO.			RECORD FOLDER NO.			DATE REPORTED			DATE/TIME RECEIVED			LAB NO.			DETACH THIS PORTION AND AFFIX THIS NUMBER ON SPECIMEN TUBE <b style="color: red; font-size: 2em;">D710605 SPECIMEN CONTROL NO.																																									
PATIENT'S NAME - LAST, FIRST, MIDDLE						SPOUSE - FIRST NAME						COLLECTION DATE															CO. NO.			CLINIC NO.			PROGRAM			RACE			ETHNICITY			SEX			AGE											
STREET AND NUMBER												Y															N			M			F																							
TOWN				STATE				ZIP				FAMILY PLANNING															PRENATAL			OTHER			PREGNANT																							
DATE OF BIRTH			RACE			ETHNICITY			SEX			PHONE NO.															SCREENING/DIAGNOSIS			CONTACT			INITIAL			REVISIT			FIRST			SECOND			Y			N								
COUNTY NO.			COUNTY NAME			SITE NO.			SOURCE OF SPECIMEN			ENDOCERVICAL															UROGENITAL			URINE			SIGNS AND SYMPTOMS			TREATMENT																				
SEND REPORT TO:			NAME			ADDRESS			CITY			STATE															ZIP CODE			1			2			3			A			M			U			P			Y			N		
PH-3179 REV. 12-08			TENNESSEE DEPT. OF HEALTH LABORATORY SERVICES DR. DAVID L. SMALLEY, PhD, MSS, BCLD, DIRECTOR			<input type="checkbox"/> K <input type="checkbox"/> J <input type="checkbox"/> N			LABORATORY PERFORMING EXAMINATION			EXAMINED BY:															RDA-1160																													

USE THE NUCLEIC ACID AMPLIFICATION TEST (NAAT) TRANSPORT PROVIDED, AFTER COLLECTION STORE AT 2° - 30° C.
 TRANSPORT SWAB SPECIMENS AND *URINE TRANSPORT TUBES TO THE STATE LABORATORY WITHIN 15 DAYS OF COLLECTION.
 *URINE SPECIMENS MUST BE TRANSFERRED TO THE URINE TRANSPORT TUBE WITHIN 24 HOURS OF COLLECTION.
 PATIENT'S NAME AND DATE OF COLLECTION MUST BE INCLUDED ON SPECIMEN CONTAINER.

SIGNS AND SYMPTOM CODES	RACE	ETHNICITY
A = ASYMPTOMATIC	ASIAN = A	HISPANIC? = U
M = MUCOPURULENT CERVICITIS	BLACK = B	Y = YES
U = URETHRITIS	NATIVE AM = N	N = NO
P = SUSPECTED PELVIC INFLAMMATORY DISEASE	OTHER = O	
	UNDECIDED = U	
	UNKNOWN = U	
	WHITE = W	

A SPECIMEN IS CONSIDERED INDETERMINATE WHEN THE INITIAL SCREENING TEST IS POSITIVE; HOWEVER, THERE IS NOT ENOUGH SAMPLE REMAINING FOR CONFIRMATION TESTING. A REPEAT SPECIMEN IS SUGGESTED.

TESTING LABORATORY LOCATION CODES

J = JACKSON BRANCH LAB, 295 SUMMAR DRIVE, JACKSON, TN - DR. ORISTYNE E. WALKER, PhD, HCLD, DIRECTOR
 K = KNOXVILLE BRANCH LAB, 1522 CHEROKEE TRAIL, KNOXVILLE, TN - DR. DAVID L. SMALLEY, PhD, MSS, BCLD, DIRECTOR
 N = NASHVILLE REFERENCE LAB, 630 HART LANE, NASHVILLE, TN - DR. DAVID L. SMALLEY, PhD, MSS, BCLD, DIRECTOR

BACK

Gonorrhea, Culture

Neisseria gonorrhoeae, Gonococcus, GC



Introduction

Screening for the presence of *Neisseria gonorrhoeae* is routinely performed using a nucleic acid amplification technology. Refer to the GONORRHEA AND CHLAMYDIA BY NUCLEIC ACID AMPLIFICATION METHOD, Section II. The culture method is recommended for antimicrobial sensitivity testing and for testing other sites. The Tennessee Department of Health (TDH) Laboratories in Jackson, Knoxville and Nashville accept specimens from public health clinics for primary isolation of *N. gonorrhoeae*. Reference cultures are accepted from public and private health care providers for confirmation.

Gonorrhea is isolated and then identified by biochemical and direct fluorescent antibody (DFA) methods. Oxidase-positive, gram-negative diplococci (GNDC) from routine specimens are confirmed as *N. gonorrhoeae* by DFA. Oxidase-positive, DFA-positive GNDC, cultures from throat cultures or children (age 12-years or less) are confirmed by a second confirmatory method. The API NHID System rapid carbohydrate test is used in the TDH Laboratories.

GNDC, DFA-negative organisms from locations other than oral sites are identified using biochemicals. GNDC, DFA-negative organisms from oral sites are tested using the superoxol test. If these are positive, they are also identified biochemically.

Antimicrobial tests are not performed in the TDH Laboratories. In special circumstances cultures can be forwarded to the Centers for Disease Control and Prevention (CDC) for further antimicrobial susceptibility studies.

Specimen Collection

The culture plates, Martin-Lewis Medium (MLM), need to be stored sealed in plastic sleeves in the refrigerator at 2-8°C. The storage life of the culture plates is six weeks. The expiration date is noted inside each sleeve. The plates should be used before the expiration date and be at room temperature before inoculation. Allow excessive moisture on the surface to evaporate before use. Dried media that has pulled away from the sides, has cracked or has severe stress marks should not be used. Likewise, media that has growth present should not be used. Sites that may be cultured are the cervix, anus, urethra, vagina and oropharynx. If more than one site is cultured, a separate laboratory request form and culture plate are required for each culture.

Endocervical

Insert the speculum into the vagina using only water as a lubricant and visualize the cervix. Remove excessive cervical mucus if present. Insert cotton-tipped swab into the endocervical canal. Move from side to side. Allow 15 to 30 seconds for secretions to be absorbed.

Rectal

Insert a cotton-tipped swab 2 to 3 cm into the anal canal. If the cotton tip is inadvertently pushed into feces, use another swab to obtain a specimen. Move the swab from side to side in the anal canal. Allow several seconds for secretions to be absorbed.

Urethral

Obtain fresh exudate from the meatus on sterile cotton-tipped swab or if no exudate is present, use a calcium alginate swab inserted 2 to 3 cm into the anterior urethra.

Gonorrhea Culture Method (Continued)

Oropharynx

Swab the posterior pharynx and the region of the tonsillar crypts.

Procedure

1. Mark the bottom of the culture plate (not the lid) with the numbered tear strip with accession number from the laboratory request form.
2. Wear disposable gloves. Collect the specimen from site to be cultured.
3. Roll the swab in a large "Z" pattern on the culture place. With a sterile wire loop or sterile wooden applicator stick, cross-streak immediately with a second "z" at a different angle.
4. Immediately place the specimen into a CO₂ enriched environment using the CO₂ Tablet/Plastic Bag method.
 - a. Place the culture into the bag with the CO₂ generating tablet within 15 minutes of inoculation.
 - b. When using individually wrapped tablets, tear the foil just enough to expose the tablet and place it in that fashion in the bag as fast as possible. Do not open the tablet until ready to put it into the bag.
 - c. Quickly expel excess air from the bag itself; seal the bag tightly.
 - d. Incubate plates within 1 to 2 hours at 35 - 37°C overnight.
5. Transport the specimen to the laboratory as soon as possible to arrive within the 72-hour limit. Suggested transport is as follows:
 - a. Hand deliver or courier the same day;
 - b. Incubate under appropriate temperature and CO₂ conditions for 18 to 24 hours. Mail the specimen to the nearest TDH laboratory.
 - c. For Friday clinics proceed as for 5.a. or 5.b. mailing specimens on Saturday to arrive on Monday or incubate all specimens except those from "STD" clinics all weekend (72 hours) and mail on Monday to arrive on Tuesday. The latter suggestion is aimed at areas having problems with specimens mailed on Saturday and delayed in transit as long as Tuesday and Wednesday. When using this alternative method (incubating 72 hours), **all specimens should be clearly marked as having been incubated for 72 hours**. If not marked, they will be reported as "unsatisfactory - too long in transit." Contact the laboratory before instituting this procedure.

Gonorrhea Culture Method (Continued)

Chart II - 4
HANDLING OF NEISSERIA GONORRHOEAE CULTURES
BY HEALTH CARE PROVIDERS
MARTIN-LEWIS PLATES

Temperature of Medium When Inoculated ROOM TEMPERATURE. The plates should be taken from refrigerator storage at least one hour before use. Tempered plates are preferable, but specimens may be inoculated on cold plates if time does not permit tempering.

Shelf Life of Medium Six weeks when sealed in plastic and refrigerated. Preparation and expiration dates of the medium will be indicated on package. Do not use beyond expiration date. DO NOT USE DEHYDRATED MEDIUM

Laboratory Form and Labeling the MLM Plate Use special **Gonococcus Culture Form** PH-1583. Complete the form with all information requested. The tearstrip number on the form should be fixed to the BOTTOM (AGAR SIDE) of the plate. IT IS IMPERATIVE THAT THE DATE COLLECTED BE INDICATED ON THE FORM, otherwise an **UNSATISFACTORY** report will result.

Inoculation of Martin-Lewis Plates ROLL the swab on the plate in "Z" pattern. ROLL THE SIDE OF THE SWAB for adequate exposure of swab to plate for transfer of microorganisms. Specimens from asymptomatic females may contain exceedingly small numbers of demonstrable gonococci. DO NOT USE THE TIP OF THE SWAB TO INOCULATE PLATE.

How Plates Should Be Cross-Streaked (Not required for the GonoPak.) The "Z" inoculum should be cross-streaked with a sterile wire loop before incubation. This effects a greater dilution of the bacterial flora of secretions, especially from the female patient. DO NOT cross-streak the entire surface of the medium.

How Cultures Are Handled after Inoculation The inoculated plates (GonoPak plates) should be placed into the accompanying whirlpack plastic bag. Drop a CO₂-generating tablet into the bag. (Tear the foil. Do not remove pill from foil). Quickly seal the bag securely to prevent leakage of CO₂. Moisture from plates will activate the CO₂ tablet.)

Gonorrhea Culture Method (Continued)

How Cultures Are Handled after Inoculation (Continued)

DO NOT ADD WATER TO THE BAG). DO NOT PUT THE FORM INTO THE BAG. *N. gonorrhoeae* must have a CO₂ atmosphere for growth. It is a fastidious microorganism that must be handled under optimum conditions to maintain its viability.

Incubate plates for 16 to 24 hours at 35° to 37° C. in an inverted (media side up) position. Allow the specimen to remain in the incubator until ready to package for transport to the laboratory.

For LOW-RISK FRIDAY CLINIC ONLY - Specimens may be incubated over the weekend and mailed on Monday. Clearly indicate ON THE FORM that the specimen has had 72 hours incubation.

When a holiday falls on Monday, the specimens should be mailed Saturday. DO NOT HOLD SPECIMENS LONGER THAN 72 HOURS.

Shipment of Specimens

Package as you would any other specimen to be mailed. The form must be properly filled out and enclosed in the outer mailing container.

Do not send any other type of specimen (blood, serum, etc.) in the package with Martin-Lewis plates.

Use a GonoPak mailing label for First-class postpaid delivery to the laboratory. Please note the duration of incubation on the outside container. The incubation time of weekend cultures must be indicated on the forms.

Gonorrhea Culture Method (Continued)

Specimen Identification

1. Complete **all** the provider and patient information areas on the Gonococcus Culture Form PH-1583. Include pertinent clinical information with each specimen.
2. Using indelible ink, label each specimen with the date of collection and the patient's first and last name. Attach the control number on the tear strip to the **BOTTOM (AGAR SIDE)** of the plate. Unlabeled specimens or specimens where the patient identifier on the specimen does not exactly match the identifier on the form will not be tested.

Specimen Shipment

1. Incubate the specimen according to information in Chart II - 4, HANDLING OF NEISSERIA GONORRHOEAE CULTURES BY HEALTH CARE PROVIDERS.
2. Transport the specimen to arrive at the laboratory within 72 hours after collection.
3. Place the form in outer container. Place the cap on securely.
3. Affix the GONOPAK mailing label, return address and follow instructions for packaging Category B, Biological Substances in Section 7, Page 5. (Postage is not required when the GONOPAK label is used.)
4. Ship the specimen to the nearest Tennessee Department of Health Laboratory **in Jackson, Knoxville or Nashville.**

REFERENCE CULTURES

To submit reference cultures of *Neisseria gonorrhoeae*, transfer a well-isolated colony from the primary isolation plate to a fresh GONOPAK plate. Incubate under CO₂ overnight or until growth is visible. Place the culture in a CO₂ environmental transport system (available with the GONOPAK kit). Pack as above. Ship to the nearest TDH Laboratory.

Reporting and Interpretation of Results

Positive specimens are reported within 1 to 4 working days after arrival in the laboratory. Negative and unsatisfactory specimens are incubated for a total of 72 hours before reporting.

Positive cultures of *Neisseria gonorrhoeae* on children 12-years-old or younger are reported immediately by telephone.

Gonorrhea results are reported as
Negative: <i>Neisseria gonorrhoeae</i> not confirmed by culture.
Positive: <i>Neisseria gonorrhoeae</i> confirmed by culture.

Gonorrhea Culture Method (Continued)

Reporting of unsatisfactory specimens: Unsatisfactory specimens are examined for a total of 72 hours. Any unsatisfactory specimen that can be identified and reported as positive, regardless of the unsatisfactory condition, will be reported as positive. (Unlabeled specimens or specimens where the patient identifier on the specimen does not exactly match the identifier on the form will not be tested.) Negative unsatisfactory specimens will be reported as unsatisfactory with the reason given.

The results of all specimens are reported to the provider who submitted the specimen. In addition, the TDH Sexually Transmitted Disease (STD) Control, the regional STD control representative and the health department in the county where the patient lives are sent reports on specimens positive for gonorrhea.

Special Reports:

Any GNDC (gram-negative diplococci) from a cervical or urethral source isolated in the TDH Laboratory that is identified as a *Neisseria meningitidis* is reported as a supplemental report. The provider is given an oral report by telephone, followed by a written report.

Criteria for Unacceptable Specimens

Specimens are reported as unsatisfactory for the isolation of *Neisseria gonorrhoeae* according to the following key. (This key appears on the back of the Gonococcus Culture Form, PH-1583.)

- a. Loss of Carbon Dioxide
 - 1) No CO₂ generating tablet.
 - 2) GONOPAK bag was not sealed.
 - 3) Improper CO₂ bag was used.

- b. Media Conditions
 - 1) Out of date.
 - 2) Frozen.
 - 3) Dehydrated.

- c. Incubation
 - 1) Too long in transit.
 - 2) Collected and mailed on same day.

- d. Other
 - 1) No specimen received.
 - 2) Improper inoculation.
 - 3) No apparent inoculation.
 - 4) No date of collection.
 - 5) OTHER _____
 - a) Overgrowth by contaminants.
 - b) Failure to properly identify specimen.
 - c) Broken in transit.
 - d) Failure to place specimen in bag.
 - e) Failure to place candle in candle jar.
 - f) Laboratory accident.

Gonorrhea Culture Method (Continued)

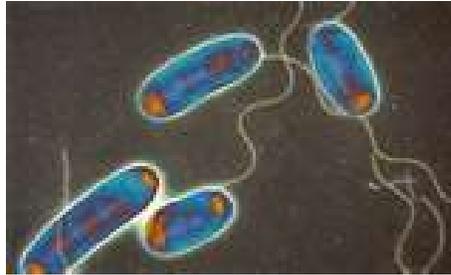
Gonococcus Culture Form PH-1583

FRONT

SOCIAL SECURITY NO.		TENNCARE NO.		MCO		GONOCOCCUS CULTURE				C0512731					
MEDICARE NO.				RECORD FOLDER NO.				DATE REPORTED		DATE/TIME RECD		LAB NO.			
PATIENTS NAME - LAST, FIRST, MIDDLE						SPOUSE - FIRST NAME									
STREET AND NUMBER															
TOWN				STATE		ZIP		COLLECTION DATE		SEX		MARITAL STATUS		RACE	
DATE OF BIRTH		RACE		ETHNICITY		SEX		PHONE NO.		CLINIC NO.		CO. NO.		AGE	
COUNTY NO.		COUNTY NAME				SITE NO.		REASON FOR EXAM		SURVEY		DIAGNOSTIC		RX CONTROL	
NAME		ADDRESS		CITY		STATE		ZIP CODE		1		2		3	
RDA-1160		TENNESSEE DEPT. OF HEALTH LABORATORY SERVICES DR. DAVID L. SMALLEY, PH.D., M.S.S., B.C.L.D., DIRECTOR				LABORATORY PERFORMING EXAMINATION		EXAMINED BY:		RDA-1160		NEISSERIA GONORRHOEA CONFIRMED		YES NO	
PH-1583 REV 11-06										1		2		3	
UNSATISFACTORY												1		2	
3 see key on back															
TREATMENT Yes <input checked="" type="checkbox"/> No <input checked="" type="checkbox"/> UNK <input checked="" type="checkbox"/> Treatment Date _____															
TREATMENT RECEIVED _____															
LABORATORY RESULTS															
NEISSERIA GONORRHOEA CONFIRMED												YES		NO	
												1		2	
UNSATISFACTORY															
3 see key on back															
EXAMINED BY:												RDA-1160			
RDA-1160															

Unsatisfactory Key for Gonorrhea Reporting			
A. Loss of Carbon Dioxide		C. Incubation	
1. No CO ₂ generating tablet		1. Too long in transit	
2. Gono-Pak bag not sealed		2. Collected and mailed on same day	
3. Improper CO ₂ bag used			
B. Media Conditions		D. Other	
1. Out of Date		1. No specimen received	
2. Frozen		2. Improper inoculation	
3. Dehydrated		3. No apparent inoculation	
		4. No date of collection	
		5. _____	
All specimens are incubated for a total of 72 hours and examined for typical growth. No gonorrhea was isolated. Because of the condition indicated above, this specimen is reported as unsatisfactory.			
TESTING LABORATORY LOCATION CODES: J - JACKSON BRANCH LAB, 245 SUMMAR DRIVE, JACKSON, TN - DR. CRISTYNE E. WALKER, PH.D., HCLD., DIRECTOR K - KNOXVILLE BRANCH LAB, 1522 CHEROKEE TRAIL, KNOXVILLE, TN - DR. DAVID L. SMALLEY, PH.D., M.S.S., B.C.L.D., DIRECTOR N - NASHVILLE REFERENCE LAB, 630 HART LANE, NASHVILLE, TN - DR. DAVID L. SMALLEY, PH.D., M.S.S., B.C.L.D., DIRECTOR			

BACK



Legionella

Introduction

Legionella are ubiquitous, fresh-water bacteria that infect humans sporadically or as epidemics. Although direct proof is lacking, transmission appears to occur primarily through airborne water droplets such as those from cooling towers or other heat-rejection systems. Laboratory data are relied on to confirm legionellosis because clinical signs are nonspecific. The organism can be isolated and identified from unfixed tissue and respiratory secretions or can be detected in fixed or unfixed tissue and body fluids with direct fluorescent antibody (DFA) staining. Culture is the recommended diagnostic procedure and should be attempted with other methodologies.

There are currently 34 *Legionella* species comprising 54 serogroups. Twelve of the species have been documented as etiologic agents of human disease by culture. Treatment decisions are based on identifying the genus since all species tested so far are susceptible to erythromycin and rifampin.

The Bacteriology Section offers culture and DFA staining of clinical specimens and reference cultures to public and private health care providers. Environmental samples that are directly related to a documented outbreak of legionellosis can be accepted for direct testing of *Legionella* when requested by Communicable and Environmental Disease Services (CEDS).

Refer to INFECTIOUS DISEASES SEROLOGY, Section V for serological tests available.

The Tennessee Medical Laboratory Act requires Laboratory Directors to submit *Legionella* species to the Tennessee Department of Health Laboratory for confirmation for typing and/or antibiotic sensitivity testing.

Specimen Collection

Clinical Specimens

Recommended specimens for culture include respiratory tract secretions, tissues, sputum, pleural fluid, transtracheal aspirates, bronchial washings and biopsies. **Do not use saline** to collect or dilute specimens for *Legionella* culture as the saline may inhibit growth. Use sterile broth or sterile distilled water.

Collect all specimens using aseptic technique following the protocols appropriate to the type of specimen. Place the specimen in a sterile screw-capped plastic centrifuge tube, such as the TB plastic conical tube. Seal the container securely to prevent leakage. Flexible plastic specimen collection cups are not acceptable for transporting specimens.

Prepare smears for DFA staining from respiratory specimens and formalized tissue by making thin films of respiratory fluids, sputum or tissue homogenates on glass slides. Formalized tissue may be scraped gently with a sterile scalpel to obtain material for smear preparation. Alternatively, a cut edge may be pressed gently onto a glass slide. Smears should be air-dried, gently heat-fixed or alcohol-fixed, flooded with 10% neutral formalin for 10 minutes, rinsed with distilled water and air-dried. **Formalin fixation is required as a safety precaution.** Submit a minimum of four separate smears per specimen.

Legionella (Continued)

Reference Specimens

Isolated organisms for identification of *Legionella* species should be pure cultures on charcoal-yeast extract agar slants incubated sufficiently to allow good visible growth. A single block of agar cut from fresh media may be placed in a sterile tube or on the surface of a charcoal agar slant.

Environmental Specimens

Contact the laboratory about the proper method for submitting environmental samples. Acceptance of environmental specimens at the request of CEDS.

Specimen Identification

1. Complete all the provider and patient information areas on the Miscellaneous Exam Form PH-1573. Include pertinent clinical information with each specimen.
3. Using indelible ink, label each specimen with the date of collection and the patient's first and last name. Attach the control number on the tear strip to the specimen and secure it with transparent tape. Unlabeled specimens or specimens where the patient identifier on the specimen does not exactly match the identifier on the form will not be tested.

Shipment of Specimens

1. Ship tissue, sputum and body fluid specimens **on cold packs as Category B, Biological Substance.**

Ship formalinized tissue, prepared slides, *Legionella* isolates or environmental samples using Packing Instructions 602. Place the form in the outer container. Follow the shipping guidelines of your current carrier or method of shipment.
2. Affix the mailing label (PH-0838), return address and infectious substance (etiologic agent) or clinical (diagnostic) specimen label to the outer container.
3. Ship to the Tennessee Department of Health Laboratory in **Nashville.**
4. Use first-class postage on US mail.
5. Contact the Bacteriology Section when an outbreak is suspected and **before** shipping the specimens.

Legionella (Continued)

Reporting and Interpretation of Results

Results of positive DFA examinations are telephoned immediately, followed by a mailed report, usually on the day of receipt or the following work day. Positive cultures are reported by telephone and by mail as soon as the growth is identified. Cultures are held for 10 days before being reported as negative.

Legionella is identified in culture and smears by specific DFA staining. DFA staining is a presumptive test. Cross-reactivity may occur among *Legionellaceae*.

Neither a negative DFA stain nor a negative culture rules out *Legionella* infection. Negative results may occur due to low numbers of organisms present, improper specimen or smear handling and previous antimicrobial therapy.

Legionella isolates requiring definitive identification are forwarded to the Centers for Disease Control and Prevention.

Smears for <i>Legionella</i> from lung tissue are reported:	
Result	Report
25 or more strongly fluorescing * bacteria per smear	DFA presumptive positive
Less than 25 strongly fluorescing * bacteria per smear	The number of fluorescing bacteria per smear is reported.
No strongly fluorescing bacteria per smear	FA presumptive negative

Smears for <i>Legionella</i> from sites other than lung tissue are reported:	
Result	Report
5 or more strongly fluorescing * bacteria per smear	DFA presumptive positive
Less than 5 strongly fluorescing * bacteria per smear	The number of fluorescing bacteria per smear is reported.
No strongly fluorescing bacteria per smear	FA presumptive negative

* Cells with morphology typical of *Legionella*.

Legionella (Continued)

Culture results are reported as:
Negative for <i>Legionella</i> species.
Positive for <i>Legionella</i> species.

The results of all specimens are reported to the health care provider who submitted the specimen. In addition, positive results are reported to the TDH Communicable and Environmental Disease Services and the health department in the county where the patient lives.

Criteria for Unacceptable Specimens

All specimens

1. The specimen not properly identified with the patient's name and the tear strip control number.
2. The patient identifier on the specimen did not exactly match the identifier on the form.
3. The specimen or the slide was broken in transit.

Clinical specimens

1. The specimen was shipped unrefrigerated or unfrozen.

Environmental samples

1. The environmental sample was not directly related to a documented outbreak of legionellosis or requested by CEDS.

Reference specimens

1. The specimen was non-viable.
2. A mixed specimen was submitted.

Miscellaneous Exam Form PH-1573

FRONT

SOCIAL SECURITY NO.		TENNCARE NO.	MCO	MISCELLANEOUS EXAM		A418027	
MEDICARE NO.		RECORD FOLDER NO.					
PATIENTS NAME - LAST, FIRST, MIDDLE			SPOUSE - FIRST NAME			DATE REPORTED	DATE/TIME RECEIVED
STREET AND NUMBER				COLLECTION DATE	DATE OF ONSET		
TOWN	STATE	ZIP		SOURCE: <input type="checkbox"/> BLOOD <input type="checkbox"/> URINE <input type="checkbox"/> FECES <input type="checkbox"/> THROAT <input type="checkbox"/> OTHER _____			
DATE OF BIRTH	RACE	ETHNICITY	SEX	PATHOGEN/DISEASE SUSPECTED/SYMPTOMS			
COUNTY NO.	COUNTY NAME		SITE NO.	EXAMINATION REQUESTED			
NAME				RECENT TRAVEL 1 <input type="checkbox"/> YES 2 <input type="checkbox"/> NO STATE/COUNTRY			
ADDRESS				EXAMINATION RESULTS			
CITY				<input type="checkbox"/> SUBMITTED TO REFERENCE LABORATORY FOR EXAMINATION. RESULTS FORTHCOMING.			
STATE				EXAMINED BY:			
ZIP CODE				LABORATORY PERFORMING EXAMINATION			
PH-1573 REV. 11/06				TENNESSEE DEPT. OF HEALTH LABORATORY SERVICES DAVID L. SMALLEY, PhD, MSS, BCLD, DIRECTOR			

**TIGHTEN CAPS SECURELY
 SUBMIT IN DOUBLE MAILING CONTAINER**

STOOL CULTURES FOR SALMONELLA, SHIGELLA AND CAMPYLOBACTER
 Place two (2) cotton tipped swabs dipped into feces or other specimens and insert into Amies, Stuarts, or Cary-Blair transport medium. Submit the transport medium refrigerated within two (2) days of collection.

INTESTINAL PARASITES: Place amount of feces equal to volume of formalin in container designated for intestinal parasites (5% Formalin).

CULTURES FOR IDENTIFICATION - Submit pure cultures on non-selective media such as trypticase soy agar slants or enriched slants (Blood or Chocolate) when required.

ANAEROBIC ORGANISMS - Submit in semi-solid media such as thioglycollate, overlaid with sterile vaspar to prevent exposing culture to oxygen.

TESTING LABORATORY LOCATION CODES
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K = KNOXVILLE BRANCH LAB, 1522 CHEROKEE TRAIL, KNOXVILLE, TN - DR. DAVID L. SMALLEY, PhD, MSS, BCLD
N = NASHVILLE REFERENCE LAB, 630 HART LANE, NASHVILLE, TN - DR. DAVID L. SMALLEY, PhD, MSS, BCLD

BACK

Anaerobic Bacteriology

Introduction *Clostridium tetani* spores

Anaerobic bacteria are a frequent cause of serious infections. The Anaerobic Bacteriology Section generally accepts only clinical isolates for identification. In certain cases, clinical material for primary isolation is accepted for cultivation of pathogenic microorganisms. Contact the Anaerobic Bacteriology Section regarding submission of these specimens.

Isolation and identification techniques used include cultural procedures, morphological and biochemical characterization, gas-liquid chromatography and toxigenicity tests for some *Clostridium* species. Anaerobic isolates are not tested for antimicrobial susceptibilities.

Laboratory Services performs toxigenicity testing and primary isolation for *Clostridium botulinum*. Refer to BOTULISM in Section II. The laboratory also performs primary isolation of *Clostridium perfringens* from suspected food-poisoning cases. Refer to FOODBORNE ILLNESS in Section II.

The Tennessee Medical Laboratory Act requires that laboratory directors submit isolates of *Clostridium tetani* and *Clostridium botulinum* to the Tennessee Department of Health Laboratory for confirmation and surveillance purposes.

Specimen Collection

Since anaerobic organisms make up a major part of the body's indigenous flora, clinical specimens for anaerobic culture must be collected by methods that avoid contamination with normal flora. Aspirates collected with a syringe or tissue specimens are recommended for anaerobic culture.

The TDH Laboratory accepts anaerobic organisms isolated from the following sources:

- Aspirated pus.
- Tissue (biopsy, surgery, autopsy).
- Transtracheal aspirates.
- Direct lung aspirates.
- Body fluids.
- Sulfur granules from suspected cases of actinomycosis.

Anaerobic organisms isolated from the sources listed below are unacceptable for testing. If you submit an isolate from one of these sources, include information that establishes its clinical significance.

- Throat, gingival or nasopharyngeal swabs.
- Skin.
- Voided urine.
- Sputum or gastric contents.
- Superficial wounds.
- Rectal swabs, feces or small bowel contents (except for special testing).
- Vaginal or cervical swabs.

Anaerobic Bacteriology (Continued)

The culture must be maintained in an anaerobic environment. Submit a PURE, actively growing culture in a screw-cap tube of liquid or semi-solid media such as motility media, thioglycollate or chopped meat broth. Pick a single colony and inoculate a tube of media. At the same time, check the oxygen requirement of the organism by streaking a single colony to an aerobic blood plate. Incubate the confirmed anaerobe for 24 to 48 hours or until visible growth is present. Overlay with 3/4 inch of sterile Vaspar *. Tighten the cap and seal securely with parafilm or waterproof tape to prevent leakage.

* Vaspar: Melt together equal portions (w/w) Vaseline and paraffin. Dispense in 3-ml amounts and autoclave at 121°C for 30 minutes. Store at room temperature and melt for use as needed.

Specimen Identification

1. Complete all the provider and patient information areas on the Miscellaneous Exam Form PH-1573. Include pertinent clinical, biochemical and epidemiological information with each specimen.
2. Using indelible ink, label each specimen with the date of collection and the patient's first and last name. Attach the control number on the tear strip to the specimen and secure it with transparent tape. Unlabeled specimens or specimens where the patient identifier on the specimen does not exactly match the identifier on the form will not be tested.

Shipment of Specimens

1. Packing and shipping specimens to the state public health laboratory requires personnel trained in current regulations. Follow the shipping guidelines for Category B substances and any additional guidelines from your current carrier or method of shipment. See Section 7, page 5 for instructions.
2. Use first-class postage on US mail.
3. Ship to the Tennessee Department of Health Laboratory in Nashville.

Note: Do not mail specimens on plates. Specimens submitted on plates are acceptable only if they are properly closed in an anaerobic transport bag and delivered by courier to the laboratory.

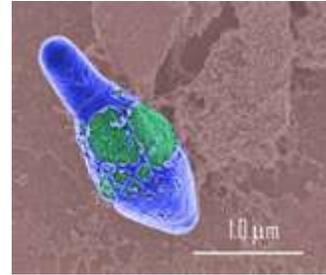
Reporting Procedures and Interpretation of Results

Reporting of results
Organisms are reported by genus and species and subspecies when appropriate.

Organisms are identified to genus, species and subspecies level when appropriate and only if culture, morphology, biochemical and gas-liquid chromatographic test results support the identification. Genus, species and subspecies designations are consistent with designations in the Virginia Polytechnic Institute's *Anaerobic Laboratory Manual*, the American Society for *Microbiology's Manual of Clinical Microbiology* and the *International Code of Nomenclature of Bacteria*. Some anaerobes, particularly members of the genus *Clostridium* and many of the non-spore forming gram-positive rods, can be identified accurately only to the genus level. Generally, *Lactobacillus* organisms are identified only to the genus level.

Botulism

(*Clostridium botulinum*)



Clostridium botulinum

Introduction

Botulism is a neuroparalytic disease caused by the neurotoxin produced by *Clostridium botulinum*. The classical disease is foodborne and results from the ingestion of food in which *C. botulinum* has grown and produced toxin. In rare cases, botulism may also result from the production of toxin by *C. botulinum* growing in a wound. A third type of botulism, referred to as infant botulism, seems to result from ingestion of the organism or its spores. There is evidence that indicates the ingested organisms grow and produce toxin in the infant's gut.

Laboratory Services performs toxin assay tests and procedures for the isolation and identification of *Clostridium botulinum*. When a diagnosis of botulism is considered, the physician or laboratory personnel should call the Tennessee Department of Health (TDH) Epidemiologist at (615) 741-7247. After hours, contact the Communicable Disease Consultation Line at (615) 741-7247 or 1-(800) 404-3006 or the TDH Laboratory at (615) 262-6300. Epidemiological aid and emergency laboratory services are available 24 hours a day when botulism is suspected.

Serum, feces, vomitus, gastric contents and pus or wound biopsies are tested when botulism is a possible diagnosis. Suspected foods are tested only when patient testing has resulted in a confirmed case of botulism. Foods are rarely tested in cases of infant botulism. Possible sources of spores for infants are multiple including dust and foods.

The Tennessee Medical Laboratory Act requires that Laboratory Directors send isolates of *Clostridium botulinum* to the TDH Laboratory for confirmation, typing and surveillance purposes.

Specimen Collection

For clinical specimens and foods -- Refer to Chart II - 2 COLLECTION OF SPECIMENS FOR BOTULISM TESTING.

Culture isolates -- Submit a pure, actively growing culture in a screw-capped tube of liquid or semi-solid medium such as motility medium, thioglycollate or chopped meat broth.

Specimen Identification

1. Complete **all** the provider and patient information areas on the Miscellaneous Exam Form PH-1573. Include pertinent clinical information with each specimen.
2. Using indelible ink, label each specimen with the date of collection and the patient's first and last name. Attach the control number on the tear strip to the specimen and secure it with transparent tape. Unlabeled specimens or specimens where the patient identifier on the specimen does not exactly match the identifier on the form **will not be tested**.

Botulism (Continued)

Chart II - 2

Collection of Specimens for Botulism Testing

Clinical Diagnosis	Specimen	Amount of Specimen	Test(s) Performed
Foodborne	Serum	10 to 15 ml optimal. 2 ml minimum.	Neurotoxin assay.
	Food	100 gm if available. Do not remove from container.	Toxin assay. Isolation and identification of <i>C. botulinum</i> .
	Feces	100 gm if available or as much as possible.	
	Gastric contents	100 gm if available.	
	Vomitus	100 gm if available.	
Wound	Serum	10 to 15 ml optimal. 2 ml minimum.	Neurotoxin assay.
	Feces	100 gm if available or as much as possible.	Neurotoxin assay. Isolation and identification of <i>C. botulinum</i> .
	Pus from wound or biopsied material	Collect in anaerobic collector. DO NOT REFRIGERATE.	Isolation and identification of <i>C. botulinum</i> .
Infant	Feces	25 gm if available.	Neurotoxin assay. Isolation and identification of <i>C. botulinum</i> .
	Bowel	Use water enema, 20 ml.	

- Collect specimens before antitoxin is given.
- Collect feces, vomitus and gastric contents in sterile containers. Do not use Cary-Blair transport medium.
- Leave suspect foods in original containers. Leave unopened containers sealed.
- Separate serum from blood cells.
- Collect specimens for wound botulism in an anaerobic collector. DO NOT REFRIGERATE.

Botulism (Continued)

Shipment of Specimens

1. Serum, food, feces, gastric contents, vomitus and bowel specimens

Packing and shipping specimens to the state public health laboratory requires personnel trained in current regulations. Follow the shipping guidelines for Category B substances and any additional guidelines from your current carrier or method of shipment. See Section 7, page 5 for instructions.

Wound botulism specimens

Place the pus or biopsied specimen from wound botulism in an anaerobic collector. DO NOT REFRIGERATE.

Reference isolates

Submit a PURE, actively growing culture in screw-cap tube of liquid or semi-solid medium such as motility medium, thioglycollate or chopped meat broth that has been overlaid with Vaspar *. Follow the instructions in Section 7, page 5 for shipping Category B Specimens to the TDH Laboratory. DO NOT REFRIGERATE OR SHIP ISOLATES WITH COLD PACKS.

2. Ship the specimen by the quickest means available to the TDH Laboratory in Nashville. Suggestions for rapid delivery include courier service, taxi, bus or plane.
4. Notify the Anaerobic Bacteriology Section at (615) 262-6362 or 262-6300 as to the method of transportation and when the specimens are scheduled to arrive.

* Vaspar: Melt together equal portions (w/w) Vaseline and paraffin. Dispense in 3 ml amounts and autoclave at 121°C for 30 minutes. Store at room temperature and melt for use as needed.

Reporting Procedures and Interpretation of Results

The TDH Laboratory reports preliminary results to the TDH epidemiologist and the patient's physician by telephone immediately, usually within 24 hours. Communication continues until the testing is complete. Additional specimens may be requested and additional tests performed depending on the patient's condition and laboratory results. A written report is made when all tests are complete.

Results of the toxin tests are reported
No botulinum toxin A, B, C, D, E, F or G detected.
Presumptive positive for botulinum toxin [insert toxin type (s)]. Assay must be confirmed by mouse bioassay.

Botulism (Continued)

Results of cultures (original specimen or isolate) are reported
No <i>Clostridium botulinum</i> detected by mouse bioassay and culture.
<i>Clostridium botulinum</i> , Type __ Detected By Mouse Bioassay. <i>Clostridium botulinum</i> , Type __ Detected By Culture.

Neurotoxin is identified according to the Centers for Disease Control and Prevention's (CDC) Laboratory Response Network Protocols and the guidelines set forth by the CDC/APHIS Select Agent Program. Seven toxigenic types of *C. botulinum* are recognized based on the antigenically distinct neurotoxins produced by the different strains classified in this species (A, B, C, D, E, F and G). Cases of human botulism are usually associated with neurotoxin Types A, B and E. Type E is usually associated with foodborne outbreaks involving seafood. Infant botulism is predominately Type A or B.

Isolates are identified according to CDC procedures. Confirmation of species and type is determined by neurotoxin assay tests.

The results of all specimen requests are reported to the health care provider who submitted the specimen, the TDH Communicable and Environmental Disease Services and the health department in the county where the patient lives.

Criteria for Unacceptable Specimens

1. The specimen was not properly identified.
2. The identifier on the specimen does not exactly match the identifier on the form.
3. The specimen was broken or leaked in transit.
4. The specimen submitted was an improper type.
5. The patient's symptoms do not warrant performance of the test requested as determined by CEDS.
6. The specimen was submitted improperly.
7. A sample of stool, serum, food, gastric contents or vomitus was not submitted with cold packs.
8. A wound culture or isolate was not submitted under anaerobic conditions.
9. A wound culture specimen or isolate was submitted refrigerated.

Miscellaneous Exam Form PH-1573

FRONT

SOCIAL SECURITY NO.		TENNCARE NO.		MCO		MISCELLANEOUS EXAM		A418027		
MEDICARE NO.		RECORD FOLDER NO.		DATE REPORTED						DATE/TIME RECEIVED
PATIENTS NAME - LAST, FIRST, MIDDLE				SPOUSE - FIRST NAME				LAB NO. ▼		
STREET AND NUMBER						COLLECTION DATE		DATE OF ONSET		
TOWN			STATE		ZIP		SOURCE: <input type="checkbox"/> BLOOD <input type="checkbox"/> URINE <input type="checkbox"/> FECES <input type="checkbox"/> THROAT <input type="checkbox"/> OTHER _____			
DATE OF BIRTH		RACE	ETHNICITY	SEX	PHONE NO.					
COUNTY NO.		COUNTY NAME			SITE NO.		PATHOGEN/DISEASE SUSPECTED/SYMPTOMS			
NAME		EXAMINATION REQUESTED								
ADDRESS		RECENT TRAVEL 1 <input type="checkbox"/> YES 2 <input type="checkbox"/> NO STATE/COUNTRY								
CITY		STATE		EXAMINATION RESULTS						
ZIP CODE		<input type="checkbox"/> SUBMITTED TO REFERENCE LABORATORY FOR EXAMINATION. RESULTS FORTHCOMING.								
PH-1573 REV. 11/06		TENNESSEE DEPT. OF HEALTH LABORATORY SERVICES DAVID L. SMALLEY, PhD, MSS, BCLD, DIRECTOR				<input type="checkbox"/> K <input type="checkbox"/> J <input type="checkbox"/> N		EXAMINED BY:		RDA-1160

**TIGHTEN CAPS SECURELY
 SUBMIT IN DOUBLE MAILING CONTAINER**

STOOL CULTURES FOR SALMONELLA, SHIGELLA AND CAMPYLOBACTER
 Place two (2) cotton tipped swabs dipped into feces or other specimens and insert into Amies, Stuarts, or Cary-Blair transport medium. Submit the transport medium refrigerated within two (2) days of collection.

INTESTINAL PARASITES: Place amount of feces equal to volume of formalin in container designated for intestinal parasites (5% Formalin).

CULTURES FOR IDENTIFICATION - Submit pure cultures on non-selective media such as trypticase soy agar slants or enriched slants (Blood or Chocolate) when required.

ANAEROBIC ORGANISMS - Submit in semi-solid media such as thioglycollate, overlaid with sterile vaspar to prevent exposing culture to oxygen.

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BACK



Foodborne Illness

Introduction

Testing for foodborne illness is available at the Tennessee Department of Health (TDH) Laboratories in Jackson, Knoxville and Nashville. Testing for botulism and intestinal parasites, such as *Cryptosporidium* and *Giardia*, is available only at the Nashville Laboratory (see section 3). The laboratory examines food samples for the presence of disease-producing bacteria only in the case of documented illness under investigation by public health officials.

A foodborne disease outbreak is defined as three or more persons with vomiting or diarrhea who attended the same event or consumed the same meal. Single isolated cases or complaints are not considered outbreaks. EXCEPTION: ONE CASE OF BOTULISM IS SUBJECT TO NOTIFICATION AND INVESTIGATION. Refer to BOTULISM in Section 2.

When you suspect a possible foodborne disease, notify your county health department immediately so investigation procedures and sample collection can be started if necessary. Contact your county health department whenever any enteric disease outbreak is suspected in a daycare center, a restaurant or other facility. Additional assistance in the investigation is available by contacting the TDH Communicable and Environmental Disease Service Section at (615) 741-7247.

Outbreak investigation involves the cooperation of several disciplines within the health department, including the epidemiologist, the county health department and the laboratory. The investigation requires interviewing patients, collecting food samples and clinical specimens and laboratory testing. Communication between the various members is essential for the prompt and precise handling of an outbreak.

A wide variety of organisms can cause gastrointestinal illness. Listed below are some of the organisms that are implicated in foodborne outbreaks:

- *Bacillus cereus*
- *Campylobacter jejuni*
- *Clostridium botulinum*
- *Clostridium perfringens*
- *Escherichia coli* 0157
- *Listeria monocytogenes*
- *Salmonella* species
- *Staphylococcus aureus*
- *Vibrio* species
- *Yersinia enterocolitica*

Organisms that can cause an enteric illness outbreak not associated with food include:

- *Cryptosporidium*
- *Giardia*
- *Shigella* species

Foodborne Illness (Continued)

Collection and Shipment of Specimens

The county health department should be notified when an outbreak is suspected. A public health official should conduct an investigation and collect samples following the steps in the Tennessee Department of Health's *Foodborne Disease Outbreak Investigation Manual*.

The laboratory accepts food samples, environmental samples and clinical specimens as deemed necessary by the investigating official. Clinical specimens should be collected from a representative number of ill persons and an equal number of exposed but well persons.

If there is a danger to the community, the public health official will take action to prevent further spread of the disease.

The **Tennessee Department of Health Laboratories in Jackson, Knoxville and Nashville** examine specimens in cases of a suspected foodborne illness. Alert the nearest TDH Laboratory when a foodborne illness is suspected so that preparations for handling the food samples and associated specimens can begin.

Complete a Miscellaneous Exam Form PH-1573 for the food samples and an appropriate form for each clinical specimen. Deliver the foods and clinical specimens to the laboratory quickly following the guidelines in the *Foodborne Disease Outbreak Investigation Manual*.

Reporting Procedures and Interpretation of Results

Communications among the TDH public health officials, the TDH Laboratory and the health care providers are continuous from the time an outbreak is reported until the results are reported. Work-up of specimens requires a constant exchange of information between the laboratory and the epidemiology team. Additional testing is performed as needed.

Examination of food samples requires from 2 to 7 working days depending upon the suspected pathogen. Examination of foods heavily contaminated with *Staphylococcus* may be completed in 48 hours. The presence of low numbers of pathogenic organisms or organisms damaged by processing may take up to 2 weeks for isolation, identification and/or serotyping. Isolation and identification of *Salmonella* species, *Shigella* species, *C. perfringens* and other more commonly encountered organisms usually require 1 week to identify. Environmental samples and swabs from food handlers are usually reported within 1 to 3 days.

Pulsed field gel electrophoresis for *S. aureus* (from documented outbreaks) is performed on a representative sample of isolates (3-5 isolates) and requires additional time for completion.

Reporting of results of food samples
Positive Results: The name of the pathogen is reported by genus and species. A bacterial count of microorganisms per gram is reported if available.
Negative Results: No (SPECIFIC FOR THE ORGANISM(S) TESTED) isolated. A bacterial count of microorganisms per gram is reported if available.

Foodborne Illness (Continued)

See individual sections for reporting of results of clinical samples.

Confirmation that a food is involved in an outbreak is made by isolating the same pathogen or toxin in ill patients' specimens and in the implicated food(s). Without clinical specimens, a food can be confirmed as the vehicle of infection if toxins are detected in it. In addition, a food can be epidemiologically suspect if food-specific attack rates are significantly higher in persons who have consumed a food as opposed to those who have not. In addition, confirmation of a foodborne disease can be made if significant numbers of pathogens known to cause food poisoning syndrome are isolated from the food or if an enteric pathogen such as *Salmonella* or *Shigella* is present in any number.

The results of all specimens and food samples are reported to the health care provider who submitted the specimen. In addition, The Tennessee Department of Health Communicable and Environmental Disease Services and the health department in the county where the patient lives are sent reports if a foodborne illness is detected.

Criteria for Unacceptable Specimens

Unsatisfactory specimens will be assessed on an individual basis. The specimen should be properly identified and the specimen identifier should match the information on the form.

Miscellaneous Exam Form PH-1573

FRONT

PLEASE FILL OUT SHADED AREA COMPLETELY TN, DEPT. OF HEALTH DIV. OF LAB SERVICES PH-1573 REV. 11/06		SOCIAL SECURITY NO.	TENNACARE NO.	MCO	MISCELLANEOUS EXAM		A418027	A418027	
PATIENTS NAME - LAST, FIRST, MIDDLE		RECORD FOLDER NO.		DATE REPORTED		DATE/TIME RECEIVED			LAB NO.
STREET AND NUMBER		SPOUSE - FIRST NAME		COLLECTION DATE		DATE OF ONSET			SOURCE: <input type="checkbox"/> BLOOD <input type="checkbox"/> URINE <input type="checkbox"/> FECES <input type="checkbox"/> THROAT <input type="checkbox"/> OTHER _____
TOWN		STATE	ZIP	PATHOGEN/DISEASE SUSPECTED/SYMPTOMS					
DATE OF BIRTH		RACE	ETHNICITY	SEX	PHONE NO.		EXAMINATION REQUESTED		
COUNTY NO.		COUNTY NAME		SITE NO.		RECENT TRAVEL 1 <input type="checkbox"/> YES 2 <input type="checkbox"/> NO STATE/COUNTRY			EXAMINATION RESULTS
NAME		ADDRESS		CITY		STATE			
Tennessee Dept. of Health Laboratory Services		DAVID L. SMALLEY, PhD, MSS, BCLD, DIRECTOR		<input type="checkbox"/> K <input type="checkbox"/> J <input type="checkbox"/> N		LABORATORY PERFORMING EXAMINATION			EXAMINED BY:
PH-1573 REV. 11/06		Tennessee Dept. of Health Laboratory Services		DAVID L. SMALLEY, PhD, MSS, BCLD, DIRECTOR		LABORATORY PERFORMING EXAMINATION			RDA-1160

BACK

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Water Microbiology



Introduction

The Environmental Microbiology Section performs microbiological analyses of water and wastewater samples. As the only direct measures of pollution by man and other warm-blooded animals, microbiological parameters contribute unique information on water and wastewater quality and the public health risk from waterborne disease. Samples are tested for the presence or absence of the coliform group of bacteria, which are indicators of fecal contamination. Microbiological analyses are conducted to:

- Determine the safety of public drinking water supplies.
- Monitor ambient water quality for recreational, industrial, agricultural and water supply uses.
- Monitor municipal and industrial discharges.
- Identify the sources of bacterial pollutants.
- Evaluate water resources.

The Tennessee Department of Health (TDH) Laboratory accepts samples from both public and private water systems. The procedures used are those specified in *Standard Methods for the Examination of Water and Wastewater* and EPA's *Microbiological Methods for Monitoring the Environment*. The laboratory uses the chromogenic substrate coliform test procedure for total coliform, *Escherichia coli* and *Enterococcus*. The laboratory uses the membrane filtration procedure for fecal coliform in wastewater. These methods provide the most probable number of bacterial colonies in a 100mL sample.

The most probable number (MPN) procedure estimates the number of specific organisms in water and wastewater by the use of probability tables. The MPN method is an alternative to the Colilert procedure and is used for total and fecal coliform tests primarily with problem water that might contain sediments, sludges or muds.

The tests commonly performed include:

Community water supplies	Total coliform and <i>Escherichia coli</i>
Non-community water systems	Total coliform and <i>Escherichia coli</i>
Private waters	Total coliform and <i>Escherichia coli</i>
Swimming pools	Total coliform and <i>Escherichia coli</i>
Natural swimming areas	Fecal coliform <i>Escherichia coli</i> and <i>Enterococcus</i>
Wastewater effluents	Fecal coliform <i>Escherichia coli</i> , <i>Enterococcus</i>

Water samples are tested for pathogens when documentation for isolation of the specific organism is present and water had been epidemiologically indicated by an investigation. For additional information on this testing, contact the Environmental Microbiology Section.

Distribution of Water Sample Collection Bottles

Community Water Supplies: The TDH Laboratory ships water sample bottles to water supply facility monitored under the Safe Drinking Water Act. The Tennessee Department of Environment and Conservation (TDEC) Division of Water Supply determine the number of bottles each water facility needs.

Water Microbiology (Continued)

Non-Community Water Systems: The TDH Laboratory ships sample bottles to each system being monitored. One bottle is sent monthly or quarterly as determined by the TDEC Division of Water Supply.

Private drinking water supplies, swimming pools, natural swimming areas, etc.: Bottles are provided to local health departments and the Tennessee Department Agriculture for support of programs that are not covered by the Safe Drinking Water Act.

Sample Collection (Standard method 9060)

1. A qualified environmentalist or a certified employee of a water treatment plant must collect the samples by the procedures designated in the current edition of *Standard Methods for the Examination of Water and Wastewater*.
2. Collect the sample in a water sample bottle (a sterile 4-oz polypropylene screw-cap bottle containing a dechlorinating and chelating agent). Do not lay the cap down. Be extremely careful not to allow anything to touch the inside of the sample bottle or the bottle cap.
3. Collect 100 ml by filling to the fill line at the shoulder of the bottle. Recap and mix by shaking the sample.

Sample Identification

1. Complete the Water Sample Request Form PH-1575. The form must contain:
 - Water system name, address, telephone number, county and location where the sample was collected.
 - Date and military time (see Appendix 2) of collection.
 - Chlorine residual.
 - Sample collector's name and title.
 - Sample type/source.
 - Examination requested.
 - Public water system identification number (PWS ID), if applicable.

The form also has an ID number that can be removed and attached to the bottle for additional sample identification.

The procedure for collection of the water sample, filling out form and the sample type key is on the back of the Water Sample Request Form. The key for the sample type is:

D-Distribution
R-Repeat
N-New Lines
S-Special
Q-Quality Control

Shipment of Sample

1. Place the form around the water sample bottle and place the sample in a mailing container. Follow the packing Instructions 650, Section I-18.
2. Affix the mailing label (PH-0838) and the return address to the container.
3. Ship the sample to the TDH Laboratory in **Jackson, Knoxville or Nashville**.
4. Use first-class postage on US mail.
5. The holding/transit time between sampling and examination for total coliform analysis must not exceed 30 hours. If possible, samples should be refrigerated (4 to 10°C) during transit. Mail samples so that they do not arrive at the laboratory on Friday afternoon, holidays or weekends.

Water Microbiology (Continued)

6. Samples for *Escherichia coli*, Fecal coliform or *Enterococcus* analysis must be refrigerated from the time they are collected until they are analyzed and must arrive in the laboratory within 6 hours after collection.

Reporting Procedures and Interpretation of Results

A water analysis report refers only to the sample as received. It should not be regarded as a complete report on the water supply.

The turn around time for tests performed by the TDH Laboratory is:

Test	Negative Sample	Positive Sample
Total Coliform, Colilert	1 day	1 day
Total Coliform, MPN	3 days	7 days
Fecal Coliform, MF and MPN	3 days	5 days
<i>Enterococcus</i> , Enterolert	1 day	1 day
<i>Escherichia</i> , Colilert	1 day	1 day

Results are reported

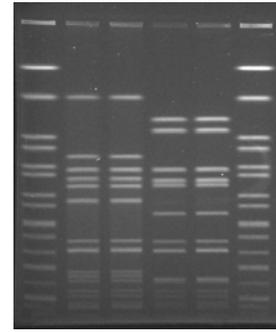
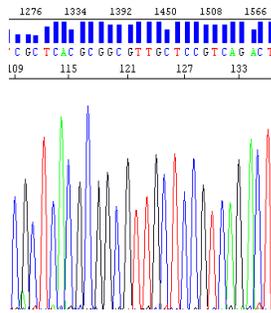
	Total coliform	<i>Escherichia coli</i>	<i>Enterococcus</i>	Fecal coliform
Negative Results	<1 cfu/100 mL	<1 cfu/100 mL	<1 cfu/100 mL	<1 cfu/100 mL
Positive Results	Positive for total coliform	Positive for <i>Escherichia coli</i>	Positive for <i>Enterococcus</i>	Positive for Fecal coliform
Method Used	SM 9223B SM 9221B	SM 9223B SM 9221B	Enterolert	SM 9221E SM 9222D

If coliform bacteria are present in a water sample, the water is considered unsafe for drinking purposes. The water supply must meet the following requirements for positive samples:

Community and non-community water systems: Phone calls are made on all positive water samples. The supply facility must submit 4 repeat samples immediately.

If the result of the sample is unsatisfactory, a new sample must be submitted immediately.

Results of all tests are reported to the provider. Results from community supplies and non-community water systems are reported to the TDEC Division of Water Supply and the provider's Water Pollution Basin Office. Depending on the source of the sample, results from other providers are reported to any required official agency.



Molecular Biology

The field of molecular biology overlaps with areas of biology, chemistry and physics. The purpose of a molecular biology laboratory in a public health setting is to provide State-of-the-Art testing and surveillance of infectious diseases. Testing is method-driven and consists of Pulsed-Field Gel Electrophoresis (PFGE), Sequencing and real-time Polymerase Chain Reaction (PCR).

The Molecular Biology department provides the following services either at the request of Communicable and Environmental Disease Services (CEDs) or as a component of routine identification.

Real-time reverse transcription polymerase chain reaction (RT-PCR) for Norovirus G1 and G2 is performed at all Tennessee state public health laboratories. A minimum submission of 3 samples is required and is performed at the direction of CEDs. Contact your local health department.

Real-time PCR for *Bordetella pertussis* is performed at the Nashville laboratory only and is performed on all *Bordetella pertussis* samples.

Real-time PCR for Shiga-toxin producing *Escherichia coli* (STEC) Shiga toxin 1 and 2 is performed in Nashville on all enteric *E. coli* samples. The Jackson and Knoxville Regional Laboratories are expected to begin testing in mid-2008.

Real-time PCR for Methicillin-resistant *Staphylococcus aureus* (MRSA) is performed in Nashville and Knoxville exclusively for outbreak situations at the direction of CEDs.

Real-time PCR for *Neisseria meningitidis* group A, B, C, W-135, X and Y is performed in Nashville only and is performed at the direction of CEDs.

Conventional nested PCR for *Shigella* spp. is performed in Nashville exclusively on food sources involved in an outbreak.

Real-time RT-PCR for influenza is performed in Jackson, Knoxville, Memphis and Nashville exclusively on outbreak situations at the direction of CEDs.

Pulsed-Field Gel Electrophoresis (PFGE) is performed in Nashville as a routine part of the PulseNet surveillance program and in outbreak situations at the direction of CEDs. Knoxville Regional Laboratory is expected to begin PFGE in 2008.