

SCOPE

A Guide To Laboratory Services



**North Carolina State Laboratory of Public Health
NC Department of Health and Human Services**

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A Guide to Services

**North Carolina State Laboratory of Public Health
Division of Public Health
Department of Health and Human Services**

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Beverly Eaves Perdue, Governor
State of North Carolina

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N.C. Department of Health and Human Services

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SCOPE, A Guide to Laboratory Services, provides descriptions of testing services, special instructions for specific tests, and explanation of reports, when necessary. It is impossible to address all situations in a guide. Efforts have been made to be concise. For more detailed information, please contact the appropriate unit.

Administration	919-733-7834
Quality Assessment	919-807-8747
Cancer Cytology	919-733-7146
Environmental Sciences	919-733-7308
Laboratory Improvement	919-733-7186
Laboratory Preparedness	
Bioterrorism & Emerging Pathogens	919-807-8765
Chemical Terrorism	919-807-8771
Microbiology	919-733-7367
Newborn Screening/Clinical Chemistry	919-733-3937
Virology/Serology	919-733-7544

North Carolina State Laboratory of Public Health (NCSLPH)

Mission

The State Laboratory of Public Health provides certain medical and environmental laboratory services (testing, consultation and training) to public and private health provider organizations responsible for the promotion, protection and assurance of the health of North Carolina citizens.

Administration

Director:	Leslie A. Wolf, PhD, HCLD
Assistant Director – Technical Services:	David E. Keller, PhD
Assistant Director - Operations:	Michael Kaufman, M.A.

General Information

Location

Bath Building
306 N. Wilmington St.
Raleigh, NC 27601-1059

Postal Address

State Laboratory of Public Health
PO Box 28047
Raleigh, NC 27611-8047
NC Courier MSC #1918

CLIA #

34D0692393

Federal EIN #

562033116

Main Number

(919) 733-7834

Web Address

<http://slph.ncpublichealth.com>

Official Business Hours

8:00 a.m. to 5:00 p.m., Monday-Friday.

Parking

- 7:00 a.m. to 5 p.m., Visitor's parking lots on Jones or Salisbury Streets.
- Check NCSLPH website at <http://slph.ncpublichealth.com/doc/DrivingParking-> for current rates and parking lot information.

Delivery of Samples

- Postal Services: Daily, although samples arriving on weekends are refrigerated. The laboratory does not accept "POSTAGE DUE" samples.
- UPS: Monday through Friday
- State Courier Service: Daily except Sundays
- Delivery in Person: From 8:00 a.m. to 5:00 p.m., Monday-Friday. Bring samples to laboratory lobby. After hours, weekends, and holidays, put in the refrigerated wall depository in the back of the building at the loading dock.
- Suspected bioterrorism (BT) environmental samples must be delivered in person by law enforcement agents or local health department staff to maintain chain of custody. Suspected BT clinical samples must be packaged appropriately prior to shipment. Please contact the Bioterrorism and Emerging Pathogens Lab at (919) 807-8765 (Mon.-Fri. 8:00 am -5:00 pm) or the BT 24/7 Number at (919) 807-8600 **prior** to submitting/delivering samples.
- Clinical samples submitted to NCSLPH for chemical agent analysis/suspected chemical terrorism agents can be delivered in person by law enforcement agents, local health department or hospital staff, or can be properly packaged and shipped by FedEx or air transport. Chain of custody must be maintained by the submitting and receiving agencies. Please contact the Chemical Terrorism Lab at 919-807-8771 or the 24/7 Number at (919) 602-2481 **prior** to submitting or delivering the specimen.

NCSLPH Objectives

- Provide quality assured laboratory services
- Assist other North Carolina laboratories in developing and strengthening their laboratory services
- Serve as North Carolina's primary Laboratory Response Network laboratory in response to acts of bioterrorism, chemical terrorism, and to address emerging public health issues
- Serve the entire state as a reference laboratory for difficult, unusual or otherwise unavailable laboratory services
- Serve as a resource of information on laboratory practice
- Test human and related animal samples and environmental samples
- Assist in the development, evaluation and standardization of medical and environmental laboratory testing procedures
- Participate in special studies and research projects
- Provide training, consultation and information updates to improve and assure quality services in other laboratories
- Certify milk and water laboratories and milk analysts

Policies and Limitations

The Laboratory receives consultation on policy matters from the State Health Director, the Epidemiology Liaison Committee of the Association of Local Health Directors, Advisory Committees to Departmental Programs and the Directors of the Departmental Agencies. Public health needs, available resources and whether or not the services are available from other laboratories determine services offered by the State Laboratory. Most public health programs are directed toward prevention of illness and require laboratory support for disease surveillance and diagnosis or monitoring and enforcement of environmental health programs. Some services are available only to Local Health Departments and State-operated health facilities.

Clinical Samples must be properly labeled. **Every tube, vial, or other sample container must be labeled with two identifiers: the patient's name and either date of birth or social security number.** Unlabeled clinical samples will be discarded. Use waterproof ink (unless otherwise indicated) to prevent smearing and washing off. Requisitions must be filled out completely and clearly; print legibly if labels are not used. Results may be delayed if all fields are not completed.

The State Laboratory, in collaboration with public health officials, reserves the right to decide whether or not to analyze samples. The Director or appropriate Unit Managers should be contacted before collecting or sending unusual numbers of samples/samples (as in epidemics, investigations or surveys). This is necessary for determining whether or not the samples can be analyzed and if so, for preparing to do so.

Samples must be submitted through a local health department, physician or other authorized sender, as defined in the N.C. Administrative Code.* Private citizens are authorized to submit animals or animal heads for rabies examination. The report of results is sent to the authorized sender of the specimen. Copies of laboratory results may be furnished to another authorized sender upon request of the actual sender. Certain results are furnished to health programs for follow-up or epidemiologic purposes.

*"Authorized sender of clinical samples" refers to any individual who, by virtue of a license to practice medicine, dentistry, veterinary medicine, nursing, etc. in the State of North Carolina, is authorized to manipulate a patient for the purpose of collecting blood, spinal fluid and other body materials for analysis. It may also refer to an agency such as a hospital, local health department, clinic, etc. which employs persons to perform such services under the direction of a licensed individual as described in this subsection. In some cases this is limited by program guidelines. "Authorized sender of environmental samples" is any individual who has been designated by law, rules and regulations, or professional position to collect and submit environmental material for analysis. In some cases this is limited by program guidelines.

Consultation

Please direct general or policy questions, comments or suggestions, and feedback on State Laboratory services to the Director's office. Each Unit may be contacted about specific problems or to obtain information concerning specific services or explanation of results, etc. The State Laboratory recognizes its special relationship with local health departments. The Laboratory Improvement Unit provides consultation for laboratory services, management and technical operations of local health departments. On-site consultation can be arranged upon request by telephoning (919) 733-7186.

Quality Assessment

The purpose of the Quality Assessment Unit (QA) is to define and implement the quality tools necessary for monitoring, assessing and improving the quality of services provided at the State Laboratory. The QA unit encompasses both clinical and environmental functions at the State Laboratory. Functions of the QA unit include: review of Federal Regulations for guidance and compliance; monitoring tasks to assess potential problems; developing, evaluating and standardizing lab procedures and tools; and providing support to all lab areas to ensure quality laboratory testing. Questions may be directed to the QA unit, (919) 807-8747.

Specimen and Sample Mailers

Laboratory Mailroom (919) 733-7656

The State Laboratory furnishes, either free or at cost, mailers for collection and shipment of laboratory specimens and environmental samples. These mailers are carefully selected by the Laboratory to meet U.S. Postal Service/DOT diagnostic specimen shipping and packaging regulations to minimize problems such as leakage or breakage, and to identify the type of specimen or sample through color-coding. Color-coding speeds up the process of sorting and routing of thousands of specimens and samples received daily. Therefore, the Laboratory prefers receiving specimens and samples in these mailers. The mailers are provided for shipping specimens and samples only to the State Laboratory and not elsewhere.

Ordering

The NCSLPH Online Supply Ordering System must be used to order supplies. Supplies may be ordered by going through the NCSLPH website, <http://slph.ncpublichealth.com/labportal>. Some services of this Laboratory are mandated by the Legislature or other funding source to be provided to both public and private providers. Many services are restricted by the Legislature, Department Programs, or other funding sources to only local health departments and state-operated facilities. The latter does not include federally funded facilities, county facilities that are not part of the health department, or private facilities even if they serve indigent patients. Some services are further restricted to certain patients seen in local health departments, such

as pregnant women, children of certain ages, patients with symptoms of certain conditions, etc. Even though a particular testing service may be available to facilities other than local health departments, the same supplies are not available to others. Certain funds are provided to the Laboratory by Department Programs or the Legislature for the purpose of furnishing only to local health departments certain items at no cost or at a very low cost (state contract price and recovery of handling costs only) to support specific tests on particular patients.

Ordering Supplies/Forms

Supplies	Order on line, http://slph.ncpublichealth.com/labportal
Clinical Specimen Submission Forms	Download and print from web site, http://slph.ncpublichealth.com <i>Note: HIV, Newborn Screening, and Hemoglobinopathy forms are not available on the State Lab Web Site.</i>
Environmental Submission Forms	<i>Environmental submission forms, except for Environmental Lead, are not available on the State Lab Web Site. These forms are included with the collection kit.</i>

Biologicals

Rabies Vaccine and Rabies Immune Globulin (RIG) are available to physicians and health departments. These items are very expensive and are not usually stockpiled by the end-user. The person ordering is financially responsible for the cost of the treatment. Once purchased, rabies treatment (vaccine and RIG) may not be returned for credit or refund. Prior to ordering vaccine, consultation with one of the authorized persons in the Occupational and Environmental Epidemiology (OEE) Branch is required.

Authorized Personnel/Rabies Treatment

Veterinarian, OEE Branch (919) 733-7419
State Epidemiologist (919) 733-3419

The above personnel may be reached after hours, nights, weekends or holidays by calling (919) 733-3419.

Shipment of rabies treatment is usually made by using UPS or FedEx. In very rare emergency situations, it may be relayed by the State Highway Patrol. This method will not be used unless absolutely necessary.

Botulism Antitoxin

The NCSLPH does not supply antitoxin for treatment of botulism. The antitoxin is available only from the Centers for Disease Control and Prevention in Atlanta, GA, and is released to physicians after consultation with a state epidemiologist or physician specialist on call to determine the validity of the diagnosis. To obtain antitoxin for treatment of botulism, contact State Epidemiologist, Communicable Disease Branch, Epidemiology Section at (919) 733-3419. This number is also used after hours, nights, weekends and holidays to reach the epidemiologist on call.

Payments and Prices

Invoices are sent via e-mail and hard copy immediately upon shipment of the entire order. Invoices can also be viewed on the NCSLPH website, mailroom ordering, while logged in to your account on-line. Prices for all laboratory supplies, specimen containers and biological products are updated as necessary and subject to change without notice.

SERVICES FROM OTHER LABORATORIES
(Not Performed at NC State Laboratory of Public Health)

<p>Criminal case tests – (919) 662-4500 (Must be referred through law enforcement) 3320 Old Garner Road Raleigh, NC 27610</p>	<p>NC Department of Justice State Bureau of Investigation</p>
<p>Food tests (not associated with human illness) Constable Laboratory – (919) 733-7366 4000 Reedy Creek Road, PO Box 27647 Raleigh, NC 27607</p>	<p>NC Department of Agriculture & Consumer Services</p>
<p>Animal diseases (except Rabies) (919) 733-3986 Rollins Diagnostic Laboratory 2101 Blue Ridge Road, PO Box 12223 Raleigh, NC 27605</p>	<p>NC Department of Agriculture & Consumer Services</p>
<p>Chromosome Studies (Karyotype) (Refer to the Genetic Counseling Program in the Department of Pediatrics at the listed medical centers)</p>	<p>Carolinas Medical Center PO Box 32816 Charlotte, NC 28232 (704) 355-3159</p> <p>ECU School of Medicine Greenville, NC 27834 (252) 744-2525</p> <p>UNC School of Medicine Pediatric Genetics and Metabolism Chapel Hill, NC 27599 (919) 966-4202</p> <p>WFU Bowman Gray School of Medicine Medical Center Boulevard Winston-Salem, NC 27157 (336) 713-4500</p>
<p>Centers for Disease Control and Prevention (CDC)</p>	<p>NOTE: Submission of specimens to CDC must be sent via the State Laboratory. In special cases the State Laboratory can arrange for direct submission of specimens to CDC.</p>

Cancer Cytology

(919) 733-7146

Introduction

The Pap test is a non-sterile cellular specimen obtained to screen for abnormal cellular changes suggestive of intraepithelial lesion or malignancy. The State Laboratory of Public Health accepts only gynecological ThinPrep™ PAP specimens from local health departments and state-operated health facilities (public health patients). The Pap test is a screening test for an asymptomatic population. Symptoms, which may be due to malignancy, should be completely evaluated: The Pap test in this situation is not appropriate management. False negative tests may occur due to sampling errors, screening difficulties inherent in PAP tests or due to the subjective nature of cytodiagnosis. Patients should have PAP tests performed on a routine schedule.

Following the latest American Society of Colposcopy and Cervical Pathology (ASCCP) guidelines published in October 2007

(<http://www.asccp.org/consensus/cytological.shtml>) the State Laboratory will automatically perform a reflex HPV test on patients twenty years of age or older with ASC-US (Atypical Squamous Cells of Undetermined Significance) pap results for the presence of high risk HPV genotypes using the original ThinPrep specimen vial. This assay detects several of the high risk genotypes (HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) but does not distinguish between them. The HPV report will state that high risk genotypes were or were not detected in the patient's sample.

Specimen Collection and Identification

Detailed information on materials needed and illustrated instructions for collecting, packaging and shipping specimens can be found in the current issue of the DHHS document, *Cervical Screening Manual: A Guide for Health Departments and Providers (July, 2008)*. This manual is accessible from the link found on the State Lab website (<http://slph.ncpublichealth.com>)

Order of collection of gynecologic specimens: the Pap test can be collected anytime after the cervix has been cleaned. Collect gonorrhea, chlamydia and Pap specimens according to local protocol using review of patient symptoms and clinic requirements.

Note: Collecting any other test sample before collecting the Pap test may remove the diagnostic cells needed for the Pap test and render a false negative report.

Specimen Identification

- Specimen vials must be properly labeled.
- Mislabeled specimens are not processed.
- PRINT patient's first and last name and date of birth on the PreservCyt vial. A computer-generated label is preferred.
- Place the label toward top of vial with the name horizontal to the vial lid.

Reminders for completing the Pap test screening form (DHHS 1010)

- All requested information on the form is vital.
- It is essential that the patient's correct first and last name be clearly printed on the form and vial. Please note any name changes from previous submissions by circling the change in red and/or writing the previous name in the appropriate space on the form.
- A letter requesting clarification of name discrepancies will be mailed if the name change is not provided on the form at submission. Results will not be released until name conflicts are resolved.
- The Social Security number must be on the form for correct identification. Reports will not be released without this data.
- The Medicaid number, if applicable, must be on the DHHS 1010 for billing purposes; therefore, it is imperative Medicaid numbers are submitted to the laboratory.
- Collection date, date of birth, first day of last menstrual period (LMP), birth control method and other pertinent history are essential for evaluation of the Pap test. Failure to provide this data may result in a sub-optimal report. CLIA '88 requires inclusion of this data.
- EIN and submitter address must be included to avoid lost or delayed Pap reports.
- Health Departments with imprinters should clarify the letters "H", "M", and "N". Please check for legibility when imprinting over printed areas of the Form 1010.
- The most common errors on the form are:
 - Social Security number is not provided
 - Provider return address is not provided
 - A name change or alias is not indicated
 - Patient history is incomplete
 - Writing is illegible or printing is smudged on the form

Unsatisfactory specimens

Unsatisfactory cytological specimens fall into two categories:

- Unsatisfactory Rejected:
 - Illegible writing or printer smudges on vial or form.
 - Name on vial and name on the form do not match.
 - The expiration date on the vial has passed.
 - No patient name on the form or vial.
- Unsatisfactory Examined: Insufficient number of cells caused by:
 - Failure to properly rinse collection devices in vial of PreservCyt solution
 - Submission of bloody specimen
 - Contamination with lubricant
 - Excessive mucus/inflammation

Shipment

Refer to the NC State Laboratory web site, <http://slph.ncpublichealth.com>. Click on Cancer Cytology, Cytology Shipping Guidelines document. This document provides explicit shipping details for cancer cytology specimens.

Reporting Procedures and Interpretation

- The Cancer Cytology Unit uses The Bethesda 01 Pap Reporting System.
- Negative results are reported upon completion of quality control procedures. Turn around time is five business days for negative reports.
- The Cytology Unit maintains two contracts for overflow Pap screening services to prevent backlogs.
- Abnormal results must be confirmed and signed by a pathologist (CLIA '88 regulation). The Cancer Cytology Unit contracts for pathology services thus requiring additional turn around time for pathologist review and courier service.
- Reports are available on the SLPH website and a computer-generated report is also returned to the health department.

- **Follow up Form # 1011 for Abnormal Pap Reports**
 - DHHS Form #1011 (for the collection of follow-up data) is sent with all Pap results of dysplasia (LSIL, HSIL), ASC-H or more severe findings or second consecutive ASCUS (Atypical Squamous Cells of Undetermined Significance).
 - Please return this form when all colposcopy/ biopsy reports are completed or when **no** referral is planned for the patient.
 - A copy of the biopsy/colposcopy report is acceptable in lieu of the report when the physician does not complete the 1011. Please include the accession number or patient's Social Security number if not returning the 1011 form.
 - Case follow-up is a requirement of CLIA '88 and is an essential part of laboratory quality assurance and continuing education.
 - Recommendations on interpretation of Pap test results, patient treatment and follow-up can be found in the DHHS document, *Cervical Screening Manual: A Guide for Health Departments and Providers (July, 2008)*.

- **Critical Value (Panic Values) Notification**
 - CLIA '88 requires a notification of all critical values reports.
 - The Cancer Cytology Unit defines critical values as all HSIL (high grade intraepithelial lesions), cancer, herpes infection in pregnancy and all corrected reports.

- These notices are made to protect the patient from delay of treatment in the event the report is lost.
- **Records**
 - DHHS #1010 forms are retained for two years.
 - Reports are recorded electronically and retained for ten years.
 - Slides are retained for five years.
 - Physicians should be advised that the slides are available for review when patients are referred for follow-up. Review slides should be returned to the lab within 60 days. A copy of all biopsy reports is requested with the returned slides.

Cytology Quality Assurance Procedures at the State Laboratory of Public Health

- To assure valid examinations and Pap reports, the Cytology Unit employs a number of quality assurance procedures. Staff members are registered Cytotechnologists trained in examination of ThinPrep™ slides. Equipment, instruments, reagents, and stains are monitored daily.
- Senior Cytotechnologists randomly rescreen 10% of all negatives.
- The diagnostic trends of each individual Cytotechnologist are statistically monitored.
- The Chief Cytotechnologist works individually with a Cytotechnologist when quality control procedures indicate the need.
- All cellular changes suggestive of repair, ASCUS, dysplasia and more severe findings are referred for Pathologist confirmation and sign out.
- All previous Paps reported as negative are rescreened when the current Pap has changes suggestive of HSIL.
- Cytology reports are correlated with clinical and surgical follow-up reports (DHHS form #1011). The slides are reviewed when pathology and cytology reports differ.
- Continuing Education seminars are conducted to keep staff informed of new developments in cytology and to review difficult or unusual cases. Cytotechnologists participate in cytology teleconferences and keep up with new developments on Internet cytology sites. Cytotechnologists are encouraged to attend local, state, and national cytology meetings.
- Cytology testing personnel participate in a CMS approved Proficiency Testing Program.
- Cytology Unit participates on the NCSLPH Quality Assurance Team and prepares a monthly report for the Laboratory Director.

References

Cervical Screening Manual: A Guide for Local Health Departments and Providers, NC Department of Health and Human Services, July 2008.

Improving the Quality of Clinician Pap Smear Technique and Management, Client Pap Smear Education, and Evaluation of Pap Smear Laboratory Testing: Resource Guide for Title X Family Planning Projects, US Dept. of Health and Human Services, September 1989.

Don't Wipe the Cervix Before Taking a Pap, Dr. Vivien Hansen, Cytoc Corp. ThinPrep™ product insert, 1999.

Consultation with staff of NC Comprehensive Breast and Cervical Control Program, Women and Children Health and STD Program

Cytoc Collection Training Bulletin

Environmental Sciences Unit

(919) 733-7308

Environmental Sciences (ES) provides consultation and laboratory support for environmental and health related programs in the Department of Health and Human Services. ES offers comprehensive analysis of drinking water for local health departments and authorized health care providers. ES is also responsible for accrediting/certifying milk and drinking water laboratories.

Environmental Sciences is organized into five lab areas:

- Environmental Inorganic Chemistry
- Environmental Organic Chemistry
- Environmental Microbiology
- Environmental Radiochemistry
- Laboratory Certification

The mission of ES is to provide timely and cost effective environmental analytical laboratory services to local health departments and supported programs.

Environmental Inorganic Chemistry

(919) 733-7308

Introduction

The Environmental Inorganic Chemistry Laboratory analyzes a variety of samples such as water and soils. Water samples from both public and private water systems are examined for chemical and/or physical parameters.

Inorganic Chemical Analysis

To obtain a chemical analysis, the homeowner must submit samples through the local health department. These samples are routinely analyzed for alkalinity, arsenic, calcium, chloride, copper, hardness, lead, iron, magnesium, manganese, pH, fluoride, and zinc. Additional parameters can be performed upon request. See **Optional Parameters** information regarding these analyses.

Fluoride Analysis

A private water system can obtain a fluoride analysis if the sample is submitted through a local health department, a dentist or a physician. The report form must contain the collection date and the patient's name. Fluoride results can only be reported to the health department, dentist or physician.

Nitrate/Nitrite Analyses

Nitrate/Nitrite analyses require a special sample kit. The kit consists of a small Styrofoam cooler with two ice packs. The ice packs must be removed from the kit and placed in a freezer for at least 24 hours prior to collecting the samples. Samples must be cooled to 4° Celsius upon collection; therefore it is recommended that the samples be placed in a cooler containing ice packs or ice upon collection and refrigerated until it is placed in the Styrofoam cooler for shipment to the laboratory. Prior to shipment, make certain that the sample is placed between the two frozen ice packs inside the Styrofoam cooler. The analysis of the sample must begin within 48 hours of collection (plan collection time and transportation accordingly). Samples received at room temperature or are greater than 48 hours old will be rejected.

Optional Parameters

A private water system can request additional testing, as necessary, for the following optional parameters by indicating the request on DHHS form #1441: aluminum, antimony, barium, beryllium, cadmium, chromium, cobalt, mercury, nickel, potassium, silver, sodium, selenium, thallium, vanadium, acidity, phosphate, conductivity, settleable solids, sulfate, total dissolved solids, total suspended solids, turbidity and surfactants.

Ammonia and cyanide analyses may also be requested but require special sampling kits and preservation. Please contact the lab supervisor to order these sample kits.

Sample Collection and Identification

All samples must be collected in sampling containers supplied by the laboratory. Complete directions for sample collection and shipment are found on the back of the request form included with each sample kit. Each sample must be properly identified with a completed form. Please write legibly on the form. Place the submitter's name on the first line of the inorganic chemical analysis form. All the information on the form must be complete. Incomplete or illegible information may lead to sample rejection.

Reasons for Sample Rejection by the Laboratory

- Samples submitted without DHHS forms or samples submitted with blank forms.
- Samples submitted without a collection date, collection time, county, or "Report to:" information on the DHHS form.
- Samples submitted by a Public Water Supply to be used for compliance with the Safe Drinking Water Act.
- Samples collected for nitrate/nitrite analyses that are more than 48 hours old or do not meet temperature requirements.
- Fluoride only samples not submitted by a doctor, dentist, or health department.
- Fluoride samples that exceed the 28 days holding time.

Shipment

Samples should be mailed as soon as possible after collection.

Reporting Procedures and Interpretation

Sample analysis time will vary from one to fourteen days, depending upon the number of parameters requested for the sample. The submitter should receive a copy of the analytical results within three weeks of the date of sample collection. Public and private water systems laboratory reports are held for five years, then destroyed.

The laboratory report contains results for each parameter tested followed by a unit of measurement. Most of the analyses are reported in parts per million (ppm) or milligrams per liter (mg/L) that are equivalent. If the laboratory does not detect the parameter in the sample, then the laboratory will report a result preceded by a less than symbol (<). These "less than" results are based on the lowest concentration of the analyte that the laboratory can satisfactorily quantify with the method and the instrumentation in use.

The recommended limits or the maximum contaminant levels (MCLs) listed are for informational purposes only to provide guidance in interpreting an inorganic chemical analysis. These limits have been established for public water systems by the Environmental Protection Agency (EPA) under the Safe Drinking Water Act. Questions or concerns about the health effects of any of these contaminants should be addressed to the Occupational and Environmental Epidemiology Branch.

The recommended limits or maximum contaminant levels for inorganic contaminants in public water supplies established by the EPA are as follows:

Antimony – MCL = 0.006 mg/L. Antimony may decrease growth and longevity. Potential sources are industrial discharges or from tin/antimony solder used in plumbing.

Arsenic – MCL = 0.010 mg/L. Carcinogenic properties have been ascribed to arsenic. Its presence may be due to natural deposits, industrial discharges or pesticides.

Barium – MCL = 2 mg/L. Barium occurs only in trace amounts in drinking water and rarely exceeds 1 mg/L.

Beryllium – MCL = 0.004 mg/L. Beryllium is very poisonous. It is used in atomic reactors, aircraft, rockets and missile fuels. It is through industrial discharges that it may enter water.

Cadmium – MCL = 0.005 mg/L. Cadmium is toxic and may be carcinogenic. It may enter water as a result of industrial pollution or deterioration of galvanized pipe.

Chromium – MCL = 0.10 mg/L. Chromium salts are used in industrial processes and may enter a water supply through industrial discharge.

Copper – MCL = 1.3 mg/L. Copper may impart a metallic taste to water and cause greenish stains on faucets and plumbing fixtures.

Cyanide – MCL = 0.2 mg/L. Cyanide can cause spleen, brain and liver damage. It is used in electroplating, steel processing, plastics, synthetic fibers, fertilizer and farm products.

Fluoride - MCL = 4.0 mg/L. Fluorides are found mostly in groundwater as a natural constituent.

Iron – MCL = 0.3 mg/L. Iron in water can cause staining of laundry and porcelain. It may give the water an astringent taste.

Lead – MCL = 0.015 mg/L. Lead is a cumulative poison. In a water supply it may occur where piping material or pipe joint compound contains lead.

Manganese – MCL = 0.05 mg/L. Manganese can cause objectionable stains to laundry and fixtures.

Mercury – MCL = 0.002 mg/L. Mercury is very toxic and its presence may be associated with industrial water and agricultural applications.

Nitrate – MCL = 10 mg/L (as nitrogen). Serious poisonings in infants have occurred following ingestion of well water containing nitrogen in the form of nitrate at concentrations greater than 10 mg/L. This problem is known as methemoglobinemia (blue-baby syndrome) and is generally confined to infants less than three months old. The presence of nitrates is usually due to animal wastes and fertilizers. Boiling water does not remove nitrates but instead concentrates them.

Nitrite - MCL = 1mg/L (as nitrogen). Nitrite is the actual etiologic agent of methemoglobinemia. It results from oxidation of ammonia or reduction of nitrates. May occur in natural water or water distribution systems.

pH – MCL = 6.5 – 8.5. Soft acid water may leach metals from plumbing causing staining problems, metallic tastes or deleterious health effects.

Selenium – MCL = 0.05 mg/L. Selenium is an essential trace nutrient, but may be toxic above trace levels. Natural levels in groundwater may be due to soil types. Selenium may be leached from coal ash and fly ash at electric power plants that burn seleniferous coal.

Thallium – MCL = 0.002 mg/L. Thallium affects the brain, kidneys, and liver. Its presence may be associated with electronics or glass industries.

The limits listed for the contaminants below are recommended limits that the EPA has established for public water systems. These recommended limits are based on the cosmetic effects (such as skin or tooth discoloration) or the aesthetic effects (such as taste, odor or color) they have in drinking water.

Aluminum – 0.05 to 0.2 mg/L. Aluminum may cause discoloration of the water and may contribute to scaling or sedimentation in pipes.

Chloride – 250 mg/L. High chloride levels may harm pipes, as well as impart an unpleasant salty taste.

Nickel – 0.1 mg/L. Nickel may affect the heart and liver. Can enter water supplies through discharges from batteries, ceramics, or glass production.

Total Dissolved Solids – 500 mg/L. Waters with high dissolved solids are unpalatable and may be unsuitable for many industrial applications.

Silver – 0.10 mg/L. Exposure to silver in drinking water may cause argyria (a discoloration of the skin). Health effects are only cosmetic.

Sulfate – 250 mg/L. Sulfate may naturally be present in groundwater. Its sodium and magnesium salts exert a cathartic action.

Zinc – 5 mg/L. Zinc may cause a bitter astringent taste and opalescence in alkaline water. It most often enters the water supply through the deterioration of galvanized iron pipes.

Environmental Microbiology

(919) 733-7308

Introduction

The Environmental Microbiology Lab performs bacteriological analyses on water samples from both public and private water systems. Samples are examined for the presence of the coliform group of bacteria, which are indicators of fecal contamination. Water is not examined for pathogenic bacteria, as the prospect of isolating them from water is very remote.

Public water system samples are submitted to this Laboratory by the Public Water Supply Section. Samples from private wells will be analyzed for coliform bacteria only if the sample is submitted through a local health department. The well should be inspected at the time the sample is collected by a health department representative. No sample for sanitary analysis should be submitted from an open well, an unprotected spring, or from any source where there is visible evidence of contamination. Such supplies are unsafe for drinking purposes, regardless of laboratory findings.

Samples of non-drinking water, such as those from lakes, streams, rivers, and ponds that are submitted by health departments may also be examined for total and fecal coliform bacteria to determine the degree of contamination.

Sample Collection and Identification

A. Coliform

All samples for coliform analysis must be collected in regulation, sterile bottles supplied by this Laboratory. Complete directions for collecting a proper sample are found on the back of the request form included with each sample kit. Directions must be followed closely to ensure that the sample is not contaminated during collection. Each sample must be properly identified with a completed form. A minimum of 100 mL is required for drinking water samples submitted for testing of total coliforms (fill to or slightly above the line). For non-drinking water samples submitted for both fecal coliform and fecal *Streptococcus*, the bottle should be filled to the neck.

B. Other Tests

With the exception of the Sulfate Reducing/Sulfur Bacteria and Iron Bacteria tests, please call the Laboratory before submitting samples for the following tests:

1. Heterotrophic Plate Count

This procedure enumerates nearly all of the bacteria present in a water sample. Results will be reported as the number of colony forming units (CFUs) per milliliter (mL) of sample.

2. **Pseudomonas**
This analysis confirms the presence of *Pseudomonas aeruginosa*. An opportunistic pathogen, this organism has been associated with eye, ear, nose, throat, skin, and urinary and intestinal tract infections. Results will be reported as the number of *Pseudomonas* organisms present in 100 mL of sample.
3. **Fecal *Streptococcus***
The fecal *Streptococcus* analysis is usually done in conjunction with the fecal Coliform analysis to determine the relative sanitary quality of non-potable waters. Results will be reported as the number of fecal Coliform and the number of fecal *Streptococcus* organisms per 100 mL of sample.
 - *Enterococcus* – The enterococcus group is a subgroup of the fecal streptococci group and detects enterococci in fresh and marine waters. Enterococci is considered a valuable bacterial indicator for determining the extent of fecal contamination of recreational surface waters. Results will be reported as the number of enterococci per 100mL of sample.
4. **Sulfate Reducing and Sulfur Bacteria**
The presence of Sulfate Reducing and/or Sulfur Bacteria in a water source may cause taste, odor, and pipe corrosion problems. These bacteria are considered “nuisance organisms” and are not pathogenic. Both tests can be performed using the same sample. Results will be reported one calendar month from initiation of sample analysis. Results are reported as either Positive or Negative for each of these bacteria.
5. **Microscopics**
 - a. **Iron Bacteria**
Iron Bacteria may produce taste, odor, and pipe corrosion problems. Iron Bacteria and Sulfate Reducing/Sulfur Bacteria tests can be performed using the same sample. Results for Iron Bacteria examinations will be reported as Positive or Negative for Iron Bacteria. If there is no visible sediment or particulate matter or reddish tinge in the water, it is unlikely that Iron Bacteria are present.
 - b. **Algae**
Samples for algae examinations must be received within 24 hours of collection and should be kept on ice during transit. If algae are found in the sample, results will include the types and genus names of the algae present.

- c. Fungi, Protozoan, and Miscellaneous Materials
Microscopic examinations will be made to identify the material or organism. Samples should be transported to the Laboratory as soon as possible after collection using the same form and bottle used for other microscopics.
- d. Giardia and Cryptosporidium
This Lab does not examine water samples for Giardia or Cryptosporidium. Water samples submitted for these parasites will be analyzed for Total and Fecal Coliform organisms. If the Total and Fecal Coliform tests are Negative, the presence of these parasites is unlikely. If the Total and Fecal Coliform tests are Positive, this information will be forwarded to the (Clinical) Microbiology Unit. Special arrangements must be made before sending samples to the Microbiology Unit for testing.

C. Milk Microbiology

The Environmental Microbiology Unit provides analyses of dairy products on reference samples received from the Milk Sanitation Branch. Proper shipping measures must be observed to maintain integrity of samples and to meet the regulatory requirements of the National Conference of Interstate Milk Shippers (NCIMS). Dairy products may be analyzed by the following procedures:

- Standard Plate Count
- Coliform Plate Count
- Inhibitory Substances Test (Antibodies)
- Direct Microscopic Somatic Cell Count
- Alkaline Phosphatase Test

Sample Shipment:

Note: Samples for coliform analysis must reach this Laboratory and be processed within a maximum of 30 hours after collection. Samples arriving after 30 hours will be rejected as unsuitable for analysis.

Non-drinking water samples should be refrigerated during a maximum transport time of six hours. A special courier may be required to deliver the samples to this Laboratory. Arrangements for these analyses should be made with the Laboratory by telephone at least 24 hours in advance.

Reporting Procedures and Interpretation

Test results for drinking water analyses are sent within two working days after the Laboratory receives the samples. If coliform bacteria are present, the water is considered unsafe for drinking purposes. Results are reported as the presence or absence of both Total Coliform and E.coli bacteria. An analysis refers only to the

sample as received and should not be regarded as a complete report on the water supply. Non-drinking water sample results are forwarded as soon as complete, typically 4-5 days after receipt of the sample and initiation of testing. Laboratory reports for private water systems are held for five years and then destroyed. Reports for public water systems are held for one year in the Laboratory then transferred to Environmental Health Central Files.

Environmental Organic Chemistry

(919) 733-7308

Introduction

The Environmental Organic Chemistry Lab analyzes water for a variety of organic chemicals. Eligible submitters include health departments and certain governmental agencies.

Sample Collection and Identification

In general, all water samples should be taken in a one (1) liter amber bottles or 40 mL glass vials supplied by the Laboratory.

- A. Petroleum Products and Volatile Organic Compounds (VOC)
Petroleum products fall into two categories: 1) solvents and gasoline; and 2) heavy oils and greases. If the suspected petroleum contaminant is a solvent or gasoline, request a Volatile Organic Compound (VOC) Kit. VOC samples are collected in 40 mL vials; all kits are available on the State Laboratory web site, <http://slph.ncpublichealth.com>. If the suspected contaminant is a heavy oil or grease, request a Petroleum Kit (also from Environmental Sciences). Petroleum Kit samples are analyzed for both volatiles and extractables. Petroleum product samples are collected in clean one-liter amber bottles and 40 mL vials. VOC and Petroleum Kits are supplied only to health departments upon request. Follow all instructions on the label or request sheet when sampling. Screw the cap tightly, making sure the cap seals. This analysis is to determine a potential health hazard of the supply and will not necessarily determine the source of contamination by identifying the compound(s). The person submitting the sample should make note of any odors or possible sources of contamination on the request sheet. Please fill in all blanks on request sheet DHHS form #2364. Print legibly.
- B. Pesticides
Samples to be analyzed for the presence of pesticides are sampled in two (2) one-liter amber glass bottles. These bottles/kits are available on the State Laboratory website, <http://slph.ncpublichealth.com>. Private samples should list suspected compound(s) on the report sheet DHHS form #2364. The Laboratory is unable to analyze for every pesticide (herbicides, fungicides, etc), so before sampling, check with the Laboratory for availability of testing. Rinse and fill the bottle with the water sample and seal with Teflon lined cap. Make sure the cap seals completely. Follow all instructions on the label or report sheet when sampling. Mail immediately to the Laboratory. Remember to complete all information on the submission form and print legibly.

Shipment

For results to be valid, it is necessary to ship samples using frozen ice packs. After collection, mail samples immediately in styrofoam mailers to the Laboratory.

Reporting Procedures and Interpretation

Organic analyses are diverse in nature and vary greatly in complexity and analytical requirements. It is difficult to state precisely when a report for a particular test will be completed. Some samples may receive priority treatment because of a critical health concern, an imminent hazard in the workplace, the instability of a particular sample, or other factors. Generally, results are complete within three weeks of the sample collection date. Public and private water system laboratory reports are retained for five years and then destroyed.

Environmental Radiochemistry

(919) 733-7308

Introduction

The Environmental Radiochemistry Lab analyzes environmental samples submitted by public water systems; local health departments; and the Divisions of Environmental Health, Solid Waste Management, and Radiation Protection. Currently, natural and manmade radiation levels in air, water, milk, food and other media, are monitored.

These environmental surveillance programs are outlined below. All parameters are not tested for every sample.

Air Filters

Gross Alpha

Gross Beta

Gamma

Air Cartridges

Gamma

Water/Rainfall

Gross Beta

Tritium

Strontium

Surface Supplies, Sewage Effluent

Gross Alpha

Gross Beta

Gamma

Strontium 89/90

Radium 226/228

Tritium

Iodine – 131 (low levels)

Uranium (total)

Public drinking water samples

Gross Alpha

Gross Beta

Radium 226/228

Uranium (total)

Ground Supplies (not public)

Gross Alpha
Gross Beta
Tritium
Radium 226/228
Uranium (total)
Gamma

Silt/Soil

Gross Alpha
Gross Beta
Tritium
Uranium (total)
Radium 226/228

Milk

Gamma
Iodine 131 (Low level)
Strontium 89/90

Edible Products

Same as Silt/Soil

Wipe Samples (Leak Test)

Isotopes as requested

Sample Collection and Identification

Samples submitted for compliance under the North Carolina Safe Drinking Water Act must be collected in containers provided by the Laboratory.

Note: Public Water Supply (PWS) systems choosing this Laboratory to analyze their water samples must complete and return the required PWS form with the appropriate fees to this Laboratory.

Other eligible submitters should complete DHHS form #2006 and indicate desired parameters.

Shipment

Use the shipment guidelines on the back of the requisition form or contact Environmental Sciences (919) 733-7308 with any questions.

Reporting Procedures and Interpretation

The variety of sample types, analytical methodologies, and current sample loads make it difficult to predict the time required for reporting. Best estimates, based on the individual situation, can be made at the time of sample submission to the Laboratory.

Crisis samples will receive priority over routine monitoring samples. Radiological laboratory reports are retained for 10 years, and then destroyed.

Note: For radiation contamination problems other than routine monitoring, please contact the DHHS/Radiation Protection Section (919) 571-4141.

Laboratory Certification

(919) 733-7308

Introduction

Laboratory Certification evaluates laboratories that analyze water from public water supplies, which are subject to regulation under the North Carolina Drinking Water Act. Laboratories and analysts that test milk under the Grade A Pasteurized Milk Ordinance (PMO) are also evaluated. Certification is granted to qualified laboratories and personnel that meet State and Federal requirements. In addition, Laboratory Certification provides consultation and guidance to Laboratories involved in milk and water testing and offers training through seminars and workshops.

Accreditation of Milk Laboratories

For a milk laboratory to be accredited, the following requirements must be met:

Laboratory facilities must meet the criteria as described in Official Milk Laboratory Evaluation Forms (FD-2400). An on-site evaluation determines compliance. When an accredited laboratory changes location or undergoes substantial remodeling, the Laboratory Evaluation Officer must be notified and facilities must be re-evaluated within three months. No evaluation of personnel or procedures is required at this time.

The analyst(s) working at the milk laboratory must be certified/approved as outlined below. All official examinations required by the Grade A Pasteurized Milk Ordinance must be performed by a certified/approved analyst.

When a certified analyst resigns from an accredited laboratory, the laboratory certification officer must be notified since loss of a certified analyst could result in loss of accreditation. For example, a laboratory having only one certified analyst would lose accreditation if that analyst resigns. No official samples could be tested until a new analyst becomes certified.

Certification or Approval of Milk Analyst

An analyst may be certified to perform analysis of raw or processed milk and milk products to meet the testing requirements of Section 6 of the PMO. Analysts may be approved for screening raw milk for the presence of antibiotic residues.

Full Certification

Three criteria must be achieved for an analyst to become fully certified:

1. The laboratory facilities must meet the requirements.
2. The analyst's performance must be evaluated during an on-site visit at least once every two years.

3. The analyst must participate annually in the split sample program and must demonstrate acceptable performance.

When all three criteria are met, the analyst is fully certified.

Conditional Certification

For initial certification, an analyst not meeting all three criteria may be granted conditional approval to conduct official examinations when 1 and 2 OR 1 and 3 are met.

If a conditionally approved analyst does not perform satisfactorily on split samples or does not meet performance standards during an on-site evaluation, his/her certification status will be revoked.

Provision Certification

A fully certified analyst who (1) fails to satisfactorily participate in the split sample program annually or (2) fails on on-site evaluation will be placed on provisional status. Failure to participate in the next split sample evaluation or to meet satisfactory performance levels on the repeat on-site evaluation will result in withdrawal of certification for that test.

An analyst who loses certification for some or all tests cannot examine official samples using those tests for which certification has been withdrawn.

Reinstatement of Decertified Analyst

An analyst who has lost certification must participate in a training program acceptable to the certifying authority before requesting recertification.

Recertification after training is based on the analyst's meeting the three criteria previously described.

Certification of Water Laboratories

For a water laboratory to be certified, three requirements must be met:

Laboratory facilities must meet the criteria as described in the regulations (10A-42D-.0200). An on-site evaluation determines compliance.

Performance test (PT) samples must be analyzed for each analyte and by each method for which certification is requested. For chemical parameters, Heterotrophic Plate Count and *E. Coli* enumeration, two of the previous three PT results must be acceptable. For the coliform bacteria group, acceptable results must be reported on 80% of the samples in each set.

Certification fees must be paid for each analyte group for which certification is desired.

Certification activities for both milk and water can be initiated by contacting:

North Carolina State Laboratory of Public Health
Laboratory Certification - Drinking Water
PO Box 28047
Raleigh, North Carolina 27611-8047.
Phone: 919-807-8879

Laboratory Improvement

(919) 733-7186

Laboratory Improvement conducts and coordinates diverse activities which promote and contribute to the quality-assurance of laboratory services. The general responsibilities of the branch are described below.

Consultation

The Laboratory Improvement consultants have knowledge and experience in many technical areas. Information is provided to local laboratory managers, laboratorians, and nursing staff concerning laboratory management, operations, technical procedures, biosafety, packaging and shipping, and quality assurance guidelines. Consultation is provided to public health programs concerning laboratory services needed to support program objectives. Arrangements for on-site reviews can be made upon request to Laboratory Improvement.

New federal mandates in the past several years have expanded the Laboratory Improvement consultant's roles. The consultants have assisted the local health departments in complying with the Occupational Safety and Health Administration (OSHA) regulations, as well as the Clinical Laboratory Improvement Amendments (CLIA '88). This has been achieved by providing more on-site visits as well as developing new training courses to address the needs of the laboratorian. Continued monitoring of the local health departments is an on-going commitment of this Unit.

Training

A survey to identify training needs is conducted periodically and used to guide the development of workshops and training activities. In addition, training activities may be developed in response to specific requests from individuals and groups. Workshops are presented on clinical, environmental and management topics; they are designed to give "hands-on" experience with methods and techniques. Instructors are selected on the basis of competency, experience, and the ability to communicate with participants. Workshops are announced annually in the Laboratory Improvement Training Bulletin. Additions to the workshop calendar are announced as they are scheduled; periodic updates from Laboratory Improvement include all training activities.

Laboratory Improvement is also an active member of the National Laboratory Training Network (NLTN). The NLTN is a cooperative training agreement between the Association of Public Health Laboratories (APHL) and the Centers for Disease Control and Prevention (CDC). The purpose of the network is to address the need for effective laboratory information and management systems to assist state health agencies to develop, promote, and deliver quality laboratory training. The network functions as a training service delivery program that utilizes available resources and conducts regionalized training based on documented needs.

Bench training can be arranged by contacting the appropriate technical unit at the State Laboratory, i.e. Microbiology, Virology/Serology, and Environmental Sciences

Laboratory Advisor to the Gonorrhea Control Program

Training consultation and quality control related to the statewide gonorrhea control program are provided. For information about laboratory methods and available workshops in this program contact Laboratory Improvement.

Control Cultures

Microbiological cultures useful in quality control of media and reagents are available on a limited basis. To order control cultures, use the "Stock Culture Order Form" on the NCSLPH public website. This form is found in the "Documents" section of the "Forms, Newsletters & Documents" tab on the home page.

Regional Laboratory Consultants

Laboratory Improvement consultants are assigned to four regional offices:

Black Mountain

Phone: (828) 289-8519

Winston-Salem

Phone: (336) 896-7944

Fayetteville

Phone: (910) 322-8120

Greenville

Phone: (252) 355-9084

Laboratory Preparedness

Bioterrorism and Emerging Pathogens (919-807-8765)

Chemical Terrorism (919-807- 8771)

The Laboratory Preparedness Unit houses both biological and chemical labs that test for agents of terrorism. Both labs are members of the Laboratory Response Network (LRN). The LRN was established by the Department of Health and Human Services and the Centers for Disease Control and Prevention (CDC). The LRN founding partners are the Federal Bureau of Investigation (FBI), the Association of Public Health Laboratories (APHL) and the CDC. The objective for establishing the LRN was to ensure an effective laboratory response to bioterrorism by helping to improve the nation's public health infrastructure. Today, the LRN maintains an integrated network of state and local public health, federal, military and international laboratories that can respond to bioterrorism, chemical terrorism and other public health emergencies. The CDC provides to all LRN members validated protocols for the testing of agents of terrorism.

Bioterrorism and Emerging Pathogens

(919) 807-8765

Introduction

The mission of Bioterrorism and Emerging Pathogens (BTEP) is to sustain laboratory capacity for the detection of biological weapons and emerging infectious diseases and to strengthen crisis response within the Division of Public Health. BTEP is a member of the Laboratory Response Network, (LRN) and also a member of the recently organized Food Emergency Response Network or FERN. The LRN and FERN provide standardized protocols for the testing of biothreat agents and emerging pathogens in clinical, environmental and food samples. BTEP functions as a referral laboratory to all labs and agencies in NC for possible Select Agent viruses, bacteria and some toxins. BTEP accepts environmental samples and food from law enforcement agencies where a biothreat agent or toxin is indicated or a credible threat is suspected in environmental situations. BTEP is a Smallpox surge capacity laboratory for the CDC.

Regional Bioterrorism Response labs, located in Pitt, Mecklenburg and Buncombe Counties, provide most of the same response services as the BTEP in Raleigh. All BTEP staff may be contacted 24/7 by:

Duty Phone: 919-807-8600 *or*
BT Pager (24/7): 919-310-4243

Facilities with requests for emerging pathogens or viral agents must first contact the State Epidemiologist or on-call staff in the Communicable Disease Branch (CDB) at 919-733-3419 for assessment and prior approval before submission of samples.

Specimen Collection and Submission

NOTE: All submission forms are located on the NCSLPH web site, <http://slph.ncpublichealth.com> under Bioterrorism Information.

Currently, three types of specimens may be submitted for analysis:

- A. Suspicious Substances – These are often environmental samples and must be submitted through a law enforcement agency or through the Office of Public Health Preparedness and Response (PHP&R). Individuals should NOT attempt collection. Suspicious substances are generally transported to the State Laboratory under ambient conditions by the submitting enforcement agency or a PHP&R representative using Chain of Custody documentation. All samples should be securely bagged, clearly identified and prescreened for radioactive substances and explosives. Notify BTEP prior to submission by phoning the duty phone or the 24/7 pager. An environmental submission form must be completed for each sample. If multiple samples are submitted, be prepared to prioritize samples for testing.

- B. Clinical Samples – **Prior to submission**, call 919-807-8600 for guidance on collection and transport of samples and labeling of packages. Private courier service is available if transport to a regional lab is required. Acceptable clinical isolates/samples include those from a hospital or other clinical lab either public or private in North Carolina. Submit isolates/samples if available microbiological methods are unable to rule out a possible bio-threat or Select Agent. Primary specimens must be collected aseptically and placed into leak-proof containers. Isolated bacterial or viral organisms should be pure and must be shipped on media or using conditions that will support the transport of the isolate. Shipment must be in a leak-proof containment system such as a screw-capped tube or vial. Bacterial isolates should NEVER be sent on plated medium. All submitters of samples should include 24/7 contact information. Submitters should call BTEP for guidance on the appropriate samples and collection for testing. Transport all samples immediately or as soon as possible to the lab. Samples for bacterial testing should be sent at ambient temperatures; samples for viral testing should be sent on cold packs. Call for transportation requirements for toxins.

For known Select Agents all submitters are required to first complete the transfer forms found in the Code of Federal Regulations (see regulations 7 CFR 331.16, 9 CFR 121.16, and 42 CFR 73.16) and receive approval from the Select Agent Program and the NCSLPH prior to transfer.

- C. Food – If food items are suspected of containing bio-threat Select Agents or toxins, contact BTEP immediately. If botulism is suspected, contact CDB at (919) 733-3419. No food samples can be submitted to the NCSLPH unless received through a law enforcement agency, the Office of Public Health Preparedness and Response (PHP&R) or by special request from the State Department of Agriculture. Transport the samples using Chain of Custody documentation. Complete and submit an environmental submission form for each food item submitted. All food items must be collected aseptically and placed into leak-proof containers, being careful not to touch the food items with hands. Collect at least 25 grams of solid food sample and at least 25 mL of liquid food sample (See table 3 for more details). All samples should be promptly refrigerated and transported on cold packs in insulated containers. DO NOT FREEZE samples. If samples are already frozen, keep frozen during transport.

Reporting Procedure and Interpretation

- A. Suspicious Substances – Presumptive and final test results are phoned to the submitter at the 24/7 contact number listed on the submission form. Final reports are sent to the submitter and the director of the county health department where the incident occurred. Final reports on BTEP environmental samples are NOT available on the NCSLPH LIMS secure web site. Requests for additional copies of reports must be made directly to BTEP. All samples received from a law enforcement agency are handled as evidence and stored in secure areas until

released to the submitters or destroyed. An internal Chain of Custody form is maintained and copies are given to the submitter when the completed sample is released. Upon request, digital photos of the materials submitted or threat letters contained within the samples, can be attempted and electronically mailed to the submitted agency. After all testing is completed, the submitting agency may claim their samples between the hours of 8 a.m. to 5 p.m., Monday-Friday. All sample material is securely stored for at least 60 days. After 60 days and without further notice, BTEP periodically destroys unclaimed samples.

- B. Clinical specimens – All positive results are called immediately to the submitter, the NCSLPH Laboratory Director and the State Epidemiologist. Negative results are called to the submitter upon request. Final reports are sent to the submitter and the State Epidemiologist; these reports are available to the submitter on the NCSLPH LIMS secure web site. Positive results are reported to the Select Agent Program at CDC.
- C. Food – Presumptive and final test results are phoned to the submitter. Final reports are sent to the submitter at the address listed on the submission form and also to the director of the county health department where the food was recovered. Final reports are NOT available on the NCSLPH LIMS secure web site. Requests for additional copies must be made to the BTEP section. Food samples submitted to BTEP are treated as environmental samples and subject to the same Chain of Custody, and storage and release requirements.

Table 1

SUSPICIOUS SUBSTANCES (ENVIRONMENTAL)

Note: As each incident is unique, call the BTEP Lab for specific details.

ORGANISM/ TOXIN	SAMPLE	TESTING PERFORMED	COLLECTION (All items)	REQUIREMENTS (All items)
<i>Bacillus anthracis</i>	Powder, swabs, wipes, envelopes suspected of or containing threats of bio-threat agents, various environmental samples including water, animal tissues, etc.	Culture, phage, PCR, other conventional microbiology tests	See PHP&R Powder Protocol guidelines at: http://www.epi.state.nc.us/epi/phpr/protocolguide.html	<ul style="list-style-type: none"> •Accepted from law enforcement or PHP&R representatives only •Prescreen for radioactivity and explosives •Securely bag and label each item separately •Transport using ambient conditions unless food •Complete BT environmental submission form, see: http://siph.ncpublichealth.com/forms.asp#bioterrorism •Describe incident and all items to be tested •Be prepared to prioritize samples should multiple samples require testing; •Be prepared to provide all known information and guidance in regards to possible agents involved; •<u>NOTIFY lab prior to arrival</u> (discussion should include possible agents and types of samples to be submitted) •<u>NOTIFY lab of approximate arrival time</u>
<i>Brucella species</i>		Culture, PCR, other conventional microbiology tests		
<i>Burkholderia mallei</i>		Culture, PCR, other conventional microbiology tests		
<i>Burkholderia pseudomallei</i>		Culture, PCR, other conventional microbiology tests		
<i>Coxiella burnetii</i> <i>Francisella tularensis</i>		Culture, DFA, PCR, slide agglutination, other conventional microbiology tests		

<p><i>Yersinia pestis</i></p> <p><i>Ricin, Ricin toxin</i></p> <p><i>Botulinum toxin</i> Call the NCSLPH</p>	<p>Food and various environmental samples</p>	<p>Culture, phage, PCR, other conventional microbiology tests PCR, ELISA (toxin) ELISA</p>	<p>Call the NCSLPH</p>	<p>Contact the NCSLPH at (919) 807-8765 and the CDB at (919) 733-3419 for further instructions.</p>
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Table 2

SUSPICIOUS SUBSTANCES: CLINICAL

ORGANISM/ AGENT/TOXIN	SAMPLE	TESTING PERFORMED	COLLECTION	SHIPPING REQUIREMENTS
<i>Bacillus anthracis</i>	Isolated organism; swabs of lesions; tissues; sputum	Culture, phage, PCR, other conventional microbiology tests	Isolated 18-24 hr. culture of unknown gram positive <i>Bacillus</i> bacteria, nonmotile & nonhemolytic on Sheep's Blood agar. Use extreme caution. Subculture without aerosolization in BSC Class II or higher.	<ul style="list-style-type: none"> •Agar slant in screw-capped leak-proof tube •Ship in ambient conditions; •Use current shipping guidelines for a diagnostic sample (if primary sample) & infectious Substance (if isolate)
<i>Brucella species</i>	Isolated organism; blood in purple or yellow-top tube	PCR, other conventional microbiology tests	Isolated 24-72 hr. culture of unknown Gram negative bacteria, where submitter is unable to rule-out <i>Brucella</i> . Use extreme caution. Subculture without aerosolization in BSC Class II or higher.	<ul style="list-style-type: none"> •Agar slant in screw-capped leak-proof tube or blood; •Ship in ambient conditions; •Use current shipping guidelines for a diagnostic sample (if primary sample) & infectious Substance (if isolate)
<i>Botulinum toxin</i>	Contact the NCSLPH at (919) 807-8600 and the CDB at (919) 733-3419	Not performed at the NCSLPH	Contact the NCSLPH at (919) 807-8600 and the CDB at (919) 733-3419	Contact the NCSLPH and the CDB (919) 733-3419. Testing performed at CDC or the Virginia LRN Laboratory
<i>Burkholderia mallei,</i>	Isolated organism; swabs of lesions; tissues; blood	Culture, PCR, other conventional microbiology tests	Isolated 24-72 hr. culture of unknown Gram negative bacteria, where submitter is unable to rule-out <i>B. mallei</i> . Use extreme caution. Subculture without aerosolization in BSC Class II or higher.	<ul style="list-style-type: none"> •Agar slant in screw-capped leak-proof tube or blood; •Ship in ambient conditions; •Use current shipping guidelines for a diagnostic sample (if primary sample) & infectious Substance (if isolate)
<i>Burkholderia pseudomallei</i>	Isolated organism; swabs of lesions; tissues; whole blood	Culture, PCR, other conventional microbiology tests	Isolated 24-72 hr. culture of unknown Gram negative bacteria, Where submitter is unable to rule-out <i>B. pseudomallei</i> . Use extreme caution. Subculture without aerosolization in BSC Class II or higher.	<ul style="list-style-type: none"> •Agar slant in screw-capped leak-proof tube or blood; •Ship in ambient conditions; •Use current shipping guidelines for a diagnostic sample (if primary sample) & infectious Substance (if isolate)
<i>Coxiella burnetii</i>	Whole blood	PCR	Collect sample in purple or yellow-topped blood collection tube for PCR; for serology, use red top tube.	<ul style="list-style-type: none"> •Ship in ambient conditions; •Use current shipping guidelines for a diagnostic sample

<i>Francisella tularensis</i>	Isolated organism; whole blood; swabs of lesions.	DFAs PCR, other conventional microbiology tests	Contact the NCSLPH and the CDB at (919) 733-3419	<ul style="list-style-type: none"> •Agar slant in screw-capped leak-proof tube or blood; •Ship in ambient conditions; •Use current shipping guidelines for a diagnostic sample (if primary sample) & infectious Substance (if isolate)
<i>Yersinia pestis</i>	Isolated organism; whole blood; tissue; swabs of lesions	Culture, phage, DFAs, PCR, other conventional microbiology tests	Isolated 24-72 hr. culture of unknown Gram negative bacteria, where submitter is unable to rule-out <i>Y. pestis</i> . Use extreme caution. Subculture without aerosolization in BSC Class II or higher.	<ul style="list-style-type: none"> •Agar slant in screw-capped leak-proof tube or blood; •Ship in ambient conditions; •Use current shipping guidelines for a diagnostic sample (if primary sample) & infectious Substance (if isolate)
<i>Avian influenza</i>	Contact the NCSLPH and the CDB at (919) 733-3419	Not performed at NCSLPH. The SLPH will contact the CDC	Contact the NCSLPH and the CDB (919) 733-3419	
<i>SARS</i>	Contact the NCSLPH and the CDB at (919) 733-3419	Not performed at NCSLPH. The SLPH will contact the CDC	Contact the NCSLPH and the CDB at (919) 733-3419	
<i>Monkeypox</i>	Contact the NCSLPH and the CDB at (919) 733-3419	Not performed at NCSLPH. The SLPH will contact the CDC	Contact the NCSLPH and the CDB (919) 733-3419	
<i>Smallpox, Orthopox, Non-orthopox, & VZV</i>	Contact the NCSLPH and the CDB at (919) 733-3419	Culture & PCR; & Electron Microscopy if needed	Contact the NCSLPH and the CDB (919) 733-3419	
<i>Viral hemorrhagic viruses</i> Notify the NCSLPH immediately if viral hemorrhagic fever (VHF) is suspected	Contact the NCSLPH and the CDB at (919) 733-3419 10-12 cc of serum to CDC	NOT performed by the NC SLPH. The NCSLPH will contact the CDC.	Avoid manipulation of specimen material and DO NOT attempt culture of material. Collect serum for shipment to CDC. Store at < 4° C	<ul style="list-style-type: none"> •Immediately call the NCSLPH and CDB •Complete CDC DASH form # 50.34 •Ship serum to CDC •Ship on cold packs using current guidelines for transport of an infectious substance
<i>Ricin</i>	Not performed on clinical samples.			Detection of human metabolites for Ricin is performed by the NCSLPH Chemical Terrorism Lab

Table 3

FOOD

SAMPLE TYPE	COLLECTION & PRESERVATION	PACKAGING & SHIPPING
Solid food >50 grams	Cut or separate portions of food with sterile knife or other implement. Aseptically collect a representative sample; transfer to sealable plastic bag or other leak-proof sterile container and refrigerate until transport.	Label each food item; pack in an insulated container with cold packs and take to the NCSLPH as soon as possible.
Liquid food >50 mls	Stir or shake liquid to mix contents. Aseptically collect sample in a leak-proof sterile container and refrigerate until transport.	Label each food item; pack in an insulated container with cold packs and take to the NCSLPH as soon as possible.
Dehydrated food >50 grams	Aseptically collect a representative sample using a sterile implement. Transfer to a sealable plastic bag or other leak-proof sterile container and refrigerate until transport.	Label each food item; pack in an insulated container with cold packs and take to the NCSLPH as soon as possible.
Frozen food >50 grams	Chip food with a sterile implement. Aseptically collect a representative sample; transfer to sealable plastic bag or other leak-proof sterile container and refrigerate until transport.	Label each food item; pack in an insulated container with cold packs or dry ice and take to the NCSLPH as soon as possible.

Chemical Terrorism

(919) 807-8771

Introduction

The Chemical Terrorism lab (CT) is part of the Laboratory Preparedness Unit at the NC State Laboratory of Public Health and is an LRN level 2 laboratory. The CT lab serves as a surge capacity laboratory for other state CT labs and for the CDC in qualified methodology.

In the event of a chemical exposure, the NCSLPH laboratory will be able to provide instruction for and assistance with the proper collection, packaging and shipping of clinical specimens either to CDC, the NCSLPH or another state CT lab. The current menu provides analyses of clinical samples for heavy metals, cyanide, volatile organic compounds, tetramine, nerve agent metabolites, ricinine/abrine, metabolic toxins, and HNPA (metabolite for tetranitromethane). In the future, the menu will be expanded to provide the capability to analyze for acrylonitriles CVAA (lewisite) and azides. CT staff may be contacted 24/7 by:

Contact Number for CT: 919-807-8771
24 hour contact number: 919-602-2481

Sample Collection and Identification

Submit the following specimens for each patient:

1. Either three (3) 5 mL or 7 mL or four (4) 3.5 mL purple top tubes of blood. Mark the first one drawn with a "1" using indelible ink. This tube will be used to analyze for blood metals.
2. One (1) 3.5 mL, 5 mL, or 7 mL green or gray top tube of blood *and*
3. At least 25 mL of urine (freeze prior to shipping)
4. Blanks – Two (2) empty, unopened purple top tubes; two (2) empty unopened green or gray top tubes; and two (2) empty, unopened urine cups from each lot of containers used must also accompany the specimens to determine background contamination.

Pediatric patients should have only urine submitted unless otherwise instructed by a physician. All specimens submitted **must** have a chain of custody accompanying them to preserve the integrity of potential evidence because all acts of terrorism are a federal offense and are subject to litigation. Specimens must be evidence taped and initialed according to CDC guidelines. Proper evidence preservation is critical. The samples also must follow CDC protocol for collection, packaging and shipping. Detailed CDC protocols for sample collection, packaging and shipping can be found at the following link <http://www.bt.cdc.gov/chemical/lab.asp>

Shipment

Blood should be shipped in an insulated shipper with cold packs at 4° C. Urine samples should be frozen before shipping and shipped on dry ice. Group Patient samples together keeping purple together. Shipping must conform to IATA guidelines for packaging and shipping of diagnostic specimens category B by air.

Submission forms, chain of custody forms, and sample manifest forms are obtainable from the NCSLPH CT Lab website.

Reporting Procedure and Interpretation

Results are reported to the NCSLPH Laboratory Director, to CDC via the LRN, and to the submitter by phone or mail.

Microbiology

(919-733-7367)

The mission of the Microbiology Unit is to provide clinical and reference microbiological services to public and private laboratories in North Carolina. A wide variety of specimen types are examined. Many of the services performed here are available only at the NCSLPH and the [Centers for Disease Control and Prevention \(CDC\)](#) in Atlanta, GA.

The Microbiology Unit is organized into four labs:

- Bacteriology
- Mycobacteriology
- Mycology
- Parasitology

Anaerobic Bacteriology
(919) 733-7367

Laboratory services in anaerobic bacteriology are not available at the NCSLPH.

Botulism (*Clostridium botulinum*)

The NCSLPH does not perform botulism-related testing.

Cases of suspected botulism constitute a health emergency and are handled according to protocols of the Division of Epidemiology and the CDC. The patient's physician **MUST FIRST** contact the Communicable Disease Branch (CDB), Epidemiology Section of the Division of Public Health at (919) 733-3419. This telephone number provides assistance on a 24-hour basis and includes recorded instructions for after-hours emergencies.

An epidemiologist in this Section must discuss the case with the patient's physician. If botulism is a probable diagnosis, the State Epidemiologist will then contact the CDC to arrange shipment of botulism antitoxin to the patient's physician. Clinical specimens also may be forwarded to the CDC for culture or toxin testing. These test results may be delayed, although they can confirm the diagnosis.

Recommended specimens for botulism examination include fresh stool specimens (25g), serum (15 ml) and any implicated food items shipped refrigerated in an insulated container.

Botulism-related specimens may be submitted to the CDC only after approval by the CDB and the CDC. Instructions for shipping specimens will be provided at that time.

Bordetella Pertussis

(919) 733-7367

Introduction

Specimens for isolation or polymerase chain reaction (PCR) screening for *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 are recommended for *Bordetella* examination, since a carrier state in asymptomatic persons has not been demonstrated as an important source of transmission due to the lack of coughing symptoms. Reference cultures are accepted for confirmation of *Bordetella pertussis*, *B. parapertussis* and *B. bronchiseptica*. Consultation and bench training are provided upon request.

Specimen Collection and Identification

Nasopharyngeal swabs should be collected as soon as possible after onset of symptoms, and prior to antibiotic treatment. There is a greater likelihood of positive cultures and/or PCR in the first two weeks of symptomatic infection than during later weeks of illness. However, PCR may detect organisms for a prolonged period of time regardless of viability.

A mailer containing materials and instructions necessary for collecting and shipping nasopharyngeal specimens is available from the Laboratory Mailroom. Order online at <https://slphreporting.ncpublichealth.com/labportal>. Transport medium in the mailer has a shelf life of two months. **Notify the Microbiology Unit before submitting large numbers of specimens.** Regan-Lowe Transport Medium (RLTM) and the DNase Free microcentrifuge tube included in the mailer should be labeled with two identifiers: patient's name and either date of birth or Social Security number accompanied by a completed DHHS form # T806. **Please do not place adhesive labels on the microcentrifuge tube.** Unlabeled specimens will not be tested. Follow collection instructions included in the mailer. The following additional clinical information should be entered on the **back of the form**: nature of symptoms, date of onset, immunization history, contact with other cases of whooping cough, any antibiotic therapy prior to specimen collection and other pertinent information.

Note: Specimens received without the **submitter's return address** are subject to rejection!

Isolated organisms for identification should be subcultured to appropriate media and incubated until growth is apparent before shipping. Bordet-Gengou or Regan-Lowe Agar is recommended for *B. pertussis*; blood, chocolate or heart infusion agar is satisfactory for other bordetellae. Agar slants are preferred. Plates are discouraged, but if necessary, may be used if they are taped closed, sealed in leak-proof bags and securely packaged in a **crush-proof** container. Growth from culture plates also may be suspended in RLTM for shipment or used to prepare smears for DFA confirmation staining.

Shipment of Specimens

Specimens should be shipped as soon as possible after collection. Clinical specimens for pertussis culture should be shipped **refrigerated** in cold RLTM using frozen cold packs provided in the insulated mailer. Nasopharyngeal swabs may be held, if necessary, in refrigerated RLTM up to 2-3 days before shipping.

It is essential for culture specimens to be kept cold after collection and during transit to the Laboratory. Swabs for PCR inside a DNase Free microcentrifuge tube should be included with culture specimens in the return mailer.

Label "Pertussis" on the outside of the package. When shipping by U.S. mail, use first-class postage. Be sure to place **return address** on outside of container, regardless of shipping method.

Reporting Procedures and Interpretation

PCR tests are batched twice per week and positive results are telephoned to the submitter on the day of completion. Positive culture results will also be called to the submitter; negatives will be held for 7 days before reporting. Positive PCR and culture results are reported to the Women's and Children's Health Section, Division of Public Health, for surveillance purposes. **All results are available via the website, <http://slph.ncpublichealth.com>.**

PCR results are reported as presumptive while culture is considered the gold standard and is used for confirmation. However, culture can be less sensitive than PCR, since PCR is not dependent on viability and may detect fewer organisms present. Discrepant PCR and culture reports may occur. Low numbers of organisms may be detected by PCR but may be overgrown by normal flora or non-viable in culture. This PCR has been known to cross-react with *Bordetella holmesii*.

Cultures indicating growth consistent with *Bordetella*, are stained with the DFA conjugate to confirm. A rare *B. bronchiseptica* may cross-react with the DFA conjugate.

Both culture and PCR may fail to detect *B. pertussis*. Positive PCR are valuable for early diagnosis of pertussis but should be accompanied by culture since culture is the recommended diagnostic method. As the disease process may continue for weeks or months after viable organisms no longer remain in the nasopharynx, a negative culture does not rule out infection, especially if specimens were collected late in the course of illness. Organisms present in low numbers may be difficult to detect by either method. Prior antibiotic therapy, overgrowth of contaminants or failure to keep specimens cold after collection and during transit may result in a negative culture. Cultures performed at the local level using commercial agar plates may be negative due to insufficient moisture in the medium. Accuracy in both tests is dependent on correctly collected specimens.

Reports are returned only to the submitting agency; the submitter is responsible for sending copies to any other agency. Copies of reports are retained at the NCSLPH. The submitting agency is responsible for maintaining reports in the patient's file.

Cholera (*Vibrio cholerae*)
(919) 733-7367 or (919) 807-8606

Strains of *Vibrio cholerae* possessing the somatic O1 or O139 antigen (“*V. cholerae*:O1” or “*V.cholerae*: O139”) are associated with epidemic cholera, while those lacking this antigen (“*V. cholerae non-O1*”, “non-cholera vibrio”), cause sporadic diarrheal disease and do not present a public health threat. Although cholera is not endemic in the U.S., cases may be imported by travelers returning from countries where the disease is prevalent. Sporadic cases of non-cholera gastroenteritis are associated with salt water exposure or consumption of raw or insufficiently cooked contaminated seafood.

Please telephone the Enteric Lab before submitting stool or food specimens when cholera or other *Vibro*-associated diarrheal disease is suspected.

Submit refrigerated but not frozen food samples as quickly as possible after collection in an insulated container with a completed DHHS form #1814 (Food/Environmental Sample Collection Report).

Note: Direct reference isolates of *Vibrio* spp. to the Atypical Bacteriology Lab with a completed DHHS form #T806.

Isolates of *V. cholerae* are tested in the Atypical Bacteriology Lab at the SLPH for the presence of the O1 and O139 antigens; those presumptively identified as *V. cholerae* O1 or O139 are forwarded to the CDC for definitive identification and toxin testing. The Foodborne Disease Epidemiologist in the Communicable Disease Branch is notified of potential cholera cases. Confirmed isolates of non-*V.cholerae* are also sent to CDC and epidemiologically investigated

Diphtheria (*Corynebacterium diphtheriae*)

919-733-7367

Introduction

The diagnosis of Diphtheria is primarily a clinical one; a thorough evaluation of the patient history should be made before deciding to culture and submit to the NC State Laboratory of Public Health for analysis. Often the patient has thrush, which can mimic the signs of Diphtheria; therefore, it is recommended that a routine bacteriological culture be performed initially.

All confirmed cases of Diphtheria must be reported to the State Epidemiology Division at 919-733-3419.

Specimen collection and Identification

At the local level:

- Specimen collection - swabs of the nasopharynx, throat, wound or membranes.
- Transport – use Amies, Stuarts, or other readily available transport medium.
- Culture – set up on blood agar and, if available, on cystine blood tellurite (CBT) agar, and a Loeffler's slant for production of polar bodies. Incubate cultures at 35-37°C preferably in CO₂ for 18-24 hours and examine the plates for predominant coryneform-like colonies. On CBT agar, *C.diphtheriae* forms small, dark "gunmetal"-gray opaque colonies with a pronounced garlic odor. On blood and other plates, colony morphology is not distinctive.
- Verify morphology by gram stain and, if possible, by the Loeffler methylene blue stain. (Apply methylene blue stain for 30-60 seconds, rinse, dry, and examine slides for unusually pleomorphic, beaded rods with swollen ends and reddish-purple metachromatic granules.)
Note: Look for beta strep and yeast as well, to rule out these organisms as the pathogen.
Gram stain – *C.diphtheriae* is typically extremely pleomorphic. Cells may exhibit elongated and exaggerated "dumbbell" shapes that usually appear beaded or barred in the central area. (This morphology is exhibited best by methylene blue stain of organisms grown on serum-containing media such as Loeffler or Pai Egg Yolk agar).
- After the gram stain, either perform biochemical screening tests for identification, or subculture and forward to the NC State Laboratory of Public Health. If isolate appears to be *C.diphtheriae*, it is advisable to send to the NC State Laboratory for confirmation.

At the NC State Laboratory:

- Telephone the Atypical Bacteriology Laboratory at 919-733-7367 or 919-807-8793 **prior** to submitting diphtheria specimens. Please include the patient's clinical history when submitting suspected diphtheria specimens to the NCSLPH.

At the CDC:

- According to the CDC Web Site (February 2011): "CDC does not perform PCR to rule out diphtheria unless diphtheria anti-toxin (DAT) has been requested to treat the patient."
- Toxigenicity testing – available at the CDC – suspect isolates from a fresh pure culture may be sent on blood, or tryptic soy agar slants. Other readily available transport media may also be used. Isolates should be shipped at room temperature.
- All specimens sent to CDC must be accompanied by a CDC Form 50.34.
- NOTE: For confirmed cases, physicians can acquire anti-toxin directly from the CDC. The earlier this is given, the more favorable the outcome for the patient. Suspect cases can be discussed with the CDC by calling 404-639-8765.

Shipment of Specimens

Submit swab specimens in a swab transport system such as Culturette®. Alternatively, place swabs in a sterile screw-capped tube in a few drops of sterile broth or saline. Seal in plastic bag, cushion with paper towels, and place in a box or other closed container. Swabs inoculated onto Loeffler slants locally may be forwarded after overnight incubation.

Submit reference isolates preferably on Loeffler agar slants; infusion, blood trypticase, or chocolate is satisfactory. Package tightly capped slant (may also seal cap with Parafilm®) wrapped in paper towels inside a metal tube placed inside a second metal tube (Microbiology Reference Culture Container available from the NCSLPH mailroom 919-807-8575). Forward to the NCSLPH either by courier or mail with a Special Bacteriology form T806 (<http://slph.ncpublichealth.com/Forms/dhhs-t806.pdf>)

****Avoid shipping packages to arrive over the weekend****.

Reporting Results and Interpretations

Reports are returned only to the submitting agency; the submitter is responsible for sending copies to any other agency. The submitting agency is responsible for maintaining reports in the patient's file.

Enteric Bacteriology

(919) 733-7367

Introduction

Clinical specimens for the isolation of enteric microorganisms are accepted only from public health care providers. Fecal specimens are examined for the presence of enteric pathogens including *Salmonella typhi*, other *Salmonella* serotypes, *Shigella*, *Campylobacter*, *Yersinia*, *Escherichia coli* (*E. coli*) 0157:H7 and other STEC. Reference isolates are accepted from public and private health care providers for identification and/or serotyping. The SLPH is the North Carolina serotyping center for *Salmonella*, *Shigella* and *E. coli* 0157:H7 and participates in the national surveillance programs of the CDC.

Feces and food specimens associated with food-borne illness are screened for disease agents (see **Foodborne Illness**).

Consultation and bench training are provided upon request.

Please telephone the Enteric Bacteriology Lab to discuss outbreak-related specimens or to coordinate specimen handling in unusual circumstances. The Communicable Disease Control Nurse for your county should also be contacted.

Sample Collection and Identification

Each specimen must be clearly labeled with the patient's name and accompanied by DHHS form #3390. Unlabeled specimens will not be tested. Specimens should be collected early in the course of enteric disease and before antimicrobial therapy is begun. **Please indicate if the patient has bloody diarrhea** and if a specific disease agent is suspected.

A. Feces Specimens

Collect specimen so that feces is free of foreign matter, following instructions in Enteric Culture mailer or equivalent. (Do not use the Parasitology mailer: it contains formalin which kills bacteria.) Using the scoop, place feces in the vial of transport medium until the level of liquid reaches the fill line marked on the label. Do not overfill vial. Break up any large pieces with the scoop. Stir well; replace the top tightly on the vial. Label with patient's name.

B. Rectal Swabs (2) **Note: FECES SPECIMENS PREFERRED**

Collect specimens by inserting two sterile swabs into rectum (best results are obtained if fecal material is observed on swab), avoiding contact with skin of perianal area. Use Enteric Culture mailer or equivalent. Place swabs in the vial of transport medium and break or cut off ends so that swabs fit into vial. Label with two identifiers: patient's name and either date of birth or Social Security number.

- C. Blood Cultures
Following incubation and subculture, isolates may be forwarded for reference identification.
- D. Reference Cultures
Reference cultures for further identification should meet the following criteria for inclusion in the family Enterobacteriaceae: gram-negative non-sporeforming rods which grow aerobically and anaerobically, grow on MacConkey agar, ferment glucose, reduce nitrates, are oxidase negative, do not require NaCl and are catalase positive.

Use the Microbiology Reference mailer or equivalent to ship pure cultures. Agar slants are preferred. Plates are discouraged, but if necessary, may be used if they are taped closed, sealed in leak-proof bags and securely packaged in a **crush- proof** container. On the form, indicate preliminary test results or presumptive identification and patient clinical information.

Note: Reference cultures of nonfermentative gram- negative organisms as well as fermenters **NOT INCLUDED** in the family, Enterobacteriaceae (ex: *Pasteurella*, *Aeromonas*, *Actinobacillus*, *Vibrio*) should be directed to the Atypical Bacteriology Unit and should be accompanied by Special/Atypical Bacteriology DHHS form #T806.

Shipment

Mailers for submitting fecal specimens and reference cultures are available on-line at <https://slph.state.nc.us/labportal>. To submit specimens:

1. Write patient's name and other identifier on specimen tube. Unlabeled specimens will NOT be tested.
2. Place completed Enteric Bacteriology DHHS form #3390 (one form for each specimen) in outer container to avoid contamination in case of breakage or leakage.
3. Use double-walled or equivalent shipping containers that meet safety requirements. Multiple tubes or specimens should be wrapped individually in absorbent cushioning material and securely packaged in a leak-proof container. Agar slants are preferred. Plates are discouraged, but if necessary, may be used if they are taped closed, sealed in leak-proof bags and securely packaged in a **crush-proof** container. Mailers should be clearly labeled "Enteric Bacteriology" on the outside of the container.
4. Ship clinical specimens as soon as possible after collection. Refrigeration is recommended for Enteric Culture mailers, particularly specimens submitted for isolation of *E. coli* 0157:H7 and other STEC.

5. When shipping by U.S. mail, use first-class postage. Be sure to place return address on outside of container, regardless of shipping method.
6. Telephone the Enteric Bacteriology Lab before shipping large numbers of specimens, such as in an outbreak situation, or those requiring urgent attention.

Reporting Procedures and Interpretation

Negative culture results are reported within one to three work days after receipt of the specimen. Serotyping and biochemical identification results usually are reported within three to five work days. Final results on isolates referred to the CDC for further testing may be delayed up to several months.

A. *Salmonella*

Salmonella species are reported according to the following designations:

- *Salmonella typhi* -- includes only this agent of typhoid fever.
- *Salmonella choleraesuis* -- includes *S. choleraesuis* and *S. choleraesuis* bioserotype Kunzendorf.
- Other *Salmonella* serotypes -- all other serotypes are reported using the traditional designation (ex.: *S. typhimurium*, *S. heidelberg*, etc.) or by antigenic formula if monophasic, although taxonomically they are classified as bioserotypes of *S. enteritidis*.
- *Salmonella arizona* -- the organism formerly known as *Arizona hinshawii* has been reclassified as a bioserotype of *S. enteritidis*. These isolates are reported as *Salmonella arizona*.

Note: All species of *Salmonella* can cause enteric disease (salmonellosis).

B. *Shigella*

Species of the genus *Shigella* are reported as follows:

- *Shigella dysenteriae* or serotype A (12 subgroups)
- *Shigella flexneri* or serotype B (6 subgroups)
- *Shigella boydii* or serotype C (18 subgroups)
- *Shigella sonnei* or serotype D (2 subgroups)

Note: All species of *Shigella* can cause enteric disease (shigellosis).

C. *E. coli* 0157:H7/STEC

E. coli 0157:H7 (sorbitol negative) is associated with hemorrhagic colitis and Hemolytic Uremic Syndrome (HUS). Stool specimens should be collected in Enteric culture containers and should be refrigerated after collection and during **transport with freezer packs in an insulated container**. Indicate on the Enteric Bacteriology, DHHS form # 3390, that examination for *E. coli* 0156:H7 is requested. Please telephone the Enteric Bacteriology Lab at (919) 733-7367 prior to submitting specimens associated with outbreaks. Contact the

Epidemiology and Communicable Disease Section at (919) 733-3419 for epidemiological assistance.

Broths submitted for STEC testing must be shipped within 7 days on ice. Broths and non-0157 isolates will be tested for Shiga-toxin. If positive, we will look for the top 6 serotypes (O26, O103, O111, O121, O45, and O145). If sample is Shiga-toxin positive, but not one of the top 6, it will be sent to CDC for further testing.

Questions concerning epidemiological investigation of these illnesses should be directed to the General and Communicable Disease Control Branch (CDB) at (919) 733-3419.

D. Other Enterobacteriaceae

The SLPH reports all confirmed *Salmonella*, *Shigella*, *Campylobacter*, *Vibrio*, and *E. coli* 0157:H7 isolates to the Communicable Disease Control Branch of the Epidemiology Communicable Disease Section for surveillance purposes.

Results are reported on computer-generated forms which are returned to the submitting agency. Bacteriology DHHS form #3390 accompanying specimens are retained in the Unit for two years. Reports are returned only to the submitting agency; the submitter is responsible for sending copies to any other agency. The submitting agency is responsible for maintaining records in patient files.

Note: Local health departments should telephone the Communicable Disease Branch at (919) 733-3419 when enteric disease outbreaks are suspected in a day care center, nursing home or restaurant. In addition, the Food, Lodging and Institutional Sanitation Branch of the Division of Environmental Health should be notified at (919) 733-2905 when restaurant- or institution-associated illness is suspected.

Foodborne Illness

(919) 733-7367

Introduction

Food samples are examined for the presence of disease-producing bacteria only in cases of documented illness involving at least two persons. Consumer complaints, foods suspected of adulteration or those not associated with illness are referred to the Food and Drug Administration through the N.C. Department of Agriculture (919-733-7366). Food samples are accepted only when submitted through the local health department. The local health department should always be notified of suspected foodborne illness so that an epidemiological investigation can be conducted. Feces and other specimens relating to foodborne disease also are accepted. The Microbiology Unit should be alerted at (919) 733-7367 as soon as possible after illness is reported. Contact the Communicable Disease Branch (CDB) at (919) 733-3419 for assistance in investigating foodborne disease.

Sample Collection and Identification

Each food item should be clearly labeled; different batches should be individually identified. Environmental samples should be labeled as to individual source. Fecal or other specimens should be clearly labeled with the patient's name; requisition forms should indicate their association with foodborne illness.

A. Food and Related Environmental Samples

Collect food samples aseptically taking care not to touch the food items with the hands or non-sterile equipment. Samples should be placed in sterilized jars or sealable plastic bags and promptly refrigerated. Packaging and shipping methods should maintain the integrity of the food sample as closely as possible to its condition when sampled. Use a separate DHHS form #1814 for each food item; when submitting multiple samples at least one form should be completed with all requested information.

If botulism is suspected immediately contact the Communicable Disease Branch at (919) 733-3419.

B. Food Handlers

To culture potential carriers of *Staphylococcus*, carefully rub sterile swab over infected area, avoiding contact with adjacent skin, or swab anterior nasal membranes. Use DHHS form #T806, SPECIAL BACTERIOLOGY.

C. Fecal Specimens

See **Enteric Bacteriology**, for instructions for collecting specimens for bacteriological culture. See **Virus Culture**, for collecting specimens for viral culture.

Shipment

Place food samples in a waterproof container inside an insulated shipping container with cold packs (do not use wet ice) and send to the NCSLPH as quickly as possible after collection. Notify the Microbiology Unit of the expected arrival time. Outbreak-associated stool specimens may be shipped separately in Enteric Culture Mailers with DHHS form # 3390.

Reporting Procedures and Interpretation

Bacteriological examination of food requires one to seven work days, depending on the etiologic agent and the type of food processing involved. Foods are implicated as vehicles of disease transmission under one or more of the following circumstances:

- confirmation of the same pathogen or toxin in ill patients' specimens and in the epidemiologically implicated food
- confirmation of the presence of bacterial toxin in the food in the absence of patient clinical specimens
- confirmation of the presence of certain enteric pathogens such as *Salmonella* in the food
- food-specific attack rates significantly higher in persons who have consumed the food compared to those who have not

Note: Local health departments should notify the Communicable Disease Branch (919) 733-3419 when enteric disease outbreaks are suspected in a daycare center, nursing home or restaurant. Additionally, the Food, Lodging and Institutional Sanitation Branch of the Division of Environmental Health should be notified at (919) 733-2905 when foodborne illness is suspected in a restaurant or institution.

Collection and Shipment of Specimens for Foodborne Illness

Sample	Collection and Preservation	Packing and Shipping
Solid food > 25 grams	Cut or separate portions of food with sterile knife or other implement. Aseptically collect a representative sample; transfer to sealable plastic bag or sterile jar and refrigerate.	Label; pack in insulated container with cold packs. Seal forms in waterproof bag. Take or ship to the NCSLPH.
Liquid food > 25 grams	Stir or shake. Use sterile implement or pour representative sample into sterile container and refrigerate.	Same as above.
Dehydrated food > 25 grams	Use sterile implement to transfer representative sample to sterile jar or sealable plastic bag.	Same as above.
Environmental or equipment surface swab	Preferably use commercially available swab collection/transport system. Or moisten swab with sterile water, rub environmental or equipment surfaces and place swab in sterile jar, plastic tube or sealable plastic bag.	Same as above.
Frozen food >25 grams	Place frozen food in sterile jars or sealable plastic bag or use sterile implement to chip food and transfer chips to container.	Keep frozen if possible with dry ice or cold pack as listed above.

Note: Most local health departments maintain a supply of sputum mailers for tuberculosis testing. These mailers contain sterile screw-capped plastic centrifuge tubes which also are suitable containers for food samples or environmental swabs.

Legionella

(919) 733-7367

Introduction

Legionellosis is diagnosed by a combination of culture and direct fluorescent antibody (DFA) staining in conjunction with the patient's clinical history. Culture is the recommended diagnostic procedure. The Bacteriology Lab offers culture and DFA staining of clinical specimens and reference cultures to public and private health care providers. Urinary antigen detection and DNA probe procedures are NOT available in this Laboratory. Environmental specimens are not tested. Consultation and bench training are available upon request.

Sample Collection and Identification

Recommended specimens for culture include respiratory tract secretions, tissues, fluids such as sputum, pleural fluid, transtracheal aspirates, bronchial washings and lung biopsies. Saline is not recommended to collect or dilute specimens for *Legionella* culture as it may inhibit growth; use sterile broth or sterile distilled water. If saline must be used to collect specimens, it may be centrifuged and the pellet resuspended in sterile distilled water or broth.

Collect specimens aseptically and place in a sterile screw-capped plastic centrifuge tube (such as those in NCSLPH sputum mailers); seal containers securely to prevent leakage. Flexible "in-house" suction tube collection cups are not acceptable for shipping specimens. DFA smears should be air dried, heat fixed, and 10% formalin fixed before mailing or packaging.

Each specimen must be clearly labeled with two identifiers and accompanied by a completed DHHS form #T806. Two forms are required for paired specimens.

NOTE: Specimens received without the **submitter's return address** are subject to rejection!

Isolated cultures for identification of *Legionella* sp. should be grown on charcoal yeast extract agar slants or plates.

Shipment

Clinical specimens for *Legionella* culture should be shipped refrigerated with cold packs in insulated containers. Specimens that must be held longer than three days should be frozen and shipped with dry ice or cold packs in insulated containers. Identification forms should be enclosed in sealed plastic bags to prevent wetting or contamination. Formalinized smears should be shipped in rigid slide mailers to prevent crushing. Formalinized tissue for DFA staining should be shipped in screw-capped containers and should be labeled as formalinized specimens. Sputum mailers are available on line at <http://slph.ncpublichealth.com>

Reference cultures of *Legionella* should be shipped in the Microbiology Reference Culture mailer or equivalent container that meets safety requirements. Agar slants are preferred. Plates are discouraged, but if necessary, may be used if they are taped closed, sealed in leak-proof bags and securely packaged in a **crush-proof** container.

Ship specimens as soon as possible after collection. When shipping by U.S. Mail, use first-class postage. Be sure to place return address on outside of container, regardless of shipping method, and plainly label "*Legionella*" on the outside of the container. Prior to shipping large numbers of specimens, telephone the Microbiology Unit at (919) 733-7367.

Reporting Procedures and Interpretation

Legionella is identified from culture and smears by specific DFA staining. At least 33 species of legionellae have been described; approximately half of human infections are associated with *L. pneumophila* serogroup 1. This Laboratory examines smears for *L. pneumophila* serogroups 1-14 and for 25 other species. DFA staining is a presumptive test. Cross-reactivity may occur among legionellae.

Neither a negative DFA stain nor a negative culture rules out *Legionella* infection. Low numbers of organisms, improper specimen/smear handling and/or previous antimicrobial therapy can influence test results.

Legionella isolates requiring definitive identification are forwarded to the CDC.

Smears are reported according to the number of strongly fluorescing cells with typical morphology seen. The CDC criteria for reporting the results of DFA staining are as follows:

- Smears from lung tissue: 25 or more organisms per smear = DFA positive
- Smears from other respiratory specimens: five or more organisms per smear = DFA positive

If the number of fluorescing cells seen is fewer than the minimum needed for a positive DFA report, the number of cells seen is reported. The results of serologic and culture tests along with the patient's clinical history may be useful in interpreting the DFA stain report.

Results of DFA examinations are available on the day of testing or the next work day, and can be accessed via the secure web page for results at <https://slphreporting.ncpublichealth.com/lms> and are followed by telephone and by mail as soon as growth is identified. Cultures are held for three weeks before being reported as negative.

All positive DFA results are reported to Epidemiology.

Reports are returned only to the submitting agency; the submitter is responsible for sending copies to any other agency. The submitting agency is responsible for maintaining reports in the patient's file.

Mycobacteriology

(919) 733-7367 or (919) 807-8620

Introduction

Specimens for isolation and identification of all *Mycobacteria* species (including *Mycobacterium tuberculosis* complex and other nontuberculous mycobacteria) are accepted from public and private health care providers. Positive isolations or identifications of *M. tuberculosis* must be reported by the submitter to the NC Tuberculosis Control program and Communicable Disease Branch (CDB) in accordance with State Law. (Refer to NC TB Policy Manual for guidance)

Sputum and specimens from other sources are concentrated and stained with fluorochrome and/or Kinyoun's stain, and are cultured for the isolation and identification of mycobacteria. No smear report will be generated for blood or bone marrows, as they are not appropriate samples for staining. They will be set up for culture and identification.

Real-time polymerase chain reaction (PCR) testing for *Mycobacterium tuberculosis* (TB) complex on selected samples is offered (see *Appendix A, Mycobacteriology* for additional information). Real time PCR is designed to supplement, not replace, standard mycobacterial culture for confirmation of diagnosis and the test is not suitable for all specimens. It is performed on undigested primary clinical samples.

Species identification is accomplished using high-pressure liquid chromatography (HPLC), nucleic acid probe tests, and/or routine biochemical characterization. Reference specimens for confirmation, identification, and/or susceptibility testing are also accepted. Consultation and bench training are provided upon request.

All isolates of *Mycobacterium tuberculosis* complex are tested for susceptibility to five primary drugs: streptomycin, isoniazid, ethambutol, rifampin and pyrazinamide. Additional susceptibility testing for *M.kansasii*, *M. marinum* and resistant strains of *M. tuberculosis* complex are available.

Specimen Collection and Identification

CDC recommends samples are received by the laboratory within 24 hours of collection. Due to shipment delays, some specimens may exceed this timeline. However, any specimen received more than 7 days after collection will not be testing. In order to ensure quality results, specimen containers must be labeled with two identifiers: the patient's first and last name and date of birth or SSN. This information must match the requisition form. A local medical record number may also be used on the specimen and requisition for one of the identifiers. Any specimens without two unique identifiers will be rejected and discarded. The following data items are essential to our computerized record handling system: patient name, date of birth or SSN or internal medical record number, submitter Federal Tax Number, Medicaid number, if eligible, submitter return address and phone number, county code, specimen collection date (CLIA requirement)

and specimen source. Without these data, specimen records cannot be entered in the computer, nor can a report of results be printed. Other data are required for follow-up and for statistical purposes. For more information, contact the Microbiology Lab at (919) 733-7367 or refer to the NC TB Control Policy Manual.

A. Sputum

A series of three specimens is recommended. Collect in the early morning on consecutive days. A volume of 1 to 5 mL is adequate for each specimen. Induced (or nebulized) sputum specimens are usually very watery, and unless indicated on the requisition form, may be mistaken for saliva, which is an inappropriate specimen. Sputum swabs are unsatisfactory. Do not use any transport medium. Use Sputum Mailer or equivalent that meets safety requirements.

B. Bronchoalveolar Lavage Fluids and Bronchial Washings

Collect at least 5 mL in a sterile container. Avoid contaminating bronchoscope with tap water. Saprophytic mycobacteria may produce false-positive culture or smear results. Frequently, bronchoscopy causes the patient to produce sputum naturally for several days after the procedure, and specimens collected a day or two after bronchoscopy enhance detection of mycobacteria. Do not use any transport medium. Use Sputum Mailer or equivalent that meets safety requirements.

C. Gastric lavage

Collect 5 to 10 mL of fluid in a sterile container without a preservative, either early in the morning or eight hours after eating or drug therapy. A series of three specimens is recommended. Neutralize as soon as possible with 100 mg of sodium carbonate powder (Na_2CO_3). Do not use any transport medium. Use Sputum Mailer or equivalent that meets safety requirements.

D. Tissue

Collect 1g of tissue, if possible, aseptically. Select a caseous portion, if available. Do not immerse the specimen in saline (or other fluid) or wrap in gauze. Freezing decreases yield. A sterile container with a small amount of sterile water or sterile saline (to keep the specimen moist) is acceptable. Do not use any transport medium, preservative or fixative. Use Sputum Mailer or equivalent that meets safety requirements.

E. Urine

Collect catheterized or mid-stream urine voided in early morning. A minimum of 40mL is recommended. Submit a series of three specimens, taken on three different days. Twenty-four hour cumulative specimens are unsatisfactory. Do not use any transport medium. Use Sputum Mailer or equivalent that meets safety requirements.

- F. **Blood and Bone Marrow**
Collect 5-10 mL for blood and as much as possible for bone marrow in a sterile tube containing heparin (green top) or sodium polyanetholsulfonate (SPS-yellow top). Blood collected in EDTA or blood that is coagulated is not acceptable. Use Sputum Mailer or equivalent that meets safety requirements.
- G. **Stools**
NCSLPH will only accept stools for suspected gastrointestinal tuberculosis (GI-TB). Prior approval from the Mycobacteriology laboratory is required for all stool samples. Any stool specimen received without prior approval will be rejected and discarded. Stool is not a recommended specimen for identification of disseminated MAC and has a poor recovery rate. Therefore, we will only accept stools from patients with highly suspect GI-TB.
- H. **Body Fluids (CSF, pleural, peritoneal, pericardial, etc.)**
Collect aseptically following proper procedure for type of specimen; collect as much as possible (10-15 mL minimum) in a sterile container. The recommendation for CSF is at least 2 mL. Bloody specimens may be anticoagulated with SPS or heparin. Please phone prior to submission if you have any questions. Do not use any transport medium. Use Sputum Mailer or equivalent that meets safety requirements.
- I. **Abscess Contents, Aspirated Fluid, Skin Lesions, Wounds**
Aspirate as much material as possible into a syringe with a luer tip cap. If the volume is insufficient for aspiration by syringe, collect the specimen on a swab and place in transport medium (Amies or Stuart's). For cutaneous lesions, aspirate material from under the margin of the lesion. Dry swabs are not acceptable. Use Sputum Mailer or equivalent that meets safety requirements.
- J. **Reference Specimens**
Select organisms or subcultures which show good growth and appear in **pure culture**. NCSLPH will no longer accept mixed or contaminated reference cultures. If a laboratory is unable to isolate the colonies, contact the NCSLPH for guidance (919-807-8620). Any culture received that is mixed with yeast or other bacteria will be rejected. Label the media with two identifiers and wrap carefully, securing screw cap. If liquid media is used, pack with enough absorbent material to absorb the entire contents in case of breakage or leakage. Do not seal cap with paraffin, as it may contaminate culture and interfere with processing. Do not wrap DHHS form #1247 around culture tube, but place in outer container of culture mailer. Use Microbiology Culture Mailer or equivalent that meets safety requirements.

Shipment

Specimens should be submitted in double-walled mailing containers. Glass tubes should be wrapped in absorbent cushioning material before they are inserted in mailing

containers. Mailers for submitting clinical specimens and for reference cultures are available from the Laboratory mail room. To facilitate safe handling, the following general suggestions are made:

1. Label specimen with two identifiers: patient's first and last name and either date of birth, Social Security number, or internal medical record number. The same identifiers must be included on the requisition. Unlabeled specimens will not be tested; they will be discarded.
2. Screw caps on tubes tightly. This is especially important with the plastic-capped centrifuge tubes in the Sputum Mailer. These plastic caps must be turned to the point of total resistance to prevent leakage. If caps are sufficiently tight, sealing with separate material, such as tape (never use paraffin, as it interferes with processing) will not be necessary. If tube appears to be leaking after cap is tightened, transfer to another tube. For safety reasons, leaking and broken specimens will not be tested.
3. Place properly completed DHHS form #1247, for each specimen or isolate in the outer container to avoid contamination in case of breakage or leakage. Screw cap on properly. Do not use any kind of tape to secure cap.
4. When shipping by U.S. mail; use first-class postage and place return address of submitting agency on the outside of the container. Do not write any patient information on the outside of the container.
5. Do not put any patient information on the outside of the containers.
6. Mail specimens as soon as possible after collection to avoid overgrowth of possible contaminants. CDC recommends specimens be sent to the lab within 24 hours of collection. Refrigerate if mailing is delayed longer than a few hours. Any specimen received more than 7 days after collection will not be tested.
7. Do not submit subcultures until good growth occurs. Do not send mixed or contaminated cultures. **Use the Orange Labeled cans for TB/Mycrobacteriology Specimens for shipment. These are available from the NCSLPH mailroom and may be ordered on-line at <http://slph.ncpublichealth.com>.**

Reporting Procedures and Interpretation

Results are reported on computer-generated forms and returned to the submitting agency. Results are also available on the NCSLPH website, <https://slph.ncpublichealth.com>. Duplicate reports for appropriate notification of results

are the responsibility of the agency submitting the specimen. Refer to the NC TB Policy Manual for reporting regulations.

A. Microscopic (Smear) Report

A smear of the concentrated clinical specimen is examined and reported within 24 hours of receipt in the laboratory. Smears are not performed on blood or bone marrow specimens. Reports may be as follows:

Found -- indicates the presence of acid-fast organisms in the smear.

Per Field -- indicates the approximate number of acid-fast organisms seen per microscopic field.

Not Found -- indicates the absence of acid-fast organisms in the smear.

B. Real-time PCR (PCR)

- a. AFB smear positive sample – Real time PCR will be performed on the first AFB smear positive sample for each patient.
 - i. If the PCR result is positive on any AFB smear positive sample, PCR will not be performed on subsequent samples. The sample, however, will be cultured and TB isolates tested for drug susceptibility as usual.
 - ii. If the PCR result is negative on any AFB smear positive sample, then testing for inhibitors will be performed.
- b. AFB smear negative samples- PCR will only be performed on patients who are at an increased risk of tuberculosis and who demonstrate signs and symptoms consistent with pulmonary TB (see *Appendix A Mycobacteriology*). The Mycobacteriology lab will determine which samples qualify to be tested.
 - i. If the PCR test is positive on any AFB smear-negative sample, up to two more samples will be tested.
 - ii. If the PCR test is negative on all three AFB smear-negative samples, then the AFB culture is the final definitive result.

C. Culture Report

Cultures are incubated for a maximum of six weeks. If growth occurs, organisms are identified, if possible, by high pressure liquid chromatography or nucleic acid probes. Identification of some organisms may necessitate subculturing for biochemical tests or susceptibility testing which may require up to several additional weeks.

A report of "no growth" indicates that no acid-fast organisms have grown by the end of six weeks. If "growth resembling mycobacteria" is observed, identification testing is performed as quickly as possible. If there is overgrowth of other

bacteria, the specimen is reported "Contaminated". Reports of "no growth" require six weeks from receipt of the specimen.

Final identification reports (including susceptibility results, where appropriate) may require three to twelve weeks for all tests to be completed. If isolates are submitted to the CDC for further testing or confirmation, additional time will be required.

D. Drug Susceptibility Tests

Indirect drug susceptibility tests for *M. tuberculosis* complex are performed using five first-line drugs. Second-line drugs are tested when resistance is seen on first-line testing. Selected drug screenings are performed for clinically significant isolates of *M. kansasii* and *M. marinum*.

E. High Pressure Liquid Chromatography (HPLC)

HPLC is currently our standard method of identification. This test is correlated with morphological data and other identification methods (DNA probes and/or biochemical testing) when necessary.

Appendix A, Mycobacteriology

Real-time PCR (PCR) will be performed on undigested primary clinical samples only.

1. PCR will be performed on the first AFB smear-positive sample for each patient.
2. PCR will be performed on smear-negative samples from patients who are at increased risk of tuberculosis and who demonstrate signs or symptoms consistent with pulmonary TB. **No more than three (3) smear negative samples will be tested per patient.**

The NCSLPH Mycobacteriology Laboratory will determine which specimens qualify for testing using the criteria outlined below. It is imperative that all fields on the Mycobacteriology (TB) submission form, DHHS 1247, are completed accurately, and includes all information specifically related to:

1. "Previously Diagnosed"
2. "Current Condition/Pertinent Date"
3. "Drug Therapy", *and*
4. "Source of Specimen"

If tuberculosis is suspected, indicate on the Mycobacteriology (TB) submission form, DHHS 1247, which signs or symptoms are present and which risk factors apply to the patient. **If this information is not supplied, RT-PCR will not be run if the sample is AFB smear-negative.**

Signs/Symptoms

(At least 2 must be present)

Cough
Fever, chills or night sweats
Significant weight loss
Hemoptysis

Risk Factors

HIV infection
Cough present for more than 2 weeks
Immigrant from high-incident country
Immunosuppressive medications (includes TNF alpha inhibitors)
Contact with known TB case in the last 2 years
Leukemia, lymphoma, or cancer of the head and neck or lung
Diabetes mellitus
Silicosis
Gastrectomy or jejunioileal bypass
Injection drug use

Also, include the following information:

1. Is patient in respiration isolation?
2. Is patient currently on TB medication? If so, which drugs and for how long?
3. Previously diagnoses
 - a. TB – date: _____
 - b. Other mycobacterium – Which: _____ When: _____

The following disclaimer for PCR tests will be included on all laboratory reports generated by the NCSLPH:

****Disclaimer: This test is not cleared by the U.S. Food and Drug Administration. It has been validated for use by the NC State Laboratory of Public Health, Mycobacteriology Lab, as preliminary identification of *M. tuberculosis* complex from primary clinical samples. Identification should not be based solely on the test results, but must be confirmed by colony morphology.**

Mycology

(919) 733-7367

Introduction

Specimens for isolation and identification of medically important fungi from body tissues and fluids are accepted from public and private health care providers. Reference cultures are also accepted for identification of yeasts, molds, and aerobic actinomycetes. Clinical specimens for "fungal" culture should be limited to those actually implicated in fungal disease. Antimicrobial susceptibility testing is not performed in this laboratory. Consultation and bench training in mycology are provided upon request.

Sample Collection and Identification

Specimens should be inoculated to isolation media within 24 hours of collection. Viability of most fungal pathogens decreases significantly with delay in processing specimens; for example, viability of *Histoplasma capsulatum* is lost after 24 hours regardless of how the specimen is handled. For this reason, it is preferable to initiate primary isolation at the local level. It is not recommended, however, that primary isolation of systemic fungi be attempted without using a biological safety cabinet for specimen processing. Appropriate culture media are available commercially; consult reference manuals for recommended isolation methods.

Blood, bone marrow, spinal fluid, biopsy material, aspirates, and other clinical specimens should be collected aseptically. Sputum for fungus culture should be an early morning specimen collected after rinsing the mouth with water. Bronchial washings and brushings and other body fluids should be submitted in the centrifuge tubes found in the sputum mailer for TB. Tissue from fungal lesions should be obtained from the center and the wall of the lesion. Skin, hair and nail clinical samples are no longer accepted.

Label specimen with patient's name, date of birth or Social Security number, or the local laboratory number. Unlabeled specimens will not be tested. It is particularly important that pertinent clinical information be sent with each specimen since it is used in selecting appropriate isolation procedures. For safety reasons, please do not submit a single clinical specimen for primary isolation of both fungi and *Mycobacterium tuberculosis*; however, please indicate if tuberculosis is suspected in addition to fungal disease.

Place properly completed identification DHHS form #2010 (one form for each specimen) in the outer container of the shipping packaged to avoid contamination in case of breakage or leakage. Place caps on tightly and secure with tape to avoid leakage. Leaking specimens constitute a biological hazard and may not be tested.

To submit reference cultures, isolated pure colonies from primary culture media should be subcultured to fresh media slants, and incubated until visible growth appears before

shipment. If necessary, initial cultures believed to be clinically significant may be submitted on primary isolation slants. **Culture plates should not be submitted.** Each specimen should be clearly labeled with two identifiers and accompanied by DHHS form #2010. **Note: Specimens received without the submitter's return address are subject to rejection!**

Shipment

Always use double-walled shipping containers, or equivalents that meet safety and current USPS shipping requirements. Several types are available from the Laboratory Mailroom. Multiple tubes or specimens should be wrapped individually in absorbent cushioning material and securely packaged in a leak-proof container. Mailers or packages not supplied by the State Laboratory should have "Mycology" plainly marked on the outside of the package. This ensures that packages and mail will be delivered directly to the Mycology Unit, eliminating needless and possibly hazardous exposure of non-technical staff.

Ship specimens as soon as possible after collection. Use first-class postage on U.S. mail. Be sure to place return address on the outside of the container, regardless of shipping method. When large numbers of specimens, unusual specimens, or potentially hazardous specimens are being submitted, telephone the Microbiology Unit at (919) 733-7367 prior to shipping.

Reference cultures may be submitted on any appropriate fungal culture medium slants after growth is visible. Use Microbiology Reference Culture mailer or equivalent for shipping. Please telephone the Microbiology Unit before mailing clinical material or cultures of *Histoplasma capsulatum*, *Blastomyces dermatitidis*, or *Coccidioides immitis*. **Known cultures of these organisms must be shipped according to Federal Regulations for Diagnostic or Infectious substances. Be aware: *Coccidioides immitis* is considered a Select Agent and shipment must comply with Federal Regulations.**

Reporting Procedures and Interpretation

Yeasts and some other fungi may be identified and reported within three to ten working days, while others may require longer time. Cultures are held four weeks before being reported as negative. Preliminary reports are sent out on all clinical specimens.

Most medically important fungi are identified to the species level (e.g., *Microsporium gypseum*, *Trichophyton mentagrophytes*). Genus and species designations are those consistent with designations in the Manual of Clinical Microbiology. Most saprophytic fungi are identified to genus level only.

Computer generated final reports are returned to the submitting agency only; therefore, the submitter is responsible for sending copies and/or making reports to any other agency. The submitting agency is responsible for maintaining reports in the patient's file.

Results are also available via the website, <http://slph.ncpublichealth.com>.

Collection and Shipment of Mycology Specimens

Specimen	Collection	Isolation Medium and/or Container*
<i>Subcutaneous and systemic mycoses</i>		
Blood	Aseptic, blood culture venipuncture. Collect with heparin anticoagulant.	Sabouraud Agar or other isolation medium.
Bone marrow	Collect aseptically	Same as above or sterile container or TB mailer.
Bronchial washings and aspirates	Collect through bronchoscopy procedure	Same as above.
Pus or exudates	Aspirate with sterile syringe	Same as above.
Spinal fluid	Routine spinal tap	Same as above.
Sputum, early morning	Allow patient to cough up and discard drainage accumulated during the night, then collect specimen in sterile container. May be obtained following inhalation of saline aerosol.	Same as above.
<i>Yeast Infections</i>	Collect as for bacteriological specimens, using aseptic technique.	Isolate on Sabouraud Agar or submit in TB sputum mailer.
<i>Reference Cultures</i>		
All except <i>Nocardia</i> sp.	Select and subculture colonies from isolation medium which show good growth and are in pure culture. Incubate until growth appears.	Sabouraud Agar slant or other fungus isolation medium.
<i>Nocardia</i> sp.	Select pure colony, as above. Incubate until growth appears	Sabouraud agar, LJ, 7H10 or 7H11 agar.

* Reference cultures should be mailed in Microbiology Reference Culture Mailer or equivalent.

Neisseria Gonorrhoeae

(919) 733-7367

Introduction

Clinical specimens such as cervical, rectal or throat swabs are not accepted by the NCSLPH for primary isolation of *Neisseria gonorrhoeae* (GC). Primary culture is available through the local health department Sexually Transmitted Disease (HIV/STD) Program. Reference cultures are accepted from public and private health care providers for confirmation. Cultures may be forwarded to the CDC for antimicrobial susceptibility studies in special circumstances.

Suspected cultures of GC should be confirmed in the following instances: 1) cultures from anatomic sources other than urogenital sites in symptomatic patients, 2) rectal cultures in homosexual males, 3) cases involving children, 4) any other legal case, or 5) if there is any question regarding the local laboratory's interpretation of biochemical or microscopic test results. Since penicillin is no longer recommended therapy for gonococcal infections, penicillin resistance testing is unnecessary at the local or State level.

Sample Collection and Identification

To submit reference cultures transfer a **well-isolated** colony from the primary isolation plate to a fresh JEMBEC plate (see also SHIPMENT OF SPECIMENS, below). Martin-Lewis, Thayer-Martin, GC-Lect® and Chocolate agar slants or plates are also satisfactory. Isolates of *Neisseria* other than gonococci may be submitted on blood, chocolate or infusion agar.

Clearly label each specimen with the patient's name and either date of birth or Social Security number; submit with a Special Bacteriology DHHS form #T806. Unlabeled specimens will not be tested. Please indicate if a specimen is a legal case.

Note: Specimens received without the submitter's return address are subject to rejection!

Shipment of Specimens

Cultures should be incubated overnight or until growth is visible before shipment. Slant cultures should be overlaid with sterile broth (such as infusion broth) to within one inch of the top of the tube, sealed with tape and placed in a leakproof container before shipping to help preserve organism viability. The submitting laboratory should maintain an additional culture in the event the isolate does not survive shipment. Do not ship on Fridays or holiday week-ends.

JEMBEC plates or commercially available media for gonococci are suitable for submitting cultures to the NCSLPH for confirmation. Health department STD clinics may obtain mailers for JEMBEC plates from the NCSLPH, Laboratory Improvement by

telephoning (919) 733-7186. Cultures must be maintained in a CO₂ atmosphere during shipment. Plate cultures in a CO₂ environmental transport system should be cushioned against breakage. Use Microbiology Reference Culture mailer for isolates submitted on tubed media; use double-walled or equivalent containers that meet safety requirements.

Place completed identification DHHS form #T806 in the outer container, or in a sealed plastic bag to prevent wetting and contamination in case of leakage. Multiple specimens should be wrapped individually in absorbent cushioning material and securely packaged in a leak-proof container, crush-proof container.

Plainly label "*Neisseria gonorrhoeae*," or "GC culture" and "DO NOT REFRIGERATE" on the outside of the package, and address to "Atypical Bacteriology". Every effort should be made to protect cultures from temperature extremes during shipment; cultures should never be refrigerated. Shipment should be timed so that cultures do not arrive on Fridays or weekends.

Ship specimens as soon as possible after collection. When shipping by U.S. Mail, use first-class postage. Be sure to place return address on outside of container, regardless of shipping method. Label "Atypical" on the outside of the package. Telephone the Microbiology Unit prior to shipping large numbers of specimens or those requiring urgent attention.

Reporting Procedures and Interpretation

Gonococcal cultures are reported as "*Neisseria gonorrhoeae* confirmed." Non-gonococcal neisseriae are reported as "*No Neisseria gonorrhoeae* isolated" or may be identified to the genus or species level as appropriate. Results usually are reported within 3-4 working days unless difficulty is encountered in growing the organism or isolating it from a mixed culture.

Reports are returned only to the submitting agency; the submitter is responsible for sending copies to any other agency. The submitting agency is responsible for maintaining reports in the patient's file.

Parasitology

(919) 733-7367

Introduction

Diagnostic specimens for examination for the presence of human parasites are accepted from public health care providers only, and only from symptomatic patients. Reference specimens for confirmation of parasite identity or further identification are accepted from all laboratories.

Feces and other specimens are examined for eggs, cysts and larvae of the intestinal parasitic worms and protozoa. Specimens are also accepted for examination in less frequently encountered parasitic infections, such as blood smears for parasitic blood diseases. Reference specimens preserved in polyvinyl alcohol (PVA) fixative or stained slides prepared from preserved material are also accepted.

Arthropods are referred to the Entomology Department at NC State University through the Insect and Plant Disease Clinic (919-515-9530) for identification for a fee of \$30. Submitter should contact the clinic directly to arrange.

Testing for *Cryptosporidium* and *Cyclospora* are offered upon request; testing for *Microsporidium* is NOT available at this time.

Specimen Collection and Identification

Clearly label each specimen with patient's name and either date of birth or Social Security number and fill out DHHS form #1245 completely. Unlabeled specimens will NOT be examined.

A. Intestinal Parasites

Fecal Specimens: Collect specimen following instructions in the Parasitology mailer supplied by this Laboratory, or in any commercially available parasite collection kit containing 10% formalin as a preservative. Do not contaminate with dirt, urine or paper. Place feces in a vial of 10% formalin, such as provided in the kits available from the NCSLPH Mailroom. Break up any large pieces by shaking or stirring well. **Do Not Overfill.** Place caps on securely to avoid leakage. Leaking specimens constitute a biological hazard and will not be tested. Label tube with two identifiers. Three specimens collected on alternate days are recommended, e.g., Monday, Wednesday and Friday. If three (3) specimens are collected, mail all three at the same time.

PVA Preserved Fecal Specimens: Please consult the Microbiology Unit for additional information. Collect feces in clean container. Avoid contamination with urine. Add feces in an amount up to the "fill line" in a small bottle of PVA fixative. Break up large pieces and stir well. Label bottle with patient's name.

Transparent Tape Slides for Pinworms: Tear off a piece of tape about 2 inches long. **DO NOT** use frosted or "magic" tape. Frosted tape is not transparent and cannot be read with the microscope. Fold tape over the end of the finger or a tongue depressor with the sticky side out. Do not let the tape wrinkle. Spread the patient's buttocks to expose anus. (Preferably take the specimen immediately after waking. Do not clean anal area before taking specimen.) Press sticky side of tape gently to anus two or three times. Lay tape smoothly on a clean glass slide, sticky side down. Press gently to slide using a piece of tissue or gauze. Cut off the tape that overhangs the slide. Label slide with patient's name. Place slide in plastic or styrofoam or other rigid container for mailing. Do not use envelopes for mailing glass slides as they are likely to break in transit. **WASH HANDS IMMEDIATELY.**

Other Clinical Materials: Collect specimen aseptically following proper procedure for type of specimen. Place in sterile container; label with two identifiers.

Whole Worms or Proglottids: Whole worms should be preserved in 70% alcohol, if possible. Place in plastic or glass container; label with patient's name. Proglottids may be preserved in 10% formalin or placed in saline or 70% alcohol. Parasitology mailer may be used if it is large enough, as it contains 10% formalin.

B. Blood and Tissue Parasites

Blood Smears for Malaria and Other Blood Parasites: Collect blood from a finger stick or venipuncture. Prepare two thin smears and two thick smears. A thin smear is made using less than a drop of blood and is "feathered" with the feather near the center of the glass slide. The thick smear is simply a drop of blood placed in the center of the glass slide with slight spreading. Smears should be allowed to air dry. Do not use fixative. Label the slides with the patient's name using a diamond tipped glass marker or with a pencil if using frosted end slides.

Corneal Scrapings for Acanthamoebae: Contact the Microbiology Unit at least 24 hours prior to taking specimen.

Shipment of Specimens

Always use triple packaging that meets DOT and USPS requirements. Mailers for submitting formalin-preserved specimens are available on-line at <http://slph.ncpublichealth.com>.

Multiple tubes or specimens should be packaged individually in leak-proof containers so as not to contaminate the requisitions. Mailers or packages should have "Parasitology" plainly marked on the outside of the package. This ensures that packages and mail will be delivered directly to the proper unit, and eliminates needless and possibly hazardous exposure of non-technical staff, as well as lost or delayed samples. To facilitate handling, the following general suggestions are made:

1. Write patient's name on specimen tube or slide. Unlabeled specimens will NOT be tested.
2. Place sealed primary container (specimen tube) inside secondary container (metal silver can) with absorbent material. Seal.
3. Place properly completed identification DHHS form #1245 around sealed secondary container to avoid contamination in case of breakage or leakage.
4. Place secondary container into outer mailing container. Please place return address on mailing container.
6. When using U.S. Mail, use first-class postage, and place return address on the outside of the container.
7. When unusually large numbers of specimens are anticipated (as an outbreak), the Microbiology Unit should be alerted by telephone at (919) 733-7367 so that preparations may be made.

Reporting Procedures and Interpretation

Specimen results are usually reported within two to three days of receipt. Reference specimens submitted to the CDC may require several weeks for analysis.

A report of *Entamoeba coli* is not to be confused with *E. histolytica*. *E. coli* is a non-pathogenic commensal amoeba often found in the human gastrointestinal tract, and is reported only as an indication of unsanitary conditions relating to the patient, such as poor personal hygiene.

Reports of "no parasites found" in water samples for *G. intestinalis (lamblia)* are not significant due to the low recovery rate of the cysts from water and the unavailability of a satisfactory test for use on water samples.

Reports are returned to the submitting agency only; therefore, the submitter is responsible for sending copies and/or making reports to any other agency. The submitting agency is responsible for maintaining reports in the patient's file.

Special and Atypical Bacteriology

(919) 733-7367

Introduction

Special Bacteriology

The Special Bacteriology lab serves primarily as a referral laboratory for bacteria that are unusual or difficult to identify. In this context, “Special Bacteriology” refers to the examination of a variety of microorganisms including the following: *Bordetella*, *Legionella*, and gram positive cocci. Certain clinical specimens are accepted for primary isolation; otherwise, pure isolates are required for identification or serotyping. Specimens are accepted from public and private health care providers. Cultures from animal or environmental sources must be associated with human illness. Anaerobic culture and antimicrobial susceptibility testing are not performed in this laboratory. Consultation and bench training are provided upon request.

Services available in the Special Bacteriology lab include:

- PCR testing for *Bordetella pertussis*
- culture and DFA staining for *Bordetella pertussis* and *B. parapertussis*
- culture and DFA staining for *Legionella*
- grouping of beta hemolytic streptococci and identification of clinically significant isolates of other gram-positive cocci

Vancomycin Intermediate and Vancomycin Resistant *Staph aureus* (VISA/VRSA):

–These isolates should be sent to the NCSLPH for minimum inhibitory concentrations (MICs) and resistant organisms will then be sent to the CDC for final confirmation. VISA and VRSA are reportable to both the CDC and the state of North Carolina through the Communicable Disease Branch at 919-733-3419. Subculture and save a copy of the isolate in-house.

***Streptococcus pneumoniae* (Pneumococcus) typing:** Unless there is an outbreak situation, these isolates are no longer routinely accepted by the CDC.⁸² Refer to section “Services available through the CDC” on the next page.

Note: Bordetella holmesii identification is performed in Atypical Bacteriology.

Atypical Bacteriology

The Atypical Bacteriology lab serves primarily as a referral laboratory for bacteria that are unusual or difficult to identify. In this context, “Atypical Bacteriology” refers to the examination of a wide variety of microorganisms including the following: *Bacillus*, *Corynebacterium diphtheriae*, *Haemophilus*, *Neisseria*, *Pasteurella*, *Pseudomonas* and similar organisms and “unclassified” bacteria. Certain clinical specimens are accepted for primary isolation; otherwise, pure isolates are required for identification or

serotyping. Specimens are accepted from public and private health care providers. Culture from animal or environmental sources must be associated with human illness. Anaerobic cultures and antimicrobial susceptibility testing are not performed in this laboratory. Consultation and bench training are provided upon request.

Services available in the Atypical Bacteriology Lab include:

- confirmation and serotyping of *Neisseria meningitidis* and *Haemophilus influenza* from sterile body sites (**see note below**)
- confirmation of *Neisseria gonorrhoeae*
- identification of non-fermentative gram-negative bacilli
- identification of gram-negative fermentative bacilli not included in the family Enterobacteriaceae
- identification of gram-positive *Bacillus sp.* and coryneform rods
- identification or referral of cultures which are unidentifiable at the local level due to special growth requirements, atypical test results or the hazardous nature of the suspected organism.

Please Note: The North Carolina Communicable Disease Control rules (10A NCAC 41A.0209) state that laboratories isolating *Neisseria meningitidis* and *Haemophilus influenza* from a normally sterile site, shall test the organism for specific serogroup or send the isolate to the State Public Health Laboratory for serogrouping.

Other -

The hazardous nature of certain suspected organisms such as *Francisella tularensis*, *Bacillus anthracis*, *Yersinia pestis* and *Brucella sp.* require submission to the Bioterrorism and Emerging Pathogens Lab (BTEP) and may be routed through the Atypical Bacteriology laboratory. Please call the BTEP Lab at 919-807-8600 if one of these organisms is to be submitted.

Services available through the CDC by referral from the NCSLPH include:

- identification of *Legionella* isolates
- specialized serotyping and strain characterization of meningococci from outbreaks
- serotyping of *Streptococcus pneumoniae* (Pneumococcus) cultures: unless there is an outbreak situation, these isolates are no longer routinely accepted by the CDC unless discussed in advance with CDC to obtain prior approval for testing. Contact the CDC *Streptococcus* Laboratory via this web submission site: www.cdc.gov/ncidod/biotech/strep/references/html.
- antimicrobial susceptibility testing of Pneumococci found to be penicillin-resistant by oxacillin disk screening

Note: All specimens forwarded to the CDC must be accompanied by a DASH form and a clinical history documenting the need for testing.

Specimen Collection and Identification

Specimens should be collected aseptically and cultured at the local laboratory. Only pure cultures should be submitted; mixed cultures are subject to rejection. To assure purity, isolates should be subcultured onto appropriate media before referral to the NCSLPH. Each specimen should be clearly labeled with the patient's name and either date of birth or Social Security number accompanied by a completed Special Bacteriology requisition DHHS form #T806. Use separate forms for individual specimens. Unlabeled specimens will not be tested. Place forms in the outer container to avoid contamination in case of specimen leakage.

Note: Specimens received without **submitter return address** are subject to rejection!

Note: CLIA regulations require the following information on all **test requisitions**:

- patient name
- name and address of submitting agency
- test requested
- date specimen collected and time, if appropriate
- source of specimen, if appropriate
- sex and date of birth

On the form indicate presumptive identification or preliminary test results and patient clinical information.

Telephone the Microbiology Lab at (919) 733-7367 to make special arrangements in urgent or unusual circumstances. Telephone before submitting large numbers of isolates or highly infectious organisms.

Shipment of Specimens

Isolated organisms other than those requiring special handling preferably should be submitted on carbohydrate-free agar slants such as infusion, nutrient, trypticase soy, blood or chocolate. Agar slants are preferred. Plates are discouraged, but if necessary, may be used if they are taped closed, sealed in leak-proof bags and securely packaged in a **crush- proof** container. Use the Microbiology Reference Mailer for agar slant cultures. Use double-walled or equivalent containers; ordinarily cultures do not require refrigeration in transit. When submitting large numbers of isolates, tubes should be wrapped individually in absorbent cushioning material and packaged together, securing against breakage.

Plainly label "Special Bacteriology" on the outside of all mailers. Ship specimens as soon as possible after collection. When shipping by U.S. Mail, use first-class postage. Be sure to place return address on outside of container, regardless of shipping method.

Reporting Procedures and Interpretation

Most culture identifications are reported within five to seven work days; mixed cultures or fastidious bacteria may require longer for identification. Reports on isolates referred to the CDC may be delayed up to several months.

Organisms are identified to a genus and species level only when cultural, morphological and biochemical test results indicate a good species correlation. Some organisms can be identified accurately only to the genus level. Organisms normally encountered as contaminants or those lacking clinical significance also may be reported only to the genus level. Test reactions of atypical organisms may fail to correlate with those of known cultures. Reports reflect any similarity to characterized bacterial strains.

Organisms reported as "unidentified" do not correspond to recognized genera and/or species. These cultures are not routinely forwarded to the CDC unless 1) the nature of the isolate, source and/or patient clinical history warrant further study, or 2) a special request is made for referral. The submitting laboratory may need to clear this request with CDC staff prior to forwarding the isolate to the NCSLPH for referral to the CDC.

Reports are returned only to the submitting agency; the submitter is responsible for sending copies to any other agency. Copies of reports are maintained in this Laboratory. The submitting agency is responsible for maintaining reports in the patient's file. Reports of *Haemophilus influenzae* and *Neisseria meningitidis* from cases of invasive disease are forwarded to the Women's and Children's Section and Communicable Disease Branch, respectively.

Specimens Requiring Special Handling

Organism or Disease	Collection Instructions	Shipping Requirements	Special Requirements
<i>Bacillus anthracis</i> *BT*	Aseptically collect specimens from lesion, contaminated hair products, sputum, or blood. Subculture isolates to agar slants. Use extreme caution: use biological safety hood class II. Smears should be fixed in absolute methanol or formalin.	Microbiology Reference mailer for isolates. Label as possible " <i>B. anthracis</i> "	Notify Unit prior to shipping. Receipted* mail required.
<i>Bordetella pertussis</i> (whooping cough): see separate listing			
<i>Brucella sp.</i> *BT*	Aseptically collect multiple blood samples, infected tissues, liver biopsies or bone marrow. Subculture to blood, infusion or Brucella agar. Extended incubation in 5-10% CO ₂ may be required. Use extreme caution: use biological safety hood class II.	Microbiology Reference mailer for isolates. Label as possible "Brucella"	Notify Unit prior to shipping. Receipted* mail required.
<i>Burkholderia</i> (formerly <i>Pseudomonas mallei</i>) (Glanders) *BT*	Aseptically collect blood, sputum, tissue or abscess specimens. Subculture to agar slants. Use extreme caution: use biological safety hood class II.	Microbiology Reference mailer for isolates. Label as possible " <i>B. mallei</i> "	Notify Unit prior to shipping. Receipted* mail required.
<i>Burkholderia</i> (formerly <i>Pseudomonas pseudomallei</i>) (melioidosis) *BT*	Aseptically collect blood, sputum, tissue or abscess specimens. Subculture to agar slants. Use extreme caution: use biological safety hood class II.	Microbiology Reference mailer for isolates. Label as possible " <i>B. pseudomallei</i> "	Notify Unit prior to shipping. Receipted* mail required.
<i>Corynebacterium diphtheriae</i>	Collect throat or skin lesion swabs (2 preferred); place in swab transport system (e.g. Amies or Stuarts) or subculture to Loeffler or other agar slants. CDC does PCR on throat or skin swabs and/or biopsy tissue. Use sterile, dry swabs and transport at room temp. or 4° C. (Recommended to submit culture if PCR is requested)	Microbiology Reference mailer for isolates or swab transport system.	Notify Unit prior to shipping. Toxigenicity testing performed at the CDC.
<i>Francisella Tularensis</i> *BT*	NOTE: Tularemia is most frequently confirmed by serology refer to VIROLOGY/SEROLOGY. Do not attempt culture before consulting Section. Aseptically collect specimens from Lesions, lymph nodes, sputum or gastric Aspirates. Smears should be fixed in Absolute methanol or formalin. Use Extreme caution: use biological safety hood class II.	Microbiology Reference mailer for isolates. Label as possible " <i>F. tularensis</i> "	Notify Unit prior to shipping. Receipted* mail required.

Organism or Disease	Collection Instructions	Shipping Requirements	Special Requirements
<i>Haemophilus ducreyi</i> (chancroid)	NOTE: Culture is seldom successful; diagnosis usually is made by clinical evidence and Exclusion of other STD agents associated with lesions. Collect specimens from lesions of Inguinal bubo and inoculate onto chocolate agar (CA) or CA + vancomycin; incubate at 33-35° in 5 – 10% CO ₂ <i>PCR testing performed at CDC; contact Section for information (919-733-7367) or (919-807-8774)</i>	Reference culture or heavy growth from CA on sterile swabs stabbed into CA. Microbiology Reference mailer.	Primary culture recommended at local level.
<i>Legionella</i>	See separate listing		
<i>Leptospira</i>	Note: Only serological testing at the CDC is available; refer to Virology/Serology		
<i>Listeria</i>	Agar Slant Isolates from blood or CSF. Microbiology Reference Mailer. Notify PFGE lab prior to submitting.		
<i>Neisseria gonorrhoeae</i> : See separate listing			
<i>Pseudomonas mallei</i>	See <i>Burkholderia</i> (formerly <i>Pseudomonas</i>) <i>mallei</i>		
<i>Pseudomonas pseudomallei</i>	See <i>Burkholderia</i> (formerly <i>Pseudomonas</i>) <i>pseudomallei</i>		
<i>Staphylococcus aureus</i>	Isolates of coagulase positive staphylococci from documented outbreaks.	Microbiology Reference mailer.	Notify Unit prior to shipping. Referred to the CDC.
<i>Yersinia pestis</i> *BT*	Aspirate fluids from lymph nodes or bubo; at least two blood samples in blood culture bottles. Subculture to blood agar. Smears should be fixed in absolute methanol or formalin to kill organisms. Use extreme caution: use biological safety hood class II.	Microbiology Reference mailer for isolates. Label as possible " <i>Y. pestis</i> "	Notify Unit prior to shipping. Receipted* mail required.

* Federal regulations require that these organisms must be shipped by a system that allows tracking and prompt location of packages and notification of receipt, such as certified or registered mail. Biohazard labeling is required on the outside of the container.

BT BT specimens require shipment on slanted media, not plated media.

Turnaround Times and Record Retention for NCSLPH Bacteriology

REPORTING SCHEDULE FOR TURN-AROUND TIMES:

Turn-around times for all in-house testing has been established (see below) and the information can be found in the SCOPE document for the NCSLPH readily available to laboratory and clerical personnel, and accessible via the internet by submitters or other interested parties.

Specimen check-in and initial patient data entry should not take any longer than one working day after receiving the specimen in the laboratory for analysis.

Similarly, specimen test results should be mailed out within one day of completion of testing and result validation.

The turn-around times for in-house testing are general guidelines and varies by the individual test:

TEST	TURNAROUND TIME
<i>Bordetella</i> PCR	Batched twice a week; endeavor for 4-5 working day TAT
<i>Bordetella</i> DFA	Day of receipt or next work day
<i>Bordetella</i> culture	7 days after receipt
<i>Legionella</i> DFA	Day of receipt or next work day
<i>Legionella</i> culture	3 weeks after receipt
Negative enteric cultures	3-4 days after receipt
Serotyping or phenotyping of isolates	4-7 work days after receipt
Food cultures	1-7 work days after receipt
<i>Neisseria gonorrhoeae</i> confirmations	2-4 work days after receipt
Bacterial isolates for identification	5-7 work days
Yeast identifications	2 weeks
Mold identifications	4 weeks
Actinomycetes identifications	6-8 weeks
Ova & protozoa concentrations	5-7 work days
Malarial and other smears	5-7 work days
Mixed, fastidious, or particularly difficult to identify isolates may take longer.	
Identifications referred to CDC may take several months.	

RECORDS RETENTION:

Laboratory records will be retained for a period of 2 years (or longer) satisfying both the requirements of state codes as well as those of CLIA '88.

Newborn Screening/Clinical Chemistry

(919) 733-3937

NCSLPH offers a Newborn Screening program for babies born in North Carolina. This program includes screening for over 30 metabolic and genetic disorders.

The Newborn Screening/Clinical Chemistry Unit consists of four labs:

MS/MS

FIA/GAL/BIO

Hemoglobinopathy/Cystic Fibrosis

HemaChem

Blood Grouping and Typing

Blood Lead

Blood Grouping and Rh Typing

(919) 733-3937

Introduction

Services for ABO blood grouping, Rh typing and antibody screening are available only to women seen in the prenatal clinics at local health departments. These services are not available to other patients of local health departments or other health care providers. Specimens must be received within 5 days of collection.

Sample Collection and Identification

- A. Specimens must be accompanied by DHHS form #2828 which is available on the NCSLPH website at <http://slph.ncpublichealth.com/forms.asp#specimen>.
- B. Specimen collection device kits can also be ordered on-line at <http://slph.ncpublichealth.com/forms.asp#mailroom>.
- C. Complete all information and identification on DHHS form #2828. It is imperative that all of the following information be completed: last name, first name, middle initial, patient number (social security number), address, date of birth, race, Medicaid number, submitter name, address and tax identification number (EIN#), specimen collection date, week of gestation, clinical information (initial screen, previously detected antibody, previous abortion/pregnancy, blood transfusion, Rh₀ Immune Globulin injection).
- D. **NOTE: DRAW BLOOD BEFORE RHOGAM INJECTION.** Collect one (1) purple top vacutainer tube (4-6 mL), containing EDTA. Mix blood with EDTA immediately by gently inverting tube 8-10 times. DO NOT SHAKE.
- E. Label the purple top vacutainer tube with patient's name, date of birth and the date collected.

NOTE: Laboratory testing(s) will not be performed unless the tube is labeled with patient identification. The name on the tube must match the name on the requisition.

- F. Refrigerate specimen until transported.

Shipment

- A. Place completed form #2828 with specimen into the mailing container. Do NOT wrap forms around individual tube(s). Ensure return address is on the form and also on the outside of the mailing container.

- B. Mail the specimens on the day of collection. Specimens for ABO, Rh and antibody screen must be tested within 5 days after collection. Specimens may be routinely collected Monday-Friday.

Reporting Procedure and Interpretation

- A. Copies of results are returned to the submitting agency.
- B. Records of results are filed by date of receipt in this Laboratory. Records are retained for five (5) years.

Note: At the present time, only two (2) antibody screens are available from this Laboratory per Rh-negative patient per pregnancy. The first screen should be done early in the pregnancy. The second screen should be performed after 24 weeks gestation.

Blood Lead Screening

(919) 733-3937

Introduction

Childhood lead poisoning is one of the most common pediatric health problems in the United States, even though it is entirely preventable. The persistence of lead poisoning, in light of present knowledge about the sources, pathways and prevention of lead exposure, presents a direct challenge to clinicians and public health authorities.

As a result of industrialization, lead is common in the environment. It has no known physiologic value and children are particularly susceptible to its toxic effects. Most poisoned children have no apparent symptoms and many cases go undiagnosed and untreated. Lead poisoning is widespread and is not solely a problem of inner city or minority children. No socioeconomic group, geographic area, racial or ethnic population is spared its effects.

New data indicate adverse effects of lead exposure in children at blood lead levels previously believed to be safe. Toxic effects have been documented at blood levels as low as 10 micrograms per deciliter (ug/dL) of whole blood. As a result, the Centers for Disease Control (CDC) 1985 intervention level of 25 ug/dL have been lowered to 10 ug/dL.

Because the erythrocyte protoporphyrin test is not sensitive enough to identify children with blood lead levels below 25 ug/dL, direct blood lead measurement is now the initial screening test. In addition, a multi-tier approach to follow-up has been adopted with an overall goal of reducing children's blood lead levels to below 10 ug/dL, effective 1993.

Who and When to Screen

All children seen at local health departments for health maintenance visits (Well Child and Well Baby Clinics; Early Periodic Screening Diagnosis Treatment (EPSDT) clinics; Pediatric Supervisory Clinics; WIC Children, etc.) and all children receiving services through private providers are to be screened at least once before the age of six without regard to risk determination.

Ideally, children should be tested between 12 and 24 months of age, or upon their first entry to the health care system at a later age. Children identified as high risk should be rescreened in 12 months.

The screening specimen should be collected by the child's primary care provider. Referral to a provider solely for the purpose of lead screening is discouraged.

Screening Test and Methodology

Direct blood lead measurement is the screening test of choice. Finger-stick, capillary blood specimens are adequate for the initial screening test, provided

that precautions are taken to minimize the risk of contamination. Venous blood specimens should be collected for confirmation of all elevated blood lead results.

The State Laboratory is available to analyze blood specimens collected by local health departments and blood specimens on all children 6 months - 6 years of age who are seen by private providers.

Sample Identification and Collection

- A. Specimens must be accompanied by DHHS form #3707 which is available on the NCSLPH website at <http://slph.ncpublichealth.com/forms.asp#specimen>. Specimen collection device kits can also be ordered on-line at <http://slph.ncpublichealth.com/forms.asp#mailroom>.
- B. Complete all identification and requested information on DHHS form # 3707. It is imperative that all of the following information be completed:
- Patient last name, first name, middle initial
 - Patient number (social security number)
 - Address
 - Date of birth
 - Race and sex
 - Medicaid number,
 - Submitter name, address and tax identification number (EIN#)
 - Specimen collection date,
 - Indicate whether Initial or follow-up blood lead test.

Submit a microtainer or EDTA blood specimen (full, unopened tube) labeled with patient name and date of birth.

C. Preparation of Child

1. Wash child's hand with soap and water, using hand brush. Rinse well. Dry.
2. Grasp the child's hand so that the blood drawer's thumb is across the top of the child's fingers.
3. Hold the child's hand so that the palm faces up.
4. Use child's middle or ring finger for sample collection.
5. Using an alcohol wipe, briskly scrub area on the child's fingertip for 20 seconds.
6. Wipe scrubbed area once, using dry gauze.
7. Use lancet to stick finger slightly left of center.

8. Use dry gauze to wipe off the first drop of blood.

Note: After specimen collection, care of puncture site should be consistent with your institution's procedures.

D. Collection of Blood Sample

1. Continuing to grasp the finger, touch the capillary tip of the collection device to the beaded drop of blood.
2. Capillary must be held continuously in a horizontal position during specimen collection to prevent air bubbles from forming in the capillary tube.
3. Dispense the full capillary of blood (200 – 250 μ L) into the microtainer.
4. Turn capillary/tube unit immediately to a vertical position to allow the blood in the capillary to flow into the tube.
5. Remove capillary with holder at the same time. Close microtainer with attached cap.
6. Agitate the specimen to mix the anticoagulant through the blood.
7. Label microtainer with patient's first and last name and date of birth and refrigerate until shipping.

*Laboratory testing will NOT be performed unless the information on the specimen tube **exactly** matches information on the collection form.

Shipment

- A. The Laboratory must receive the specimen within 28 days of collection; however, immediate shipping is recommended to ensure specimen integrity and suitability for analysis.
- B. If not shipped immediately, store in refrigerator.

Reporting Procedures and Interpretation

Children are classified according to the risk for adverse effects of lead based solely on blood lead measurement. The urgency and type of follow-up required are based on a child's risk classification.

FOLLOW-UP SCHEDULE FOR DIAGNOSTIC / CONFIRMED LEAD LEVELS	
Blood Lead Level	Response
<10 µg/dL	<ul style="list-style-type: none"> • Blood lead test or reassessment at age 2 • Report blood lead test results to parent and document • No additional action necessary unless risk of exposure increases
10-19 µg/dL (Diagnostic test within 3 months)	<ul style="list-style-type: none"> • Continue testing every 2-3 months until 2 consecutive venous or capillary tests are <10 µg/dL. • Provide family lead education. • Take environmental history to identify obvious sources of exposure and offer environmental investigation. • Refer to WIC Program
20-44 µg/dL (Diagnostic test Within 1 week)	<ul style="list-style-type: none"> • Provide clinical management, including family lead education. • Provide environmental investigation and lead hazard control. • Refer to WIC program. • Children ages birth to 35 months refer to CDSA Early Intervention. • Children ages 3-5 refer to CSC • Refer to Social Services as needed for housing or additional medical assistance. • Continue testing every 2-3 months until 2 consecutive tests are <10µg/dL.
45-69 µg/dL (45-69 Diagnostic test within 48 hours) (60-69 Diagnostic test within 24 hours)	<ul style="list-style-type: none"> • Provide clinical management, including family lead education. • Provide environmental investigation and lead hazard control. • Refer to WIC Program • Refer children ages birth to 36 months to CDSA Early Intervention. • Refer children ages 3-5 to CSC • Refer to Social Services as needed for housing or additional medical assistance • Continue testing every 2-3 months until 2 consecutive tests are < 10 µg/dL.
>70 µg/dL (Diagnostic test immediately as emergency lab test)	<ul style="list-style-type: none"> • Hospitalize child and begin medical treatment immediately. • Provide clinical management, including family lead education. • Provide environmental investigation and lead hazard control. • Refer to WIC program. • Refer children ages birth to 36 months to CDSA Early Intervention • Refer children ages 3-5 to CSC • Refer to Social Services as needed for housing or additional medical assistance • Continue testing every 2-3 months until 2 consecutive tests are <10µg/dL.

Note: Each block in the above table represents a risk category. Each blood lead test performed must be confirmed within that category. If the diagnostic blood lead test moves to a higher risk category, then confirmation must be done in the higher risk category.

Additional information can be found at
http://www.deh.enr.state.nc.us/Children_Health/2009printedversionleadmanual.pdf

Hemoglobinopathies

(919) 733-3937

Introduction

Newborn Screening includes a screening test for abnormal hemoglobins S, C, and E and is performed only on infants six months of age or younger.

Hemoglobinopathy testing is offered as a follow-up test on specimens reported as abnormal by Newborn Screening and on infants greater than six months of age. It tests only for hemoglobin identification. This test is also used to screen blood samples from individuals and family studies for hemoglobin S (sickle cell) and other hemoglobinopathies. Isoelectric focusing electrophoresis (IEF) is used as a screening test and high performance liquid chromatography (HPLC) is performed on specimens determined to be abnormal by IEF. These services are available to public and private providers for the purposes of prenatal screening, family studies and follow-up testing.

Sample Identification, Collection and Shipment

- A. A hemoglobin electrophoresis filter paper collection DHHS form #1859 can be ordered on line at <http://slph.ncpublichealth.com>
- B. Complete the entire identification section on the DHHS form #1859 with ballpoint pen or type, making sure all copies are legible. It is imperative that the following information is completed: patient's name, social security number, address, sex, race, birth date, blood specimen collection date, transfusion information, Medicaid number, if applicable, complete name and address of submitter, and EIN #.

Note: This form should be used for specimen collection on children greater than 6 months of age to adult.
- C. Follow your institution's procedures for performing heel or finger punctures. After skin is cleansed with an antiseptic, wipe dry with a sterile gauze pad and puncture heel or finger with sterile lancet.
- D. Fill each circle on the form with blood, making sure it soaks completely through the paper.
- E. Allow the sample to dry for a minimum of three hours at room temperature before mailing. Do not expose the sample to temperature extremes (heating or freezing), as this will render the sample unsatisfactory for use in the testing procedures.
- F. Mail specimen within 24 hours of collection. Write return address on envelope and add First Class postage. Do not mail specimens in plastic bags.

Note: Filter paper specimens should not be submitted for detecting thalassemia. A whole blood specimen is required when thalassemia is suspected. (Please follow whole blood testing guidelines).

Specimen Collection and Identification for Whole Blood Specimens

- A. The laboratory may request an EDTA whole blood sample in order to perform follow-up testing for certain previously reported hemoglobin screening results. Samples from the patient and/or both of the patient's biological parents are necessary in order to provide definitive results. **Whole blood testing is NOT to be used as a screening tool.** Blood spots should be submitted for screening purposes.
- B. Listed below are the conditions by which whole blood family study/follow-up testing is requested:
- Hemoglobin Disease states
 - FA+ Variant or A+ Variant
 - Not Definitive results
 - Trait patients who are pregnant. Whole blood testing on the partners can be requested.
 - Diseased patients being monitored by medical facilities
 - Abnormal results on original patient. Whole blood testing may be requested by physician when sibling/parent studies are needed.
 - Suspected Beta Thalassemia due to family history (Please add requesting physician's name to form.)
- C. The whole blood methodology requires a longer time for completion than that of blood spot testing. Please allow a MINIMUM of 14 business days, from the time of receipt in the lab, before expecting patient results.
- D. Complete the DHHS form #1859 for each specimen collected. Include patient name, patient number, address, birth date, race, sex, Medicaid number, patient phone number, date specimen collected, blood transfusion information (if applicable), complete name and address of submitter and EIN#. For family study specimen submission, provide the original laboratory reference number, original name as submitted for newborn screening and date of birth of the infant. This information will allow the laboratory personnel to reference and link the family study results to each other. It is IMPERATIVE that the forms are filled out completely. Any missing information could result in longer turn around time or unsatisfactory reports.
- E. Submit 5-7 mL of well-mixed blood collected in EDTA (lavender top) specimen collection tube. If the patient is an infant or young child, submit 0.5- 1 mL of blood collected in EDTA (lavender top) microtainer specimen collection device. DO NOT apply blood to the filter portion of the form. Write patient name and date specimen collected on the specimen tube label. If using an adhesive label, do not cover up the tube expiration date or obscure view of the specimen because laboratory personnel

must assess specimen integrity before testing. Clotted blood is unsatisfactory for use. EDTA blood received greater than 7 days after collection is unacceptable. Blood submitted in expired EDTA tubes is unacceptable.

- F. Mail the specimen(s) on the same day of collection, if possible. Refrigerate at 2-8° Celsius until the specimen can be transported. If the specimen could be subjected to extreme temperatures in transit during the summer, place a cold gel pack with the specimen in an insulated box for transport to the NCSLPH.

Reporting Procedures and Interpretations

- A. Normal results are reported within 3 days after receipt in the Laboratory. Abnormal results are reported after further testing. A copy of each diseased patient report is sent to the Sickle Cell Program and Regional Counselors for follow-up.
- B. The whole blood methodology requires a longer time for completion than that of blood spot testing. Please allow a minimum of 14 business days after specimen receipt in the lab, before expecting patient results.
- C. There are testing limitations with identification of some hemoglobin variants. In these instances, the lab suggests referrals to a local hematologist.

Newborn Screening

(919) 733-3937

Introduction

NCSLPH offers Newborn Screening testing to all babies born in North Carolina. These tests are performed on a filter paper blood spot sample collected from the newborn baby. This sample is tested for diseases that may cause mental retardation or death, if untreated. To prevent early effects of disease the sample should be drawn during the infant's first 48 to 72 hours of life. Present protocol includes testing for:

- Primary Hypothyroidism: Both thyroxine (T4) and thyroid-stimulating hormone (TSH) are measured. Both analytes are measured by time-resolved fluoroimmunoassay (FIA).
- Hemoglobinopathies: A hemolyzed filter paper specimen is examined by isoelectric focusing for the presence of abnormal hemoglobins. The abnormal hemoglobins are confirmed by High Performance Liquid Chromatography (HPLC).
- Galactosemia: Galactose and galactose-1-phosphate are measured by continuous flow. A qualitative and/or quantitative test for uridyl transferase is performed on all specimens with abnormal results and on all specimens collected from an infant who has been fed non-lactose containing formula.
- Congenital Adrenal Hyperplasia (CAH): 17-alpha Hydroxy Progesterone (17-OH-P) is measured by time-resolved FIA.
- Amino Acid Disorders: Amino acids (Phenylalanine, Tyrosine, Valine, Leucine, Methionine, and Citrulline) are measured by Tandem Mass Spectrometry (MS/MS).
- Fatty Acid Oxidation Disorders: Acylcarnitines from C0 (Free Carnitine) to C18 are measured by MS/MS.
- Organic Acid Disorders: Acylcarnitines from C0 (Free Carnitine) to C18 are measured by MS/MS.
- Biotinidase Deficiency: Biotinidase enzyme activity is measured using semi-quantitative colorimetric continuous flow analysis.
- Cystic Fibrosis (CF): Immunoreactive trypsinogen (IRT) is measured by time-resolved FIA. The daily top 5% of specimens with the highest IRT values undergo DNA testing using a panel of over 40 common CF mutations. All abnormal results are referred for follow-up by sweat chloride testing.

Sample Collection and Identification

A. Newborn Screening Specimen collection DHHS form #3105 and pre-addressed mailing envelopes can be ordered on-line at <https://slphreporting.ncpublichealth.com/labportal>

B. Time of Collection

- 1) A blood specimen (heel stick) should be obtained from every infant prior to discharge or transfer to another hospital regardless of age. The number or type of feedings (breast or formula) will not affect this rule. Optimum time for specimen collection is 48-72 hours of age.
- 2) Infants screened prior to 24 hours of age should have a repeat specimen collected by one week of age. It is the responsibility of the infant's health care provider whose name is noted on the newborn screening form to obtain this second specimen in a timely manner. (Parents should be informed that the infant is being retested because of early discharge from the hospital, not because the infant has an increased risk for a metabolic disease.)
- 3) Premature or ill infants or infants receiving parenteral feeding should be screened between 24-72 hours of age. The status of feedings will not affect this policy. The sample should not be obtained from a central line when an amino acid solution is being infused.
- 4) Any infant transferred from a local hospital to a major medical center for post-natal care should have a blood specimen collected for screening within 48 hours of arrival at the major medical center. Optimally, this specimen should be collected before the infant is transfused. When screening for hemoglobinopathies, a repeat specimen should be collected four months after the last transfusion.
- 5) All infants less than or equal to 1500 grams (Very Low Birthweight) shall have a repeat specimen collected at 4-6 weeks of age. If the infant is discharged prior to this time, a repeat specimen shall be collected at the time of discharge, with an additional repeat specimen collected at 4-6 weeks of age.
- 6) If a blood specimen cannot be collected due to parental refusal or other reasons, a newborn screening form with the baby's demographic data should be submitted to the State Laboratory.

Note: Limits for blood spot specimen submission are based on the baby's age at specimen collection. MS/MS and CF and FIA/GALBIO are limited to babies less than 6 months of age at the time of collection. Sickle Cell testing can be done on babies greater than six months of age by submitting a blood spot sample on DHHS form #1859 (See Hemoglobinopathies).

- C. Identification and Collection of Newborn Screening Specimen. It is recommended that blood be obtained from the heel of the infant (heel stick).
- D. Complete all information and identification on Newborn Screening Form #3105. It is imperative that all of the following information be given: newborn name, newborn birth date/time, specimen collection date/time, race, sex, type of feeding, birth weight in grams, transfusion date/time, mother's name, social security number and address, hospital/submitter name and tax identification number (EIN) and doctor/health care provider name and tax identification number (EIN).
- 1) Do not contaminate filter paper circles by allowing the circles to come in contact with spillage or by touching before or after blood collection.
 - 2) "Keep for your records" portion of the form should be retained by the hospital/submitter.
 - 3) Warm heel with a soft cloth, moistened with warm water up to 41° Celsius, for three to five minutes or use an approved commercial warmer according to manufacturer's instructions.
 - 4) Cleanse site with 70% isopropyl alcohol prep pad. Wipe site dry with sterile gauze pad.
 - 5) Puncture heel with lancet and wipe away first blood drop with another sterile gauze pad. Allow another LARGE blood drop to form.
 - 6) Lightly touch filter paper circle to the LARGE blood drop. Allow blood to soak through and completely fill circle with SINGLE application of the LARGE blood drop. Only apply blood once to one side of filter paper. Fill remaining circles in the same manner, with additional blood drops. Care of puncture site should be consistent with your institution's procedures.
 - 7) Allow blood spots to air-dry thoroughly for a minimum of three hours at room temperature on a flat non-absorbent surface. Keep away from direct sunlight and heat.

Mail Blood Spots for the Laboratory

- A. Mail completed form to the NCSLPH laboratory within 24 hours of collection. **DO NOT HOLD SAMPLES.** Several specimens may be mailed in the same envelope. Include return address and sufficient first-class postage. DO NOT DELAY mailing specimen by waiting for a batch to accumulate or to complete hearing screening.
- B. **Do not package blood spot collection forms in plastic bags for mailing.**

Reporting Procedures and Interpretation

- A. **Primary Hypothyroidism:** Thyroid results are reported as normal, borderline, or abnormal. For borderline results, a repeat filter specimen is requested by confirmation mail. For abnormal results, the infant's healthcare provider is contacted by telephone by Women's and Children's Section or the Newborn Screening Unit. For abnormal values, immediate serum thyroid testing (T4, TSH) is requested to be done locally. If there is no response within 14 days, a Confirmation letter is sent to the mother requesting a new specimen.
- B. **Galactosemia:** Galactose results are reported as normal or abnormal. Results are reported normal if the results are less than 10 mg/dL. All samples with galactose results greater than 10 mg/dL will have a qualitative galactose-1-phosphate uridyl transferase (GALT) test performed. If uridyl transferase activity is absent, quantitative uridyl transferase and phosphoglucomutase tests are performed to rule out the possibility of sample deterioration. Uridyl transferase testing is also performed on all samples from infants with soy formula, parenteral or unknown feeding. All abnormal results are called to the region's medical geneticist who will contact the baby's health care provider about follow up. If there is no response within 14 days, a Confirmation letter is sent to the mother requesting a new specimen.
- C. **Congenital Adrenal Hyperplasia (CAH):** 17-OH-Progesterone results are reported normal, borderline or abnormal. The baby's health care provider is contacted by the Women's and Children's Health Section or the Newborn Screening Unit regarding abnormal results. If there is no response within 14 days, a Confirmation letter is sent to the mother requesting a new specimen.
- D. **Hemoglobinopathies (Sickle Cell):** Hemoglobinopathies results are reported as normal if no abnormal hemoglobin is detected. Heterozygotes S, C and E results are reported as trait, and a letter is sent to the baby's health care provider. Abnormal hemoglobin disease states are reported to the baby's health care provider and the North Carolina Sickle Cell Syndrome Program. Appropriate follow up is requested which include additional testing using whole blood samples from the infant and biological parents. If there is no response within 14 days, a Confirmation letter is sent to the mother requesting a new specimen.
- E. **Tandem Mass Spectrometry (MS/MS) screening:** An amino acid profile and acylcarnitine profile are measured to detect disorders in amino acid, fatty acid oxidation and organic acid metabolism. Results from each profile are reported as normal, borderline or abnormal. Normal results require no further specimen submission, unless clinically indicated. For borderline results a repeat blood spot specimen is requested by confirmation mail to be collected and submitted by the baby's health care provider. Abnormal results are called to a Metabolic specialist who contacts the baby's health care provider to arrange for clinical evaluation and

an additional specimen to be collected for clinical diagnosis. If there is no response within 14 days, a Confirmation letter is sent to the mother requesting a new specimen.

- F. **Biotinodase deficiency:** Biotinodase results are reported as normal, borderline, or abnormal. On borderline results, a repeat filter specimen is requested by confirmation mail. For abnormal results, the infant's healthcare provider is contacted by telephone by Women's and Children's Section or the Newborn Screening Unit. If there is no response within 14 days, a Confirmation letter is sent to the mother requesting a new specimen.
- G. **Cystic Fibrosis:** Results with IRT values that do not fall in the daily top 5% are reported normal for CF with no additional testing required. IRT values greater than the 95th percentile are reflexed to a second tier DNA test. Results with no mutations and an IRT value ≤ 175 ng/mL are reported normal for CF. Results with one or two mutations, or with an IRT value >175 and no mutations are reported as abnormal for CF. Abnormal results will contain the actual IRT value and the specific mutations detected. All abnormal results are called to the CF Follow-up Coordinator who contacts the infant's health care provider to arrange for sweat chloride testing at an accredited CF center.
- H. **Insufficient or Unsatisfactory Specimens:** A letter is sent to the baby's health care provider and submitter to request a repeat specimen. If there is no response in 14 days, a Confirmation letter is sent to the mother requesting a new specimen.

The integrity of the infant's newborn screening results are dependent upon the timely collection and quality of application of a blood specimen on the filter paper form. DO NOT DETATCH and re-attach the filter portion of the form. Taking the time to accurately complete the information and identification on the filter form, preparing the site for blood collection and properly applying the blood specimen on the filter form saves time, resources and the need for a repeat blood spot collection. Insufficient and unsatisfactory specimen submissions are totally avoidable.

- I. Records of laboratory results are filed by date of birth and baby's name. Records are retained for at least five (5) years in the Newborn Screening computer database.

Virology/Serology

(919) 733-7544

Virology/Serology (VS) performs highly complex laboratory tests to identify infections with a variety of bacterial and viral pathogens of public health significance. The majority of reports generated by this unit are used by state and local health officials in the diagnosis, treatment, surveillance, and control of communicable disease.

Virology/Serology is organized into four laboratory areas:

- Bacterial STD
- Serology
- Special Serology
- Viral Culture/Rabies

The mission of VS is to provide quality-assured laboratory services to public and private health provider organizations and to assist other Public Health program partners responsible for communicable disease prevention and control.

Arbovirus

(919) 733-7544

Introduction

Diagnostic serologic assays are performed on serum suspected for Arbovirus. The Arbovirus panel includes Eastern Equine Encephalitis (EEE), Western Equine Encephalitis (WEE), St. Louis Encephalitis (SLE), LaCrosse Encephalitis (LAC), and West Nile Virus (WNV). All serum received will be tested for IgG antibodies to EEE, WEE, LAC, SLE, and WNV by IFA and IgM antibodies to WNV and LAC by EIA. For more information about these viruses, go to <http://www.cdc.gov/>

Sample Collection and Identification

Only serum and CSF may be submitted for serologic testing. Clearly label each sample vial with the patient's name (first and last) and either the date of birth or Social Security number. Be sure to label vials with date collected for paired serum samples. Complete a DHHS #3445 submission form specifying all required patient information and which infectious agents are suspected. Failure to supply the requested patient information may result in significantly delayed specimen testing. Assure an onset date, a collection date, a return address, symptoms, travel history, and vaccination history are given. This information is crucial for accurate interpretation of results. Tests must be requested by name. Nonspecific requests for "viral studies" or "viral serologies" will not be accepted. Consult with the laboratory if there is a question as to which test is appropriate.

The serodiagnosis of a current or recent infection generally requires the simultaneous testing of paired serum samples, principally acute and convalescent serum samples. The acute serum should be collected no later than 3-5 days after the onset of illness. The convalescent serum should be collected 2-4 weeks after onset. Since paired sera are advised for all arboviral studies, it is to the advantage of both the submitter and this laboratory if the acute serum is stored frozen by the submitter until the convalescent serum is collected. Both serum samples may be submitted with one submission form. Antibody determinations on cerebrospinal fluid may be of value in diagnosing viral encephalitis and other central nervous system diseases. **Cerebrospinal fluids for serologies should always be accompanied by a serum collected the same day.**

Note: "Specific laboratory arbovirus testing is available at the State Laboratory of Public Health in Raleigh for patients satisfying one or more of the WNV associated clinical syndromes. Classical WNV fever is often associated with headache, lymphadenopathy, nausea, vomiting, and fatigue. WNV Central Nervous System Infection is associated with meningitis, encephalitis, meningoencephalitis, and/or acute flaccid paralysis resembling Guillian-Barre syndrome." (Memo from State Epidemiologist)

Note: "Serum specimens should be sent for antibody detection during the acute illness. Acute CSF for antibody detection, if desired, must be accompanied by a

companion serum collected at approximately the same time. Convalescent serum should be sent 2-3 weeks after onset of illness, or at the time of hospital discharge, for confirmation of probable cases. Samples should be sent with an accompanying completed DHHS form #3445. Tests may be ordered as an "Arbovirus Panel" since the State Laboratory will automatically test for all mosquito-borne viral encephalitides." (Memo from State Epidemiologist)

Testing of horses is no longer available at the State Laboratory of Public Health. Specimens should be submitted through the Rollins Animal Diagnostic Laboratory.

Shipment

Send the properly identified vials of patient sera and the completed DHHS form #3445 in the "Special Serology" (blue colored) mailing containers via the State Courier or U.S. Mail. Specimens may be shipped refrigerated or at ambient temperature.

Reporting Procedure and Interpretation

Failure to detect a significant antibody response may be the result of a number of factors including improperly collected specimens, specimens collected too early or too late during the immune response, selection of the incorrect infectious agent for testing, or lack of sensitivity in the serological system being used.

The following chart lists the arboviral assays performed by this lab. A brief statement of the "normal" values for each assay is given under the heading "Negative Reference Range". The test method, specimen requirements and turn-around-times are also listed for each assay performed.

Arboviral Assays

Test	Test Method	Negative Reference Range	Specimen Requirement	Turn Around Time
California Encephalitis (LAC), IgG	IFA Quant	<1:16	2 mL serum/CSF PSA	10 calendar days
LAC, IgM	IFA Quant EIA Qual	<1:16 Negative	2 mL serum/CSF PSA	10 calendar days
Eastern Equine Encephalitis (EEE), IgG	IFA Quant	<1:16	2 mL serum/CSF PSA	10 calendar days
EEE, IgM	IFA Quant	<1:16	2 mL serum/CSF PSA	10 calendar days
St. Louis Encephalitis (SLE), IgG	IFA Quant	<1:16	2 mL serum/CSF PSA	10 calendar days
SLE, IgM	IFA Quant	<1:16	2 mL serum/CSF PSA	10 calendar days
Western Equine Encephalitis (WEE), IgG	IFA Quant	<1:16	2 mL serum/CSF PSA	10 calendar days
WEE, IgM	IFA Quant	<1:16	2mL serum/CSF PSA	10 calendar days
West Nile Virus (WNV), IgG	IFA Quant	<1:16	2mL serum/CSF PSA	10 calendar days
WNV, IgM	IFA Quant EIA Qual	<1:16 Negative	2mL serum/CSF PSA	10 calendar days

Abbreviations:

EIA Enzyme Immunoassay
 IFA Indirect Fluorescent Antibody
 IgG Immunoglobulin G
 IgM Immunoglobulin M
 PSA Paired Sera Advised
 Quant Quantitative
 Qual Qualitative

Chlamydia/Gonorrhea

(919) 733-7544

Introduction

Chlamydia trachomatis and *Neisseria gonorrhoeae* infections are two of the most common sexually transmitted infections worldwide. In the United States alone a total of 1,244,180 cases of *C. trachomatis* and 301,174 cases of *N. gonorrhoeae* infections were reported in 2009.

Chlamydia are nonmotile, gram-negative, obligate intracellular bacteria. The *C. trachomatis* species consists of a group of 15 different serovars that can cause disease in humans. The serovars D through K are the major cause of genital chlamydial infections in men and women. *C. trachomatis* can cause assorted urogenital infections in addition to asymptomatic infection, which if undiagnosed could lead to pelvic inflammatory disease (PID), ectopic pregnancy, and infertility in women. Children born to infected mothers are at significantly higher risk for inclusion conjunctivitis and chlamydial pneumonia.

N. gonorrhoeae is the causative agent of gonorrheal disease. *N. gonorrhoeae* are non-motile, gram-negative diplococci. The majority of gonorrheal infections are uncomplicated lower genital tract infections and may be asymptomatic. However, if left untreated in women, infections can ascend and cause PID. PID can manifest as endometritis, salpingitis, pelvic peritonitis, and tubo-ovarian abscesses. A smaller percentage of persons with gonococcal infections may develop Disseminated Gonococcal Infection (DGI).

The diagnostic testing for *C. trachomatis* and *N. gonorrhoeae* at the NC State Laboratory of Public Health is a nucleic acid amplification test (NAAT) that dually detects the presence of *C. trachomatis* RNA and/or *N. gonorrhoeae* RNA on a single vaginal swab specimen. Chlamydia cell culture is no longer performed at the NC State Laboratory of Public Health; this procedure is available from commercial reference laboratories.

Urine testing for *C. trachomatis* and *N. gonorrhoeae* is available on a limited basis to pre-approved, select sites. Diagnostic testing is the same as for vaginal swab specimens.

Sample Collection and Identification

In addition to the instructions below, an instructional powerpoint presentation “Chlamydia/Gonorrhea Vaginal Specimen Collection and Form Training” can be accessed and viewed at the State Lab website (www.ncpublichealth.com). The purpose of the presentation is to assist in training people who collect and submit vaginal samples to the NCSLPH for Chlamydia/Gonorrhea testing. Following the instructions should result in optimal quality of test samples and the expeditious

reporting of test results. The presentation may be reviewed for guidance or continuing education.

Clearly label each vial of chlamydia/gonorrhea detection transport medium with the patient's name (first and last) and either the date of birth or Social Security number. Complete submission form DHHS 4011 "Chlamydia/Gonorrhea Detection".

A. Vaginal swab specimens (clinician-collected) are obtained by the following procedure:

1. Partially peel open the swab package. Do not touch the soft tip or lay the swab down. If the soft tip is touched, the swab is laid down, or the swab is dropped, use a new APTIMA Vaginal Swab Specimen Collection Kit.
2. Remove the swab.
3. Hold the swab, placing your thumb and forefinger in the middle of the swab shaft.
4. Carefully insert the swab into the vagina about 2 inches (5 cm) past the introitus and gently rotate the swab for 10 to 30 seconds. Make sure the swab touches the walls of the vagina so that moisture is absorbed by the swab.
5. Withdraw the swab without touching the skin.
6. While holding the swab in the same hand, unscrew the cap from the tube. Do not spill the contents of the tube. If the contents of the tube are spilled, use a new APTIMA Vaginal Swab Specimen Collection Kit.
7. Immediately place the swab into the transport tube so that the tip of the swab is visible below the tube label.
8. Carefully break the swab shaft at the scoreline against the side of the tube and discard the top portion of the swab shaft. Do not spill the contents of the tube. If the contents of the tube are spilled, use a new APTIMA Vaginal Swab Specimen Collection Kit.
9. Tightly screw the cap onto the tube.

B. Patients who wish to collect their own vaginal swab specimens should be instructed as follows:

1. Partially peel open the swab package. *Do not touch the soft tip or lay the swab down. If the soft tip is touched, the swab is laid down, or the swab is dropped, request a new APTIMA Vaginal Swab Specimen Collection Kit.*
2. Remove the swab.
3. Hold the swab in your hand, placing your thumb and forefinger in the middle of the swab shaft.
4. Carefully insert the swab into your vagina about two inches inside the opening of the vagina and gently rotate the swab for 10 to 30 seconds. Make sure the swab touches the walls of the vagina so that moisture is absorbed by the swab.
5. Withdraw the swab without touching the skin.
6. While holding the swab in the same hand, unscrew the cap from the tube. *Do not spill the contents of the tube. If the contents of the tube are spilled, request a new APTIMA Vaginal Swab Specimen Collection Kit.*
7. Immediately place the swab into the transport tube so that the tip of the swab is visible below the tube label.
8. Carefully break the swab shaft at the score line against the side of the tube and discard the top portion of the swab shaft.
9. Tightly screw the cap onto the tube. Return the tube as instructed by your doctor, nurse, or care-provider.

C. Urine specimens are obtained by the following procedure:

1. The patient should not have urinated for at least 1 hour prior to specimen collection.
2. Direct patient to provide a first-catch urine (approximately 20 to 30 mL of the initial urine stream) into a urine collection cup free of any preservatives. Collection of larger volumes of urine may result in specimen dilution.
3. Remove the cap and transfer 2 mL of urine into the urine specimen transport tube using the disposable pipette provided. The correct volume of urine has been added when the fluid level is between the black fill lines on the urine specimen transport tube label.

4. Re-cap the urine specimen transport tube tightly. This is now known as the processed urine specimen.

Note: *Chlamydia trachomatis/Neisseria gonorrhoeae* laboratory services are subject to the following guidelines which have been developed to ensure proper patient management and efficient utilization of limited resources. Information regarding health care provider eligibility and patient selection is stated below. Specimens submitted to the Virology/Serology laboratory must be accompanied by a fully completed submission form, DHHS #4011. Failure to supply the requested patient information may result in significantly delayed specimen testing or in specimen rejection. Specimens for diagnostic testing not labeled with correct patient identification information will not be tested. Minimal patient specimen identification includes two identifiers: full first and last name and either the date of birth or Social Security number. Specimens which, for any reason, are deemed unsuitable or inappropriate for diagnostic testing will not be tested. Rejected specimens will be properly stored for thirty days pending verbal and/or written notification of the submitter. Unless alternate arrangements are initiated by the submitter upon notification of specimen rejection, the specimen will be discarded at the end of the holding period.

Eligible Health Care Providers: Local Health Departments.

Patient Selection: Only the following specimens will be accepted:

1. Vaginal swab specimens from women with syndromes compatible with *C. trachomatis* and/or *N. gonorrhoeae* infection.
2. Vaginal swab specimens from pregnant females.
3. Vaginal swab specimens from asymptomatic women, 24 years old and younger seen in either Family Planning or Sexually Transmitted Disease clinics.
4. Vaginal swab specimens from women for retest for Chlamydia/Gonorrhea at three months post-treatment.
5. Vaginal swab specimens from women due to sex partner referral.
6. Vaginal swab specimens from women with high risk history (i.e. new partner, multiple partners, etc.)
7. Vaginal swab specimens for Chlamydia testing prior to IUD insertion.

Shipment

Properly identified specimen transport vials and completed submission forms are sent to the Laboratory at ambient temperature in goldenrod colored specimen mailers labeled CHLAMYDIA/GONORRHEA DETECTION. Ship at ambient temperature by the State Courier or U.S. Mail. Vaginal swab specimens are stable for up to 60 days at room temperature after collection and urine specimens are stable for up to 30 days at room

temperature after collection; however, it is advisable to ship as soon as possible to avoid delays in turn-around time of test results.

Reporting Procedures and Interpretation

Since the Chlamydia/Gonorrhea NAAT test methodology performed at the NCSLPH is a dual detection assay, both test results will be reported for each clinical specimen. Specimens that are determined to be positive for *C. trachomatis* will be reported as “*C. trachomatis* RNA detected”. Specimens that are determined to be positive for *N. gonorrhoeae* will be reported as “*N. gonorrhoeae* RNA detected”. Negative laboratory results will be reported as “*C. trachomatis* RNA not detected” and “*N. gonorrhoeae* RNA not detected”, respectively. If the test result for either agent is determined to be equivocal, that result will be reported as “Indeterminate, a new specimen should be collected”; in these cases, another specimen should be properly collected and submitted to resolve the status of the patient. Results are available within two working days after receipt of the specimen. Results on specimens requiring repeat testing will be available within three working days after receipt. Results should be interpreted in conjunction with patient history and clinical findings.

Data indicates that both the sensitivity and specificity of the nucleic acid amplification test (NAAT) approach 100%. Although these values are quite impressive for laboratory tests, it must be remembered that the results of this test are not 100% predictive of every patient’s true infected status, and that both false negative and false positive results are a possibility.

Hepatitis Serology

(919) 733-7544

Introduction

Hepatitis B serologies are available on a limited basis for diagnosis of acute and chronic disease, for monitoring the course of disease and the effectiveness of therapy, and for screening select patient populations. Hepatitis A IgM testing is available on a limited basis for the diagnosis of acute disease.

Three types of testing panels are available: diagnostic, screening, and monitoring. The available panels, the markers used with specific patient populations, and the rationale for testing are detailed in the chart at the end. Serologic testing for hepatitis infection is available only to patients who are seen in local health departments and state-operated health care facilities

Hepatitis B virus testing is available to the following patient populations:

1. Symptomatic patients
2. Prenatal patients
3. Refugees
4. Sexual or needle sharing contacts of known infected persons
5. Patients who are household contacts of hepatitis B carriers or acute cases and are candidates for vaccine
6. Infants born to infected mothers
7. Known previous HBsAg positives
8. Previously vaccinated health department employees with percutaneous exposure to hepatitis B virus
9. Source patient of percutaneous exposure

Hepatitis A virus serology is available to patients who are:

1. Symptomatic without an epidemiological link to another case of known hepatitis A infection
2. Suspected cases, whether or not epidemiologically-linked, who are:
 - food handlers
 - health care workers
 - day care attendees
 - day care workers
 - at risk of liver disease through IV drug use, alcohol abuse, etc.
3. Associated with an outbreak situation (prior approval required)

Routine testing for either hepatitis A or B is limited to those groups listed above; however, if you have special needs that are not addressed in the acceptance criteria, please call (919) 733-7544. Special arrangements for testing can be made on an individual basis.

Note: Hepatitis B immune status testing will not be performed to determine immune status of health care workers, dental workers, etc. who are candidates for routine vaccination or to establish routine post-vaccination immunity.

Specimen Collection and Identification (for Hepatitis A or B)

A full 3 mL of serum should be submitted for hepatitis testing. Serum transport tubes should not be overfilled past the 3.0 mL line on the tubes. Submit the serum in a well constructed plastic screw-capped vial with threads on the outside. Excessively hemolyzed, grossly contaminated, or extremely lipemic sera are unacceptable for hepatitis assays.

Clearly label each vial of serum with the patient's first and last name and either the date of birth or Social Security number. Complete a submission form DHHS #3722. All items on this form must be completed before the specimen can be processed.

Only serum may be submitted for serologic testing. Specimens submitted to the Virology/Serology Unit must be accompanied by a fully completed submission form DHHS #3722. Failure to supply the requested patient information may result in significantly delayed specimen testing.

Specimens submitted for testing that are not labeled with two identifiers will not be tested. Specimens which, for any reason, are deemed unsuitable or inappropriate for serologic testing will not be tested. Rejected specimens will be properly stored for ten days pending verbal and/or written notification of the submitter. Unless alternate arrangements are initiated by the submitter upon notification of specimen rejection, the specimen will be discarded at the end of the holding period.

Shipment

Send the properly identified vials of patient sera and the completed form DHHS #3722 in the "HEP SEROLOGY" (buff colored) mailing containers via the State Courier Service.

Specimens should be shipped immediately and should arrive in the laboratory within 48 hours of collection. If transport to the Laboratory is to be delayed, specimens can be refrigerated up to seven days or frozen. Specimens can be mailed at ambient temperature.

Reporting Procedures and Interpretation

The following chart provides information regarding turn-around-times, test methods and negative reference ranges.

Description	Test Method	Negative Reference Range	Turn-Around-Time
Hepatitis B virus surface antigen	EIA-Qualitative Screen	Antigen not detected	4 working days
Hepatitis B virus surface antigen	EIA-Confirmatory	Interpreted by report	7 working days
Hepatitis B virus core-IgM antibody	EIA-Qualitative	No antibody detected	7 working days
Hepatitis B virus core-total antibody	EIA-Qualitative	No antibody detected	7 working days
Hepatitis B virus surface antibody	EIA-Qualitative	No antibody detected	7 working days
Hepatitis A IgM antibody	EIA-Qualitative	No antibody detected	2 working days

Abbreviations:

EIA Enzyme Immunoassay

IgM Immunoglobulin M

Hepatitis Testing Panels and Corresponding Markers

Type of test	Population	Panel Markers	Purpose for testing
Diagnostic	Symptomatic person	HBsAg anti-HAV-IgM anti-HBc-IgM	To separate and identify the type of viral hepatitis for diagnostic purposes
Screen	Prenatal	HBsAg anti-HBc-IgM (if HBsAg is positive)	To identify HBsAg positive pregnant women and thus allow treatment of their newborns with hepatitis B vaccine
	Refugee	HBsAg anti-HBc-IgM (if HBsAg is positive) anti-HBc-Total	To identify HBV carriers in order to reduce the risk of HBV infection in NC refugee population
	Sexual or needle sharing contact of known infected person	HBsAg anti-HBc-IgM (if HBsAg is positive) anti-HBs (if HbsAg is negative)	To determine susceptibility to HBV Infection, assess the need for prophylaxis, or determine the source of infection
	Household contact of chronic HBV carrier	HBsAg anti-HBc-IgM (if HBsAg is positive) anti-HBs (if HBsAg is negative)	To determine susceptibility to HBV infection, and thus allow treatment with hepatitis B vaccine
	Source patient of percutaneous exposure	HBsAg anti-HBc-IgM (if HBsAg is positive)	To determine HBsAg status of source patient in order to assess need for prophylaxis of exposed person
Monitor	Follow-up of infant born to an infected mother	HBsAg anti-HBs	To monitor the effectiveness of therapy
	Follow-up previous HBsAg positive person	HBsAg anti-HBc-Total anti-HBs	To determine the course of the disease, i.e., has infection been resolved or progressed to chronic carrier state
	Previously vaccinated contact of known infected person	anti-HBs	To determine antibody level and thus allow revaccination if the antibody level is inadequate (negative by EIA)

Abbreviations

HBV	Hepatitis B virus
HAV	Hepatitis A virus
anti-HBs	Antibody to hepatitis B surface antigen
anti-HBc-IgM	IgM Antibody to hepatitis B core antigen
anti-HAV-IgM	IgM antibody to hepatitis A virus
HBsAg	Hepatitis B surface antigen
anti-HBc-total	Total antibody to hepatitis B core antigen

Human Immunodeficiency Virus Serology

(919) 733-7544

Introduction

Serologic screening for human immunodeficiency virus (HIV infection is available only through designated counseling and testing sites. Three serologic assays are available for the detection of antibodies to HIV-1 (including Group O and subtypes) and HIV-2. Initial screening for HIV antibodies is performed using an enzyme immunoassay (EIA) which detects HIV-1 (Groups M and O) and HIV-2. All reactive EIAs are repeated in duplicate to verify the initially reactive test result. All repeatedly reactive EIA tests (two or more reactive) are tested by the Western blot HIV-1 (WB) assay. Specific interpretation of the laboratory findings are an integral part of the laboratory report.

The Western blot supplemental serologic test for HIV-1 infection is available as a reference service to both public and private health care providers. Sera will be accepted for testing provided that the submitter states that serologic screening test results are repeatedly reactive.

All HIV specimens that test nonreactive for HIV antibodies by the EIA screening assay are testing for HIV RNA using multi-staged pooling strategies and molecular methodology to detect acute HIV infections. Samples that test repeatedly reactive on the screening assay but fail to test as reactive /positive by HIV-1 WB or HIV-1 RNA are further tested by a third serologic assay that differentiates HIV-1 and HIV-2. If the test results indicate HIV-2 reactivity, the samples is referred to CDC for HIV-2 confirmation.

At least 3 mL of serum is required for the complete HIV testing protocol.

Sample Collection and Identification

Submit a full 3 mL of serum in a well constructed plastic screw-capped vial with threads on the outside. Serum transport tubes should not be overfilled past the 3.0 mL line on the tubes. Excessively hemolyzed or extremely lipemic sera are unacceptable for HIV assays.

Label each vial of serum with the patient's first and last name and either the date of birth or Social Security number. A pre-printed HSIS label may be used. Complete the HIV submission form in its entirety. All items on this form must be completed before the specimen can be processed.

Only serum samples are acceptable for HIV testing on a routine basis. Specimens submitted to the Virology/Serology Unit must be accompanied by a fully completed HIV OCR scannable submission form. If two identifiers, (the patient's first and last name, and either date of birth or Social Security number), are not present on the HIV scannable form, the specimen is deemed "Unsatisfactory" for HIV testing and the specimen is discarded. A minimum of two identifiers on the patient specimen must

match the identifiers on the form exactly or the specimen will be discarded and reported as “Unsatisfactory” for HIV testing. HIV OCR Forms submitted without a specimen will be held for ten days pending verbal and/or written notification of the submitter. Unless alternate arrangements are initiated by the submitter upon notification of the missing specimen, the paperwork will be deemed “Unsatisfactory” at the end of the holding period.

Shipment

Scannable forms should be placed inside an envelope with cardboard to ensure that the form arrives at the lab without folds, tears, or wrinkles and the return address and collection date should be recorded on the envelope in the marked space in the upper left-hand corner. Specimens should be placed inside a plastic bag and then placed into the mailer marked “CTS Serology” and return address and date of collection entered on the outside in the marked space. If courier service is used, the appropriate barcode should be affixed to the front of the envelope and both the envelope with the scannable forms and the specimen mailer should then be placed inside a large plastic bag and sealed. Specimens for other laboratory services should not be placed in this bag. Specimens can be shipped at ambient temperatures.

Scannable HIV forms are available to Counseling and Testing Sites through the Virology/Serology Unit Office, telephone (919) 733-7544. Submitters of reference specimens for Western Blot serology should use DHHS form #3445 and the SPECIAL SEROLOGY mailer.

Reporting Procedures and Interpretation

The following chart provides information regarding test methods and turn-around-times. A brief statement of the “normal” values for each assay is given under the heading “Negative Reference Range”.

Description	Test Procedure	Negative Reference Range	Turn-Around-Time
Human Immunodeficiency Virus type 1 (Groups M & O and type 2 antibodies)	EIA- Qualitative	No antibody detected	10 working days
Human Immunodeficiency Virus type 1 antibody	Western blot- Qualitative	No antibody detected	5 working days
Human Immunodeficiency Virus type 1 RNA	Nucleic Acid Amplification Test (NAAT)- Qualitative	No HIV-1 RNA detected	10 working days
Human Immunodeficiency Virus type 1 (Groups M&O) and type 2 antibodies	Rapid EIA- Qualitative	No antibody detected	10 working days

The State Laboratory of Public Health uses the APHL/CDC criteria* shown below for the interpretation of the Western blot.

Interpretation	Criteria
Negative	The absence of any and all bands – not just viral bands.
Indeterminate	The presence of any other band or bands that fail to meet the positive criteria.
Positive	The Presence of any two of the following bands: p24 gp41 gp120/gp160

* Centers for Disease Control and Prevention. Interpretation and Use of the Western Blot Assay for Serodiagnosis of Human Immunodeficiency Virus Type 1 Infections. MMWR 1989; 38:1-7.

The following recommendations are made regarding follow-up specimens:

1. If patient HIV infection status is inconclusive based upon test results, submit another specimen for testing within a month. If test results for the second specimen are also inconclusive for HIV infection status, the patient should be tested again at six months.
2. When a patient receives the first positive test result, a verification specimen should be collected at the time the patient is given the results of the first test.

HIV COUNSELING AND TESTING REPORT FORM INSTRUCTIONS

INSTRUCTIONS FOR COMPLETING THE HIV COUNSELING AND TESTING REPORT FORM

PURPOSE: The form is to be completed for all patients who undergo HIV counseling and testing events. Do not complete forms for patients who decline testing. Completed forms and specimens are to be sent to the State Laboratory of Public Health at the address on the top of the form. This is a two-sided form. Please complete both sides of this form.

FRONT SIDE: (Please use **X** instead of \surd for check boxes.)

[1] Bar Code: This is the pre-printed bar code number. Do **NOT** mark in this area.

[2] Label: Please affix the standardized Patient Information Label/HSIS Laboratory Label if one is available. The standard label format is available from Virology/Serology. If you use the standardized label, do not enter information in [3]. Please align the label in the box on the right top of the form. The label must fit within the box, not touch the lines on the edge of the box, and the printed information must be parallel to the top of the box. Labels placed at an angle will not be read accurately.

[3] Patient Demographic Information: Complete the information if the standard label/HSIS Laboratory Label is not attached. See example at bottom of page 2. Note: Birth Sex must be marked if different from Current Gender.

- Patient Last Name, First Name, Middle Initial
- County - patient's residence – North Carolina numeric county code, 101 for counties outside of NC.
- State - patient's residence – Alpha state abbreviation, i.e. NC for North Carolina
- Zip Code - patient's residence zip code
- Is the patient on Medicaid? - Mark one box. If patient is unsure, look up Medicaid eligibility if possible before completing.
- Medicaid ID - Medicaid ID number on patient Medicaid card. Look up Medicaid patient number if patient does not have the card.
- Other ID – Patient ID defined for local use, i.e. chart number.
- SSN – Social Security Number of the patient
- Date of Birth – Month/Day/Year (use a 4-digit year)
- Ethnicity – Mark one box.
- Race – Mark all that apply.
- Current Gender – Mark one box.
- Birth Sex – Mark one box.

[4] Visit Information

- Site Number - assigned by the HIV/STD Prevention and Care Branch
- EIN Number – Employment Identification Number with the **SLPH assigned suffix for specific sites**
- Site Type – Indicate site of counseling/testing – Mark one box.
- Date of Visit – Month/Day/Year (use a 4-digit year)

[5] Testing Information

5.1 Patient Previously Tested/Result?

- Mark only one box .

- Most recent test date known? – If patient was previously tested, do they know the month and year of the test?
- If yes, Most recent test date? – Month /Year (4-digit year) of previous test. No day is required.

5.2 Lab Testing –

- A. Patient Tested This Visit & Sample Sent to Lab? - If the patient was tested this visit mark yes if specimen is to be submitted to the SPLH, if not, mark no and go to C.
- B. Type of Sample – Mark one box. *Note: Serum is the only type of sample accepted by the SPLH without prior authorization.*
- C. If Not Tested This Visit, Indicate Reason – If a specimen has not been drawn or will no be sent to the lab, indicate why this was not done. Mark one box.

5.3 Preliminary Testing –

- Preliminary Rapid Test Performed - Mark one box - If yes, complete the rest of section 5.3. **If no, proceed to Section 8.**
- Rapid Test Used – Indicate test brand by marking OraQuick, Reveal, Uni-Gold, or Other. If ‘Other’ is marked, write in name of brand in the blocks provided labeled “Rapid Test Brand”.
- Lot Number – Write in the Lot number of the rapid test used.
- Rapid Test Brand – If the Rapid Test Used list does not include the brand used in testing, indicate the brand name of the test.
- Type of Specimen – Mark the type of specimen collected for the rapid test.
- Rapid Test Results This Visit – Mark one box.
- Rapid Test Results Provided to Client – Did patient receive rapid test results? Mark one box.

[6] Do Not Remove Bar Codes - for State Laboratory Use only

BACK SIDE

[8] Risk Data Information

- Pretest Counselor (number assigned by your agency) – Enter five digits using leading zeros if necessary.
 - Client Counseled – Was patient counseled/educated this visit? Mark yes or no.
 - STARHS Consent – Do not check, no longer applicable.
- If Female, Is Patient Pregnant – mark yes, no or unknown.
- If Pregnant, In Prenatal Care – If the patient is pregnant, mark one box.
 - Outreach Venue - Mark yes or no. Regardless of your site type, indicate if this is part of an outreach activity. An outreach venue is defined as testing services provided outside of a fixed site and/or clinical setting.
 - Reason for the Visit – Mark all that apply.
 - Risk Behaviors in last **12 months** – Mark all that apply.

[9] Additional Demographic Information

- Primary Language – Mark the primary language of the patient, if not English or Spanish, mark Other and enter the name of the primary language in the blocks provided.

Please Note: This form is designed to be scanned by a computer. A data entry person will manually verify any letters or numbers that the computer cannot interpret. Please help us to save time and improve accuracy by writing carefully and following the instructions below. Please use X instead of √ for check boxes.

For optimum accuracy, please print in capital letters and avoid contact with the edge of the box. Follow the sample letters and numbers as closely as possible.	A	B	C	D	E	F	G	H	I	J	K	L	M
	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
	1	2	3	4	5	6	7	8	9	0			

Rabies Virus

(919) 733-7544

Introduction

The State Laboratory of Public Health (SLPH) is the sole source for rabies diagnostic testing in North Carolina. This service is available to all health care providers within the state. Submission of specimens for rabies testing must meet the established testing criteria. Specimens submitted for testing that fail to meet the testing policy will be rejected and destroyed.

Testing resources are reserved for situations where the testing outcome will influence patient management decisions. Terrestrial animal submissions are limited to significant rabies vector species that expose humans, livestock, or unvaccinated pets. Exposure is defined as a bite that breaks the skin or contact of mucous membranes or broken skin with either animal saliva or nervous tissue. Significant rabies vector terrestrial species include raccoons, skunks, foxes, most other carnivores, and woodchucks. **Domestic animals exhibiting signs of rabies and wild animals that have potentially exposed a person, unvaccinated pet, or livestock to rabies should be submitted for testing without delay.**

Dogs, cats, and ferrets that do not exhibit signs of rabies and which bite people, pets or livestock should not be euthanized, but rather should be confined and observed for 10 days, unless circumstances demand otherwise. Observation is of value because the length of time that virus may be excreted in saliva prior to the onset of signs can be predicted. It is known that dogs, cats, and ferrets may excrete rabies virus up to five days prior to the onset of signs. The ten-day observation period for dogs, cats, and ferrets is thus twice the predicted time, allowing a 100% margin of safety. If a dog, cat, or ferret shows no clinical signs of rabies after ten days of observations, one can be assured that the animal was not shedding virus at the time of the exposure. Dogs, cats, and ferrets that survive the 10-day quarantine period should not be submitted to the rabies laboratory for testing. Conversely, if the dog, cat, or ferret does not survive the 10-day quarantine period, the specimen should be submitted to the rabies laboratory for testing.

Wild animals (unlike dogs, cats, and ferrets) do not have a predictable time for shedding of rabies virus prior to presentation of symptoms. Therefore, animals in this group should not be held for observation following an exposure. These animals should be caught, euthanized immediately, and the head submitted for rabies virus detection.

Bats that have interaction with humans should be submitted for testing only if the contact involves: 1) a bite; 2) handling where a bite cannot be ruled out; or 3) are found in a domicile with access to humans while they were asleep, unconscious, or incapacitated. If one or more bats escape capture, do not submit the remaining bats since recommendations regarding post-exposure prophylaxis will not be altered by testing only some of the bats. The State Public Health Veterinarian or epidemiologist

on-call should be consulted regarding any decisions to treat potentially exposed individuals.

Surveillance animals will be tested only with prior approval. Low risk animals (i.e., rabbits, squirrels, opossums, and small rodents) rarely require testing and should not be submitted without prior approval from either our laboratory or the State Public Health Veterinarian at (919) 733-3419.

Routine testing is available Monday through Friday (7:30 a.m. to 4:00 p.m.). Weekend/holiday testing will be handled via a “duty cell phone on-call system” and restricted to emergency situations only. The circumstances constituting an emergency situation for human exposure to suspected rabies must satisfy one of the following criteria:

1. Unprovoked bite from a wild animal, such as a raccoon, fox, skunk, bobcat, etc.
2. Unprovoked bite from an unvaccinated dog or cat.
3. Bite (provoked or not) resulting in skin breakage on either the head or neck.
4. Bites from bats.
5. Bat(s) found in a domicile where people were asleep.

The laboratory on-call person can be reached at (919) 733-7544 during regular hours of operation or by telephoning the duty cell phone at (919) 280-8915 between 4:30 p.m. Friday and noon on Saturday. Specimens received after noon on Saturday (without prior approval) will be tested on the following routine work day, i.e. usually Monday.

Specimen Collection and Identification

Animals should be euthanized in a manner that will not destroy the brain tissue which is examined in the diagnosis of rabies. Thus, only the animal’s head should be submitted for diagnostic purposes. The animal’s neck should be severed at the midpoint between the base of the skull and the shoulders. Small animals no larger than a squirrel may be submitted whole. For bats, the whole dead animal must be submitted and should be secured in a clear container such as a zip-lock bag or equivalent. **Treat any specimens for fleas, ticks, maggots, ants, etc. prior to packing.**

Submitters need to fully complete the submission form (DHHS # 1614) indicating the species of animal, date of bite or other significant exposure, anatomical area exposed, and county where exposed. Also list the name of the individual who will be responsible for contacting this patient, if necessary. Include telephone numbers with area code where the responsible individual can be reached during working hours and nights, weekends, or holidays. If a specimen is received on the weekend or holiday without this information, the specimen will be held and tested on the next routine work day. Seal the rabies submission form in a separate plastic bag and enclose within the specimen container. Complete one form per specimen submitted.

Shipment

Specimens being shipped for rabies testing must meet standards set forth as detailed in 49 CFR 173.199 including:

1. Clear watertight primary, i.e. inner, container. (A clear plastic bag that can be sealed to be leak-proof should suffice.)
2. Absorbent material between the specimen and primary container must be sufficient to absorb all liquids in the primary container. (A butchers meat packaging absorbent pad or equivalent should suffice.)
3. Watertight secondary container. (Another plastic bag that can be sealed to be leak-proof should suffice.).
4. An insulated tertiary container with lid should be utilized, since refrigeration is needed.
5. The last inner container must be marked with the International Biohazard symbol (39 CFR part 111 8.6).
6. Sturdy outer packaging tested to meet the standards must secure the above items. (An ordinary cardboard box does not meet the requirements set forth in 49 CFR.)
7. The outer shipping container must be clearly and durably marked "Biological Substance, category B UN3373".
8. A label must be securely affixed to the outer shipping container that lists complete information about both the shipper and consignee.

Enclose refrigerants to keep the specimen cold and tightly seal. Specimens should be kept cold but NOT FROZEN. DO NOT USE LOOSE WET ICE OR DRY ICE. Specimens inadvertently frozen are still suitable for testing; however, testing may be delayed due to thawing. Submit specimens to the rabies laboratory at the N. C. State Laboratory of Public Health as soon as possible. If shipment will be delayed, refrigerate specimens prior to shipment.

Large animal heads such as cows, horses, deer, large dogs, etc. should be submitted to our rabies laboratory via the Dept. of Agriculture's Rollins Animal Disease Diagnostics Laboratory in Raleigh (919) 733-3986 or one of their satellite laboratories throughout the state:

Hoyle C. Griffin Animal Diagnostic Lab (Monroe)	(704) 289-6448
Northwestern Animal Disease Diagnostic Lab (Elkin)	(336) 526-2499
Western Animal Disease Diagnostic Lab (Arden)	(828) 684-8188

These laboratories will remove the brain tissue and forward the tissue to the SLPH rabies laboratory for testing. Contact the agriculture labs directly for specimen submission information. The anatomical tissues that the SLPH requires for a satisfactory rabies test include either hippocampus or cerebellum and a complete cross section of the brain stem. Specimens fixed in formalin cannot be tested at the SLPH and will be reported as unsatisfactory. (These specimens may be tested at the CDC; the submitter must contact the CDC regarding testing.)

Shipment via State Courier Service is usually the most rapid mode of transit. Personal conveyance may be used when courier service is unsuitable. The laboratory should be informed in advance of the manner of shipment to be used for samples that have been approved for weekend testing. Address all shipping containers using the special label (white with red lettering) available from the SLPH mailing room. This label instructs the transporting service to call the SLPH upon arrival and will assure proper handling of the specimen. If you do not have a specific mailing label, the following information should be clearly visible on the exterior of the mailing container containing the animal head:

TO: State Laboratory of Public Health
306 N. Wilmington St.
Raleigh NC 27601
MSC 1918

“This package contains an animal head suspected of having rabies.”

To transporting company:

“Call on arrival (919) 733-7656 Weekdays (8:00 a.m. – 5:00 p.m.).”

Specimens may be brought to the lobby of the Bath Building, 306 N. Wilmington Street, Raleigh, during working hours (8:00 a.m. to 5:00 p.m.), Monday through Friday. At all other times, specimens should be placed in the specimen drop chute at the back of the building. Packages which are too large for the drop chute must be delivered to the laboratory only during working hours. The maximum size package that will fit into the drop chute is 14 inches x 14 inches x 11 inches (i.e. correlates to Tegrant Thermo Safe Box #38-1KD). DO NOT leave unattended packages on the loading dock, even if arrangements have been made for after-hours testing.

Return of shipping containers: Please contact the Rabies laboratory at (919) 733-7544.

Reporting Procedures and Interpretation

Test results for any animal positive for rabies or any unsatisfactory test result will be telephoned automatically by laboratory staff to the appropriate parties (Public Health veterinarians, submitter, and county animal control) at the numbers provided. **IT IS THE RESPONSIBILITY OF THE SUBMITTER, NOT THE LABORATORY, TO NOTIFY THE**

PERSON EXPOSED. All test results will be sent via US Mail or the State Courier System to the submitter and county health department director in the county where the animal specimen was obtained. It should be noted that although the fluorescent antibody test is very reliable, a negative test does not completely exclude the possibility of the animal being rabid.

All Rabies results are also available on-line to the submitter (<http://slph.ncpublichealth.com>). Go to "login" on the home page. If you are a new user, follow the link at the bottom of the page to request a new account.

Note: Human Rabies Testing:

All suspected cases of rabies in humans are handled on a case-by-case basis. Contact the laboratory at (919) 733-7544 for special instructions on specimen collection criteria and shipping directions. Hospital infection control consultation should be obtained Monday-Friday, 8:00 a.m. to 5:00 p.m., from the rabies public health veterinarians at (919) 733-3419. Consultation services are available after working hours and during weekends or holidays. Leaving a message in the voice mail-box at (919) 733-3419, will automatically activate a beeper for the on-call individual.

Rabies Virus Serology:

Rabies virus antibody testing is available through commercial laboratories. Testing of specimens should be arranged directly with those laboratories. The following laboratory is known to offer the Rapid Fluorescent Focus Inhibition Test for rabies virus antibody:

Rapid Fluorescent Focus Inhibition Test
Department of Veterinary Diagnosis
Veterinary Medical Center
Kansas State University
Manhattan, Kansas 66506
(785) 532-4483
www.vet.ksu.edu/depts/dmp/service/rabies/index.htm

Post-Exposure Prophylaxis:

Consultation prior to post-exposure prophylaxis should be obtained Monday-Friday, 8:00 a.m. to 5:00 p.m., from one of the Public Health Veterinarians at (919) 733-3419

Consultation services are available after work hours and during weekends or holidays. Leaving a message in the voice mail box at (919) 733-3419 will automatically activate a beeper for the on-call individual.

Rubella Serology

(919) 733-7544

Introduction

Immune status testing for rubella antibody is available only to local health departments for prenatal patients with no documentation of vaccination or previous immune status testing. Immune status testing for rubella is also available for both clients and health department employees when vaccination is contraindicated (e.g., pregnancy, immunosuppression, or allergy to vaccine components). Reason for contraindication must be noted on the test request.

Sample Collection and Identification

Submit 2 mL of serum in a plastic screw-capped vial. Serum transport tubes should not be overfilled past the 3.0 mL line on the tubes. Hemolyzed, icteric, or lipemic serum may be unacceptable for certain serologic assays.

Clearly label each vial of serum with the patient's name (first and last) and either the date of birth or Social Security number. Complete submission form DHHS #1188 (immune status testing) or DHHS #3445 (serodiagnosis of current or recent infection). Please note that all suspect or probable rubella cases must be reported to the Immunization Branch at (919)707-5550 for prior approval of Rubella IgM laboratory testing. Failure to supply the requested patient information may result in significantly delayed specimen testing. Please note that all suspect or probable rubella cases must be reported to the Immunization Branch at (919) 707-5550 for prior approval of Rubella IgM laboratory testing.

Specimens submitted for testing that are not labeled with two identifiers will not be tested. Specimens which, for any reason, are deemed unsuitable or inappropriate for serologic testing will not be tested. Rejected specimens will be properly stored for ten days pending verbal and/or written notification of the submitter. Unless alternate arrangements are initiated by the submitter upon notification of specimen rejection, the specimen will be discarded at the end of the holding period.

Although the serodiagnosis of many current or recent viral infections requires the simultaneous testing of paired sera, rubella IgM assays on a single acute serum specimen may provide evidence of a recent rubella infection. Immune status determinations for rubella also require only a single serum sample.

Shipment

Send the properly identified vial of patient serum and the completed submission form DHHS #1188 or DHHS #3445 in the "Special Serology" (blue-colored) mailing containers via the State Courier or U.S. Mail.

Specimens may be shipped refrigerated or at ambient temperature.

Reporting Procedures and Interpretation

The following chart provides information regarding test methods, serum requirements, turn-around times, and negative reference ranges.

Description of Antibody Test	Test Method	Negative Reference Range	Specimen Requirements	Turn-Around-Time
Rubella, Immune Status, IgG	EIA-Qual	Interpreted by report	2mL serum	2 working days
Rubella, IgM	EIA-Qual	Interpreted by report	2 mL serum	2 working days

Abbreviations:

EIA Enzyme Immunoassay
IgG Immunoglobulin G
IgM Immunoglobulin M
Qual Qualitative

Serological Tests Referred to the Centers for Disease Control and Prevention (CDC) through the NC State Laboratory of Public Health

(919) 733-7544

Introduction

Serologic tests for antibodies to some bacterial, fungal, parasitic, chlamydial, rickettsial, and viral agents not performed at this laboratory are available from the Centers for Disease Control and Prevention (CDC), Atlanta, Georgia.

Sample Collection and Identification

Submit 2mL of serum in a plastic screw-capped vial. Hemolyzed, icteric, or lipemic serum may be unacceptable for certain serologic assays. Clearly label each vial of serum with the patient's name (first and last), date collected, and either the date of birth or Social Security number. Complete a DHHS #3445 specifying all required patient information and which infectious agents are suspected. Specimens sent to the CDC for testing also require a fully completed CDC 50.34 (DASH form).

Services are available to all health care providers. Only serum may be submitted for serologic testing. Specimens must be submitted through the State Laboratory of Public Health, Virology/Serology Unit in the same manner as those for special serology specimens. Specific requirements for specimen submission vary depending upon the nature of the infectious agent involved and the assay requested. In general, all specimens submitted to the State Laboratory to be forwarded to the CDC must include the patient's age, sex, the date of the onset of illness, collection date, pertinent history, and clinical information.

Specimens submitted for diagnostic testing labeled with incorrect patient identification information will not be tested. Patient identification includes full first and last name and either date of birth or SSN. Specimens that, for any reason, are deemed unsuitable or inappropriate for diagnostic testing will not be tested. Rejected specimens will be properly stored for seven days pending verbal and/or written notification of the submitter. Unless alternate arrangements are initiated by the submitter upon notification of specimen rejection, the specimen will be discarded at the end of the holding period.

Shipment

Send the properly identified vial of patient serum and both completed forms in the "Special Serology" (blue-colored) mailing containers via the State Courier or U.S. Mail. Specimens may be shipped refrigerated or at ambient temperature.

Reporting Procedures and Interpretation

The average turn-around-time in which results can be expected back from the CDC is about three weeks. Interpretation of test results is included in the report, if sufficient clinical information was included on the submission form.

**Special Serology Testing:
Measles, Mumps, Varicella Zoster, Rocky Mountain Spotted Fever**
(919) 733-7544

Introduction

Diagnostic and immune status serologic assays are performed for various rickettsial and viral agents. Assay methods vary depending upon the specific test requested. For hepatitis, syphilis, rubella, and HIV serologies, see separate sections.

Screening for immunity to measles, mumps, and varicella zoster is not available on a routine basis. Exceptions to this policy apply to local health departments only and include the following:

1. All suspect or probable cases of vaccine preventable diseases (measles, mumps, varicella zoster) must be reported to the Immunization Branch at (919)707-5550 for prior approval of laboratory testing.
2. “Stat” varicella zoster virus (VZV) immune status testing is available for prenatal clients **only** who lack a clear history of varicella zoster infection or whose immune status is unknown and have been exposed to a known case of VZV. In cases in which testing is appropriate and results are urgently needed, the submitter must contact the Women’s Health Nurse Consultant or Maternal Health Nurse Consultant to arrange for testing at the State Laboratory. The Consultant will then contact either the Virology/Serology Unit Supervisor or the Special Serology Laboratory Supervisor at (919) 733-7544 so that testing can be scheduled for timely results, preferably during normal business hours. Use DHHS #3445 and provide a contact name and telephone number for the person who is to receive the test result. If required, every effort will be made to provide “stat” VZV testing during off hours, weekends, and holidays; however, this testing is dependent upon the availability of limited trained personnel who are not designated “on call”.
3. Immune status testing for measles is available for clients when vaccination is contraindicated (e.g., pregnancy, immunosuppression, or allergy to vaccine components). Reason for contraindication must be noted on the test request. Use DHHS #3445.

Sample Collection and Identification

Submit 2-3 mL of serum in a plastic screw-capped vial. Hemolyzed, icteric, or lipemic serum may be unacceptable for certain serologic assays.

Clearly label each vial of serum with the patient’s name (first and last), date collected, and either the date of birth or Social Security number. Complete a DHHS #3445

submission form specifying all required patient information and which infectious agents are suspected.

Specimens submitted to the Virology/Serology Unit must be accompanied by a fully completed submission DHHS #3445. Failure to supply the requested patient information may result in significantly delayed specimen testing. Tests must be requested by name. Nonspecific requests for “viral studies” or “viral serologies” will not be accepted. Consult with the laboratory if there is a question as to which test is appropriate.

Specimens submitted for testing that are not labeled with correct patient identification information will not be tested. Patient identification includes two identifiers. Specimens which, for any reason, are deemed unsuitable or inappropriate for serologic testing will not be tested. Rejected specimens will be properly stored for seven days pending verbal and/or written notification of the submitter. Unless alternate arrangements are initiated by the submitter upon notification of specimen rejection, the specimen will be discarded at the end of the holding period.

Note: The serodiagnosis of a current or recent infection generally requires the simultaneous testing of paired serum samples, acute and convalescent serum samples. The acute serum should be collected no later than 3-5 days after the onset of illness. The convalescent serum should be collected 2-4 weeks after onset. Where paired sera are advised or required, it is to the advantage of both the submitter and this Laboratory if the acute serum is stored frozen by the submitter until the convalescent serum is collected. Both serum samples may be submitted with one submission form.

Serologic diagnosis of mumps between acute and convalescent sera can be made by demonstrating a four fold or greater rise in titer. For certain agents, such as measles, specific IgM assays on a single acute serum specimen may provide evidence of a recent infection. Additionally, single “high” antibody titers to viral, and rickettsial agents may be considered presumptive evidence of recent infection. Immune status determinations require a single serum sample only and should be clearly designated on the request form.

Shipment

Send the properly identified vials of patient sera and the completed DHHS #3445 in the “Special Serology” (blue-colored) mailing containers via the State Courier or U.S. Mail. Specimens may be shipped refrigerated or at ambient temperature.

Reporting Procedures and Interpretation

Failure to detect a significant antibody response may be the result of a number of factors including improperly collected specimens, specimens collected too early or too

late during the immune response, selection of the incorrect infectious agent for testing, or lack of sensitivity in the serological system being used.

The following chart lists the special serologic assays performed by this laboratory. A brief statement of the “normal” values for each assay is given under the heading “Negative Reference Range”. The test method, specimen requirements, and turn-around-times are also listed for each assay performed.

Special Serology Assays

Description of Antibody Test	Test Method	Negative Reference Range	Specimen Requirements	Turn-Around-Time
<i>Coxiella burnetti</i> (Q fever), IgG	IFA-Quan	<1:64	2 mL serum; PSA	7 calendar days
<i>Ehrlichia chaffeensis</i> , IgG	IFA-Quan	<1:64	2 mL serum	7 calendar days
Measles, IgM	IFA-Qual	Interpreted by Report	2 mL serum	3 calendar days
Measles, IgG	IFA-Qual	Interpreted by Report	2 mL serum	7 calendar days
Mumps, Diagnostic IgG	IFA-Quan	Interpreted by Report	2 mL serum; PSA	7 calendar days
<i>Rickettsia rickettsii</i> (RMSF), IgG	IFA-Quan	<1:64	2 mL serum	7 calendar days
<i>Rickettsia typhi</i> (Typhus), IgG	IFA-Quan	<1:64	2 mL serum	7 calendar days
<i>Varicella zoster</i> , IgG	IFA-Qual	Interpreted by Report	2 mL serum	7 calendar days

Abbreviations:

- IgG Immunoglobulin G
- IgM Immunoglobulin M
- IFA Indirect Fluorescent Antibody
- Quan Quantitative
- Qual Qualitative
- PSA Paired Sera Advised

Syphilis Serology

(919) 733-7544

Introduction

Syphilis, a disease caused by infection with the bacterium *Treponema pallidum*, can be readily diagnosed by serologic methods. Serologic assays used to screen patients for syphilis are non-treponemal tests. The nontreponemal test performed in this laboratory is the Tolidine Red Unheated Serum Test (TRUST). Confirmation of reactive screening test results (TRUST) is obtained through the use of specific treponemal tests for syphilis. The TREP-SURE EIA test is performed in this laboratory to confirm syphilis screening test results when appropriate. The Venereal Disease Research Laboratory (VDRL) and the Fluorescent Treponema Antibody Absorption (FTA-ABS) assays are no longer performed at the NC State Laboratory of Public Health. These procedures are available from commercial reference laboratories.

Sample Collection and Identification

The non-treponemal test for syphilis (TRUST) performed on serum is available only to local health departments and state-operated health facilities. Although the specific treponemal test for syphilis (TREP-SURE) is available to all health care providers, it is not designed to be a screening procedure and thus is only performed when required for proper patient management.

Submit 2-3 mL of serum in a plastic screw-capped vial. Grossly hemolyzed, icteric, or lipemic serum is unacceptable for syphilis serologic assays. Clearly label each vial of serum with the patient's name (first and last), and either date of birth or Social Security number.

Recommended Tests for the Different Stages of Syphilis

Disease Stage	Specimen	Test to Request
Screening	Serum	TRUST
Primary	Serum	TRUST
Secondary	Serum	TRUST
Latent	Serum	TRUST, TREP-SURE
Late Neurosyphilis	Serum	TRUST, TREP-SURE
Congenital CNS Involvement	Serum	TRUST, TREP-SURE

All screening tests performed in this laboratory which are determined to be reactive will be confirmed by the TREP-SURE test, unless a previous positive TREP-SURE or other confirmatory test result is on file at the laboratory. In those cases, only the screening test results will be reported.

A request to this laboratory for a TREP-SURE test must be accompanied by a quantitative screening test result; i.e., the submitter must provide a titer. This request will yield only a qualitative TREP-SURE test result without performing a screening test. If a previous positive TREP-SURE or other confirmatory test result is on file at the laboratory, no testing will be performed.

For the purposes of evaluating patients suspected of having late syphilis, the TREP-SURE test will be performed in this laboratory on serum regardless of the screening test result. Under these circumstances, the submitter must specifically request a TREP-SURE test, state the quantitative screening test result/titer, and indicate that late syphilis is suspected.

Note: A law was ratified in June, 1981 which rescinded the North Carolina law requiring a premarital serologic test for syphilis. The Laboratory maintains premarital medical examination forms, if available, for all states that require a serologic test for syphilis. All states requiring a premarital syphilis test accept test results from the State Laboratory of Public Health. North Carolina no longer has a list of “approved laboratories” that perform premarital serologies.

Note: North Carolina Administrative Code, Chapter 19, Section .0204(e) states... *“All pregnant women shall be tested for syphilis and gonorrhea early in pregnancy and in the third trimester. Pregnant women at high risk for exposure to syphilis and gonorrhea shall also be tested for syphilis and gonorrhea at the time of delivery.”*

For TRUST screening, complete DHHS #3446; for the TREP-SURE test, complete DHHS #3445. Specimens submitted to the Virology/Serology Unit must be accompanied by a fully completed form DHHS #3445 or DHHS #3446. Failure to supply the requested patient information may result in significantly delayed specimen testing.

Only serum may be submitted for primary serologic syphilis testing. Specimens submitted for diagnostic testing not labeled with correct patient identification information will not be tested and will be discarded. Patient specimen identification includes full first and last name and either date of birth or SSN. Specimens that, for any reason, are deemed unsuitable or inappropriate for diagnostic testing will not be tested and will be discarded. Specimens received without a test requisition will be properly stored for ten days pending verbal and/or written notification of the submitter. Unless a test requisition is received, the specimen will be discarded at the end of the holding period.

Shipment

Properly identified vials of patient sera and completed submission forms are sent to the Laboratory in white-colored specimen mailers labeled SYPHILIS SEROLOGY. Ship at ambient temperature by the State Courier or U.S. Mail.

Reporting Procedures and Interpretation

Results of nontreponemal tests for syphilis (TRUST) performed on serum are available within two working days after receipt of the specimen. Treponemal specific tests (TREP-SURE) performed on serum are available within three working days after receipt of specimen.

Patients with primary syphilis may have a non-reactive TRUST and/or TREP-SURE when first seen; however, these tests will usually become reactive soon thereafter. Most patients treated for primary syphilis will have a reversion of nontreponemal tests to non-reactive within 2-3 years. The TREP-SURE test will usually remain reactive after treatment. Non-reactive serologic tests and normal clinical evaluations do not exclude incubating syphilis.

Syphilis Serology Test Results and Interpretations

TRUST Results	TREP-SURE Results	Interpretation
Reactive	Reactive	Usually indicates syphilis.
Reactive	Non-Reactive	"Biologic False Positive" reaction in reagin tests may be caused by infection, immunizations, inflammatory disease, immunity abnormalities, drug addiction, pregnancy, or aging. Tests should be repeated on a follow-up specimen if doubt exists.
Non-Reactive	Not Done	Treponemal tests are not indicated unless late syphilis is suspected according to clinical data.
Non-Reactive	Reactive	Usually indicates previously treated syphilis or late syphilis (untreated).

Virus Culture

(919) 733-7544

Introduction

Successful performance of virologic studies is in part dependent upon the cooperation of informed clinicians who will obtain proper specimens taken at the correct time during the patient's illness and provide sufficient clinical information for the laboratory to select the appropriate test or tests. Virus culture employing assorted cell culture systems and molecular assays provide a mechanism for the detection and identification of many human viruses which cause a wide variety of common illnesses. The Virus Culture lab is capable of isolating and identifying most Biological Safety Level I through III viruses that can be propagated in conventional cell culture. Molecular testing (RT-PCR; NAAT) is also routinely available for some viral agents, such as influenza.

Sample Collection and Identification

Routine Viral Cultures:

All appropriate diagnostic specimens for culture of human viruses will be accepted from both public and private providers of health care.

Viruses are obligate intracellular parasites. Consequently, diagnostic specimens for viral culture must be vigorously collected to ensure the presence of infected cells for optimal results. Specimens for viral culture should be collected as soon as possible after the onset of clinical illness (i.e., 24-72 hours). Specimens collected more than one week after onset usually do not yield live viruses. Clearly label each specimen with the patient's full name (first and last) and either the date of birth, Social Security number, or unique identifier (such as internal record number). Complete DHHS #3431, supplying all required patient information and specifying the virus agent suspected. Please provide a complete submitter's mailing address, EIN#, physician name, and telephone number. Minimal essential patient information that must be provided includes: the patient's first and last name, date of birth, either Social Security Number or unique identifier (such as internal medical record number), Medicaid number (if applicable), sex, onset date, **plus** specimen source and collection date. Also provide information on the suspected infectious agent(s) and/or provide the patient's signs and symptoms, including vaccination and/or travel history, if applicable. Failure to supply the requested patient information may result in significantly delayed specimen testing.

Specimens that, for any reason, are deemed unsuitable or inappropriate for diagnostic testing will not be tested. Rejected specimens will be properly stored for seven days pending verbal and/or written notification of the submitter. Unless alternate arrangements are initiated by the submitter upon notification of specimen rejection, the specimen will be discarded at the end of the holding period.

The source of the specimen(s) collected must be carefully matched with the virus suspected. A chart is included which describes the virus isolation service available at

the State Laboratory, the turn-around-time for virus cultures, and the specimens of choice for each virus listed. Dacron-tipped, rayon-tipped, or flocked swabs with plastic or aluminum shafts are acceptable. Cotton-tipped swabs with wooden shafts are not recommended; calcium alginate swabs are not acceptable. Most specimens can be held at 4-8°C for several days before there is a significant loss of infectivity. If transport to the laboratory will be delayed for more than several days, freezing specimens to -70°C or below will preserve viral infectivity of specimens almost indefinitely. Many viruses lose infectivity rapidly when stored at -20°C or warmer. Specimens to be tested for respiratory syncytial virus (RSV), varicella zoster virus (VZV), or cytomegalovirus (CMV) should NOT be frozen since these viruses are easily inactivated.

The following general guidelines may be used when properly collecting specimens for virus culture:

- A. **Autopsy or Biopsy**
Collect fresh, unfixed tissue from the probable sites involved using a separate sterile instrument for each sample. Place each specimen into a separate small, sterile vial of virus transport medium. Screw the cap on tightly. Keep cold (~ 4°C) pending prompt shipment on icepacks.
- B. **Cerebrospinal Fluid**
Discard the virus transport medium from a small specimen vial. Aseptically collect about 3 ml of CSF and transfer to the empty vial. Screw the cap on tightly. Keep cold (~ 4°C) pending prompt shipment on icepacks.
- C. **Feces**
Discard transport medium from small specimen vials. Place a piece of feces about 2-5 grams (approximately the size of the end of an adult thumb) into a vial. Screw the cap on tightly. Keep cold (~ 4°C) pending prompt shipment on icepacks.
- D. **Nasal/Nasopharyngeal Swab**
Pass a flexible, fine-shafted swab into the nostril/nasopharynx. Rotate slowly for 5 seconds to absorb secretions. Remove swab and place into a vial of viral transport medium. Repeat for the other nostril using a fresh swab. Place both swabs in the same transport tube.
- E. **Nasopharyngeal Aspirate or Wash**
Pass appropriately-sized tubing or catheter into the nasopharynx. Aspirate material with a small syringe. If material cannot be aspirated, tilt patient's head back about 70° and instill 3 to 7 mL of sterile saline or viral transport medium until it occludes the nostril. Re-aspirate. If < 2 mL is recovered, deposit directly into viral transport medium. If > 2 mL is recovered, no additional viral transport medium is required.

- F. **Rectal Swabs**
Generally, rectal swabs are less satisfactory than feces for the isolation of viruses. If used, rectal swabs are obtained by inserting a dry swab at least 5 cm into the anal orifice, rotating the stick and then withdrawing it. Some fecal material must be obtained on the swab tip. The swab tip is then broken off into a vial of viral transport medium. Screw the cap on tightly. Keep cold (~ 4°C) pending prompt shipment on icepacks.
- G. **Throat Swabs**
Vigorously rub the tonsils and posterior wall of the pharynx with a dry, sterile swab. The swab should not touch the tongue or buccal mucosa. Break off the swab tip into a vial of virus transport medium. Nasopharyngeal washings with about 10 ml of broth are acceptable as well. Screw the cap on tightly. Keep cold (~ 4°C) pending prompt shipment on icepacks.
- H. **Urine**
Discard the virus transport medium from the small specimen vials. Collect clean voided urine, preferably first voided morning urine. Transfer to the small specimen vials. Screw the cap on tightly. Keep cold (~ 4°C) pending prompt shipment on icepacks.
- I. **Vesicle**
Using a sterile instrument, open the fluid filled vesicle. Using firm pressure, absorb the fluid with a sterile swab and scrape the perimeter of the lesion obtaining cellular material on the swab tip. Avoid causing excessive bleeding. Break off the swab tip into a vial of virus transport medium. Screw the cap on tightly. Keep cold (~ 4°C) pending prompt shipment on icepacks.
- J. **Tissue Culture Isolates**
The Virus Culture Lab provides referral identification services for laboratories throughout North Carolina which perform viral isolation. Referral specimens should be observed microscopically at the initial laboratory until 50% or more of the available cell sheet is exhibiting viral cytopathogenic effect (CPE). These specimens may be shipped as a Biological Substance Category "B". If the virus is suspected to be a Category "A" infectious substance, as defined by the Federal Register, then ship as "dangerous goods". Samples should be frozen on dry ice and be accompanied by a completed DHHS #3431 indicating the original anatomical site and the type of cell culture which grew the viral-like agent. Please indicate the suspected virus when completing the test request form.
- K. **Buccal Swabs**
The parotid gland is located below the zygomatic arch (triangular bone of the cheek), below and in front of the ear. The parotid (Stenson's) duct drains this gland and empties into the buccal cavity opposite the second upper molar. Massage the parotid gland for 30 seconds, and then use a swab to sweep the parotid duct area of the buccal surface from the upper to the lower molars.

HSV Cultures:

The HSV culture service is available only to local health departments and other state operated health care facilities. Specimens acceptable for HSV culture are limited to the following:

1. Specimens from prenatal patients who have a suspicious lesion not previously confirmed as herpes. Routine cultures in the absence of lesions will not be accepted.
2. Specimens from patients presenting with an atypical lesion where a clinical distinction cannot be made between herpes, chancroid, and syphilis. Cultures done simply to confirm a clinical diagnosis of herpes are not available on a routine basis.

Using a sterile instrument, open the fluid filled vesicle. Using firm pressure, absorb the fluid with a sterile swab and scrape the perimeter of the lesion obtaining cellular material on the swab tip. Avoid causing excessive bleeding. Break off the swab tip into a vial of virus transport medium. Screw the cap on tightly. Keep cold (~ 4°C) pending prompt shipment on icepacks. Clearly label the specimen with the patient's full name (first and last) and either the date of birth, Social Security number, or unique identifier (such as internal medical record number).

Specimens submitted for herpes viral culture must be accompanied by a DHHS #3431 that includes the clinic in which the patient was seen and the specific reason for testing, i.e., differential diagnosis of an atypical lesion, lesions in pregnant women, etc. Submitters need to fully complete the submission form indicating patient's first and last name, date of birth, either Social Security Number or unique identifier (such as internal medical record number), Medicaid number (if applicable), sex, race, specimen source, collection date, onset date, submitter information (including clinic and contact information), pregnancy status and due date (if applicable), date specimen submitted, and patient signs and symptoms. Select Herpes Simplex Virus as the agent requested. Failure to supply the requested clinical patient information may result in significantly delayed specimen testing or rejected specimens.

Herpes virus culture from urogenital sites is limited to one specimen per patient. If more than one urogenital site is cultured, both swabs should be submitted in the same transport tube. Cultures from multiple sites submitted individually will be pooled in the laboratory at the risk of diluting out the virus. Please **DO NOT** place more than two swabs in a single viral transport medium vial.

Shipment

Seal the form in a separate plastic bag and enclose with the specimen between the secondary and tertiary container. Submit no more than three specimens per patient with each form. One form can be used for up to three different specimens from the same patient.

Keep clinical specimens cold (~ 4°C) during transit and ensure delivery to the State Laboratory within 24-48 hours of collection. Ideally, ship specimen(s) to the State Laboratory the same day collected. Although the virus transport mailer was designed for several specimens, the cost of the transport medium is negligible and unused medium can simply be discarded. Do not delay the shipment of specimens until all the vials of transport medium are used.

Specimens submitted for viral isolation should be packaged according to 49 CFR and Department of Transportation Regulations.

1. Wrap absorbent material around the primary container containing the specimen, which is properly labeled with the patient name and either the date of birth, Social Security number, or unique identifier (such as internal medical record number).
2. Place the properly identified inoculated vials of transport medium into the large conical plastic shipping tubes. If all of the transport medium is not used, return the unused large conical plastic shipping tubes to maintain a tight pack and prevent breakage. Place the two frozen ice packs into the shipping container.
3. Place the large conical plastic tubes containing specimen(s) or tubes without specimens (for a total of three tubes) between the ice packs. Place the completed forms into the plastic bag and slide into the space at the narrow end of the ice packs. Replace the styrofoam lid on the box, seal the cardboard box, and attach the return pre-addressed shipping label on top of the label used to ship the kit to you. Ship the specimen to the State Laboratory by the fastest means possible.

Report Procedures and Interpretation

Turn-around time for negative cultures varies from one to six weeks. Cultures yielding virus isolates may require more time for identification of the virus, depending upon the isolate involved. Failure to isolate a virus may be the result of a number of factors, including improperly collected specimens, specimens collected at a period in the disease when the patient is not shedding virus, improperly transported specimens, or a lack of sensitivity in the system being used for isolation. Failure to isolate a virus should not rule out the virus as a cause of the clinical illness. Conversely, since people may asymptotically carry a variety of viruses, viruses may be isolated which are unrelated to the current illness. The clinician should interpret the laboratory report in conjunction with patient history and clinical findings.

Virus Culture Service

Virus Description	Test Method	Specimen Requirements	Turn Around Time (if Negative)
Adenovirus	Cell culture	Throat washing or swab, nasal swab, nasopharyngeal washing or swab, conjunctival swab, feces, pericardial fluid	3 weeks
Arbovirus	Cell culture	Brain tissue, CSF	3 weeks
California encephalitis	Cell culture	Brain tissue, CSF	3 weeks
Coxsackievirus	Cell culture	Throat swab, feces, CSF, pericardial fluid, skin tissue	3 weeks
Cytomegalovirus	Cell culture	Urine, throat swab, lung tissue, lung aspirate	6 weeks
Eastern equine encephalitis	Cell culture	Brain tissue, CSF	3 weeks
Echovirus	Cell culture	Throat swab, feces, CSF, pericardial fluid, skin tissue	3 weeks
Encephalitis/Meningitis	Cell culture	Brain tissue, CSF	3 weeks
Enterovirus	Cell culture	Throat swab, feces, CSF, pericardial fluid, vesicle scraping	3 weeks
Herpes simplex	Cell culture	Vesicle scraping, brain biopsy, CSF, conjunctival swab	1 to 3 weeks
Influenza	Cell culture PCR	Throat washing or swab, nasal swab, nasopharyngeal washing or swab, lower respiratory specimens <i>PCR: nasal or nasopharyngeal swabs only</i>	3 weeks <i>PCR: 1 to 3 days</i>
Mumps	Cell culture PCR	Throat swab, CSF, buccal swab	3 weeks <i>PCR: 1 to 3 days</i>
Parainfluenza virus	Cell culture	Throat washing or swab, nasal swab, nasopharyngeal washing or swab	3 weeks
Poliovirus	Cell culture	Throat swab, feces, CSF	3 weeks
Respiratory syncytial	Cell culture	Nasopharyngeal washing or swab	3 weeks
Respiratory virus	Cell culture	Throat washing or swab, nasal swab, nasopharyngeal washing or swab	3 weeks
Rubella (Reference Lab)	Cell culture PCR	Nasopharyngeal swab	Up to 8 weeks
St. Louis encephalitis	Cell culture	Brain tissue, CSF	3 weeks
Varicella-zoster	Cell culture DFA	Vesicle scraping	4 weeks <i>DFA: 1 to 2 days</i>
Virus isolate identification	Cell culture	Frozen isolate	Varies
Western equine encephalitis	Cell culture	Brain tissues, CSF	3 weeks

APPENDIX A

CLINICAL HOLDING TIMES

MICROBIOLOGY

Special Bacteriology	<ul style="list-style-type: none"> • Bordetella and Legionella can be refrigerated (<u>not</u> frozen) up to several days. • Gram positive cocci reference isolates can be held at room temperature or in refrigerator for several days. Strep pneumoniae is moisture sensitive and should have a small amount of Heart Infusion Broth added to the tube.
Atypical Bacteriology	<ul style="list-style-type: none"> • Neisseria and Haemophilus are good up to 72 hours in an incubated, CO₂ atmosphere. • Gram positive and gram negative bacilli reference isolates may be good up to a week if not subjected to extreme temperatures.
Enteric Bacteriology	<ul style="list-style-type: none"> • Salmonella may be held in transport media up to 7 days at room temperature. • E. coli and Shigella may be held in transport media 4 to 5 days at room temperature, 7 to 10 days if refrigerated. • Campylobacter may be held in transport media up to 4 days at room temperature, 7 to 10 days if refrigerated. • Reference isolates may be held up to 1 month at room temperature or refrigerated.
Mycology	<ul style="list-style-type: none"> • Clinical specimens may be acceptable up to 1 week refrigerated. • Reference specimens may be acceptable up to 2 to 3 weeks unless culture is mixed and one organism overgrows another.
Parasitology	<ul style="list-style-type: none"> • Stool specimens should be preserved within 30 minutes of passing, and not frozen or incubated. Once in preservative (formalin or PVA), they keep for an extended period of time. • Blood smears need to be made within one hour of draw and must be received in the lab within 3 days. Time and heat will degrade the blood film and the quality of the stain. • Corneal scrapings for Acanthamoebae must be received in the lab within 24 hours of collection. <u>The lab must be notified 24 hours in advance of submission.</u>
Tuberculosis	<ul style="list-style-type: none"> • Specimens should be held in the refrigerator and will be viable for a limited time

VIROLOGY/SEROLOGY

Serological Specimens (i.e. either serum or a serum/CSF pair)	<ul style="list-style-type: none"> • These specimens, if aseptically collected and maintained, are stable at ambient temperature for several days or refrigerated (4-8° C) for up to four (4) weeks. They can be frozen without damage to the antibody. Examples of serological tests include hepatitis, rubella, syphilis, arboviruses, rickettsia, and assorted other special serology antigens. Sera for HIV testing must not be kept at room temperature except during transport to the laboratory. Due to the temperature labile nature of HIV RNA, serum must be immediately refrigerated or frozen until shipped.
Chlamydia/GC Genprobe swabs	<ul style="list-style-type: none"> • Must be tested within 60 days of collection and should be transported and stored at room temperature.
Chlamydia/GC Genprobe urines	<ul style="list-style-type: none"> • Must be tested within 30 days of collection and should be transported and stored at room temperature.
Viral Isolation Specimens	<ul style="list-style-type: none"> • These specimens must be kept refrigerated post collection and while in transit to our laboratory. Freezing is not recommended for clinical viral specimens but if absolutely necessary, it should be at minus 40° C or colder, i.e. dry ice temps. Prolonged storage (this varies by the virus) of clinical specimens at refrigeration or freezing will result in viable virus reduction usually by a log or more and should be avoided if possible. Do not freeze specimens for respiratory syncytial virus (RSV), varicella zoster (VZV) or cytomegalovirus (CMV).
Rabies Specimens	<ul style="list-style-type: none"> • Should be kept refrigerated post collection and in transit to our laboratory. Freezing is NOT recommended.

NEWBORN SCREENING/CLINICAL CHEMISTRY

NBS Blood Spot Filter Specimen	<ul style="list-style-type: none">• Can be held at room temperature away from sunlight, moisture, and heat and mailed immediately upon restoration of transport services. Do not mail in plastic bags.
Sickle Cell (Whole blood)	<ul style="list-style-type: none">• Can be held up to one week refrigerated.
Blood lead	<ul style="list-style-type: none">• Must be received within 28 days.
Prenatal Testing Samples	<ul style="list-style-type: none">• Must be received at the State Lab within 5 days.

CANCER CYTOLOGY

ThinPrep Pap specimens	<ul style="list-style-type: none">• Must be processed within 21 days of collection. Specimens received past that time frame are rejected. Temperature range is 4 to 37°C after specimen is put in vial.
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BIOTERRORISM/EMERGING PATHOGENS

All samples	<ul style="list-style-type: none">• All samples should either be driven to the lab or shipped next day air with FEDEX.
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APPENDIX B

ENVIRONMENTAL HOLDING TIMES

CHEMISTRY

Parameter/ Method	Preservative	Sample Holding Time	Extract Holding Time and Storage Conditions	Suggested Sample Size	Type of Container
Metals (except Hg)	HNO ₃ pH<2	6 months		1 L	Plastic or Glass
Mercury	HNO ₃ pH<2	28 days		100 mL	Plastic or Glass
Alkalinity	Cool, 4C	14 days		100 mL	Plastic or Glass
Asbestos	Cool, 4C	48 hours		1 L	Plastic or Glass
Chloride	none	28 days		100 mL	Plastic or Glass
Residual Disinfectant	none	immediately		200 mL	Plastic or Glass
Color	Cool, 4C	48 hours		100 mL	Plastic or Glass
Conductivity	Cool, 4C	28 days		100 mL	Plastic or Glass
Cyanide	Cool, 4C, Ascorbic acid (if chlorinated), NaOH pH>12	14 days		1 L	Plastic or Glass
Fluoride	none	1 month		100 mL	Plastic or Glass
Foaming Agents	Cool, 4C	48 hours			
Nitrate (chlorinated)	Cool, 4C non-acidified	14 days		100 mL	Plastic or Glass
Nitrate (non chlorinated)	Cool, 4C, non-acidified	48 hours		100 mL	Plastic or Glass
Nitrite	Cool, 4C	48 hours		100 mL	Plastic or Glass
Nitrate+ Nitrite	H ₂ SO ₄ pH<2	28 days		100 mL	Plastic or Glass

Parameter/ Method	Preservative	Sample Holding Time	Extract Holding Time and Storage Conditions	Suggested Sample Size	Type of Container
Odor	Cool, 4C	24 hours		200 mL	Glass
pH	none	immediately		25 mL	Plastic or Glass
o-Phosphate	Cool, 4C	48 hours		100 mL	Plastic or Glass
Silica	Cool, 4C	28 days		100 mL	Plastic
Solids (TDS)	Cool, 4C	7 days		100 mL	Plastic or Glass
Sulfate	Cool, 4C	28 days		100 mL	Plastic or Glass
Temperature	none	immediately		1 L	Plastic or Glass
Turbidity	Cool, 4C	48 hours		100 mL	Plastic or Glass
502.2	Sodium Thiosulfate or Ascorbic Acid, 4C, HCl pH<2	14 days		40-120 mL	Glass with PTFE Lined Septum
504.1	Sodium Thiosulfate Cool, 4C	14 days	4C, 24 hours	40 mL	Glass with PTFE Lined Septum
505	Sodium Thiosulfate Cool, 4C	14 days (7 days for Heptachlor)	4C, 24 hours	40 mL	Glass with PTFE Lined Septum
506	Sodium Thiosulfate Cool, 4C, Dark	14 days	4C, dark 14 days	1 L	Amber Glass with PTFE Lined Cap
507	Sodium Thiosulfate Cool, 4C, Dark	14 days(see method for exceptions)	4C, dark 14 days	1 L	Amber Glass with PTFE Lined Cap

508A	Cool, 4C	14 days	30 days	1 L	Amber Glass with PTFE Lined Cap
508.1	Sodium Sulfite HCl pH<2 Cool, 4C	14 days (see method for exceptions)	30 days	1 L	Glass with PTFE Lined Cap
515.1	Sodium Thiosulfate Cool, 4C, Dark	14 days	4C, dark 28 days	1 L	Amber Glass with PTFE Lined Cap
515.2	Sodium Thiosulfate or Sodium Sulfite HCl pH<2 Cool, 4C, Dark	14 days	≤4C, dark 14 days	1 L	Amber Glass with PTFE Lined Cap
515.3	Sodium Thiosulfate Cool, 4C, Dark	14 days	≤4C, dark 14 days	50 mL	Amber Glass with PTFE Lined Cap
515.4	Sodium Sulfite, dark, cool ≤10C fro first 48 hr. ≤6C thereafter	14 days	21 days at ≤0C	40 mL	Amber glass with PTFE lined septum
524.2	Ascorbic Acid or Sodium Thiosulfate HCl pH<2, Cool 4C	14 days		40-120 mL	Glass with PTFE Lined Septum
525.2	Sodium Sulfite, Dark, Cool, 4C, HCl pH<2	14 days (see method for exceptions)	30 days from collection	1 L	Amber Glass with PTFE Lined Cap
531.1, 6610	Sodium Thiosulfate, Monochloroacetic acid, pH<3, Cool, 4C	Cool 4C 28 days		60 mL	Glass with PTFE Lined Septum
531.2	Sodium Thiosulfate, Potassium Dihydrogen Citrate buffer to pH 4, dark, ≤10C for first 48 hr, <6C after that	28 days		40 mL	

Parameter/ Method	Preservative	Sample Holding Time	Extract Holding Time and Storage Conditions	Suggested Sample Size	Type of Container
547	Sodium Thiosulfate Cool, 4C	14 days(18 mo.frozen)		60 mL	Glass with PTFE Lined Septum
548.1	Sodium Thiosulfate (HCl pH 1.5-2 if high biological activity) Cool, 4C, Dark	7 days	14 days ≤4C	≥ 250 mL	Amber Glass with PTFE Lined Septum
549.2	Sodium Thiosulfate, (H ₂ SO ₄ pH<2 if biologically active) Cool, 4C, Dark	7 days	21 days	≥ 250mL	High Density Amber Plastic or Silanized Amber Glass
550, 550.1	Sodium Thiosulfate Cool, 4C, HCl pH<2	7 days	550, 30 days 550.1, 40 days Dark, 4C	1 L	Amber Glass with PTFE Lined Cap
551.1	Sodium Sulfite, Ammonium Chloride, pH 4.5-5.0 with phosphate buffer Cool, 4C	14 days		≥ 40 mL	Glass with PTFE Lined Septum
552.1	Ammonium chloride Cool, 4C, Dark	28 days	≤4C, dark 48 hours	250 mL	Amber Glass with PTFE Lined Cap
552.2	Ammonium chloride Cool, 4C, Dark	14 days	7 days ≤4C, dark 14 days ≤-10C	50mL	Amber Glass with PTFE Lined Cap
555	Sodium Sulfite HCl, pH≤2 Dark, Cool 4C	14 days		≥ 100 mL	Glass with PTFE Lined cap
1613	Sodium Thiosulfate Cool, 0-4C, Dark		Recommend 40 days	1 L	Amber Glass with PTFE Lined Cap

RADIOCHEMISTRY

Parameter	Preservative	Container	Maximum* Holding Time
Gross Alpha	Conc. HCl or HNO ₃ to pH <2	P or G	6 mo
Gross beta	Conc. HCl or HNO ₃ to pH <2	P or G	6 mo
Strontium-89	Conc. HCl or HNO ₃ to pH <2	P or G	6 mo
Strontium-90	Conc. HCl or HNO ₃ to pH <2	P or G	6 mo
Radium-226	Conc. HCl or HNO ₃ to pH <2	P or G	6 mo
Radium-228	Conc. HCl or HNO ₃ to pH <2	P or G	6 mo
Cesium-134	Conc. HCl to pH <2	P or G	6 mo
Iodine-131	None	P or G	8 days
Tritium	None	G	6 mo
Uranium	Conc. HCl or HNO ₃ to pH <2	P or G	6 mo
Photon emitters	Conc. HCl or HNO ₃ to pH <2	P or G	6 mo

*The holding time varies for non-EPA public water supply samples.

ENVIRONMENTAL MICROBIOLOGY

Total Coliforms	<ul style="list-style-type: none"> The time between sample collection and the placement of sample in the incubator must not exceed 30 hours (per regulation at 40 CFR 141.21(f)(3)). All samples received in the laboratory should be analyzed on the day of receipt. If the laboratory receives the sample late in the day, the sample may be refrigerated overnight as long as analysis begins within 30 hours of sample collection.
Total coliforms and fecal coliforms in surface water sources	<ul style="list-style-type: none"> Preferably should not exceed eight hours. The maximum time the sample should be held in the refrigerator is 24 hours at 4°C.
Coliphage analysis	<ul style="list-style-type: none"> The time between sample collection and the placement of sample in the incubator must not exceed 48 hours. The time from sewage sample collection to analysis of QC spiking suspensions may not exceed 24 hours, unless re-titered and titer has not decreased by more than 50%. If titer has not decreased by more than 50%, the sample can be stored for up to 72 hours.
Heterotrophic bacteria in drinking water	<ul style="list-style-type: none"> Preferably should not exceed eight hours. The maximum time the sample should be held in the refrigerator is 24 hours at 4°C.