SCOPE

A Guide To Laboratory Services

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

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

October 2017

Roy Cooper, Governor
State of North Carolina

Mandy Cohen, MD, MPH, Secretary
N.C. Department of Health and Human Services

Scott J. Zimmerman, Dr.PH, MPH, HCLD(ABB)
Laboratory Director

(SCOPE Manual 05.1)

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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, pleases contact the appropriate unit.

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Customer Service	919-733-3937
Environmental Sciences	919-733-7308
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North Carolina State Laboratory of Public Health

Mission

The North Carolina State Laboratory of Public Health (NCSLPH) 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

Laboratory Director:	Scott J. Zimmerman, Dr.PH, MPH, HCLD(ABB)
Assistant Director - Technical Services:	Denise Pettit, PhD
Assistant Director - Operations:	Michael Kaufman, M.A.

ADDRESS FOR USPS AND STATE COURIER DELIVERIES

State Laboratory of Public Health
4312 District Drive
1918 Mail Service Center
Raleigh, NC 27699-1918

ADDRESS FOR FEDEX, UPS, AND OTHER COMMERCIAL COURIERS

State Laboratory of Public Health
4312 District Drive
Raleigh, NC 27607

ADDRESS FOR SLPH PO BOX

(Used for Blood Lead Mailers, Fluorides, PKU Specimens and Hemoglobinopathy Specimens)

State Laboratory of Public Health
PO Box 28047
Raleigh, NC 27611-8047

CLIA Certificate of Compliance # 34D0692393

Federal EIN # 562033116

Main Phone 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: parking is available on-site in front of NCSLPH.

NCSLPH Objectives

- Provide high quality laboratory services
- Assist other North Carolina laboratories in developing and strengthening their laboratory services
- Serve as North Carolina's primary Laboratory Response Network (LRN) 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

Delivery of Specimens and Samples

- Postal Services: Daily except Sundays (specimens arriving on weekends are refrigerated, except NBS). The laboratory does not accept "POSTAGE DUE" samples.
- UPS, FedEx, and private courier: Monday through Friday and Saturday 8:00-12:00.
- State Courier Service: Daily except Sundays, Mondays and holidays (specimens arriving on weekends are refrigerated, except NBS).
- Delivery in Person: Monday – Friday, from 6:30 a.m. to 4:00 p.m. Newborn Screening samples only are accepted on Saturday 8:30 a.m. – 12:00 p.m.
- After normal business hours: Specimens/Samples are delivered to the door to the left of the Loading dock. A sign directs the deliverer to "ring buzzer for after-hours assistance." The buzzer will notify on-site Capital Police for access to the building and they will respond via the facility intercom.
- Environmental Samples for agents of bioterrorism (BT) must be delivered in person by law enforcement agents. Clinical isolates/specimens are typically delivered by private courier. BTEP staff will coordinate delivery of clinical specimens with the submitter. Please call the Bioterrorism Laboratory at 919-807-8600 (24/7 number) prior to submitting/delivering samples.
- For samples or specimens needing chemical examination for agents of chemical terrorism (CT), please contact the Chemical Terrorism Laboratory at 919-602-2481 prior to submitting/delivering samples.

Policies and Limitations

NCSLPH receives consultation on policy matters from the State Health Director, the Epidemiology/Preparedness 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.

All clinical and environmental samples submitted for testing to the NCSLPH must be accompanied by a specimen submission form (test requisition). **Every tube, vial, or other sample container must be labeled with two identifiers (e.g. the patient's name and date of birth) that exactly match identifiers on the submission form.** Unlabeled clinical specimens will be deemed "Unsatisfactory" for testing. The clinical specimen submission form must include the patient's first and last name, patient date of birth, patient demographics (sex and race), date of collection, submitter's EIN and suffix, ICD-10 code, ordering provider name and NPI, Medicaid number, if applicable, and test requested. Use waterproof ink (unless otherwise indicated) to prevent smearing and washing off. Submission forms must be filled out completely and clearly; print legibly if labels are not used. Results may be delayed if all required fields are not completed. Most specimen submission forms are available from the State Laboratory Public Health Website at http://slph.ncpublichealth.com/forms. Do not photocopy forms.

NCSLPH, in collaboration with public health officials, reserves the right to decide whether or not to analyze samples. The Director or appropriate Laboratory Managers should be contacted before collecting or sending unusual numbers of samples/samples (as in epidemics, investigations or surveys). This is necessary for determining if 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 submitter, as defined in the N.C. Administrative Code. * Private citizens are authorized to submit animals or animal heads for rabies examination. [10A NCAC 42A.0105(b)]

The report of results is sent to the authorized submitter of the clinical specimen. Copies of clinical laboratory results may be furnished to another authorized submitter upon request of the initial authorized submitter. Certain results are furnished to public health programs for follow-up or epidemiologic purposes.

*" Authorized submitter of clinical samples" [10A NCAC 42A.0102(6)] 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.

The patient or their designated personal representative can request a copy of their completed laboratory results. For privacy protection, the laboratory will require proof of identity prior to issuing the test results. This request must be made in writing on a request form available at http://slph.ncpublichealth.com/.

Consultation

Please direct general or policy questions, comments or suggestions, and feedback on NCSLPH 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. NCSLPH 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 NCSLPH. The QA unit encompasses the clinical functions at NCSLPH. Functions of the QA unit include: review of Federal Regulations for guidance and compliance; overseeing proficiency testing, 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. Quality Assessment of the environmental functions is overseen by the Environmental Sciences Manager.

Specimen and Sample Mailers

Laboratory Mailroom (919) 733-7656

NCSLPH 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/forms *Note: Newborn Screening and Hemoglobinopathy forms which require dried blood spots are not available on the State Lab Web Site and must be ordered same as supplies.*
Environmental Submission Forms	Provided as part of the sample collection kit.

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 Communicable Disease Branch (CDB) is highly recommended.

Authorized Personnel/Rabies Treatment

PH Veterinarian, Communicable Disease Branch (919) 733-7419
State Epidemiologist or Medical Consultation Unit (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

NCSLPH does not supply antitoxin for treatment of botulism. The antitoxin is available only from the Centers for Disease Control and Prevention (CDC) 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 Communicable Disease Branch (CDB) 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

Printed invoices are sent immediately upon shipment of the entire order. Invoices can also be viewed on the NCSLPH website, mailroom ordering portal, while logged in to your account. 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)

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

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, dust wipes, paint and soils. Water samples from both public (non-compliance) 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. A full well panel includes: alkalinity, arsenic, barium, cadmium, calcium, chromium, copper, hardness (Total), lead, iron, magnesium, manganese, mercury, nitrate/nitrite, pH, selenium, sodium, silver, sulfate, chloride, fluoride, and zinc. This laboratory also offers testing for hexavalent chromium, uranium and soluble/insoluble iron and manganese. Sampling kits are available for selective testing when the full panel is not required.

Fluoride Analysis

A fluoride analysis can be performed on a private well water sample if 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 are 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 three 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 after 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 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 optional parameters by indicating on the sample submittal form. These include, but may not be limited to: aluminum, antimony, beryllium, cobalt, nickel, potassium, thallium, vanadium, acidity, phosphate, conductivity, settleable solids, total dissolved solids, total suspended solids, and turbidity. The laboratory also offers a full panel for Coal Ash analytes.

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 thirteen 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 to seven years depending on program area, 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. If a limit is not listed in this column of the report, neither the EPA or the State has established an MCL for the contaminant. Questions or concerns about the health effects of any of these contaminants should be addressed to the Occupational and Environmental Epidemiology Branch.

The EPA and/or State recommended limits or maximum contaminant levels for the primary drinking water 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. Sources include discharge from metal refineries and erosion of natural deposits.

Beryllium – MCL = 0.004 mg/L. Beryllium is very poisonous. It may enter a water system through metal refineries and coal-burning factories and discharges from electrical, aerospace and defense industries.

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 and erosion of natural deposits.

Copper – MCL = 1.3 mg/L. Copper may impart a metallic taste to water and cause greenish stains on faucets and plumbing fixtures, and can lead to both short and long-term health effects. Sources include household plumbing and erosion of natural deposits.

Cyanide – MCL = 0.2 mg/L. Cyanide can cause spleen, brain and liver damage and can lead to thyroid problems. 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. It is added to water to promote strong teeth.

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 and is of special concern for infants and small children where exposure may lead to physical and mental developmental delays. 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 can lead to kidney damage. 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 = 1 mg/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 from fertilizer use as well as leaching from septic systems and sewage.

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 and are therefore considered as secondary contaminants.

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.

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 (non-compliance) and private water systems. Samples are examined for the presence of the coliform group of bacteria, including total coliform and *E. coli,* 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 or other authorized submitter. 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). Most coliform and *E. coli* results are reported as present or Absent, but enumeration using a Most Probable Number (MPN) method is available upon request.

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 does not determine a specific organism, but the aerobic bacteria present in a water sample that will grow at the temperature of incubation and on the non-selective media used. 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. Enterococcus

This test detects enterococci in fresh and marine waters. Enterococci are considered a valuable bacterial indicator for determining the extent of fecal contamination of recreational surface waters. Results will be reported as the Most Probable Number (MPN) of enterococci per 100 mL 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. Legionella

The laboratory has added testing for Legionella pneumophilia in water samples to the list of available test options. This test requires a 100-ml sample volume and is incubated for a seven-day period. Results are reported as a Most Probable Number (MPN).

6. Microscopics

a. Iron Bacteria

Iron Bacteria may produce taste, odor, and pipe corrosion problems. 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.

C. Milk Microbiology

The Environmental Microbiology Unit provides analyses of milk and dairy products on samples received from the Milk Sanitation Branch of the NC Department of Agriculture and Consumer Services. 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). Milk/Dairy products and containers may be analyzed for the following:

- Aerobic Bacteria
- Coliform Bacteria
- Inhibitory Substances, including beta-lactam and tetracycline antibiotics
- Somatic Cell Count
- Alkaline Phosphatase – residual, microbial, or reactivated

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 three working days after the Laboratory receives the samples. If *E. coli* 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. With the exception of Sulfur/Sulfate-reducing bacteria and Legionella, 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 to seven years and then destroyed. Reports for public water systems are held for one year in the Laboratory then transferred to Environmental Health Central Files. Grade A milk reports are retained for three years.

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, 60-120 mL bottle 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. Petroleum 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 by identifying the compound(s) and will not necessarily determine the source of contamination. 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 the sample submittal form provided in the kit. Print legibly.

B. Pesticides (Herbicides, Fungicides, Insecticides, etc.)

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. The Laboratory is unable to analyze for every pesticide, so before sampling, check with the Laboratory for availability of testing. Carefully 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 so that analysis can be started within the method established holding time. Remember to complete all information on the submission form and print legibly.

Multiple test procedures are used for this class of organic compounds. Clorinated pesticides, nitrogen-phosphorous pesticides, glyphosate (Round-up®) and herbicides are all analyzed by different procedures and require individual sample collection kits. Refer

to the SLPH ordering website and contact the Environmental Sciences laboratory at 919-733-7308 if you have questions.

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 to seven years and then destroyed.

Environmental Radiochemistry

(919) 733-7308

Introduction

The Environmental Radiochemistry Lab analyzes environmental samples submitted by the Radiation Protection Section (Division of Health Service Regulations/DHHS) and other approved sample submitters. 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

Surface Supplies
Gross Alpha
Gross Beta
Gamma
Tritium
Iodine – 131 (low levels)
Uranium (total)

Ground Supplies (not public)
Gross Alpha
Gross Beta
Tritium
Uranium (total)
Gamma

Silt/Soil
Gross Alpha
Gross Beta
Uranium (total)

Milk
Gamma
Iodine 131 (Low level)

Edible Products
Same as Silt/Soil

Wipe Samples (Leak Test)
Isotopes as requested

Sample Collection and Identification

Eligible submitters must provide a detailed listing of the sample source and the testing required. Formats will vary depending on the submitting agency.

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.

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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:

1. Laboratory facilities must meet the criteria as described in the regulations (10A-42D-.0200). An on-site evaluation determines compliance.
2. 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 (Total Coliform and *E. coli*), acceptable results must be reported on 90% of the samples in each set.
3. 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

Blood Lead Testing

(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 as well as pathways and prevention of lead exposure, presents a direct challenge to clinicians and public health authorities.

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.

According to the Centers for Disease Control and Prevention (CDC), there are approximately a half-million children in the United States ages 1-5 with blood lead levels above 5 micrograms per deciliter (μg/dL), the reference value at which CDC recommends public health actions be initiated. No safe blood level in children has been identified. Lead exposure can affect nearly every system in the body. Because lead exposure often occurs with no obvious symptoms, it frequently goes unrecognized.

The newest methodology at the North Carolina State Laboratory of Public Health (NCSLPH) includes ICP-MS (Inductively Coupled Plasma Mass Spectrometer). In addition, effective July 2012, a multi-tier approach to follow-up has been adopted with an overall goal of reducing children's blood lead levels below 5 μg/dL.

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 specimen should be collected by the child’s primary care provider.

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, community clinics, hospitals, and private providers on all children 6 months - 6 years of age.

Sample Identification and Collection

A. Specimens must be accompanied by DHHS form #3707 which is available on the NCSLP website at http://slph.ncpublichealth.com/forms.asp#specimen. This is a scannable form and must be printed on plain white paper from the website.

B. Specimen collection device kits can also be ordered on-line at http://slph.ncpublichealth.com/forms.asp#mailroom.

C. Complete all identification and requested information on DHHS form # 3707. It is imperative that all following information be completed:
   - First and last name of patient
   - Patient date of birth
   - Patient demographics (sex, race, etc.)
   - Patient number or Social Security Number (optional)
   - Date of Collection
   - Submitter EIN
   - ICD-10 code (reason for testing)
   - Ordering provider and National Provider Identifier (NPI)
   - Medicaid number if patient has Medicaid
   - Test requested
   - Indicate whether Initial or follow-up blood lead test.

D. Submit an EDTA lavender top capillary or venipuncture tube blood specimen. All specimens must be labeled with at least two identifiers that match exactly with the submission form.
   - First and last name of patient
   - Patient Date of Birth
   - Patient number or Social Security Number
   - Medicaid number

E. Preparation of Child for Fingerstick Specimen Collection
   a. Wash child's hand with soap and water, using hand brush. Rinse well. Dry.
   b. Grasp the child's hand so that the blood drawer's thumb is across the top of the child's fingers.
   c. Hold the child's hand so that the palm faces up.
   d. Use child's middle or ring finger for specimen collection.
   e. Using an alcohol wipe, briskly scrub area on the child's fingertip for 20 seconds.
   f. Wipe scrubbed area once, using dry gauze.
   g. Use lancet to stick finger slightly left of center.
   h. 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.

F. Collection of Blood Specimen:
   a. Continuing to grasp the finger, touch the capillary tip of the collection device to the beaded drop of blood.
   b. The capillary must be held continuously in a horizontal position during specimen collection to prevent air bubbles from forming in the capillary tube.
   c. Dispense the full capillary of blood (150 – 200 µL) into the container.
   d. Turn capillary/tube unit immediately to a vertical position to allow the blood in the capillary to flow into the tube.
   e. Remove capillary with holder at the same time. Close blood container with attached cap.
   f. Agitate the specimen to mix the EDTA through the blood.
   g. Label capillary blood tube 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

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

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.

Additional information may be found at:

http://ehs.ncpublichealth.com/hhccehb/cehu/lead/docs/2016ClinicalTrainingManualFINAL042116.pdf

Prenatal Lead Testing

The North Carolina State Laboratory of Public Health (NCSLPH) has established a Prenatal Lead Testing Program in partnership with local public health departments (LHDs) in North Carolina. Since the Centers for Disease Control and Prevention (CDC) does not recommend blood lead testing of all pregnant women in the United States, state or local public health departments should identify populations at increased risk for lead exposure and provide community specific risk factors to guide clinicians in determining the need for population-based blood lead testing.

Routine blood lead testing of pregnant women is only recommended in clinical settings that serve populations with specific risk factors for lead exposure that meet the required criteria assessed using the Lead Risk Assessment Questionnaire. Health care providers serving lower risk communities should consider the possibility of lead exposure in individual pregnant women by evaluating risk factors for exposure as part of a comprehensive occupational, environmental, and lifestyle health risk assessment of the pregnant woman, and perform blood lead testing if a single risk factor is identified.

This test is only available to local public health departments.

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. This is a scannable form and must be printed on plain white paper from the website. Do not photocopy form.

B. Complete all identification and requested information on DHHS form # 3707. It is imperative that all of the following information be completed:
   a. First and last name of patient
   b. Patient date of birth
   c. Patient demographics (sex, race, etc.)
   d. Patient number or Social Security Number (optional)
   e. Date of Collection
   f. Submitter EIN
   g. ICD-10 code (reason for testing)
   h. Ordering provider name and National Provider Identifier (NPI)
   i. Medicaid number if patient has Medicaid
   j. Test requested
   k. Indicate whether Initial or follow-up blood lead test.
   l. Assure that the Prenatal box is checked appropriately.

C. Please be advised that the specimen of choice for this testing is a venipuncture specimen (rather than fingerstick) collected in a lavender-top (EDTA) blood collection tube. All specimens must be labeled with at least two identifiers that match with the submission form.

First and last name of patient
Patient Date of Birth
Patient number or Social Security Number
Medicaid number

Shipment

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

Additional information may be found at:

http://epi.publichealth.nc.gov/oee/programs/ables.html

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 unit 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 on the NCSLPH website under Lab Improvement Training Workshops tab. 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 "Forms" section of the "Forms, Newsletters & Bulletins" tab under Lab Improvement 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) 306-4302

Fayetteville
Phone: (910) 322-8120

Greenville
Phone: (252) 414-3078

Laboratory Preparedness

Bioterrorism and Emerging Pathogens (919-807-8765)
Chemical Terrorism (919-807- 8776)

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 US 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 the Food Emergency Response Network (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 for all labs and agencies in NC for possible Select Agent viruses, bacteria and some toxins. BTEP also 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.

The BTEP Unit may be contacted for emergency situations by:

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

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 Public Health Preparedness and Response Branch (PHPR). Individuals should NOT attempt collection. Suspicious substances are generally transported to the State Laboratory under ambient conditions by the submitting law enforcement agency 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. Acceptable clinical isolates/samples include those from a hospital or other public or private clinical lab 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 Public Health Preparedness and Response Branch (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. 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 Medical Consultation Unit (MCU)/Epidemiology

Section. Negative results are called to the submitter. Final reports are sent to the submitter. Results are reported to 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. 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 1SUSPICIOUS SUBSTANCE **(ENVIRONMENTAL)**

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

ORGANISM/ TOXIN	SAMPLE (All items)	TESTING PERFORMED	COLLECTION (All items)	REQUIREMENTS (All items)
Bacillus anthracis	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	·**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://slph.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; ·**NOTIFY lab prior to arrival** (discussion should include possible agents, types and number of samples to be submitted) ·**NOTIFY lab of approximate arrival time**
Brucella species		Culture, PCR, other conventional microbiology tests		
Burkholderia mallei		Culture, PCR, other conventional microbiology tests		
Burkholderia pseudomallei		Culture, PCR, other conventional microbiology tests		
Coxiella burnetii		PCR		
Francisella tularensis		Culture, DFA, PCR, slide agglutination, other conventional microbiology tests		
Yersinia pestis		Culture, phage, PCR, other conventional microbiology tests		
Ricin toxin		Time Resolved Fluorescence		
Botulinum toxin Call the NCSLPH		***Call the NCSLPH***		

Table 2 **SUSPICIOUS SUBSTANCES: CLINICAL**

ORGANISM/ AGENT/TOXIN	SAMPLE	TESTING PERFORMED	COLLECTION	SHIPPING REQUIREMENTS
Bacillus anthracis	Isolated organism; swabs of lesions; tissues; sputum, blood (EDTA or sodium citrate), serum, plasma, pleural fluid, respiratory specimens, CSF	Culture, phage, PCR, other conventional microbiology tests	Isolated 18-24 hr. culture of unknown gram-positive *Bacillus* bacteria, nonmotile & nonhemolytic on Sheep's Blood agar. Use extreme caution. Subculture without aerosolization in BSC Class II or higher.	•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)
Brucella species	Isolated organism; whole blood or serum	PCR, other conventional microbiology tests, culture	Isolated 24-72 hr. culture of unknown gram-negative bacteria, where submitter is unable to rule-out *Brucella*. Use extreme caution. Subculture without aerosolization in BSC Class II or higher.	•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)
Botulinum toxin	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
Burkholderia mallei	Isolated organism; swabs of lesions; tissues; blood or serum	Culture, PCR, other conventional microbiology tests	Isolated 24-72 hr. culture of unknown Gram neg. bacteria, where submitter is unable to rule-out *B. mallei*. Use extreme caution. Subculture without aerosolization in BSC Class II or higher.	•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)

Table 2 SUSPICIOUS SUBSTANCES: CLINICAL

ORGANISM/ AGENT/TOXIN	SAMPLE	TESTING PERFORMED	COLLECTION	SHIPPING REQUIREMENTS
Burkholderia pseudomallei	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 *B. pseudomallei.* Use extreme caution. Subculture without aerosolization in BSC Class II or higher.	•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)
Coxiella burnetii	Whole blood	PCR	Collect sample in purple or yellow-topped blood collection tube for PCR; for serology, use red top tube.	•Ship in ambient conditions; •Use current shipping guidelines for a diagnostic sample
Francisella tularensis	Isolated organism; whole blood; swabs of lesions.	Culture, DFA, PCR, other conventional microbiology tests	Isolated 24-72 hr. culture of unknown gram-negative bacteria where submitter is unable to rule-out F. *tularensis*. Use extreme caution. Subculture without aerosolization in BSC Class II or higher.	•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)

Table 2 **SUSPICIOUS SUBSTANCES: CLINICAL**

ORGANISM/ AGENT/TOXIN	SAMPLE	TESTING PERFORMED	COLLECTION	SHIPPING REQUIREMENTS
Yersinia pestis	Isolated organism; whole blood; tissue; swabs of lesions, bronchial wash, transtracheal aspirate, sputum, nasopharyngeal swabs	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 *Y. pestis.* Use extreme caution. Subculture without aerosolization in BSC Class II or higher.	•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)
Avian influenza	Contact the NCSLPH and the CDB at (919) 733-3419	PCR (presumptive ID) – confirmation testing performed at CDC	Contact the NCSLPH and the CDB (919) 733-3419	
SARS	Contact the NCSLPH and the CDB at (919) 733-3419	Not performed at NCSLPH. The NCSLPH will contact the CDC	Contact the NCSLPH and the CDB (919) 733-3419	
Monkeypox	Contact the NCSLPH and the CDB at (919) 733-3419	Not performed at NCSLPH. The NCSLPH will contact the CDC	Contact the NCSLPH and the CDB (919) 733-3419	
Smallpox, Orthopox, Non-orthopox,& VZV	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	

Table 2 **SUSPICIOUS SUBSTANCES: CLINICAL**

ORGANISM/ AGENT/TOXIN	SAMPLE	TESTING PERFORMED	COLLECTION	SHIPPING REQUIREMENTS
Other viral hemorrhagic viruses 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.	Contact the NCSLPH and the CDB (919) 733-3419	•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
Ricin	Not performed on clinical samples.			Detection of human metabolites for Ricin is performed by the NCSLPH Chemical Terrorism Lab
Ebola	Whole blood in EDTA, serum, plasma, urine	PCR	Contact the NCSLPH and the CDB (919) 733-3419 Collect two 4ml EDTA tubes of whole blood (preferred), serum or plasma, urine in a sterile specimen cup with volume of 1-3 mLs (urine should not be sole specimen)	•Immediately call the NCSLPH and CDB •Complete CDC DASH form # 50.34 •Ship on cold packs using current guidelines for transport of an infectious substance
MERS	Lower respiratory specimen (bronchial lavage or sputum), NP and OP swab, serum	PCR	Contact the NCSLPH and the CDB (919) 733-3419	•Immediately call the NCSLPH and CDB •Complete CDC DASH form # 50.34 • Refrigerated (4^{o}C), place of cold packs if shipment is to be received within 72 hr of collection. For delays exceeding 72 hr., freeze at -70^{o}C.

Table 3 **FOOD**

SAMPLE TYPE		COLLECTION & PRESERVATION	PACKAGING & SHIPPING
Solid food >50 grams	Contact NCSLPH at 919-807-8600 first	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	Contact NCSLPH at 919-807-8600 first	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	Contact NCSLPH at 919-807-8600 first	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	Contact NCSLPH at 919-807-8600 first	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 HNPAA (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)-807-8803

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) 807-8803

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 Epidemiology Section 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) 807-8603

Introduction

Specimens for isolation of *Bordetella pertussis* and *B. parapertussis* in suspected cases of whooping cough are accepted from public and private health care providers. PCR screening is available for *Bordetella pertussis* only. 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. 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, medical record number or Social Security number accompanied by a completed DHHS form #4121. **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!

Fill out form DHHS#4121 with the following required information:

- First and last name of patient
- Patient date of birth
- Patient demographics (sex, race, etc.)
- Patient number or Social Security Number (optional)
- Date of Collection
- Submitter EIN
- ICD-10 code (reason for testing)
- Ordering provider and National Provider Identifier (NPI)
- Medicaid number if patient has Medicaid
- Test requested

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 3 days before shipping. Friday shipments are not recommended as specimens should be kept cold.

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, along with a completed DHHS 4121 form.

Reference cultures may be shipped in a microbiology reference mailer with a completed DHHS form 4121. Plates should be wrapped individually in absorbent cushioning material and securely packaged in a leak-proof, crush-proof container. 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, usually within 3 to 4 days of sample receipt. 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 Epidemiology Section, Division of Public Health, for surveillance purposes. **All results are available via the website.**

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) 807-8606

Strains of *Vibrio cholerae* possessing the somatic 01 or 0139 antigen (*"V. cholerae:01" or "V.cholerae: 0139"*) are associated with epidemic cholera, while those lacking this antigen (*"V. cholerae non-01"*, "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). Submit stool specimens in unrefrigerated Enteric culture mailers with a completed DHHS form #3990. Indicate on the form that *Vibrio* is suspected.

Note: Direct reference isolates of *Vibrio* spp. to the Atypical Bacteriology Lab with a completed DHHS form #4121.

Isolates of *V. cholerae* are tested in the Atypical Bacteriology Lab at the SLPH for the presence of the 01 and O139 antigens; those presumptively identified as *V. cholerae* 01 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) 807-8793

Introduction

Diphtheria is an upper respiratory tract illness caused by Corynebacterium diphtheriae, a facultative anaerobic, Gram-positive bacteria. Diphtheria is a contagious disease spread by direct physical contact or breathing the aerosolized secretions of infected individuals. Historically quite common, diphtheria has largely been eradicated in industrialized nations through widespread vaccination. The diphtheria-pertussis-tetanus (DPT) vaccine is recommended for all school-age children in the U.S., and boosters of the vaccine are recommended for adults, since the benefits of the vaccine decrease with age without constant re-exposure; they are particularly recommended for those traveling to areas where the disease has not been eradicated.

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 Communicable Disease Branch 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 CO_2 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 919-807-8606 **prior** to submitting diphtheria specimens. Please include the patient's clinical history when submitting suspected diphtheria specimens to the NCSLPH.

At the CDC:

- 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 DASH.
- NOTE: For confirmed cases, physicians can acquire anti-toxin (DAT) directly from the CDC. The earlier this is given, the more favorable the outcome for the patient. Clinicians can obtain DAT by calling 770-488-7100, CDC Emergency Operations Center.

Shipment of Specimens to the NC State Lab of Public Health

Submit swab specimens to the NC State Lab 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 DHHS form #4121 for Special Bacteriology. (http://slph.ncpublichealth.com/Forms/dhhs-4121.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) 807-8608

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 NCSLPH is the North Carolina serotyping center for *Salmonella, Shigella* and *E. coli* 0157:H7 and participates in the national surveillance programs of the CDC.

Please Note: The North Carolina Communicable Disease Control rules (10A NCAC 41A.0209) state that laboratories culturing stool from a person with bloody diarrhea should culture for shiga-toxin producing Escherichia coli or send the specimen to the State Public Health Laboratory for shiga-toxin testing after consultation with the Enterics lab 919-807-8608.

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 a 2nd identifier 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. Cary-Blair transport media for collection of feces or rectal swabs is available from the laboratory mailroom on-line at https://slphreporting.ncpublichealth.com/labportal.

Fill out form DHHS #3390 with the following required information:

- First and last name of patient
- Patient date of birth
- Patient demographics (sex, race, etc.)
- Patient number or Social Security Number (optional)
- Date of Collection
- Submitter EIN
- ICD-10 code (reason for testing)
- Ordering provider and National Provider Identifier (NPI)

Medicaid number if patient has Medicaid
Test requested
Specimen source

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

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 two identifiers: patient's name and either date of birth or Social Security number.

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 #4121.

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 four to seven 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 subgroup A (12 serotypes)
- *Shigella flexneri* or subgroup B (6 serotypes)
- *Shigella boydii* or subgroup C (18 serotypes)
- *Shigella sonnei* or subgroup D (2 serotypes)

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) 807-8608 prior to submitting specimens associated with outbreaks. Contact the Epidemiology and Communicable Disease Section at (919) 733-3419 for epidemiology 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.

Isolates of sorbitol-negative E. coli are tested for reactivity in somatic 0157 and flagellar H7 antisera.

Isolates of other E. coli (non-0157:H7) from documented cases of bloody diarrhea or associated with cases of hemorrhagic colitis or HUS will be confirmed and serotyped, if possible. Referral to CDC may be necessary.

Recommendations for diagnosis and follow-up of cases of disease caused by Salmonella, Shigella, E. coli 0157:H7 or other Shiga-toxin producing E. coli (STEC), and Campylobacter are outlined in Control of Communicable Diseases in Man. Questions concerning epidemiological investigation of these illnesses should be directed to the Epidemiology and Communicable Disease Section at (919)733-3419.

D. Other Enterobacteriaceae

Members of other genera in the Family Enterobacteriaceae are reported using genus and species designations consistent with descriptions in the Manual of Clinical Microbiology, or in accordance with the International Code of Nomenclature of Bacteria.

The NCSLPH reports all confirmed *Salmonella*, *Shigella*, *Campylobacter*, *Vibrio,* and *E. coli* 0157:H7 isolates to the Communicable Disease Branch of the Epidemiology 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 5 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 Protection Program of the Environmental Health Section should be notified at (919) 707-5854 when restaurant- or institution-associated illness is suspected.

Foodborne Illness

(919) 807-8608

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 and Consumer Services (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) 807-8608 or (919) 807-8803 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 #4121, 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 fecal 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 Protection Program of the Environmental Health Section should be notified at (919) 707-5854 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) 807-8603

Introduction

Legionellosis is diagnosed by a combination of culture, direct fluorescent antibody (DFA) staining, serum serologic testing (performed in Virology/Serology Unit) and other techniques in conjunction with the patient's clinical history. Culture is the recommended diagnostic procedure and should be attempted along with other methodologies. The Bacteriology Laboratory 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 #4121. Two forms are required for paired serum specimens.

Fill out form DHHS #3390 with the following required information:

- First and last name of patient
- Patient date of birth
- Patient demographics (sex, race, etc.)
- Patient number or Social Security Number (optional)
- Date of Collection
- Submitter EIN
- ICD-10 code (reason for testing)
- Ordering provider and National Provider Identifier (NPI)
- Medicaid number if patient has Medicaid
- Test requested
- Specimen source

Note: Specimens received without submitter 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) 807-8803.

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. All positive DFAs are reported to Epidemiology.

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 and are followed by a computer-generated report. Positive cultures are reported by telephone and by mail as soon as growth is identified. Cultures are held for three weeks before being reported as negative. All positive cultures 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) 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 in the Communicable Disease Branch (CDB) in accordance with State Law. (Refer to NC TB Policy Manual for guidance)

Respiratory specimens from other sources are concentrated and stained with fluorochrome and/or Kinyoun stain, and cultured for the isolation and identification of mycobacteria. No smear will be performed on blood or bone marrow, 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 respiratory samples.

Species identification is accomplished using high-performance 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 four primary drugs: isoniazid, ethambutol, rifampin and pyrazinamide. Additional susceptibility testing for resistant strains of *M. tuberculosis* complex is available.

Specimen Collection and Submission

CDC recommends specimens be received by the laboratory within 24 hours of collection. Due to shipment delays, some specimens may exceed this timeframe. However, any specimen received more than 7 days after collection will not be tested. To ensure proper patient/specimen identification and ensure accurate results reporting, specimen containers must be labeled with two identifiers: the patient's first and last name and date of birth. 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 laboratory information management system: patient name, date of birth, submitter Federal Tax Number, Medicaid number, if eligible, submitter return address and phone number, county code, provider name and NPI (National Provider Identifier), specimen collection date (CLIA requirement) and specimen

source. Without these data, a report of results cannot be printed. Other data are required for follow-up and for statistical purposes. For more information, contact the Mycobacteriology Laboratory at (919) 807-8620 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 5 mL is recommended for each specimen. A negative result may be less reliable if the specimen volume is less than 5 mL. 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 *Mycobacterium avium complex* 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 be accepted. 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.

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 date of birth. 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 sending specimens 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.

Do not submit subcultures until good growth occurs. Do not send mixed or contaminated cultures. **Use the Orange Labeled cans for TB/Mycobacteriology Specimens for shipment. These are available from the NCSLPH mailroom and may be ordered on-line at** http://slph.ncpublichealth.com.

Specimen Testing and Reporting

Results and interpretations are reported to the submitting agency via US Mail. 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. **Fluorochrome (AFB) Smear**

A smear is used as a rapid test to detect mycobacteria and many Nocardia spp. that may be causing an infection. 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.

Reporting:

Positive, Grade, Per Field -- indicates the presence of acid-fast organisms in the smear, the smear grade and 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)**

This NCSLPH method was developed for the rapid detection of *M. tuberculosis* complex (MTBc) **DNA** directly from respiratory specimens. Accurate PCR results depend on proper specimen collection and transport. PCR testing is performed based on fluorochrome (AFB) smear results:

a. AFB smear positive sample – Real-time PCR will be performed on the first AFB smear positive sample for each patient.
    i. Positive PCR result - PCR will not be performed on subsequent samples. Positive PCR results are called to the submitting agency for each first-time PCR positive patient. The sample will be cultured and TB isolates tested for drug susceptibility.
    ii. Negative PCR result - up to two more positive AFB samples will be tested using real-time PCR.
b. AFB smear negative samples- PCR will not be performed. (see *Appendix A Mycobacteriology*).

Reporting:

MTB Complex DNA – DETECTED by real-time PCR – indicates the presence of MTB complex DNA in the sample.

MTB Complex DNA NOT detected by real-time PCR – indicates that MTB complex was not detected in the sample. Negative test results do not indicate the absence of disease.

C. **High-Performance Liquid Chromatography**

HPLC is an analytical chemistry method used to identify mycobacteria by analysis of mycolic acids. HPLC is primarily used for the identification of non-tuberculous mycobacteria (NTM). This test is correlated with morphological data and other identification methods when necessary.

Reporting:

Identified organism

D. **Nucleic Acid (DNA) Probes**

Nucleic acid probes are one of the most rapid methods used for the definitive identification of mycobacteria. Nucleic acid probes are used for the identification of *M. tuberculosis complex*, *M. avium complex*, *M. gordonae*, and *M. kansasii*.

Reporting:

Accuprobe "xxxx" - Negative for "xxxx" – targeted organism was not detected from the patient sample.

Accuprobe "xxxx" - Positive for "xxxx" - targeted organism was detected from the patient sample.

E. **Drug Susceptibility Test**

Indirect drug susceptibility tests for *M. tuberculosis* complex are performed on clinical and reference samples using four first-line drugs; ethambutol, isoniazid, rifampin, and pyrazinamide. Second-line drugs are tested when resistance is seen in first-line testing. Reporting is the same for both first and second line drug susceptibility test results.

Reporting:

Pending – a preliminary result. Drug susceptibility test results are pending completion.

Susceptible – final result. *M. tuberculosis* from patient sample is susceptible to tested drug.

Resistant – final result. M. tuberculosis from patient sample is resistant to tested drug. All resistant results all called to the submitter. If resistance is found in first-line testing, second-line drug testing will be initiated.

F. **Culture/Final Report**

Cultures are incubated for a maximum of six weeks (42 days). If growth occurs, organisms are identified, if possible, by high-performance liquid chromatography or nucleic acid probes. Identification of some organisms may necessitate sub-culturing 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

Appendix A, Mycobacteriology

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

1. PCR will be performed on the first AFB smear-positive specimen for each patient.

2. PCR will be performed on smear-negative specimens 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 specimens 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)*	**Risk Factors**
Cough	HIV infection
Fever, chills or night sweats	Cough present for more than 2 weeks
Significant weight loss	Immigrant from high-incident country
Hemoptysis	Immunosuppressive medications (includes TNF alpha inhibitors)
	Contact with known TB case in last 2 years
	Leukemia, lymphoma, or cancer of the head and neck or lung
	Diabetes mellitus
	Silicosis
	Gastrectomy or jejunoileal 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) 807-8813

Introduction

Clinical specimens for isolation and identification of medically important fungi from body tissues and fluids are accepted from public and private health care providers but must be limited to those actually implicated in fungal disease. Reference cultures are also accepted for identification of yeasts, molds, and aerobic actinomycetes. 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 DHHS form #2010 (one form for each specimen) in the outer container of the shipping packaged, this helps 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 at https://slphreporting.ncpublichealth.com/labportal/. 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 Laboratory, 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 outbreak associated specimens, unusual specimens, or potentially hazardous specimens are being submitted, telephone the Microbiology Unit at (919) 807-8803 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.**

Fill out form DHHS #2010 with the following required information:

- First and last name of patient
- Patient date of birth
- Patient demographics (sex, race, etc.)
- Patient number or Social Security Number (optional)
- Date of Collection
- Submitter EIN
- ICD-10 code (reason for testing)
- Ordering provider and National Provider Identifier (NPI)
- Medicaid number if patient has Medicaid
- Test requested
- Specimen source

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

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., *Microsporum gypseum, Trichophyton mentagrophytes*). 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***
Subcutaneous and systemic mycoses		
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.
Yeast Infections	Collect as for bacteriological specimens, using aseptic technique.	Isolate on Sabouraud Agar or submit in TB sputum mailer.
Reference Cultures		
All except *Nocardia* 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.
Nocardia 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) 807-8793

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.

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 #4121. Unlabeled specimens will not be tested. Please indicate if a specimen is a legal case.

Fill out form DHHS #4121 with the following required information:

- First and last name of patient
- Patient date of birth
- Patient demographics (sex, race, etc.)
- Patient number or Social Security Number (optional)
- Date of Collection
- Submitter EIN
- ICD-10 code (reason for testing)
- Ordering provider and National Provider Identifier (NPI)
- Medicaid number if patient has Medicaid
- Test requested
- Specimen source

Note: Specimens received **without submitter 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 leak-proof 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 CO_2 atmosphere during shipment. Plate cultures in a CO_2 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 #4121 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 growth is present on the plate. Please note: Neisseria species die easily and should be freshly subbed to a chocolate slant or transport systems such as the Jembec plate. Incubation for 18 to 24 hours prior to transport enhances survival. Do not ship on a Friday or holiday weekend. 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” and “DO NOT REFRIGERATE” 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. " Non-gonococcal neisseriae are reported as “No Neisseria gonorrhoeae isolated” and may be identified to the genus or species level as appropriate. Results usually are reported within one to three work days unless difficulty is encountered in growing the organism or isolating it from a mixed culture. Results can be accessed via the secure web page for results.

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) 807-8743

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, with the exception of blood, are accepted from all laboratories.

Feces and other specimens are examined for eggs, cysts and larvae of the intestinal parasitic worms and protozoa.

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.

Testing for *Cryptosporidium* and *Cyclospora* are offered upon request; testing for *Microsporidium* is NOT available at this time. Testing for Blood Parasites is available from the Centers for Disease Control and Prevention (CDC).

Specimen Collection and Identification

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

Fill out form DHHS #1245 with the following required information:

- First and last name of patient
- Patient date of birth
- Patient demographics (sex, race, etc.)
- Patient number or Social Security Number (optional)
- Date of Collection
- Submitter EIN
- ICD-10 code (reason for testing)
- Ordering provider and National Provider Identifier (NPI)
- Medicaid number if patient has Medicaid
- Test requested
- Specimen source

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

A. Intestinal Parasites

Fecal Specimens: Collect specimen following instructions in the Parasitology mailer supplied by this Laboratory, by ordering online at https://slphreporting.ncpublichealth.com/labportal/ 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.

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. Commercial collection devices may also be used.

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

Whole Worms or Proglottids: Whole worms should be preserved in 70% alcohol, if possible. Place in plastic or glass container; label with two patient identifiers. 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. **Corneal Scrapings for Acanthamoebae: Contact the Microbiology Unit at least** 24 hours prior to taking specimen.

C. Arthopods: 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.

Shipment of Specimens

Always use double-walled shipping containers that meet 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 and date of birth on specimen vial 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 (found at http://slph.ncpublichealth.com/forms.asp#specimen) 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.

An estimate of few, moderate, or many will be reported only with certain organisms where quantity may have a correlation with worm burden (such as *Ascaris*, Trichuris, and hookworms) or be an indicator for treatment (such as *Blastocystis hominis*).

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 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 can be accessed via the secure webpage at https://slphreporting.ncpublichealth.com/lims/ClinicalLims/Login.aspx.

Special and Atypical Bacteriology

(919) 807-8793

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

Streptococcus pneumoniae typing:
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. Please call the Streptococcus Laboratory at 404-639-1237. Isolates should be submitted on a chocolate slant with a completed CDC 50.34 DASH Form (PDF, 2.5 MB) including documentation of prior communication with CDC.

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.

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, Haemophilus, Neisseria, Pasteurella, Pseudomonas* and similar organisms and "unclassified" bacteria. 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*
- confirmation of *Listeria monocytogenes*
- 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 misidentification from automated systems. Hazardous suspected organisms such as Brucella should be directed to the Bioterrorism Unit.

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

*The hazardous nature of certain suspected organisms such as *Francisella tularensis, Bacillus anthracis, Yersinia pestis, Burkholderia mallei, Burkholderia pseudomallei and Brucella spp.* require submission to the Bioterrorism and Emerging Pathogens Unit (BTEP). Please call the BTEP Unit at 919-807-8600 if one of these organisms is to be submitted.

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 #4121. 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!

Fill out form DHHS #4121 with the following required information:

First and last name of patient
Patient date of birth

Patient demographics (sex, race, etc.)
Patient number or Social Security Number (optional)
Date of Collection
Submitter EIN
ICD-10 code (reason for testing)
Ordering provider and National Provider Identifier (NPI)
Medicaid number if patient has Medicaid
Test requested
Specimen source

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

Telephone the Microbiology Laboratory at (919) 807-8803 of outbreak situations, to make special arrangements in urgent or unusual circumstances, or 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 Communicable Disease Branch.

Specimens Requiring Special Handling

Organism or Disease	Collection Instructions	Shipping Requirements	Special Requirements
Bacillus anthracis	See Section: Bioterrorism and Emerging Pathogens		
Bordetella pertussis (whooping cough)	See separate listing		
Brucella spp.	See Section: Bioterrorism and Emerging Pathogens		
Burkholderia mallei	See Section: Bioterrorism and Emerging Pathogens		
Burkholderia pseudomallei	See Section: Bioterrorism and Emerging Pathogens		
Corynebacterium diphtheriae	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. (Recommend 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.
Francisella tularensis	See Section: Bioterrorism and Emerging Pathogens		
Haemophilus ducreyi (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 33-35º in 5-10% CO_2	Reference culture of heavy growth from CA on sterile swabs stabbed into CA Microbiology Reference mailer	*Primary culture recommended at local level*
	PCR testing performed at CDC; contact Section for information (919-733-7367) or (919-807-8774)		
Legionella	See separate listing		
Leptospira	Note: Only serological testing at the CDC is available; refer to Virology/Serology		
Staphylococcus aureus	Isolates of coagulase positive staphylococci from documented outbreaks.	Microbiology Reference mailer.	Notify Unit prior to shipping. Referred to CDC
Yersinia pestis	See Section: Bioterrorism and Emerging Pathogens		

* 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

Turnaround Times for NCSLPH Bacteriology

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

TEST	TURNAROUND TIME
Bordetella PCR	Batched twice a week; usually 4-5 working day TAT
Bordetella culture	7 days after receipt
Legionella DFA	Day of receipt or next work day
Legionella culture	3 weeks after receipt
Special Reference identifications	~5-7 work days
Enteric Reference identifications	~7-10 work days
Enteric Clinical Cultures	~7-10 work days
Food cultures	1-7 work days after receipt
Neisseria gonorrhoeae confirmations	3-5 work days after receipt
Atypical Reference identifications	~5-7 work days
Yeast identifications	~2 weeks
Mold identifications	~5-8 weeks
Actinomycetes identifications	6-8 weeks
Ova & protozoa concentrations	3-5 work days
Mixed, fastidious, or particularly difficult to identify isolates may take longer.	
Identifications referred to CDC may take several months.	
TEST	**TURNAROUND TIME**
M. tuberculosis PCR	Testing: Mon, Wed, Fri (TAT 24-48 hrs.)
Fluorochrome Acid Fast staining	24 hrs. from receipt of specimen
M. tuberculosis identification	Goal TAT 14-21 days from receipt Mixed/contaminated cultures may cause delays
Liquid (1st Line) Drug Susceptibility testing for *Mtb*	Goal TAT 17 days from positive identification of M. tuberculosis. Mixed or contaminated cultures may cause delays.
Conventional (2nd Line) Drug Susceptibility testing for *Mtb* (agar proportion)	21 days from drug set up
Mycobacterium sp. (NTM) identification	7-42 days from receipt
Culture Negative (No Growths)	42 days
Mtb isolates sent to Michigan Department of Community Health (CDC GIMS Program)	7-10 days
Mixed, fastidious, or particularly difficult to identify isolates may take longer.	
Identifications referred to CDC may take several months.	

NEWBORN SCREENING

(919) 733-3937

NCSLPH offers a Newborn Screening program for babies born in North Carolina. Briefly, the sample used to perform NBS consists of an aliquot of the sample submitted from a dried blood spot (DBS) specimen collected from the baby. This program includes screening for over 40 metabolic and genetic disorders.

Newborn Screening is performed in four laboratories:

MS/MS: This DBS punch is extracted in a solvent containing known amount of internal standards for each analyte of interest. The extract is introduced into the mass spectrometer by flow injection analysis and is analyzed directly, without chromatographic separation of the specimen extract components. The tandem mass spectrometer instrument detects analytes of interest (e.g. amino acids and acylcarnitines). The results are evaluated by a set of rules to correlate the analytes concentration to metabolic disorders such as Amino Acid Disorders, Fatty Acid Oxidation Disorders, and Organic Acid Disorders

FIA/GAL/BIO: Congenital Adrenal Hyperplasia, Congenital Hypothyroidism, Galactosemia, Biotinidase Deficiency

Hemoglobinopathy/CF: Hemoglobinopathies and Cystic Fibrosis

Molecular: Here we screen for Severe Combined Immunodeficiency (SCID) using real-time PCR, Galactosemia using Amplification Refractory Mutation System-PCR and Cystic Fibrosis using NextGen Sequencing.

Hemoglobinopathies

(919) 733-3937

Introduction

Newborn Screening includes a screening test for abnormal hemoglobins S, C, D, 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) and high performance liquid chromatography (HPLC) are utilized in the testing process. These services are available to public and private providers for the purposes of prenatal screening, family studies and follow-up testing. **The laboratory does not have the capacity to perform sickle cell trait testing for the purposes of school and college athletics**.

Specimen Identification, Collection and Shipment for Filter Paper Spots

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, making sure all copies are legible. It is imperative that the following information is given: patient's name or unique identifier, patient number, address, sex, race, birth date, blood specimen collection date, transfusion information, Medicaid number, provider name and NPI#, complete name and address of submitter, and EIN #.

C. Follow your institution's procedures for performing heel or finger punctures. After skin is cleansed with alcohol, 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 3-4 hours at room temperature on a flat non-absorbent surface before mailing. DHHS #3105, newborn screening filter form, requires 3-4 hours' drying time. 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 biohazard or 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).

Whole Blood Specimen Submission and Testing

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.

B. Listed below are the conditions by which whole blood family study and/or follow-up testing may be 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.
- 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 a DHHS form #1859WB 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), provider name and NPI#, complete name and address of submitter, NPI 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. 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 will be subjected to extreme high temperatures during transit in 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 on blood spot specimens are reported within 1 week 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 respect to the 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 (DBS) collected from the newborn baby. This sample is screened for diseases that may cause intellectual or physical disabilities, or other health complications, if untreated. To prevent early effects of disease the sample should be drawn during the infant's first 24 to 48 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: The specimen is analyzeed by high performance liquid chromatography (HPLC) for the presence of abnormal hemoglobins. The abnormal hemoglobins are confirmed by isoelectric focusing (IEF).

- Galactosemia: Total Galactose and galactose-1-phosphate are measured, total galactose by a fluorescent galactose oxidase method. Galactose-1-phosphate uridyl transferase (GALT) activity is determined by measuring its reaction produced over time. Both assays are performed on all specimens. GALT PCR is a reflex test performed on low level Galactosemia Newborn Screening blood spots using a conventional PCR method for mutation detection (Tetra-prime Amplification Refractory Mutation System PCR (ARMS-PCR) or if the total galactose is $\geq$ 7.3 mg/dl and the patient is transfused.

- 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, Alanine, Succinylacteone, Citrulline, Arginine and Arginoinosuccinic Acid) are measured by flow injection analysis Tandem Mass Spectrometry (MS/MS).

- Fatty Acid Oxidation Disorders: Acylcarnitines from (Free Carnitine C0) to longer chain (C18) are measured by MS/MS.

- Organic Acid Disorders: Acylcarnitines from (Free Carnitine C0) to longer chain (C18) are measured by MS/MS.

- Biotinidase Deficiency: Biotinidase enzyme activity is measured using a colorimetric assay.

- Cystic Fibrosis (CF): Immunoreactive trypsinogen (IRT) is measured by time-resolved FIA. The daily top 4% of specimens with the highest IRT values undergo DNA testing using a panel of over 139 common CF **mutations**.

- Severe Combined Immunodeficiency (SCID): SCID is a group of inherited disorders characterized by a deficiency or absence of functional T-cells which causes a loss of cellular and humoral immunity that has a 100% mortality rate from opportunistic infections if left untreated. SCID testing is performed by measuring T-cell Receptor Excision Circles using real time PCR.

Sample Collection and Identification

A. Newborn Screening Specimen collection form DHHS #3105 can be ordered on-line at https://slphreporting.ncpublichealth.com/labportal

B. Training in specimen collection and form completion is available from the laboratory's website at http://slph.adobeconnect.com/newborn/.

C. Time of Collection

1) A blood spot specimen (heel stick) should be obtained from every infant prior to discharge or transfer to another hospital, regardless of age. In instances where a specimen was not collected prior to discharge or transfer, submit a filter form with completed demographics and without sample to the NCSLPH to document the infant in the database. Should a parent refuse screening, document internally and submit a filter form with completed demographics and without sample, to the testing laboratory to document the infant in the database. The number or type of feedings (breast or formula) will not affect this rule. Optimum time for specimen collection is 24-48 hours of age.

2) The specimen should be collected 24 hours after birth. Optimum time for specimen collection is 24-48 hours of age. If the specimen is collected prior to 24 hours of age a repeat screening specimen should be collected by one week of age. It is the responsibility of the provider whose name is listed on the form to obtain this second specimen in a timely manner. Parents should be informed that the infant is being retested because of early sample collection, not because the infant has an increased risk for a disorder.

3) The specimen should be collected pre-transfusion. If the initial specimen was collected post-transfusion, a second specimen should be collected 90 days after the last transfusion. Note that infants greater than 6 months of age at collection are no longer considered newborns and are only eligible for hemoglobinopathies testing (refer to section C5 Note).

4) Premature (gestational age less than 37 weeks or low birth weight of < 2500 grams) or ill infants receiving parenteral feeding should be screened upon admittance to the special care baby unit, regardless of age, medical condition, or status of feedings. If the first

specimen is collected at less than 24 hours of age or the infant had a birthweight of < 2000 grams, a second screen should be collected between 48-72 hours of age. At the 28th day of after birth or upon discharge, whichever is first, a third specimen should be collected on those infants whose birthweight was < 2000 grams. 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.

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.

Note: Limits for blood spot specimen submission are based on the baby's age at specimen collection. MS/MS and CF and FIA/GAL/BIO are limited to babies less than 6 months of age at the time of collection. Only Sickle Cell testing can be done on babies greater than six months of age by submitting a blood spot sample on DHHS form #1859, Hemoglobin Electrophoresis form (See Hemoglobinopathies). Do not submit a sample for hemoglobin electrophoresis on DHHS # 3105.

D. Identification and Collection of Newborn Screening Specimen. The recommended method of collection is from a heel stick; collection into a capillary or other device is not the recommended method of collection. Anticoagulants interfere with laboratory testing. Collection and transport instruction follow. Refer to online training http://slph.ncpublichealth.com/newborn/resourcesupdates.asp#formtraining for a tutorial of these processes.

E. Complete all information and identification on Newborn Screening Form #3105. It is imperative that all demographic fields are complete when submitting the filter form, even if the sample submitted is a repeat.

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 for documentation purposes.

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. Position the infant with the leg dependent for optimal venous flow.

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.

Transport Blood Spots for the Laboratory

A. After drying, send completed forms (both first tests and repeats) to the NCSLPH for *delivery* within 24 hours of collection, using an overnight transport method. **DO NOT HOLD OR BATCH SAMPLES FOR ANY REASON, INCLUDING COMPLETION OF HEARING SCREENING**. Overnight delivery preserves the integrity of the sample, decreases transit time, and allows for earlier diagnosis and treatment of an affected infant.

B. **Do not package blood spot collection forms in plastic bags for mailing.** Heat and humidity build up and can deteriorate the dried blood spot specimen.

Reporting Procedures and Interpretation In all cases where a repeat sample is requested, it should be collected as soon as possible and transported to the NCSLPH within 24 hours of collection. Do not wait until the next well-baby visit for collection.

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, it is recommended that serum testing be performed by the provider or approved laboratory. NCSLPH does not perform serum testing.

B. **Galactosemia**: Galactose results are reported as normal or abnormal. Both Galactose and Galactose-1-phosphate uridyl transferase (GALT) results are determined by the galactosemia algorithm. All urgent results are reported to the genetic coordinator who will contact the baby's health care provider.

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.

D. **Hemoglobinopathies (Sickle Cell)**: Hemoglobinopathies results are reported as normal if no abnormal hemoglobin is detected. Heterozygotes S, C, D 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.

E. **Tandem Mass Spectrometry (MS/MS) screening**: Analytes screening results of interest (e.g. amino acids profile and acylcarnitine profiles) are evaluated by a set of rules to correlate the analytes concentration to metabolic disorders such as Amino Acid Disorders, Fatty Acid Oxidation Disorders and Organic Acid Disorders. Screening results from each profile are reported as normal, borderline or abnormal. Unless clinically indicated, normal results require no further specimen submission. 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 referred 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.

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.

G. **Cystic Fibrosis**: Results with IRT values that do not fall in the daily top 4% are reported normal for CF with no additional testing required. IRT values greater than the 96^{th} percentile are reflexed to a second tier DNA test. Results with no mutations and an IRT value $\leq$ 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. Severe Combined Immunodeficiencies (SCID): The results will be reported as normal, borderline, abnormal or Unsat Inconclusive. For abnormal and borderline normal birthweight, the healthcare provider will be contacted by follow up with further recommendations. For Unsat inconclusive there was not enough DNA present to evaluate specimen and another sample will need to be sent in. For pre-term borderline results it is recommended to resubmit another sample after 14 days and every 2 weeks until results are in normal range and baby is full term.

I. **Insufficient or Unsatisfactory Specimens**: A letter is sent to the baby's health care provider and submitter (as listed on the filter form) to request a repeat specimen.

The integrity of the infant's newborn screening results is 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 and 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.

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

Virology/Serology

(919) 733-3937 or (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) 733-3937

Introduction

Diagnostic serologic assays are performed on serum and CSF 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). Classical WNV fever is often associated with headache, lymphadenopathy, nausea, vomiting, and fatigue. WNV Central Nervous System (CNS) infection is associated with meningitis, encephalitis, meningoencephalitis, and/or acute flaccid paralysis resembling Guillian-Barre syndrome. All specimens received will be tested for IgG antibodies to EEE, WEE, LAC, SLE, and WNV and IgM antibodies to WNV and LAC by immunofluorescence (IFA) or enzyme immunoassay (EIA).

Serologic and molecular testing for chikungunya, Zika, and dengue viruses is now available at the NCSLPH. All clinical and travel information, including date of onset, **must** be included on the test request form; the provider must complete the form and sign the Physician Attestation statement. Specimens from symptomatic patients with travel history collected <14 days post-illness onset will be tested first by RT-PCR. If RT-PCR is negative, these specimens will also be tested for the presence of IgM antibody by ELISA. Specimens from symptomatic travelers collected ≥14 days post-onset will receive IgM ELISA testing only. Additional testing may be performed for symptomatic or asymptomatic patients who are pregnant and have traveled to or are living in areas with active Zika transmission. All specimens with "Presumptive Positive" IgM ELISA results will be referred to CDC for confirmatory plaque reduction neutralization testing. Testing for chikungunya, Zika, and dengue will also be included with requests for Arboviral panel testing on patients with a history of travel to an endemic area. Urine, CSF, whole blood, and amniotic fluid may also be submitted for Zika molecular testing, but these specimen types must be submitted along with a paired serum specimen. Special requests for molecular testing on specimen types such as cord blood, placental tissue, or umbilical tissue can be arranged at CDC.

For more information about these viruses, go to http://www.cdc.gov/

Specimen Collection and Identification

Only serum and CSF may be submitted for arboviral serologic testing. Clearly label each specimen vial with the patient's name (first and last) and either the date of birth, Social Security number, or other unique identifier. Be sure to label vials with date collected for paired serum specimens. 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, collection date(s), submitter name and address, signs/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 specimens, principally acute and convalescent serum specimens. The acute serum should be collected no later than 3-5 days after the onset of illness. The convalescent serum should be collected 2-3 weeks after onset, or at the time of hospital discharge, for confirmation of probable cases. Since paired sera are advised for all arboviral studies (except for chikungunya, Zika and dengue viruses), 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 specimens 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.**

Equine specimens for Arborviral testing should be submitted through the Rollins Animal Diagnostic Laboratory.

Shipment

For Arborviral panel testing, 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.

For chikungunya, Zika, and dengue testing, send the properly identified specimen vials on cold packs or frozen. These specimens should be packed and shipped as Category B infectious substances.

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	6 working days
LAC, IgM	IFA Quant EIA Qual	<1:16 Negative	2 mL serum/CSF PSA	6 working days
Eastern Equine Encephalitis (EEE), IgG	IFA Quant	<1:16	2 mL serum/CSF PSA	6 working days
EEE, IgM	IFA Quant	<1:16	2 mL serum/CSF PSA	6 working days
St. Louis Encephalitis (SLE), IgG	IFA Quant	<1:16	2 mL serum/CSF PSA	6 working days
SLE, IgM	IFA Quant	<1:16	2 mL serum/CSF PSA	6 working days
Western Equine Encephalitis (WEE), IgG	IFA Quant	<1:16	2 mL serum/CSF PSA	6 working days
WEE, IgM	IFA Quant	<1:16	2mL serum/CSF PSA	6 working days
West Nile Virus (WNV), IgG	IFA Quant EIA Qual	<1:16 Negative	2mL serum/CSF PSA	6 working days
WNV, IgM	IFA Quant EIA Qual	<1:16 Negative	2mL serum/CSF PSA	6 working days
Chikungunya Virus, IgM	EIA Qual	Negative	2mL serum	6 working days
Chikungunya Virus	RT-PCR	Negative	2mL serum/CSF/ urine/amniotic fluid/whole blood (EDTA)	6 working days
Zika Virus, IgM	EIA-Qual	Negative	2mL serum	6 working days
Zika Virus	RT-PCR	Negative	2 mL serum /CSF /urine/amniotic fluid/whole blood (EDTA)	6 working days
Dengue Virus, IgM	EIA-Qual	Negative	2 mL serum	6 working days
Dengue Virus	RT-PCR	Negative	2 mL serum /CSF /urine/amniotic fluid/whole blood (EDTA)	6 working days

Abbreviations:

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

Chlamydia/Gonorrhea

(919) 733-3937

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 1,526,658 cases of *C. trachomatis* and 395,216 cases of *N. gonorrhoeae* infections were reported in 2015.

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 not performed at the NC State Laboratory of Public Health, but 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 NC State Laboratory website: (http://slph.ncpublichealth.com/labimprovement/labtraining.asp). 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, Social Security number, or other unique

identifier. Complete submission form DHHS #4011 “Chlamydia/Gonorrhea Detection”. The DHHS #4011 form is available on this website. Forms should be printed on white paper only.

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 Multitest 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 Multitest 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 Multitest 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 Multitest 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 Multitest 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, Social Security number or other unique identifier. Specimens which, for any reason, are deemed unsuitable or inappropriate for diagnostic 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.

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, 25 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 collection kits 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. Turn-around time for test results is three working days. 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 B and Hepatitis A Serology

(919) 733-3937

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 healthcare 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) 733-3937. 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, Social Security number or other unique identifier. 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	IA-Qualitative Screen	Antigen not detected	2 working days
Hepatitis B virus surface antigen	IA-Confirmatory	Interpreted by report	3 working days
Hepatitis B virus core-IgM antibody	IA-Qualitative	No antibody detected	3 working days
Hepatitis B virus core-total antibody	IA-Qualitative	No antibody detected	3 working days
Hepatitis B virus surface antibody	IA-Qualitative	No antibody detected	3 working days
Hepatitis A IgM antibody	IA-Qualitative	No antibody detected	1 working days

Abbreviations:
IA 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

Hepatitis C

(919) 733-3937

Introduction

Serologic testing for Hepatitis C (HCV) infection is available only to patients with specific risk factors. The HCV testing algorithm includes initial screening for antibodies to HCV using an immunoassay (IA). Patients who test nonreactive for HCV antibodies by the IA screening assay can be considered negative for both acute and past HCV infection. All reactive IAs are then tested for HCV RNA by nucleic acid amplification (NAAT). Patients with detectable HCV RNA should be considered as likely active HCV infections. Patients with a reactive antibody test but undetectable HCV RNA may be considered to have had a resolved past infection.

At least 3 mL of serum is required for the complete HCV testing protocol. NOTE: If also requesting HIV testing on the specimen, a single 3 mL volume is sufficient for both tests.

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

1. People who currently inject drugs (PWID)
2. People with a history of injecting drug use
3. People who are HIV positive
4. People born between 1945 and 1965 (only once unless other risk factors above are present), in accordance with CDC screening recommendations

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 HCV assays.

Label each vial of serum with the patient's first and last name and either the date of birth, Social Security number, or other unique identifier. A pre-printed HSIS label may be used. Complete the HIV/HCV submission form (DHHS #1111) in its entirety. All items on this form must be completed before the specimen can be processed.

Only serum samples are acceptable for HCV testing. Specimens submitted to the Virology/Serology Unit must be accompanied by a fully completed HIV/HCV OCR scannable submission form (DHHS #1111). If two identifiers (the patient's first and last name and either date of birth, Social Security number, or other unique identifier) are not present on the HIV/HCV scannable form, the specimen is deemed "Unsatisfactory" for HCV 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 HCV testing. HIV/HCV 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

Send the properly identified vials of patient sera and the completed form DHHS #1111 in the "CTS SEROLOGY" mailing containers via the State Courier Service. Specimens can be shipped at ambient temperature.

The DHHS #1111 scannable HIV/HCV form, along with instructions for completing the form, is available on this website. Forms should be printed directly from the website on white paper only; photocopies of the form are not acceptable.

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
HCV antibodies	IA-Qualitative	No antibody detected	2 working days
HCV RNA	Nucleic Acid Amplification Test (NAAT)-Qualitative	No HCV RNA detected	5 working days

Human Immunodeficiency Virus Serology

(919) 733-3937

Introduction

Serologic screening for human immunodeficiency virus (HIV) infection is available only through designated counseling and testing sites. Two HIV serologic assays are utilized as part of an HIV testing algorithm. Initial screening for HIV-1 p24 antigen and antibodies to HIV-1 (including Group O and subtypes) and HIV-2 is performed using an immunoassay (IA). All reactive IAs are repeated in duplicate to verify the initially reactive test result. All repeatedly reactive IA tests (two or more reactive) are tested by the Geenius HIV-1/HIV-2 discriminatory assay that differentiates HIV-1 and HIV-2**.** Patients who test HIV-1 positive on the Geenius assay should be considered HIV infected. If the test result indicates HIV-2 reactivity, the sample is referred to CDC for HIV-2 confirmation.

Patients who test nonreactive for HIV p24 antigen and HIV-1/HIV-2 antibodies by the IA screening assay can be considered negative for both acute and established HIV infection. Samples that test repeatedly reactive on the screening assay but test as either HIV negative, HIV positive-untypable (undifferentiated), HIV-2 positive with HIV-1 cross-reactivity, HIV indeterminate, HIV-1 indeterminate, HIV-2 indeterminate, or invalid by Geenius are further tested for HIV-1 RNA by nucleic acid amplification (NAAT). Patients with detectable HIV-1 RNA should be considered as likely acute HIV infections.

At least 3 mL of serum is required for the complete HIV testing protocol. protocol. NOTE: If also requesting HCV testing on the specimen, a single 3 mL volume is sufficient for both tests.

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, Social Security number, or other unique identifier. A pre-printed HSIS label may be used. Complete the HIV/HCV submission form (DHHS #1111) 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. Specimens submitted to the Virology/Serology Unit must be accompanied by a fully completed HIV/HCV OCR scannable submission form (DHHS #1111). If two identifiers (the patient's first and last name and either date of birth, Social Security number, or other unique identifier) are not present on the HIV/HCV 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/HCV 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

Send the properly identified vials of patient sera in the "CTS SEROLOGY" mailing containers. Place the container and the corresponding DHHS #1111 forms in a zip-lock specimen bag and send to the State Lab via the State Courier Service. Specimens can be shipped at ambient temperature.

The DHHS #1111 scannable HIV/HCV form, along with instructions for completing the form, is available on this website. Forms should be printed directly from the website on white paper only; photocopies of the form are not acceptable.

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; HIV p24 antigen	IA-Qualitative	No antibody or p24 antigen detected	2 working days
Human Immunodeficiency Virus type 1 (Groups M&O) and type 2 antibodies	Rapid EIA-Qualitative	No antibody detected	2 working days
Human Immunodeficiency Virus type 1 RNA	Nucleic Acid Amplification Test (NAAT)-Qualitative	No HIV-1 RNA detected	5 working days

Rabies Virus

(919) 733-3937

Introduction

The North Carolina State Laboratory of Public Health (NCSLPH) 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 multiple bat submissions

(defined as more than 1 bat) or bat infestations and will make 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

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, unconscious, or incapacitated.

The laboratory on-call person can be reached at (919) 733-3937 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.

NOTE: In addition to the instructions below, an instructional PowerPoint presentation "Guide to Rabies - Packaging and Shipping" can be accessed and viewed at the NC State Laboratory website http://www.quia.com/pages/cmiller20/rabiespackandship The purpose of the presentation is to assist in training people who collect and submit rabies samples to the NCSLPH for 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.

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. **Treat any specimens for fleas, ticks, maggots, ants, etc. prior to packing.**

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. **DO NOT SUBMIT LIVE BATS – PLEASE ENSURE THAT THE BAT HAS BEEN EFFECTIVELY EUTHANIZED BEFORE PLACING IN THE BAG.**

Submitters need to fully complete the submission form (DHHS # 1614) indicating the species of animal, vaccination history, date and type of bite or other significant exposure, anatomical area exposed, and county (including zip code and GPS location, if known) 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 NCSLPH rabies laboratory for testing. Contact the agriculture labs directly for specimen submission information. The anatomical tissues that the NCSLPH 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 NCSLPH 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 or FedEx shipment for overnight delivery 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. In addition, the outside of the box should be clearly labeled **"Approved for Weekend (or Holiday) Testing"** if the sample is to be tested on Saturday or a holiday. Address all shipping containers using the special label (white with red lettering) available from the NCSLPH mail room. This label instructs the transporting service to call the NCSLPH 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: NC State Laboratory of Public Health
4312 District Drive
Raleigh NC 27607
MSC 1918

"This package contains an animal head suspected of having rabies."

Delivery in Person: From 8:00 a.m. to 5:00 p.m., Monday-Friday
Specimens/samples are delivered to the "Specimen Receiving and Drop Off" area adjacent to the facility loading dock (please follow signs).

AFTER HOURS: Specimens/Samples are delivered to the same location, but delivery personnel must notify on-site Capital Police for access to the building via intercom. DO NOT leave unattended packages on the loading dock, even if arrangements have been made for after-hours testing.

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 (https://celr.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) 733-3937 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 Serolo

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 or the epidemiologist on-call 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) 733-3937

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, Social Security number, or other unique identifier. Complete DHHS form #1188 (immune status testing) or DHHS form #3445 (serodiagnosis of current or recent infection). Please note that all suspect or probable rubella cases must be reported to the Communicable Disease Branch at (919)733-3419 for prior approval of Rubella IgM laboratory testing. 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.

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 form #1188 or 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 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	1 working day

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) 733-3937

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, Social Security number, or other unique identifier. Complete a DHHS form #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). The CDC 50.34 form and instructions are available from the State Lab website http://slph.ncpublichealth.com/forms.asp.

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, Social Security number, or other unique identifier. Specimens that, for any reason, are deemed unsuitable or inappropriate for diagnostic 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 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) 733-3937

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 Communicable Disease Branch at (919)733-3419 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) 733-3937 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, Social Security number, or other unique identifier. Complete a DHHS form #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 form #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 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.

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 2-3 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 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 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
Ehrlichia chaffeensis, IgG	IFA-Quan	<1:64	2 mL serum, PSA	5 working days
Measles, IgM	IFA-Qual	Interpreted by Report	2 mL serum	1 working day
Measles, IgG	IFA-Qual	Interpreted by Report	2 mL serum	3 working days
Mumps, Diagnostic IgG	IFA-Quan	Interpreted by Report	2 mL serum; PSA	3 working days
Rickettsia rickettsii (RMSF), IgG	IFA-Quan	<1:64	2 mL serum, PSA	5 working days
Rickettsia typhi (Typhus), IgG	IFA-Quan	<1:64	2 mL serum, PSA	5 working days
Varicella zoster, IgG	IFA-Qual	Interpreted by Report	2 mL serum	3 working days

Abbreviations:

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

Syphilis Serology

(919) 733-3937

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 Rapid Plasma Reagin (RPR). Confirmation of reactive screening test results (RPR) 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 not performed at the NC State Laboratory of Public Health, but are available from commercial reference laboratories.

Sample Collection and Identification

The non-treponemal test for syphilis (RPR) 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. 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	RPR
Primary	Serum	RPR
Secondary	Serum	RPR
Latent	Serum	RPR, TREP-SURE
Late Neurosyphilis	Serum	RPR, TREP-SURE
Congenital CNS Involvement	Serum	RPR, 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: North Carolina law no longer requires a premarital serologic test for syphilis. Any other states requiring a premarital syphilis test will accept test results from the State Laboratory of Public Health.

Note: North Carolina Public Health Law 10A NCAC 41A.0204 requires all pregnant women to be screened at the first prenatal visit, between 28-30 weeks gestation, and at delivery.

Specimens submitted to the Virology/Serology Unit must be accompanied by a fully completed DHHS #3446 request form.

- Check "RPR (Titer and Confirmatory if Reactive)" for screening purposes. All specimens testing Reactive on the screening RPR will be automatically reflexed to a quantitative RPR (titer) and syphilis confirmatory test (TREP-SURE EIA).
- Check "*Treponema pallidum* confirmatory serology" only if requesting follow-up confirmatory testing on a previously Reactive screening test; please provide screening test (RPR/TRUST) quantitative titer results.
- When requesting both an RPR and a confirmatory test (even if the RPR is Nonreactive) because latent or late syphilis is suspected, write in this reason for testing in the "Other" section under Reason for Testing.

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, Social Security number, or other unique identifier. 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 the 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 (RPR) performed on serum are available within three working days after receipt of the specimen. Treponemal specific tests (TREP-SURE) performed on serum are available within four working days after receipt of specimen.

Patients with primary syphilis may have a non-reactive RPR 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

RPR Results	TREP-SURE Results	Interpretation
Reactive	Positive	Usually indicates syphilis.
Reactive	Negative	"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	Positive	Usually indicates previously treated syphilis or late syphilis (untreated).

Virus Culture

(919) 733-3937

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 Viral 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 by RT-PCR is also routinely available for some viral agents, such as influenza, mumps, herpes, VZV, and enteroviruses.

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 form #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 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.

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 by viral culture 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 70º 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/VZV Molecular Testing:

HSV/VZV molecular testing of cutaneous and mucocutaneous lesions is available only to local health departments and other state operated health care facilities. Specimens acceptable for HSV/VZV molecular testing are limited to the following:

1. Specimens from prenatal patients who have a suspicious lesion not previously confirmed as herpes. Routine testing 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. Testing done simply to confirm a clinical diagnosis of herpes is 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/VZV testing must be accompanied by a DHHS form #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 HSV/VZV as the agent requested. Failure to supply the requested clinical patient information may result in significantly delayed specimen testing or rejected specimens.

HSV/VZV testing from urogenital sites is limited to one specimen per patient. If more than one urogenital site is sampled, both swabs should be submitted in the same transport tube. Specimens 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 asymptomatically 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 PCR	Throat swab, feces, CSF, pericardial fluid, skin tissue	3 weeks PCR: 3 days
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 PCR	Throat swab, feces, CSF, pericardial fluid, skin tissue	3 weeks PCR: 3 days
Enterovirus	Cell culture PCR	Throat swab, feces, CSF, pericardial fluid, vesicle scraping	3 weeks PCR: 3 days
Herpes simplex	Cell culture	Brain biopsy, CSF, conjunctival swab	1 week
Herpes simplex	PCR	Vesicle scraping	2 days
Influenza	Cell culture PCR	Throat washing or swab, nasal swab, nasopharyngeal washing or swab, lower respiratory specimens	3 weeks PCR: 3 days
Measles	Cell culture PCR (Reference Lab)	Throat swab, nasopharyngeal swab, CSF	3 weeks PCR: 4 to 5 days
Mumps	Cell culture PCR	Throat swab, CSF, buccal swab	3 weeks PCR: 3 days
Parainfluenza virus	Cell culture	Throat washing or swab, nasal swab, nasopharyngeal washing or swab	3 weeks

Virus Description	Test Method	Specimen Requirements	Turn-Around Time (if Negative)
Poliovirus	Cell culture PCR	Throat swab, feces, CSF	3 weeks PCR: 3 days
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)	PCR	Nasopharyngeal swab	4 to 5 days
St. Louis encephalitis	Cell culture	Brain tissue, CSF	3 weeks
Varicella-zoster	PCR	Vesicle scraping	2 days
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	• **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.
Atypical Bacteriology	• **Neisseria and Haemophilus** are good up to 72 hours in an incubated, CO_2 atmosphere. • **Gram positive and gram negative bacilli** reference isolates may be good up to a week if not subjected to extreme temperatures.
Enteric Bacteriology	• **Reference isolates** may be held up to 1 month at room temperature or refrigerated.
Mycology	• **Clinical specimens** are optimally received within 1 or 2 days, but may be acceptable up to 1 week refrigerated. • **Reference specimens** should be received within 1 week of collection. Please submit a growing, isolated subculture; if culture is mixed and one organism overgrows another, it is unsatisfactory.
Parasitology	• **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. • **Corneal scrapings** for Acanthamoebae are referred to the CDC for testing. Submit corneal scrapings in a sterile collection tube containing 200µl saline + 200µl sterile water as preservative.
Tuberculosis	• Specimens should be held in the refrigerator and will be viable for a limited time. Send specimens within 24 hours of collection. NOTE: Any specimen received 7 days after collection will be rejected.

VIROLOGY/SEROLOGY

Serological Specimens (i.e. either serum or a serum/CSF pair)	• These specimens, if aseptically collected and maintained, are stable at ambient temperature for several days or refrigerated (4-8º 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	• Must be tested within 60 days of collection and should be transported and stored at room temperature.
Chlamydia/GC Genprobe urines	• Must be tested within 30 days of collection and should be transported and stored at room temperature.
Viral Isolation Specimens	• 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 40º 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	• 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	• Can be held at room temperature away from sunlight, moisture, and heat and sent immediately upon restoration of transport services. Do not mail in plastic bags. Specimens received 14 or more days from the date of collection cannot be tested due to the age of the specimen.
Sickle Cell (Whole blood)	• Can be held up to one week refrigerated.

HEMACHEMISTRY

Blood lead	• Must be received within 28 days.

BIOTERRORISM/EMERGING PATHOGENS

All samples	• All samples should either be driven to the lab or shipped next day air with FEDEX or contract courier.

APPENDIX B

ENVIRONMENTAL HOLDING TIMES

Reference *EPA Manual for the Certification of Drinking Water Laboratories*. Not all the environmental tests listed below are performed by the SLPH.

CHEMISTRY

Parameter/ Method	Preservative	Sample Holding Time	Extract Holding Time & 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	H2SO4 pH<2	28 days		100 mL	Plastic or Glass

Parameter/ Method	Preservative	Sample Holding Time	Extract Holding Time & 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

Parameter/ Method	Preservative	Sample Holding Time	Extract Holding Time & Storage Conditions	Suggested Sample Size	Type of Container
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 for 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 & Storage Conditions	Suggested Sample Size	Type of Container
547	Sodium Thiosulfate Cool, 4C	14 days (18 months 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_2SO_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	• 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	• 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	• 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	• Preferably should not exceed eight hours. The maximum time the sample should be held in the refrigerator is 24 hours at 4˚C.