

MICROBIOLOGY SERVICES

OVERVIEW

The Microbiology Section offers testing for infectious diseases in support of the Georgia Department of Public Health/ Division of Public Health programs and initiatives in the areas of bacteriology, immunology, parasitology, mycobacteriology, and virology. The mission of this section is to give assistance to public and private health-care providers in their investigation and control of diseases. This mission can be best accomplished by providing accurate and timely analysis of clinical and reference specimens submitted by the county health departments, hospitals, other clinical laboratories, and physicians in private practice.

Our laboratory has provided valuable information on molecular typing of a number of isolates and has identified strains that have been implicated in foodborne outbreaks by utilizing Pulsed-field gel electrophoresis (PFGE) technology. In addition, molecular testing has been implemented for detection of Chlamydia and gonorrhea, determination of viral load in HIV infected patients, diagnosis of parasitic diseases and tuberculosis.

Serological tests are offered to determine past or current infections with a variety of viruses including hepatitis and HIV among many other viruses tested. Luminex flow analyzer (BioPlex) technology is used for enhanced detection of West Nile Virus by immunoassay and detection of respiratory viruses by Polymerase Chain Reaction (PCR). In addition, viral culture is used for detection of respiratory viruses, herpes virus and other viruses listed in the "List of Laboratory Services" in this manual.

Mycobacteriology Unit offers testing for TB by MGIT 960 and HPLC for a faster turn-around time and is capable of identifying most species of mycobacteria and fungi.

In addition to clinical testing, rabies testing of suspected rabid animals is performed on the animal heads submitted through Animal Controllers. When deemed necessary, environmental specimens are tested to help investigate outbreaks of diseases. Microbiology Section also assists the Centers for Disease Control and Prevention (CDC) by participating in surveillance studies and by providing them with the results of our laboratory findings for a number of communicable diseases.

The following sections of the manual provide more detail information on testing and specimen submission requirements for each type of test offered.

Mahin M. Park, Ph.D., HCLD
Clinical Microbiology Service Director
E-mail: mpark@dhr.state.ga.us
Telephone No: (404) 327-7905

BORDETELLA PERTUSSIS
404-327-7990

INTRODUCTION

The **Bacteriology Unit** accepts cultures for the isolation of *Bordetella pertussis* and *Bordetella parapertussis*, nasopharyngeal swabs in charcoal-blood transport medium for both agents, and slides for direct fluorescent antibody (DFA) testing. Specimens may be sent from public and private health care providers.

SPECIMEN COLLECTION/LABELING/REQUISITION FORM

Nasopharyngeal secretions are the optimal specimens for isolating pertussis bacterium and obtaining a lab confirmed diagnosis. A specimen should be collected as soon as possible after onset of illness, preferably before antibiotic treatment. Dacron (not cotton) or calcium alginate swabs are superior to other types of swabs and are recommended for use in collecting nasopharyngeal specimens. Regan Lowe semisolid transport medium tube is recommended for transport to the laboratory. The medium should be stored in a refrigerator at 2-8 degrees Celsius.

Please note: Regan-Lowe medium should be removed from the refrigerator and warmed to room temperature before use.

A. Specimen for Nasopharyngeal Swab Culture:

1. Immobilize and tilt the patient's head.
2. Pass nasopharyngeal swab very gently into a nostril until it reaches the posterior nares.
3. Leave the swab in place for up to 10 seconds (this may induce a cough and in practice only a few seconds may be possible.) If resistance is encountered during insertion of the swab, remove it and attempt insertion on the opposite nostril.
4. Remove the swab slowly.
5. Streak slides (frosted side up) then insert and immerse swab into the tube of Regan-Lowe transport medium, available commercially. Cut the handle end of the swab extending above the transport tube if necessary and cap the container tightly. **Note:** If Regan-Lowe plate is used, streak the plate and then discard the swab.
6. Label slide holder with the patient's name and date of specimen collection (avoid using wax pencil or gummed labels on slides).
7. Label Regan-Lowe transport tube with the patient's name and date of specimen collection.

B. Specimen for Slides:

Prepare 2 dime-sized smears on each of 2 microscope slides (one slide per nostril). Use frosted-end slides with prestamped circles if possible (frosted side up). Label slide holder with the patient's name or other unique identifier and date of specimen collection (avoid using wax pencil or gummed labels on slides).

C. Culture (*B. pertussis* isolated at submitting laboratory):

1. Inoculate a pure culture onto a plate of charcoal agar with horse blood, such as Regan-Lowe medium, and send to GPLH within 24 hours or incubate at 35° C under high humidity. Schedule sub-culture so that time between optimal growth and transport of culture is <24 hours.
2. Examine medium beginning after 3 days incubation until confluent growth appears in the first quadrant of the plate.
3. Using a Dacron swab, aseptically harvest all growth from the first quadrant of the plate by sweeping the swab head through the area of confluent growth while rotating the swab shaft. Immerse swab containing harvested growth 2mm deep into the agar of a charcoal-blood transport tube, warmed to room temperature.
4. Aseptically cut the swab shaft flush with the top of the transport tube and replace cap.
5. Label transport tube with patient identifier information.
6. Maintain additional subculture(s) on plates at submitting laboratory until viability of referred culture is confirmed by the Bacteriology Unit.

Requisition Form

Use form #3410 for specimen submission. It is extremely important that the form is entirely completed. Please include the following information:

1. Unique patient identifier (name or number).
2. Date of specimen collection.
3. Test requested, such as culture for *B. pertussis* and *B. parapertussis* or DFA.
4. Submitter's name and address.
5. Name and telephone number of clinician to contact.
6. Source of specimen, such as nasopharyngeal.
7. Patient's race, sex, age, and address, if available.
8. Brief clinical history, if available.

The patient identifier (name, number, or both) indicated on the requisition form should match that written on the slide holder, transport tube, or culture.

Unlabeled specimens will not be tested.

SHIPMENT OF SPECIMENS AND CULTURES

Note: Always submit the specimen/culture according to current DOT and IATA guidelines.

- A. Slides: Place slides securely in the plastic slide holders provided (outfit #0525, available from Laboratory Services and Supply, 404-327-7920). Place plastic holder in a plastic biohazard bag. Insert requisition form in the pouch in the front of the bag. Place the bag with form affixed in the orange-labeled cardboard mailing container (Decatur address). Seal the lid of the outside container with tape. The specimens should be

transported (room temperature) to the Bacteriology Unit immediately by courier or via Federal Express or UPS. If the shipment is delayed, the specimens should be refrigerated at 2-8 degrees Celsius and then sent the next day on ice packs by first class mail, common carrier, or courier.

- B. Nasopharyngeal Specimens for Culture: The method of shipment is dependent upon the selection of transport media used and the length of time required arriving at the Bacteriology Unit. Inoculated plates must be incubated at 35° C with increased humidity or transported (room temperature) to the Bacteriology Unit immediately by courier. Specimens in tubes of charcoal-blood transport media should be transported (room temperature) to the Bacteriology Unit immediately by courier, or if there is any delay in shipment, they should be stored and sent refrigerated (with ice packs) by first class mail, common carrier, or courier.
- C. Isolates inoculated into charcoal-blood transport media may be shipped at room temperature within 24 hours.

REPORTING AND INTERPRETATION OF RESULTS

Results of DFA testing are completed the day of receipt or next day. Results of positive reports are telephoned to the submitter the day of testing. Nasopharyngeal smears are reported positive for *Bordetella pertussis* if there are ≥ 5 organisms present with typical cellular morphology and intense fluorescence. Due to shortage of quality reagent, *Bordetella parapertussis* DFA testing is confined to confirmation of positive cultures for this organism.

Cultures are held for 7-10 days from the date of inoculation and read daily. Nasopharyngeal swabs received in transport medium tubes are inoculated immediately onto Regan-Lowe plates when received and then incubated. After the final day of incubation, if there are no colonies typical of *B. pertussis* or *B. parapertussis* present, the culture is reported negative for these organisms. A positive culture report is based upon typical cellular and colonial morphology and is confirmed by fluorescent antibody testing. Positive cultures or cultures overgrown with mold or normal flora are reported immediately upon detection, and results are telephoned to the submitter.

Both culture and DFA procedures are recommended for diagnosis of *B. pertussis* whenever possible. "False negative" DFA results may occur from low numbers of organisms present in nasopharyngeal secretions. The DFA test is most valuable during the early phase of illness and before antibiotic treatment when the largest numbers of organisms are being shed. Positive FA results should be considered presumptive. As with any subjective test, "false positive" DFA results are possible. Only highly experienced staff in the Bacteriology Unit read slides and report results to minimize false positives. "False negative" culture results may follow from any procedures that render the organisms nonviable, such as improper handling of plates and transport medium after collection or prolonged antibiotic treatment. The DFA test will detect nonviable organisms. A positive culture result is the most reliable.

UNACCEPTABLE SLIDES AND CULTURES

1. Smears too thick or too thin for accurate interpretation.
2. Slides broken in transit.
3. Cultures overgrown with mold or normal flora.
4. Cultures or transport medium improperly submitted (see above for the recommended procedure).
5. No patient identifier on specimen or culture.

**CHLAMYDIA & GONORRHEA
NUCLEIC ACID AMPLIFICATION TEST
404-327-7990**

INTRODUCTION

The Bacteriology Unit uses Target-amplified direct nucleic acid amplification test for chlamydia and gonorrhea. *Chlamydia trachomatis* is the most commonly reported bacterial sexually transmitted disease in the US. Complications of untreated chlamydial infection in females include acute pelvic inflammatory disease, ectopic pregnancy, chronic pelvic pain, and infertility. *Neisseria gonorrhoeae* often pass unnoticed and asymptomatic carriers contribute significantly to the public health concern of gonorrhea. In women, gonorrhea is a common cause of pelvic inflammatory disease.

Client testing criteria: All female/male clients screened for chlamydia will also be screened for gonorrhea. Females with cervical stenosis should be included in the chlamydia/gonorrhea screening. If the client does not have a cervix, she will not be included in the chlamydia screening.

Family Planning Clinics. All females age 29 years or younger who receive a pelvic exam during a visit (initial, annual or comprehensive medical only) will be screened for chlamydia. A female will only be screened once per calendar year for chlamydia unless she has clinical signs or symptoms of chlamydia (cervicitis/MPC, friable cervix, cervical ectopy, abdominal pain, abnormal bleeding, dyspareunia or dysuria) or a new sex partner or multiple sex partners in the past 60 days. All females 30 years and older who meet these criteria will also be screened for chlamydia.

STD/General Clinics. All females age 10-29, who receive a pelvic exam during a visit, will be screened for chlamydia. This includes rescreening females who are returning for medical problems, receiving more than one pelvic exam a year and/or are currently on antibiotics. All females 30 years and older who present with clinical signs or symptoms of chlamydia (cervicitis/MPC, friable cervix, cervical ectopy, abdominal pain, abnormal bleeding, dyspareunia or dysuria) or have a new sex partner or multiple sex partners in the past 60 days will also be screened for chlamydia. Male clients presenting for services should also be screened for chlamydia.

Teen Clinics. All females who receive a pelvic exam will be screened for chlamydia. This includes rescreening females who are returning for medical problems, receiving more than one pelvic exam a year and/or are currently on antibiotics. Males should also be screened.

SPECIMEN COLLECTION/LABELING/REQUISITION FORM

The APTIMA Combo 2 Assay is designed to detect the presence of *C. trachomatis* and *N. gonorrhoeae* in urine, endocervical and urethral specimens. Specimen collection kits are supplied to the health districts for clients seen at the Public Health STD and Family Planning Clinics.

Endocervical swab

1. Remove excess mucus from the cervical opening and surrounding mucosa using the cleaning swab (white shaft swab in the package with red printing). Discard this swab. Note: To remove excess mucus from the cervical opening, a large-tipped cleaning swab (not provided) may be used. Discard the swab after use.
2. Insert the specimen collection swab (blue shaft) into the endocervical canal.
3. Gently rotate the swab clockwise for 10 to 30 seconds in the endocervical canal to ensure adequate sampling.
4. Withdraw the swab carefully; avoid any contact with the vaginal mucosa.
5. Remove the cap from the swab specimen transport tube and immediately place the specimen collection swab into the transport tube.
6. Carefully break the swab shaft at the score line; avoid splashing the contents.
7. Recap the swab specimen transport tube tightly. Legibly label tube with patient name, patient ID# and date of collection. Unlabeled specimens will not be tested.

Male urethral swab

1. The patient should not have urinated for at least one hour prior to specimen collection.
2. Insert the specimen collection swab (blue shaft swab in the package with the green printing) 2 to 4 cm into the urethra.
3. Gently rotate the swab clockwise for 2 to 3 seconds in the urethra to ensure adequate sampling.
4. Withdraw the swab carefully.
5. Remove the cap from the swab specimen transport tube and immediately place the specimen collection swab into the specimen transport tube.
6. Carefully break the swab shaft at the score line; avoid splashing the contents.
7. Recap the swab specimen transport tube tightly. Legibly label tube with patient name, patient ID# and date of collection. Unlabeled specimens will not be tested.

Urine

1. The patient should not have urinated for at least one hour prior to specimen collection.
2. Direct the patient to provide a first-catch urine (approximately 20 to 30 ml of the initial urine stream) into a urine collection cup which is free of any

- preservatives. Collection of larger volumes of urine may result in specimen dilution that may reduce test sensitivity. Female patients should not cleanse the labial area prior to providing the specimen.
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 transport tube label.
 4. Recap the urine specimen transport tube tightly. This specimen is now referred to as the *processed urine specimen*. Legibly label tube with patient name, patient ID# and date of collection. Unlabeled specimens will not be tested.

Requisition Form

1. Use the Chlamydia and Gonorrhea requisition form #3568.
2. Fill out the form completely by printing or typing legibly. Only legible information can be entered correctly into the laboratory database. Incomplete or illegible information may delay your results. Do not use computer-generated labels for patient information.
3. Information required is as follows:
 - a. Submitter information (submitter code, submitter address, and phone number).
 - b. Patient information (name, patient ID number, county of residence, zip code, State, race, ethnicity, gender, date of birth).
 - c. Specimen information (test requested, reason for test, date collected, source of specimen, specimen status).
4. Tear off the top section of form (white copy) and mail to the laboratory with the specimen, making sure the names on the specimen and form are exact matches. Retain the other two copies of the form (pink and yellow) for clinic and program use.
5. Chlamydia and Gonorrhea Laboratory Submission forms will be provided to the participating Public Health Clinics by the STD Program Office.

SHIPMENT OF SPECIMENS

For best results, specimens should be transported to the laboratory on the date of collection; however, if this is impossible, specimens may be kept at room temperature and shipped as soon as possible. **Urine specimens over 30 days old and/ or swab specimens over 60 days old at the time of arrival in the laboratory will be reported unsatisfactory.**

1. Chlamydia/Gonorrhea APTIMA Combo 2 specimens may be transported at room temperature. Use the specimen transport cans and Decatur address labels (available from the laboratory).
2. Be sure the caps on the transport tubes are secure, and wrap each specimen in absorbent packing material. Place the wrapped specimen inside the aluminum

- can, and close the can securely.
3. Wrap the completed requisition form around the aluminum can and secure it with a rubber band.
 4. Place the aluminum can inside the labeled (Decatur address) fiberboard can, close, and secure the lid with tape.
 5. An alternate shipping method may be utilized by substituting the biohazard bag for the inner aluminum container. If this method is chosen, the matching requisition forms should be placed in the pouch located in the front of each bag.
 6. Specimens may be mailed or shipped by the method most convenient and expedient.

REPORTING AND INTERPRETATION OF RESULTS

The goal of the Bacteriology Unit is to test and report all Chlamydia/Gonorrhea APTIMA Combo 2 specimens within a 3 days turnaround period, unless confirmation testing is required. An electronic copy of all positive Chlamydia and Gonorrhea reports is transmitted to the State Sexually Transmitted Disease (STD) Program. A hard copy of all positives is also mailed to the State STD Surveillance Office.

Results are reported as follows:

1. Positive for *C. trachomatis* and/or *N. gonorrhoeae* by Amplified Aptima Combo 2 Assay.
2. Negative for *C. trachomatis* and/or *N. gonorrhoeae* by Amplified Aptima Combo 2 Assay.
3. Indeterminate, a new specimen should be collected.
4. Unsatisfactory, specimen compromised in some manner making it unsatisfactory for testing. The reason for each unsatisfactory result will be listed on the report form.

UNACCEPTABLE SPECIMENS

Specimens will be reported unsatisfactory for the following reasons:

1. No patient identifier on the specimen, or discrepancy between identifier on the specimen and requisition form.
2. Source other than urine, cervix or male urethra.
3. Two swabs received in the collection outfit.
4. No solution in the collection outfit.
5. No swab or improper swab (not from Gen-Probe kit) in the collection outfit.
6. Urine specimens over 30 days old and/ or swab specimens over 60 days old from date of collection when received.
7. Collection kit expired.
8. No specimen received.
9. Medical/legal specimens (Aptima Combo 2 is not intended for medical/legal cases).
10. Overfilled. Liquid level in the urine transport tube must fall between the two black indicators lines.

ENTERIC BACTERIOLOGY 404-327-7990

INTRODUCTION

The **Bacteriology Unit** examines feces and other specimens for the presence of enteric pathogens, namely *Salmonella* serotypes, *Shigella* sp, *Campylobacter* sp., *Aeromonas* sp., *Yersinia enterocolitica*, and Shiga-like toxin producing *E. coli* (STEC), on a routine basis. Testing for Shiga-like toxin (SLT) will be done on all diarrheal stools and on all stools when STEC is requested. Stool specimens for other foodborne etiologic agents, such as *Bacillus cereus*, *Staphylococcus aureus*, *Vibrio* sp., and *Clostridium perfringens*, will be tested if the patient's clinical history and the epidemiological data warrant testing (see section on Foodborne Illness). Environmental samples may be accepted for enteric culture, testing only when implicated in cases of human illness or by prior consultation.

All isolates of *Salmonella*, *Shigella*, *Campylobacter*, STEC, *Yersinia enterocolitica*, and *Vibrio* recovered from specimens by other clinical laboratories in Georgia should be referred to the Bacteriology Unit, either directly or through the Emerging Infections Program (EIP) site. Referred isolates will be further characterized by various methods, such as biochemical reactions, serogrouping and serotyping, Polymerase Chain Reaction (PCR) and by Cell Wall Fatty Acid Analysis when necessary. Pulsed-Field Gel Electrophoresis (PFGE) will be performed on selected serotypes of *Salmonella* and STEC to determine if strains are related,

SPECIMEN COLLECTION/LABELING/REQUISITION FORM

- A. Feces Specimens for *Salmonella*, *Shigella*, *Campylobacter*, *Aeromonas*, *Yersinia*, STEC, *Listeria*, *Staphylococcus aureus*, and *Vibrio*
1. Feces specimens for the above agents should be collected in the Para-Pak C&S outfit, orange-colored top (do not use the Parasitology O&P kit, blue and white-colored top, because it contains formalin that kills bacteria). Collect freshly passed feces as soon as possible after onset of illness and before antimicrobial therapy has been initiated (stools for *S. aureus* must be collected within 24 hours after onset). The patient should be cautioned against the use of antacid, barium, bismuth, anti-diarrheal medication or oily laxatives prior to specimen collection. An appropriate (i.e. bloody, slimy, watery) area of stool should be selected and sampled with the collection spoon provided on the cap of the container. Add specimen only to the red line on the vial, tighten the cap securely, and invert several times. The solution in the vial should be salmon colored before the specimen is added. Three consecutive specimens collected on

different days during the acute stage of diarrheal disease are suggested (first three days). Ship at **room temperature**.

2. Rectal swabs containing detectable feces may be collected and placed in a Culturette with Stuarts, Cary-Blair, or other commercially available transport medium, if a feces specimen cannot be obtained. Ship under appropriate conditions for the transport medium and test to be performed. Please note: Pathogens are less likely to be recovered from rectal swabs than from feces.
 3. Shiga toxin positive broths (GN or MC) are acceptable and should be sent to GPHL as soon as possible if culture is not performed onsite. Ship **refrigerated** and in compliance with current DOT and IATA guidelines.
- B. Feces specimens for *Clostridium perfringens*, *C. botulinum*, and *Bacillus cereus*
1. Collect fresh stool specimens and place in a leak-proof, non-crushable, clean container (not provided by GPHL). Do not use the enteric ParaPak™ C&S stool culture outfit.
 2. For *C. perfringens* and *B.cereus*, stool specimens must be collected **within 48 hours** from the time symptoms begin.
 3. Store and ship **refrigerated**.

Requisition form

A. Referred Cultures (for identification)

Submit an overnight, pure culture of the isolated bacteria on carbohydrate-free media available commercially (TSA, TSI and LIA are acceptable). Label tube with the patient's name and complete the Isolation for Identification form #3410. If possible, please include any clinical data, cultural characteristics, biochemical reactions, or serology. The form must include the following information:

1. Patient identifier (name or number).
2. Date of collection.
3. Source of specimen.
4. Agent suspected.
5. Submitter's name and address.

B. Each stool specimen must be clearly labeled with the patient's name and accompanied by a properly completed Bacteriology form #3410. The form must include the following information:

1. Patient identifier (name or number).
2. Date of specimen collection.
3. Agent suspected, if applicable.
4. Submitter's name and address.
5. Symptoms.

The patient identifier (name, number, or both) indicated on the requisition form should match that written on the specimen or culture. **Unlabeled specimens or cultures will not be tested.**

SHIPMENT OF SPECIMENS AND REFERRED CULTURES

Note: Always submit the specimen/culture according to current DOT and IATA guidelines.

Mailing containers for submitting fecal specimens and referred enteric cultures are available from Laboratory Services and Supply (404) 327-7920. For stool culture specimens, order outfit #0555, and for culture referral for identification, order outfit #0505.

To facilitate handling, the following should be observed:

1. Wrap kyfax absorbent around the specimen/culture and place in the biohazard bag provided. Put the Bacteriology form # 3410 in the pouch located in front of the bag.
2. Place the biohazard bag in the cardboard mailing container with the Decatur address label (orange). Seal the lid of the outside can with tape.
3. Ship specimens as soon as possible after collection. If there is a delay in transport, keep the specimen at room temperature; do not refrigerate (except as noted above for stool specimens for *C. perfringens*, *C. botulinum*, and *B. cereus*).
4. Specimens and referred cultures should be mailed first class, shipped by common carrier, or delivered to the Laboratory by courier.
5. When large numbers of specimens are expected (such as in an outbreak), please alert the Bacteriology Unit by telephone (404) 327-7990 before mailing so adequate amounts of media will be available for processing.

REPORTING AND INTERPRETATION OF RESULTS

Serotyping and confirmation or identification results are usually reported within 3 to 5 working days for *Salmonella*, *Shigella*, *Aeromonas*, *Vibrio*, and *Yersinia*; within 4 to 6 working days for STEC, and up to 10 working days for *Campylobacter*.

Stool specimen results are reported within 3 to 6 working days. A negative stool culture report reflects the organisms for which the stool specimen was routinely examined: No *Salmonella*, *Shigella*, *Campylobacter*, *Aeromonas*, *Yersinia*, or STEC found. Other organisms will be reported, as appropriate, per request. The results of SLT testing will be reported when performed.

The following enteric pathogens, whether isolated from stool specimens or submitted as referred cultures are identified/confirmed to the species or serotype level:

Salmonella sp. or serotype
Shigella sp. and serotype
Campylobacter sp.
STEC

Aeromonas sp.
Vibrio sp.
Yersinia sp. and serotype

UNACCEPTABLE SPECIMENS

1. Specimens submitted in wrong preservative, e.g., PVA or 10% formalin.
2. Refrigerated specimen for *Shigella*.
3. Multiple specimens collected on the same day (only one specimen will be tested).
4. No patient identifier on specimen or culture.
5. Name or patient identifier mismatch.
6. Specimens received more than 7 days after collection. .
7. Swab submitted in Para-Pak C&S outfit.

FOODBORNE OUTBREAKS 404-327-7990

INTRODUCTION

The **Bacteriology Unit** assists physicians and county health department officials in the diagnosis and epidemiological investigation of outbreaks of suspected foodborne illness. The laboratory examines food, feces, and other epidemiologically implicated specimens for the presence of disease-producing bacteria or toxins. Food samples from single cases of suspected foodborne illness will **not** be examined with the exception of suspected botulism cases. The Georgia Environmental Health and Injury Control Branch (404-657-6534) must be notified by the County Health Department in which the suspected outbreak has occurred, unless botulism is suspected, in which case the Georgia Epidemiology and Prevention Branch (404-657-2588) should be notified. The Bacteriology Laboratory must be advised by either the Environmental Health and Injury Control Branch or the County Health Department of the forthcoming samples, the method of shipment, and the expected time of arrival. This will facilitate the necessary preparations that must be made for processing and examining the samples. Suspect food samples from commercial or retail stores should be referred to the Georgia Department of Agriculture (404-656-3621).

Pulse-field gel electrophoresis will be done on STEC, *Listeria monocytogenes*, *Salmonella*, and *Shigella* isolates, as part of the foodborne illness investigation.

SPECIMEN COLLECTION/LABELING/REQUISITION FORM

All food items must be clearly labeled as to contents and type of food. Fecal or other specimens should be clearly labeled with the patient's name or other unique identifier and indicated as being associated with the outbreak under investigation. When submitting food samples, clinical signs and symptoms, incubation periods, duration of illness, the type of food being submitted, and other pertinent clinical or epidemiological data, must be indicated. This information should also be appropriate for the suspected organism before testing will be initiated. **Unlabeled specimens will not be tested.**

A. **Food**

All food specimens (25 grams minimal, **preferably 100 grams**) should be collected under sterile conditions, placed in sterile plastic bags (whirl packs) or other suitable leak proof containers, and refrigerated. It is the responsibility of the environmental health specialist who collects the specimens to ensure that each food item is accompanied by a completed Bacteriology form #3410. The following minimum information must be included: source of sample; type of sample; date collected; incubation period; clinical symptoms; organism suspected; and submitter's name and address.

Botulism (testing performed at CDC)

- a. Contact the Bacteriology Unit (404-327-7990) for the initial coordination of specimen collection.
- b. The submitter must also contact the GA Epidemiology and Prevention Branch, (404-657-2593), to assess through epidemiological and patient clinical history and determine if testing is warranted.
- c. If approval is given, Epidemiology will notify both the Bacteriology Unit and CDC that specimens will be coming.
- d. Feces, food, and bowel contents (at autopsy) may be examined directly for *C. botulinum* specific neurotoxins A through G. These specimens may also be cultured for *C. botulinum*, and if an isolate is recovered, it may subsequently be tested for the presence of the specific neurotoxins.
- e. Collect feces and food samples in leak-proof, non-crushable containers, refrigerate immediately, and keep **refrigerated** after collection.
- f. Complete a CDC form #50.34 (available from the Bacteriology Unit) and a Bacteriology form #3410 (Environmental/ Food section) for each specimen or food sample submitted.

B. Fecal Specimen

1. Enteric Pathogens and *Staphylococcus aureus*:
Refer to the Enteric Bacteriology section of this manual for instructions pertaining to collecting and shipping specimens in the ParaPak™ stool culture outfit. **Do not refrigerate these stool specimens.** Ensure that each specimen is properly collected and labeled and submitted with form #3410 (Environmental/ Food section). Indicate the enteric pathogens suspected on this form.
2. *Clostridium perfringens* and *Bacillus cereus*:
Collect a stool specimen **within 48 hours** from the time symptoms begin, place in a leak-proof, non-crushable container and **refrigerate immediately**. Label the specimen with the patient's name or other unique identifier. Do not use the enteric ParaPak™ stool culture outfit, as this will result in erroneous quantitative count results. Form #3410 (Environmental/ Food section) must be filled out and *C. perfringens* or *B. cereus*, depending upon the request, checked the agent suspected.

C. Food Handlers

1. *S. aureus*: Swab the infected area and/or anterior nasal membranes. Place the swab in a culturette with Stuarts, Cary-Blair, or other commercially available transport medium. Label specimen with the patient's name or other unique identifier and complete form 3410 (Environmental/ Food section).
2. Enteric Pathogens: Follow procedure in section B.1, above.

D. **Environmental**

Swab the suspected area(s) and submit as in C. above.

SHIPMENT OF SPECIMENS

- A. **Food:** Keep food samples refrigerated. Do not freeze or use dry ice. However, if a food is received frozen, keep it frozen and ship on dry ice. Using a sturdy, waterproof shipping container, place samples and refrigerant in the shipper. Place form 3410 and other epidemiological and clinical information in an envelope attached to the outside of the shipper, or put the form(s) in a separate waterproof plastic bag inside the shipper with the food. Deliver sample(s) to the Bacteriology Unit by courier or ship by the most rapid method. Always notify the Bacteriology Unit of the expected time of arrival.

Botulism

- a. Specimens may be sent to the Bacteriology Unit for referral to CDC and Epidemiology should be notified.
 - b. All specimens for botulism must be shipped **refrigerated**.
- B. **Feces and Other Types of Clinical Specimens** (except those for botulism): Feces for enteric pathogens and *Staphylococcus aureus* should be shipped according to the instructions in the Enteric Bacteriology section of this manual, they should not be sent refrigerated. **Specimens for *Clostridium perfringens* and *Bacillus cereus* should be sent refrigerated** and may be delivered to the Bacteriology Unit by courier or shipped separately by the usual transportation or mailing systems available.

REPORTING AND INTERPRETATION OF RESULTS

Examination of food samples and fecal specimens may require up to seven working days depending upon the suspected etiologic agent. Positive reports will reflect the etiological agent(s) which have been detected and/or enumerated and may include one or more of the following: *Salmonella* sp. or serotype, *Shigella* sp., *Campylobacter* sp., STEC, *Clostridium perfringens*, *Staphylococcus aureus*, *Bacillus cereus*, *Vibrio* sp., or other possible pathogens. Negative reports will be issued in those cases where the suspected organism is not detected. "Test Not Performed" will be indicated when the organism does not fit the epidemiological and clinical data indicated. Testing for Toxin may be performed in appropriate cases: enterotoxin for *S. aureus* and SLT for *E. coli*, and may take an additional two to three working days. Swab results from food handlers are usually reported within three days.

Confirmation that a certain food sample is involved in an outbreak is made by detecting the same pathogen (or toxin) in patient specimens and also in suspect food(s). See Table, Guidelines for Confirmation of Foodborne-Disease Outbreaks of Bacterial Origin.

UNACCEPTABLE SPECIMENS

1. No identifier on the specimen, or discrepancy between identifier on the specimen and requisition form.
2. Specimen or transport medium improperly submitted (see above for the recommended procedure).
3. Less than 25 grams of food (preferably 100 grams).
4. Other unacceptable food specimens will be assessed on an individual basis.

Guidelines for Confirmation of Foodborne-Disease Outbreaks of Bacterial Origin

Etiological Agent	Incubation Period	Clinical Syndrome	Required Specimen	Collection Vial	Submission Form	How to Ship	Criteria for Outbreak Association
<i>Bacillus cereus</i> Emetic toxin (heat stable)	1 – 6 h	Nausea & vomiting, sometimes diarrhea. Fever rare. Duration of \leq 1 day.	Stool w/in 48 h after onset	Clean container	#3410	Refrigerated	Isolation of organism from \geq 2 people.
			Food	Zip-lock bags; clean container	#3410	Refrigerated	Isolation of $\geq 10^5$ organisms/g from implicated food, provided specimen properly handled.
<i>Bacillus cereus</i> Diarrheal toxin (heat labile)	6 – 24h	Watery diarrhea, abdominal cramps; sometimes nausea & vomiting; fever rare. Duration of \leq 1 days.	Same as for emetic toxin	Same as for emetic toxin	Same as for emetic toxin	Same as for emetic toxin	Same as for emetic toxin
<i>Campylobacter sp.</i>	2 – 10d; usually 2 – 5d	Diarrhea (often bloody), fever, abdominal pain. Duration 1 - 5 days.	Stool	Para-Pak #0555	#3410	Ambient Temp.	Isolation of organism from \geq 2 people.
			Food	Zip-lock bags; clean container	#3410	Refrigerated	Isolation of organism from implicated food.
<i>Clostridium perfringens</i>	6 – 24h, median 12h	Diarrhea, abdominal cramps; vomiting, fever, & chills rare. Short	Stool w/in 48h after onset	Clean container	#3410	Refrigerated	Isolation of $\geq 10^5$ organisms/g from \geq 2 people; Demonstration of enterotoxin from \geq 2 people.

		duration of ≤ 1 day.	Food	Zip-lock bags; clean container	#3410	Refrigerated	Isolation of $\geq 10^5$ organisms/g from implicated food; isolation of enterotoxin-producing strain of <i>C. perfringens</i>
<i>Clostridium botulinum</i> – Preapproval by Georgia Epidemiology & Prevention Branch and Prearrangement with CDC required.	2h - 8d; usually 12 - 48h	Illness of variable severity; nausea, vomiting, abdominal pain, & diarrhea may appear early. Head-ache, diplopia, blurred vision, & bulbar weakness common; paralysis, which is usually descending & bilateral may progress rapidly.	Serum Stool* Gastric Contents Food	Clean vials and/or container	CDC Form #50.34 - one per specimen & #3410	Refrigerated	Detection of botulinal toxin in serum, stool, gastric contents, or implicated food; Isolation of organism from stool or intestine. Isolation of <i>C. botulinum</i> , but not the toxin, in a food consumed by a patient with suspected botulism is generally inadequate for confirming the illness.
Shiga-like toxin producing <i>E. coli</i> (STEC)	1 – 10d; usually 3 – 4d	Diarrhea (often bloody), abdominal cramps (often severe), little or no fever.	Stool**	Para-Pak #0555	#3410	Ambient Temp.	Isolation of STEC from ≥ 2 people, same subtype (PFGE).
			Food	Zip-lock bags; clean container	#3410	Refrigerated	Isolation of STEC from implicated food, same serotype/subtype as that found from stool specimens.

*Best for infant botulism.

**Tests for detection of SLT from an overnight broth culture, or directly from a fresh stool, will be performed. The presence or absence of the SLT will be reported. These results must be interpreted according to the total clinical picture.

<i>Listeria monocytogenes</i>	Invasive disease - 2-6 wks	Meningitis, neonatal sepsis, fever	CSF, bld., placenta, amniotic fluid, fetal tissue	Sterile vial and/or Container	#3410	Refrigerated - arrive w/in 48 hr.	Isolation of organism from normally sterile site, same serotype/subtype as implicated food.
			Pure Culture	Culture Referral Outfit #0505	#3410	Ambient Temp.	Confirmation of identification of organism & of same serotype/subtype as implicated food.
	Diarrheal disease – Unknown	Diarrhea, abdominal cramps, fever	Stool	Para-Pak #0555	#3410	Ambient Temp.	Isolation of organism, same serotype /subtype: (a) from ≥ 2 people exposed to implicated food, or (b) as isolate recovered from implicated food.
			Food	Zip-lock bags; clean container	#3410	Refrigerated	Isolation of organism from implicated food, same serotype/subtype as isolates from ≥ 2 people.
<i>Salmonella sp.</i>	6h - 10d, usually 6 - 48h	Diarrhea, abdominal cramps, fever, chills, vomiting, headache, anorexia, malaise. Duration of several days.	Stool	Para-Pak #0555	#3410	Ambient Temp.	Isolation of organism, same serotype/subtype, ≥ 2 people.
			Food	Zip-lock bags; clean container	#3410	Refrigerated	Isolation of organism from implicated food, same serotype/subtype as that found from stool specimens.
<i>Staphylococcus aureus</i>	30 min - 8h, usually 2 – 4h	Vomiting, diarrhea. Short duration of not more than a day or	Stool w/in 24h after onset	Para-Pak #0555	#3410	Ambient Temp.	Isolation of organism of same subtype (PFGE) from ≥ 2 people.

		two.	Nasal or Wound Swab – food handler	Swab outfit (not supplied)	#3410	Ambient Temp.	Isolation of organism of same subtype (PFGE) as stool and/or food isolates.
			Food	Zip-lock bags; clean container	#3410	Refrigerated	Detection of enterotoxin in implicated food; isolation of enterotoxin-producing strain of <i>S. aureus</i> ; isolation of $\geq 10^5$ organisms/g from implicated food, provided specimen properly handled.
<i>Shigella sp.</i>	12h - 6d; usually 2-4d	Diarrhea (often bloody), fever, abdominal cramps. Duration of several days.	Stool	Para-Pak #0555	#3410	Ambient Temp.	Isolation of organism, same serotype/subtype, from ≥ 2 people.
			Food	Zip-lock bags; clean container	#3410	Refrigerated	Isolation of organism from implicated food, same serotype /subtype as stool isolates.
<i>Vibrio cholerae</i> O:1 and O:139	1 – 5d	Sudden onset, profuse watery diarrhea, often w/ mucus, abdominal pain, rapid dehydration.	Stool	Para-Pak #0555	#3410	Ambient Temp.	Isolation of toxigenic organism from ≥ 2 people.
			Food	Zip-lock bags; clean container	#3410	Refrigerated	Isolation of toxigenic organism from implicated food.
Etiological Agent	Incubation Period	Clinical Syndrome	Required Specimen	Collection Vial	Submission Form	How to Ship	Criteria for Outbreak Association
<i>Vibrio parahaemolyticus</i>	4 – 30 h	Diarrhea	Stool	Para-Pak #0555	#3410	Ambient Temp.	Isolation of Kanagawa-positive organism from ≥ 2 people.

			Food	Zip-lock bags; clean container	#3410	Refrigerated	Isolation of $\geq 10^5$ Kanagawa-positive organisms/g from implicated food.
<i>Yersinia enterocolitica</i>	1 – 10d, usually 4 – 6d	Diarrhea, abdominal pain (often severe) - may mimic appendicitis	Stool	Para-Pak #0555	#3410	Ambient Temp.	Isolation of pathogenic strain or serotype from ≥ 2 people.
			Food	Zip-lock bags; clean container	#3410	Refrigerated	Isolation of pathogenic strain or serotype from implicated food.

References:

1. Centers for Disease Control and Prevention. CDC Surveillance Summaries, March 17, 2000. MMWR 2000; 49(No. SS-1).
2. Bryan, F.L. 1982. *Diseases Transmitted by Foods. A Classification and Summary*, 2nd ed. Centers for Disease Control, Atlanta.
3. Bryan, F.L. 1995. Outbreaks of Food-Borne Disease, p. 209-226. In P. Murray et al. (ed.), *Manual of Clinical Microbiology*, 6th ed. ASM Press, Washington D.C.

THROAT CULTURE FOR GROUP A *STREPTOCOCCUS* **404-327-7990**

INTRODUCTION

The **Bacteriology Unit** accepts specimens from public health care providers for the detection of Group A *Streptococcus* and other beta-hemolytic streptococci from throat. Reference cultures will be accepted from public and private health care providers for identification and/or serogrouping of beta-hemolytic streptococci. Culture is the method used for isolation of the organism, and latex agglutination is used for serogrouping isolates.

SPECIMEN COLLECTION/LABELING/REQUISITION FORM

The sterile swab supplied in outfit #0560 should be used for throats. Outfits are available from Laboratory Services and Supply, 404-327-7920. The Group A *Streptococcus* outfit may be used for the isolation of any beta-hemolytic *Streptococcus* from throat.

Specimen collection

1. After adequately exposing and illuminating the pharynx, rub tonsils and pharynx with the swab provided. Be careful to obtain any exudates present and avoid the tongue and uvula tissues.
2. Place swab in the silica gel envelope, according to instructions supplied with the outfit by placing the silica gel package in the polyfoil kraft envelope.
3. Clearly write the patient's name or other unique identifier in the space provided.

Requisition form

Use requisition form #3410 and supply the following information:

1. Patient identifier (name or number).
2. Date of specimen collection.
3. Name and telephone number of clinician to contact.
4. Submitter's name and address.
5. Patient's race, sex, age, and address, if available.

The patient identifier on the form should match that on the specimen. **Unlabeled specimens will not be tested.**

SHIPMENT OF SPECIMENS

Place the polyfoil envelope and requisition form in the brown mailing envelope provided. Specimens may be sent to the Laboratory by first-class mail, common carrier, or courier. If there is any delay in shipment, hold specimens at room temperature.

REPORTING AND INTERPRETATION OF RESULTS

Culture results of beta-hemolytic *Streptococcus* will be completed within 2 days after receipt of the specimen. Any Group A *Streptococcus* detected will be reported. Other groups of beta-hemolytic *Streptococci* will be reported if they are the predominate organism present. The report will read "No Group A *Streptococcus* Found by Culture" for negatives; if no growth is detected this also will be noted. For positives, the report will read, "Group A *Streptococcus* Found by Culture," or Group B, C, F, G *Streptococcus* Found by Culture, Predominate Organism Present."

UNACCEPTABLE SPECIMENS

1. Specimens not submitted in the silica gel outfit (or other suitable transport outfit approved by the Bacteriology Unit).
2. Silica gel envelope not sealed.
3. Specimens received >4 weeks after collection.
4. No patient identifier on specimen.

SPECIAL BACTERIOLOGY 404-327-7990

INTRODUCTION

The **Bacteriology Unit** accepts reference cultures from public and private health care providers for the identification, confirmation, and/or serotyping of bacterial isolates. This includes a wide variety of aerobic, anaerobic, and facultative organisms isolated from clinical sources. Isolates should be submitted as pure cultures. Techniques used in the identification process include a combination of some or all of the following: cellular and colonial morphology, conventional biochemical tests, Cell Wall Fatty Acid Analysis (CWFAA), and DNA probes.

All isolates of *Neisseria meningitidis*, *Haemophilus influenzae* and *Listeria monocytogenes*, recovered from sterile sources should be forwarded to the Bacteriology Unit, either directly or through the Emerging Infections Program (EIP) site for grouping, typing or Pulsed-field gel electrophoresis (PFGE) testing.

Submission of Group A *Streptococcus*, Group B *Streptococcus* and *S. pneumoniae* isolates to GPLH is no longer required. Nevertheless, these organisms remain reportable to the Notifiable Diseases Unit. In addition, CDC no longer provides typing and/or molecular characterization for them, unless by prior arrangement (please call 404-327-7997 for details).

Antimicrobial susceptibilities are not performed except as an aid to identification. Such requests can be forwarded to the CDC through the Bacteriology Unit by special arrangement. Any unidentified organisms isolated from sterile sources will also be sent to the CDC for further studies, upon request.

SPECIMEN COLLECTION/LABELING/REQUISITION FORM

A. Pure cultures for **anaerobic** identification from aspirated pus, transtracheal and lung aspirates, body fluids, tissue (biopsy, surgical, and autopsy), suprapubic aspirate of bladder urine, and sulfur granules from suspected cases of actinomycosis are acceptable. Clearly label referred cultures with the patient's name or other unique identifier.

B. Pure culture for **aerobic** identification from autopsy material, tissues, urine, respiratory and urogenital tract secretions, wounds, abscesses, spinal fluid, and blood are acceptable. Clearly label referred cultures with the patient's name or other unique identifier.

See Table 1 for aerobic and anaerobic organisms requiring special handling. In all of the cases noted in Table 1, notify the Bacteriology Unit prior to shipping.

Requisition Form:

Complete form #3410 when sending referred cultures for aerobic or anaerobic identification, confirmation, and/or serotyping. Include the following information:

1. Unique patient identifier (name or number).
2. Agent suspected or test requested.
3. Submitter's name and address.
4. Name and telephone number of contact person.
5. Date of specimen collection.
6. Source of specimen.
7. Date of transplant (if applicable).
8. Brief clinical history.
9. Patient's race, sex, age, and occupation, if available.

The patient identifier (name or number) indicated on the requisition form should match that written on the specimen or culture. **Unlabeled specimens or cultures will not be tested.**

SHIPMENT OF SPECIMENS AND REFERRED CULTURES

Note: Always submit the specimen/culture according to current DOT and IATA guidelines

Submit referred cultures as pure cultures in the appropriate medium. For **anaerobic** identification, chopped meat broth, thioglycollate broth, motility test medium (inoculate near the bottom of the tube), or anaerobic blood culture bottles are suitable choices and are commercially available. For **aerobic** identification, slants of solid medium appropriate for growth of the organism in question are recommended and are commercially available.

Use screw-capped tubes and tighten securely. Place a strip of parafilm around the cap of the tube to help prevent loosening of the cap in transit. In all cases, submit young, actively growing subcultures to ensure viability. Wrap the sides and bottom of culture tubes in kyfax packing material or paper towels to prevent breakage. Use outfit #0505, "Culture Referral Isolation for Identification," available from Laboratory Services and Supply, 404-327-7920, and follow these instructions:

1. Place culture wrapped in packing material in the biohazard bag provided.
2. Put the requisition form in the pouch in the front of the bag.
3. Place the biohazard bag in the orange-labeled cardboard mailing container (Decatur address) and secure the lid with tape.
4. Place the return address on the outside of the container.
5. Send by first class mail, common carrier, or courier.

Avoid submitting cultures in petri dishes, but if necessary, cushion them with absorbent material and securely package in leak-proof containers. Seal culture plates for anaerobic identification in an anaerobic "bag" system before packaging.

REPORTING AND INTERPRETATION OF RESULTS

Organisms are identified to the genus and species level when there is agreement with all appropriate data, such as cultural, morphologic, biochemical, and cell wall fatty acid analysis. Genus and species designations are current with new bacterial nomenclature. The identification of the organism will be reported. When serotyping is requested and available, the appropriate serotype or serogroup will be reported. Unidentified organisms isolated from sterile sources may be forwarded to CDC upon request, but those isolated from nonsterile sites must be accompanied by a detailed clinical history to justify sending to CDC and requires prior approval from CDC.

Culture results for the identification of aerobic, facultative, and anaerobic bacteria are usually completed within seven working days, but may require up to fifteen working days, depending upon the organism. Mixed cultures, fastidious, slow-growing, or nutritionally-deficient bacteria may occasionally require additional time beyond the fifteen working days for a complete identification. Results of serotyping are usually completed as follows: *Neisseria meningitidis* (1-3 working days) and *Haemophilus influenzae* (2-3 working days). Cultures referred to the CDC may require up to six months for a final identification.

UNACCEPTABLE SPECIMENS

1. Cultures broken in transit.
2. Non-viable cultures: all cultures received will be subcultured upon receipt. Every attempt will be made to obtain viable growth. If no growth occurs, the submitter will be notified, a report will be issued and another subculture will be requested, if available.
3. Cultures overgrown with contaminants.
4. No patient identifier on the specimen or culture.
5. Specimens improperly collected or submitted (see section on specimen collection and shipment).

Table 1. Organisms Requiring Special Handling

Organism	Special Instructions and Requirements
<i>Clostridium botulinum</i>	See section on Foodborne Illness for further details. For wound infections, submit material from wound, refrigerated with cold pack, in an anaerobic environment system. Send suspected isolate in chopped meat broth or motility test medium (inoculate near bottom of tube) in culture referral outfit (#0505) at ambient temperature. Specimens and isolates will be forwarded to CDC for isolation, confirmation, and/or toxin testing.
<i>Clostridium perfringens</i>	See Foodborne Outbreaks Section for further details. Submit material from site of infection, refrigerated with cold pack, in an anaerobic environment system; or send suspected isolate in chopped meat broth or motility test medium (inoculate near bottom of tube) in culture referral outfit (#0505) at ambient temperature.
<i>Corynebacterium diphtheriae</i>	Collect throat or skin lesion swabs (use Strep outfit #0560 or place on Loeffler's agar slant, available commercially. Subculture suspected isolates to Loeffler or cysteine tellurite blood agar slants. Toxigenicity testing will be performed at CDC. Send suspected isolates in culture referral outfit (#0505). Contact the Bacteriology Lab prior submission as special media is required for testing.
<i>Haemophilus ducreyi</i>	Collect specimens from lesions or inguinal bubo. Inoculate onto enriched chocolate agar with vancomycin. Incubate 33-35° C in 5-10% CO ₂ in water-saturated atmosphere. Referred cultures only will be accepted.
<i>Haemophilus influenzae</i>	For serotyping and/or culture confirmation, submit 18-24 hr. subculture on chocolate slant, available commercially. Submit through the EIP site, by mail, or by courier.
<i>Leptospira</i>	Collect blood during first 10 days of illness, and urine (3 consecutive samples) from second to fourth week of illness. Obtain instructions for inoculation from the Bacteriology Unit prior to specimen collection. Ellinhausen's medium is available commercially. Immediately inoculate tubes and submit at room temperature to the Bacteriology Unit. Specimens and isolates will be forwarded to CDC for isolation and confirmation.
<i>Neisseria gonorrhoeae</i>	For culture confirmation, submit 18-24 hr. subculture on chocolate slant or emulsify fresh growth in trypticase soy broth with 20% glycerol and freeze. Submit preferably by overnight mail (slant) or frozen on dry ice (broth). See separate section for GC culture screening.
<i>Neisseria meningitidis</i>	For serogrouping and/or culture confirmation, submit 18-24 hr. subculture on chocolate slant, available commercially. Submit through the EIP site, by overnight mail, or by courier.
<i>Staphylococcus aureus</i>	Submit coagulase positive isolates from outbreaks or multiple isolates from different sources in same patient. Submit on nutrient or heart infusion agar slants.

INFECTIOUS DISEASE SEROLOGY 404-327-7970

INTRODUCTION

The **Microbial Immunology Unit** performs infectious disease serology. Serology for HIV (Human Immunodeficiency Virus), is performed in the Virology Unit. Various serologic procedures are performed for a variety of bacterial, parasitic, and viral agents. Hepatitis serology is performed for county health departments, and Georgia Department of Public Health agencies, only. Tests not performed in the Public Health Laboratory are forwarded to CDC, if they provide the requested testing.

SPECIMEN COLLECTION/LABELING/REQUISITION FORM

Collection Using Universal Precautions, and standard venipuncture technique, collect approximately six milliliters of whole blood (for serum) in a red top tube (no additive), labeled with patient's identifier (name, first and last, or number), date, and name of the submitter. Use a marker that will not fade, smear, or run during transportation. Use proper size needle (large enough to prevent hemolysis of the red blood cells) for the vein location and age of the patient. Allow blood specimen to clot, at least 30 minutes undisturbed, at room temperature, and transport, or place in the refrigerator for transporting. Collect blood specimens in, or transfer them to, non-breakable, leak resistant tubes. Specimens should be transported as soon as possible, do not hold over 7 days. Specimens over 14 days old are unacceptable. Many of the procedures we perform are not approved for use with plasma. Therefore, please submit only serum or whole blood without anticoagulants, not plasma.

Collect cerebrospinal fluids (CSF) according to proper hospital procedure. CSF contaminated with blood or grossly contaminated with bacteria is unacceptable.

Labeling All specimens must be labeled with patient identification (name or number), in acceptable testing condition, and accompanied by a completed requisition form. If the form is not specific for one test or a set of tests, the specific testing requested must be handwritten in the proper area, e.g., "viral serology" is not acceptable, the specific agent, e.g., "CMV", must be clearly requested. Failure to provide proper patient information may result in testing delays.

Requisition Form Use Form #3432 for all tests performed by the Microbial Immunology Unit. There is a single requisition form for all serological tests performed, including the RPR and EIA tests for syphilis, Hepatitis B (DCH facilities and county health departments only), and other Serology. Completely fill out the form and include the following information:

1. Unique patient identifier (name or number).
2. **TEST(S) REQUESTED (Please check only the corresponding box for test(s) requested).**

3. Date specimen collected.
4. Submitter's name, address, and code number, where applicable.
5. For hepatitis the reason for testing, e.g., routine, or prenatal.
6. Any information the submitter needs for patient identification, e.g., chart number, address.
7. The date of onset of illness, if applicable.
8. Race, sex, and age, where applicable, e.g., hepatitis testing.

SHIPMENT OF SPECIMENS

Use outfit #0500, available from Laboratory Services and Supply, 404-327-7920, and follow the specific instructions below. Specimens may be mailed or delivered to the laboratory by courier.

Shipping Instructions for USPS and Couriers:

Place the tubes of blood in protective, leak resistant, double-walled containers, e.g., aluminum and cardboard box, for transport. Wrap the requisition form around the inner (aluminum) can, secure with a rubber band and place in the outer container. If a screw-cap outer container is used, the screw-cap must be secured with tape or the Postal Service will return it for taping. Up to 50 milliliters of blood may be transported in one package (U.S. postal regulations). Therefore, an individual tube of blood may be placed in the metal can, with the requisition form secured to the outside by a rubber band, and several aluminum cans placed in one cardboard box for transporting.

Shipping Instructions for Courier Services Only:

Tubes of blood may be placed in leak proof biohazard bags. Wrap brown absorbent material around the tube, then secure with a rubber band. Place the requisition form in the sleeve located on the outside of the bag.

REPORTING AND INTERPRETATION OF RESULTS

Table 1 (pages IV-61 through IV-63) summarizes the interpretation of results for all serological tests performed in the Microbial Immunology Unit. Table 2 (page IV-64) gives the turnaround times for all of the same serological tests. The turnaround time after receipt of the specimen depends on the testing methodology and the frequency of testing. The frequency of testing depends on the demand for a specific test. Several tests are performed daily, while others are performed weekly. The turn-around time for specimens referred to CDC depends on CDC's schedule, which varies from laboratory to laboratory.

UNACCEPTABLE SPECIMENS

1. Spinal fluid obviously contaminated with bacteria or blood;
2. All specimens:

Not approved for testing by the indicated method, e.g., plasma for RPR.
Grossly hemolyzed, lipemic, turbid, or contaminated.
Over 14 days old.
Broken in transit.
Insufficient quantity for testing.
No identification on specimen.
Name on tube and form does not match.

The submitter will be notified of all rejected specimens. Most serologic services are available to both the public and private sectors. However, hepatitis B testing is limited to the public health care providers, and not available to the private providers.

Test Result Interpretation ¹						
Agent	Test	Method	Negative	Positive ²	Diagnostic	Presumptive ³
California Encephalitis	IgG ¹ IgM ¹	IFA ¹	IgG <1:16 IgM <1:16	IgG ≥1:16 IgM ≥ 1:16	Four-fold rise in titer between paired sera ⁴	≥ 1:16
Cytomegalovirus (CMV)	IgG IgM	EIA ¹	No IgG Detected No IgM Detected	IgG Detected IgM Detected	IgM detected and/or significant rise in titer between paired sera ^{4,5}	IgM Equivocal
Eastern Equine Encephalitis	IgG IgM	IFA	IgG <1:16 IgM <1:16	IgG ≥1:16 IgM ≥ 1:16	Four-fold rise in titer between paired sera ⁴	≥ 1:16
Hepatitis A Total	IgG	EIA	Negative	Positive		
	IgM	EIA	Negative	Positive	Presumptive evidence of IgM antibodies to HAV	
Hepatitis C		EIA	Negative	Positive	s/co>=10; samples confirm >=95% positive. <10; confirm with Riba positive 2 or more 1+ bands.	
Hepatitis B Virus (HBV) ⁶	HBs ¹	EIA	Negative	Positive	Positive HBs	None
	Hbe ¹		Negative	Positive		
	Anti-HBs ¹		Negative	Positive		
	Anti-HBc ¹		Negative	Positive		
Herpes Simplex Virus (HSV)	Type 1	EIA	No IgG Detected	IgG Detected	Significant difference between paired sera ^{4,5}	None
	Type 2					

Test Result Interpretation ¹						
Agent	Test	Method	Negative	Positive ²	Diagnostic	Presumptive ³
Mumps	IgG	EIA	No IgG Detected	IgG Detected	Significant difference between paired sera ^{4,5}	None
Murine typhus	IgG	IFA	<1:16	≥ 1:16	Four-fold rise in titer between paired sera ⁴	≥ 1:128
Rocky Mountain Spotted Fever (RMSF)	IgG	IFA	< 1:16	≥ 1:16	Four-fold rise in titer between paired sera ⁴	≥ 1:128
Rubeola (Measles)	IgG	EIA	No IgG Detected	IgG Detected	IgM detected and/or significant increase between paired sera ^{4,5}	IgM Equivocal
	IgM		No IgM Detected	IgM Detected		
Rubella (German Measles)	IgG	EIA	No IgG Detected	IgG Detected	IgM detected and/or significant increase between paired sera ^{4,5}	Not Applicable
	IgM		No IgM Detected	IgM Detected		
St. Louis Encephalitis	IgG IgM	IFA	IgG <1:16 IgM <1:16	IgG ≥1:16 IgM ≥ 1:16	Four-fold rise in titer between paired sera ⁴	≥ 1:16
Syphilis	RPR ¹	Agg ¹ .	Non-Reactive	Reactive ≥ 1:1	RPR and EIA Reactive	Reactive RPR and Equivocal EIA
	Confirmatory EIA	EIA	Non-Reactive	Reactive		
	FTA	IFA	Non-Reactive	Reactive	RPR, EIA, FTA	Equivocal EIA
Syphilis	VDRL (CSF ¹)	Agg ¹	Non-Reactive	Reactive	VDRL Reactive	Reactive VDRL
Toxoplas-	IgG	EIA	No IgG Detected	IgG Detected	IgM detected and/or significant increase	IgM Equivocal

mosis	IgM		No IgM Detected	IgM Detected	between paired sera ^{4,5}	
Varicella Zoster		EIA	No IgG Detected	IgG Detected	Significant increase between paired sera ^{4,5}	None
Agent	Test	Method	Negative	Positive²	Diagnostic	Presumptive³
West Nile Virus	IgG IgM	EIA	No IgG Detected No IgM Detected	IgG Detected IgM Detected	IgM detected and/or significant increase between paired sera ^{4,5}	IgM Equivocal
Western Equine Encephalitis	IgG IgM	IFA	IgG <1:16 IgM <1:16	IgG ≥ 1:16 IgM ≥ 1:16	Four-fold rise in titer between paired sera ⁴	≥ 1:16

Abbreviations (in alphabetical order)¹

Agg.	Agglutination
Anti-HBs	Hepatitis B surface antibody
CSF	Cerebrospinal Fluid
Anti-HBc	Hepatitis B core antibody
EIA	Enzyme Immunoassay
IgM	Immunoglobulin M
IgG	Immunoglobulin G
FTA	Fluorescent treponemal antibody
IFA	Indirect Fluorescent Antibody
RPR	Rapid Plasma Reagin
HBs	Hepatitis B surface antigen
VDRL	Venereal Disease Research Laboratory
HBe	Hepatitis B e antigen

²A positive result indicates natural or acquired immunity, especially for vaccine-preventable diseases

³Presumptive results identify a significant result for a single (not paired) serum, and need confirmation by clinical symptoms, recollection of specimen for retesting, or if applicable, submission of a convalescent specimen.

⁴Paired sera (acute and convalescent) dates of collection and date of onset of illness are needed for proper interpretation of results.

⁵A significant difference is determined by instructions given in individual enzyme immunoassay procedures, and may differ between manufacturers.

⁶Performed for county health departments and DCH facilities only.

**FOR ADDITIONAL INFORMATION REGARDING TEST INTERPRETATION
CALL THE MICROBIAL IMMUNOLOGY LABORATORY
AT (404) 327-7970**

Table 2. Turnaround Times

Agent	Turnaround Time
	Working Days ¹
California Encephalitis IgG ² /IgM ²	10-12 days
Cytomegalovirus IgG/IgM	7-14 days
Eastern Equine Encephalitis IgG/IgM	10-12 days
Hepatitis A	5-7 days
Hepatitis C	5-7 days
Hepatitis C (Confirmation)	7-14 days
Hepatitis B Diagnosis	
Surface antigen	5-7 days
Total antibody	5-7 days
core antibody	5-7 days
e-antigen (performed on surface antigen- (positive specimens only)	7-14 days
(positive HBs specimens)	7-14 days
Herpes simplex 1 & 2	7-14 days
Mumps	7-14 days
Murine typhus	7-14 days
Rocky Mountain Spotted Fever	5-7 days
<u>(Misc. Serology)</u>	
Rubella IgG / IgM	5-7 days
Rubeola IgG/IgM	7-14 days
St. Louis Encephalitis IgG/IgM	10-12 days

Syphilis

RPR ² Non-Reactive	3-5 days
RPR Reactive	5-7 days
Confirmatory Non-Reactive	5-7 days
Confirmatory Reactive	5-7 days
FTA ² Non-Reactive	5-7 days
FTA Reactive	5-7 days
FTA Repeats	7-14 days
Toxoplasmosis IgG /IgM	7-14 days
West Nile Virus IgG/IgM	10-12 days
Western Equine Encephalitis IgG/IgM	10-12 days
Varicella zoster	7-14 days
VDRL ² (CSF ² only)	5-7 days

¹ Special arrangements may be made in the case of an emergency.

² Abbreviations: IgG, Immunoglobulin; G; IgM, Immunoglobulin M; EIA, Enzyme Immunoassay; RPR, Rapid Plasma Reagin; FTA, Fluorescent treponemal antibody; VDRL, Veneral Disease Research Laboratory; CSF, Cerebrospinal Fluid.

ISOLATION AND IDENTIFICATION OF MYCOBACTERIA 404-327-7945

INTRODUCTION

The **Mycobacteriology Unit** accepts specimens for the isolation and identification of *Mycobacterium tuberculosis complex* and other mycobacteria which may cause disease under certain circumstances. Susceptibility testing is performed on all isolates of the *Mycobacterium tuberculosis complex*. The services of this laboratory are available to both public and private health care providers.

SPECIMEN COLLECTION/LABELLING/REQUISITION FORM

A. Clinical Specimens

Although the most common specimen received in the TB Unit is sputum, the GPLH accepts specimens of various other types: bronchial washings, gastric lavage, urine (voided early morning or catheterized), pus (aspirated or on swab), tissue (biopsied lymph node or biopsied portions of lung or other organs), bone, stool, and body fluids (cerebrospinal, pleural, pericardial, or joint).

If a case has not been diagnosed as tuberculosis, a series of three early morning sputum specimens should be collected on successive days and transported to the laboratory as soon as possible. Specimen collection outfits (Item #0550) are available at no charge from the Laboratory Supply Office (404-327-7928). The patient's name must be clearly printed on the 50 ml specimen collection tube.

Mycobacteriology Submission Form 3412 should be filled out completely, giving the following information: patient's name (please print and be sure it is evident which is the patient's first and last name), address including county of residence, race, sex, date of birth, type of specimen, date collected, and name and address of submitter. All information requested on the form should be provided on all request forms, since the laboratory information system creates a unique tracking number for each patient based on these demographics.

B. Isolates or Cultures for Identification and/or Susceptibility Testing

Isolates/cultures for identification and/or drug susceptibility testing should be submitted on solid media or in liquid media after good growth has occurred. The patient's name must be clearly printed on the media. Mycobacteriology Submission Form 3412, appropriately marked for either "Culture for identification" or "Culture for susceptibility testing," should be filled out completely and legibly and submitted with each isolate. If an isolate submitted for identification is identified as *M. tuberculosis complex*, susceptibilities will automatically be performed.

SHIPMENT

A. Clinical Specimens

GPHL specimen collection/shipping outfits (Category B) consist of a rigid mailing container that holds a specimen collection tube (50 ml plastic screw-cap tube), a biohazard bag, a square of absorbent material, and a white Tyvek envelope. The specimen collection tube is placed in the biohazard bag along with the square of absorbent material. The bag is sealed and placed inside the white Tyvek envelope. The white envelope is sealed and placed inside the outer rigid container. A Mycobacteriology Submission Form 3412, marked for "Clinical Specimen for smear, culture & susceptibility," is also placed in the rigid container. The lid of the rigid container is screwed on tightly and secured with tape. The specimen should be mailed or delivered to the laboratory as soon as possible after collection to avoid overgrowth of unwanted bacteria. If mailing is delayed overnight, the specimen should be refrigerated.

B. Isolates or Cultures for Identification and/or Susceptibility Testing

If isolates or cultures are submitted in glass tubes or bottles, they must be wrapped with absorbent cushioning material before being placed inside any shipper component.

MGIT tubes showing growth of acid-fast bacilli may be submitted for identification and drug susceptibility testing. The MGIT tube must be placed inside a plastic 50 ml conical tube, capped securely and then packaged according to federal shipping regulations. In case of breakage of the MGIT tube, the broth will still be contained inside the 50 ml conical tube, provided all caps have been securely tightened.

Category B Shippers are available from the Laboratory Supply Office (404-327-7928). Isolates which have been identified as *Mycobacterium tuberculosis complex* must be shipped as Category A. GPHL does not supply Category A shippers.

REPORTING/INTERPRETATION OF TEST RESULTS

Nucleic Acid Amplification Test for *M. tuberculosis complex* (MTD Test)

The MTD Test is a nucleic acid probe test for the detection of *Mycobacterium tuberculosis complex* rRNA in concentrated specimen sediments prepared from respiratory sources. This test is intended for use only with specimens from patients showing signs and symptoms consistent with active pulmonary tuberculosis, and who have not received antituberculosis drug therapy in the last 12 months; or if drug therapy has been started, the patient has received less than 7 days of such therapy. The MTD Test must be performed in conjunction with mycobacterial culture. MTD Test results are called to the submitters.

Microscopic Examination

Fluorochrome stained slides are prepared from all clinical specimens and are examined using a fluorescent microscope for the presence of bacilli showing a yellow-green fluorescence. Results are reported as “No AFB found”; “+/-“ (1-3 AFB/slide); “1+” (4-36 AFB/100 fields); “2+” (4-36 AFB/10 fields); “3+” (4-36 AFB/field); or “4+” (>36 AFB/field). Submitters are notified by telephone of a positive smear on a new patient.

Culture

A tube of solid medium (Lowenstein-Jensen agar) and a tube of liquid medium (BACTEC MGIT broth) are inoculated from each clinical specimen. These cultures are incubated for six weeks before a report of “No Mycobacteria Isolated” is issued. If growth in either medium occurs at any time during the six weeks, identification procedures begin. Lowenstein-Jensen agar slants are examined weekly for signs of growth and morphology.

Identification

As soon as growth has been detected, the culture is stained to determine if acid-fast organisms are present. Sometimes the growth is made up of other bacteria which are not acid-fast, and the specimen is then reported as “Contaminated.” Cultures showing acid-fast organisms are tested by High Performance Liquid Chromatography (HPLC) to determine the identification of the organism.

Drug Susceptibility Testing

Drug susceptibility testing is performed only on *M. tuberculosis* complex organisms. Each new isolate is automatically tested at the time of identification, and testing is repeated every three months if the organism is still being isolated from culture. Results are usually available within 10-14 days after identification of the organism. Susceptibility testing is performed using the BACTEC 960 MGIT procedure with a panel of three drugs (isoniazid, rifampin, and ethambutol) initially. If any of the drugs show resistance, the test is repeated, and streptomycin is added to the panel. Isolates which are resistant to one or more drugs are sent to the Centers for Disease Control and Prevention for testing with an expanded drug panel. The CDC drug testing usually requires approximately 4-6 weeks. Reports on isolates showing drug resistance for the first time are called to submitters as soon as results are available.

Results for all mycobacterial testing are reported electronically to the submitter as soon as they are available. In addition, copies of reports for positive specimens (i.e. positive AFB smears, positive TB identification, and susceptibilities) are sent to the Georgia TB Control Program. Test results will only be released to the submitter of the specimen.

UNACCEPTABLE SPECIMENS

1. No patient identifier on specimen or culture.
2. No specimen received (empty collection tube).
3. Specimen leaked or was damaged or crushed in transit.
4. Wrong type of specimen submitted.
5. Patient identifier on specimen does not match that on form.

INTESTINAL PARASITES 404-327-7961/7963

INTRODUCTION

The **Parasitology Unit** accepts fecal specimens to be examined for human parasites from all county health departments. Reference specimens for confirmation of parasite identity or further identification are accepted from all laboratories and health care providers. Diagnosis of most intestinal parasitic infections is dependent upon finding the eggs or larvae of helminthes and trophozoites or cysts of protozoa in feces by microscopic examination. If particular infections are suspected, please alert our laboratory to your suspicions.

Molecular methods are more sensitive than microscopy and may be required for the differentiation of morphologically identical *Entamoeba histolytica* and the non-pathogenic *Entamoeba dispar* as well as the different species of malaria parasites, Cyclospora and Cryptosporidium. In such instances, a fresh sample of stool preserved in potassium dichromate or unpreserved frozen specimen is preferred.

SPECIMEN COLLECTION/LABELING/REQUISITION FORM

All specimen containers should be properly labeled with the patient's name and date collected. It will be marked unsatisfactory and discarded if the patient's name is not on this container. The submission/report form should be filled out completely. When outfits are picked up or shipped in bulk the instruction sheets and submission form may be placed in the box with the specimen containers.

- A. **IP (formalin)/LV-PVA outfit:** Only specimens preserved in 5% or 10% formalin and LV-PVA should be submitted. Follow the instructions provided in the kit for collecting the stool sample. The kit systems are packaged in ziplock bags with illustrated, multilingual patient instructions to assist in safe and sanitary specimen collection by personnel and/or patients. Remove vials from ziplock bags and discard the bag. Do not contaminate specimen with dirt, urine, or paper. The ingestion of antidiarrheal compounds, antacids, bismuth, and mineral oils may also interfere with the diagnosis of parasites. Complete two **Form 3414 per patient** (1 for IP and 1 for PVA); fold completed forms in half and place in outside pouch of the biohazard bag. Three kits (per patient), collected on consecutive days that you have a bowel movement, should be sent for testing (e.g., one collected on Monday, one collected on Tuesday, and one collected on Thursday). We ask you to send in both the IP (5% or 10% formalin) and the LV-PVA because we see different organisms when these preservatives are used. If both containers are sent, we can diagnose *Giardia lamblia*, *Entamoeba histolytica*, *Dientamoeba fragilis*, *Cryptosporidium sp.*, *Cyclospora*, *Microsporidium*, and all the helminth eggs and larvae. The LV-PVA container is a must for *Dientamoeba fragilis* and *Entamoeba histolytica*. Please make sure the patient's race/nationality and foreign travel is marked on the submission form. Please write the patient's last name then the first name on the submission form to avoid confusing the two names for data entry and reports and records. Intestinal worms shed eggs intermittently and in varying numbers,

and certain protozoan cysts are shed in "showers"; therefore, we request three consecutive day specimens on each patient. An individual's report may be positive one day and negative the next day due to this shedding. If you only return 1 kit (IP/LV-PVA), we only have a 30% chance of recovering the organism, 90% chance if 3 kits are returned

- B. Potassium dichromate outfit for PCR:** Feces can be tested by molecular diagnostic procedures. Follow steps as in A above for collection of fecal specimen. Collect fecal specimens according to instructions. Do not contaminate specimen with dirt, urine, or paper. Place enough feces in container to bring the liquid up to the red line; mix thoroughly. Place in the biohazard transport bag; seal and complete appropriate form; fold completed form in half and place in outside pouch of the biohazard bag. Place biohazard transport bag in the fiberboard mailing container, secure lid and mail. Please make sure the patient's race/nationality and foreign travel is marked on the submission form. Please write the patient's last name then the first name on the submission form to avoid confusing the two names for data entry and reports and records.
- C. Pinworm Outfits:** Because the female *Enterobius vermicularis* (Pinworm) leaves the intestinal tract to lay her eggs around the anal opening, we have a special collection outfit to collect these eggs. The specimen needs to be immediately collected upon the patient's awakening in the morning since the eggs may be lost later during the day as a result of scratching, bowel movement or bathing. Collect the specimen following the printed instructions for Pinworm Slide Outfit. Do not let feces get on the tape or slide. Place slide inside the cardboard mailing container and close the top. Make sure patient's name is written on the cardboard mailing container label. Place container with slide inside the mailing envelope. Complete Form 3414.
- D. Whole Worms or Proglottids:** At times individuals will pass whole worms or small white segments (Proglottids) with feces; these should be separated from the feces and preserved in 70% alcohol. If the worm/Proglottids cannot be separated, please note on the submission form that worms or white segments were seen upon collection. If worms/proglottides were passed without feces, they should also be preserved in 70% alcohol. Place in a plastic or glass container to mail. Make sure the patient's name is on the container. Complete Form 3414.

SHIPMENT OF SPECIMENS

Specimens may be delivered to the laboratory by courier, shipped by common carrier, or mailed. Be sure specimens are placed in the correct mailing container; otherwise they may get lost in shipment or be delayed in delivery. The double-walled mailing containers for submitting fecal specimens are available from Laboratory Services and Supply (404-327-7920). The round fiberboard-mailing container is not necessary for specimens delivered by courier. Place several biohazard transport bags containing specimens in a box or a large envelope for courier delivery. Parasitologic specimens sent through the mail have to conform to postal regulations. If a screw-cap outer container is used to mail the specimen, the screw cap must be secured with tape or the Postal Service will return it for taping. It is

the responsibility of the sender to make sure any viable or preserved biological material conforms to the most recent postal regulations.

When unusually large numbers of specimens are anticipated (such as outbreak situations), the Parasitology Unit should be alerted so that preparations may be made (404-327-7961/7963).

REPORTING AND INTERPRETATION OF RESULTS

The turn around time for the diagnosis of intestinal parasitic infections is 24-72 hrs depending on the volume of specimens received. For all other miscellaneous specimens the turn around time is 12-24 hrs. Results of specimens sent to CDC for identification or confirmation may take up to two weeks.

If there is an emergency situation, the specimen will be considered stat and immediately processed, and reported. Please notify us when you have an emergency.

The Laboratory Findings section of the report form contains the results of our examination. The extent of testing informs the submitter the actual test that we performed. If the report indicates the presence of pathogenic parasites, the patient needs to be treated. Non-pathogenic parasites are also reported, but their presence indicates hand to mouth fecal contamination. Unsatisfactory results indicate the specimen was compromised in a way that might render the test results invalid. Below is the list of unsatisfactory specimen submissions.

Our new MLAB-EE laboratory data management system allows submitters with internet access to view and print results online. Laboratory results become available to the submitters upon verification by the testing personnel.

UNACCEPTABLE SPECIMENS

Formalin and LV/PVA Specimens

1. No patient identifier on specimen container.
2. No specimen (submission form only) received.
3. No feces (container only) received.
4. No preservative in container.
5. Severely leaked in transit and are considered a hazard to open.
6. Urine submitted instead of feces.
7. Multiple specimens collected on the same day (one specimen will be tested).
8. Insufficient material to examine.
9. Specimens submitted in Para-Pak C&S Outfit (for enteric bacteria).
10. Inappropriate specimen/collection outfit for test requested.
11. Refractile material interfering with diagnosis.
12. PVA solution jelled.
13. Frosted tape used for pinworm collection.
14. Pinworm tape stuck to applicator paddle.
15. Feces or powder on pinworm slide or tape.
16. Slide broken in transit.

BLOOD AND TISSUE PARASITES

404-327-7961/7963

INTRODUCTION

The **Parasitology Unit** examines specimens for blood and tissue parasites. Due to the ease of foreign travel and the influx of immigrants and refugees, it is very important to fill out patient history on the form. Some “exotic” parasites enter this country by travelers who visit or those who come from a foreign country. We need to know any recent health problems, symptoms, travel history, place of residence, typical and unusual food preferences and environmental exposure. Many parasites have well-defined geographical ranges and unless an individual has traveled or resided within an endemic or enzootic zone, infection with the parasite is unlikely. There are other situations individuals may become infected with blood parasites. Ones to consider are - blood transfusions, use of hypodermic needles contaminated by prior use, possibly congenital infection and transmission in the United States by indigenous mosquitoes that acquired the parasites from imported infections. Food fads have introduced new parasites into the human population. Some of the blood and tissue parasites that we examine are malaria, microfilaria, *Trypanosoma*, *Isospora*, and *Babesia*. Molecular methods are used to confirm a diagnosis. The advantages of this method over the traditional blood film examination include (1) ability to detect lower parasitemia (2) confirmation of false-negative results by microscopy as true positive and (3) identification of organisms to the species level even in mixed infections. Parasites in human tissue are also examined in our lab, but are often sent to a reference lab for confirmation.

SPECIMEN COLLECTION/LABELING/REQUISITION FORM

Presently, the microscopic examination of blood parasites in stained blood smears offers the most accurate and reliable method of laboratory diagnosis of malaria and other blood parasites. The morphologic features of protozoan parasites such as malarias, trypanosomas, and babesias are demonstrated in these smears. In order to diagnose the malaria parasites, **thick and thin** blood smears are required. The most favorable time for collecting blood for making the smears would be halfway between the chill/fever. Prepare three thick and three thin smears **immediately** from capillary blood (fingerstick) or **within one hour** from venous blood, using EDTA as an anticoagulant. If possible the smear should be stained with Giemsa stain. If you do not have the capability of staining, send it as soon as possible. Morphological changes can occur within one hour of taking the blood. These changes make it very difficult to diagnose the species. An EDTA tube of blood (2 ml.) should be sent with smears. If necessary the EDTA blood is used for DNA extraction for PCR procedure. The Unit will request that you submit original slides (one that is stained and at least one thin and one thick unstained slide) as well as one tube of EDTA preserved blood.

Blood Parasites

A. Thick smear preparation

1. Cleanse the finger-tip with alcohol and allow to dry thoroughly.
2. Puncture the skin deeply enough to allow the blood to well up in a large drop. Do not squeeze the finger; this will dilute the blood with tissue fluid.
3. Touch the **clean** slide to the crest of the drop of blood, or place a drop of venous blood using a Pasteur pipette, in the center of the slide.
4. With a wooden applicator stick, using a circular motion spread the blood to the size of a dime. The thick smear should just be thick enough so that newspaper print can barely be read through it. Do not place a large a drop of blood on the slide. Too much blood will cause it to flake off the slide after drying.
5. Allow the smear to air dry in a flat, horizontal position so that the blood will be evenly distributed. Protect from dust and insects (roaches enjoy eating the blood on the slide). Do not fix with alcohol.
6. Write patient identifier number or name, and the date the smear was made on the frosted-end portion of the slide. Place in the cardboard mailing container with completed Form 3415.

B. Thin Smear Preparation

1. Cleanse the fingertip with alcohol and allow to dry thoroughly.
2. Puncture the skin deeply enough to allow the blood to well up in a large drop. Do not squeeze the finger; this will dilute the blood with tissue fluid.
3. Touch the **clean** slide to the crest of the drop of blood, or place a drop of venous blood using a Pasteur pipette, at the frosted end of the slide.
4. Hold a second spreader slide at a 40-45-degree angle and touch edge of blood, allow blood to spread by capillary action along the edge of the slide.
5. Rapidly and smoothly push the spreader slide to the opposite end of the slide while pulling the blood behind it. The smear should have a feathered edge.
6. Air dry at room temperature. Protect from dust and insects. Remember that roaches will eat the blood on the slides.
7. Write patient identifier number or name, and the date the smear was made on the frosted end portion of the slide. Place in the cardboard mailing container with completed Form 3414.

Tissue Parasites

Histology preparations from biopsy material are prepared in the hospital or private laboratories and mailed to our lab for review, consultation, or confirmation. Most of these smears are H&E or Giemsa stained. Place patient identifier number or name, and the date the smear was made on the frosted end portion of the slide. Place in a cardboard mailing container with completed Form 3414.

SHIPMENT OF SPECIMENS

Specimens may be delivered to the laboratory by courier, shipped by common carrier, or mailed. Be sure specimens are placed in the correct mailing container; otherwise they may get lost in shipment or be delayed in delivery. The mailing containers for submitting blood and tissue specimens are available from Laboratory Services and Supply (404-327-7920). The round fiberboard-mailing container is not necessary for specimens delivered by courier. Place several biohazard transport bags containing specimens in a box or large envelope for courier delivery. Prepared slides can be packed in boxes, cardboard slide holders, or any other suitable container that will prevent damage or breakage. Specimens for Parasitology sent through the mail have to conform to postal regulations. If a screw-cap outer container is used to mail the specimen, the screw cap must be secured with tape or the Postal Service will return it. It is the responsibility of the sender to make sure any biological material preserved, or viable, conforms to the most recent postal regulations.

REPORTING AND INTERPRETATION OF RESULTS

Specimens are reported as quickly as possible. The specimen results may be reported the same day that they are received. We strive for a 12-24 hour turn around time. If there is an emergency situation, the specimen will be considered **stat** and immediately processed, and reported. Please notify the lab of any emergency. Malaria parasites are identified to species when possible. The four species reported are *Plasmodium malariae*, *Plasmodium ovale*, *Plasmodium vivax* and *Plasmodium falciparum*. At times when species diagnosis cannot be made from the smears a PCR test is performed if blood is available to determine the species. This may delay the reporting in some cases.

PCR results in some areas of parasitology are considered experimental and are provided for information only.

At times, we report a specimen unsatisfactory. Unsatisfactory results indicate the specimen was compromised in a way that might render the test results invalid. Below is the list of unsatisfactory specimen submissions.

UNACCEPTABLE SPECIMENS

Blood and Tissue Parasites

1. No patient identifier on specimen container.
2. No specimen received.
3. Smear is too thin or too small.
4. Smear damaged by flies or roaches.
5. Smear is improperly dried.
6. Thick smear is too thick (portion flaked off).
7. Thin smear not feathered at the end.
8. Grease on slide.
9. Smear improperly fixed.
10. Blood coagulated or dried up.

MISCELLANEOUS SPECIMENS

404-327-7961/7963

INTRODUCTION

The **Parasitology Unit** examines specimens collected from the environment as well as from humans for identification. There are many arthropods of medical importance that transmit diseases to man and other animals. Transmission may be mechanical or biological. Some of the common vectors that are sent in are flies, midges, lice, bedbugs, ticks, mites, chiggers, spiders and many more. Some specimens sent in for identification are pseudoparasites and artifacts. These need to be distinguished from the true parasite because of the physical and mental relief it has upon individuals. Parasites such as *Entamoeba histolytica*, *Giardia lamblia*, *Cryptosporidium parvum*, *Cyclospora cayetanensis* and *Microsporidia* spp. can cause waterborne and foodborne illness.

We have the capability of capturing images of parasites with our digital camera set up and transmitting them by Internet access to our submitters. This is used for reference and training purposes.

SPECIMEN COLLECTION/LABELING/SUBMISSION FORM

All specimen containers/slides should be properly labeled with the patient's name, date collected, and time of collection. It will be marked unsatisfactory and discarded if the patient's name is not on the container/slide. When outfits are picked up or shipped in bulk, the instruction sheet and the submission form may be placed in the box with the specimen containers. Complete Form 3414 for each specimen type submitted.

A. Skin scrapings: Gently scrape the skin with a scalpel. Collect the scrapping on a piece of paper and transfer into a bottle containing 70% alcohol.

B. Impression Smears: Smears from the aspirated material/tissue can be made and examined for parasites. To prepare the smear, press the material/tissue to the slide, air dry, and fix if needed.

C. Arthropods: Place in 70% alcohol or on a pad of tissue or loose cotton to avoid damaging fragile body structures. Do not place on cellophane tape. Place in plastic or glass container and mail.

D. Water Samples for Giardia/Cryptosporidium: *Water samples thought to be the source of human giardiasis or cryptosporidiosis will be accepted by special arrangement.* Please contact your local County Health Department's environmental specialist to collect the water and notify the laboratory before submitting the water sample. Three gallons of water need to be collected in sterile containers and sent to the lab. We would ask that one gallon be from the well-head, one from an inside faucet and one from an outdoor faucet. Before the water is accepted for testing, a positive test for

Giardia/Cryptosporidium infection must be confirmed in stool specimens of people who have used the water. Complete Form 3414, one for each location that water was collected.

E. Worms for Identification: When worms are found in diapers, on bed linens, in the toilet bowl, etc., they should be retrieved as carefully as possible and placed in a bottle containing 70% alcohol. Some of the worms have delicate structures used for identification and should be collected carefully. Make sure the patient's name is on the container.

SHIPMENT OF SPECIMENS

Specimens may be delivered to the laboratory by courier, shipped by common carrier, or mailed. To avoid delay, loss or damage during shipment specimens must be sent in appropriate container. It is the responsibility of the sender to ensure that any specimen sent through regular mail conforms to most recent postal regulations.

A. stool: Double-walled mailing containers are available from Lab Services and Supply (404-327-7920). The round fiberboard-mailing container is not necessary for specimens delivered by courier. Several specimens in separate biohazard transport bags may be placed in a box or a large envelope for courier delivery.

B. blood: The mailing containers for submitting blood and tissue specimens are available from Laboratory Services and Supply (404-327-7920). Prepared slides can be packed in boxes, cardboard slide holders, or any other suitable container that will prevent damage or breakage.

C. miscellaneous samples: If a screw-cap outer container is used to mail the specimen, the screw cap must be secured tightly with cellophane tape so as to avoid leakage. The Postal Service will not deliver leaky biological materials. Specimens are to be packaged in suitable boxes and sent to the lab by the sender's preferred method of shipment.

REPORTING AND INTERPRETATION OF RESULTS

Specimens are reported as quickly as possible. The specimen results may be reported the same day that they are received. We strive for a 12-24 hour turn around time. If there is an emergency situation, the specimen will be considered **stat** and immediately processed, and reported. Please notify the lab or any emergency. If we have to send it to a reference lab, it may take up to two weeks to get the results back. PCR results in some areas of parasitology are considered experimental and are provided for information only.

At times, we report a specimen as unsatisfactory. Unsatisfactory results indicate the specimen was compromised in a way that might render the test results invalid. Below is a list why the report may have been marked unsatisfactory.

UNACCEPTABLE SPECIMENS

Arthropods and Misc. Specimens

1. No patient identifier on specimen container/slide.
2. No specimen received.
3. Smear is too thin or too small.
4. Smear damaged by flies or roaches.
5. Smear is improperly dried.
6. Sample amount is insufficient for accurate diagnosis.
7. Identifying structure is missing.
8. No preservative in container.
9. Severely leaked in transit and considered a hazard to open.
10. Slide or container broken in transit.

TELE-DIAGNOSIS
404-327-7961/7963

The DPDx project

DPDx is a Web site developed and maintained by CDC's Division of Parasitic Diseases (DPD) that uses the power and speed of the Internet to assist laboratories and pathologists in parasite identification within and outside the United States. The Georgia Public Health Laboratory uses this technology to view images submitted for diagnosis by labs that are equipped to send images and was one of the first beneficiaries of the DPDx program. The diagnosis DPDx is also used to strengthen and expedite diagnosis of parasitic diseases via e-mail from minutes to hours. The consultation and assistance is provided free of charge by the DPDx staff. In addition to the diagnostic assistance, the DPDx also offers a Reference and Training function which enables you to browse through concise reviews of parasites and parasitic diseases, an image library and a review of recommended procedures for collecting, shipping, processing and examining biologic samples are available at <http://www.dpd.cdc.gov/dpdx>.

HUMAN IMMUNODEFICIENCY VIRUS SEROLOGY 404-327-7980

INTRODUCTION

Serologic assays are available for the detection of antibodies to the human immunodeficiency virus (Bio-Rad HIV-1/2 plus O). An enzyme immunoassay (EIA) is used as a screening test for antibodies to HIV. All reactive EIAs are repeated in duplicate to verify the initially reactive test result. All repeatedly reactive EIA tests (two or more reactive EIAs) are confirmed by the Western Blot (WB) Assay. If the HIV-1 WB is indeterminate or negative, HIV-2 antibody needs to be ruled out by confirming with CDC.

SPECIMEN COLLECTION/LABELING/REQUISITION FORM

Using Universal Precautions, and standard venipuncture technique collect approximately five milliliters of whole blood (or serum) in a red top tube (no additive), labeled with patient's identifier, date, and name of the submitter, use a marker that will not fade, smear, or run during transportation. Use proper size needle (large enough to prevent hemolysis of the red blood cells) for the vein location and age of the patient. Wrap the specimen in the absorbent packing paper provided to absorb any fluid from leaking or broken specimens (do not use rubber bands or tape). Place the wrapped specimen inside a biohazard bag. Place the completed Virology submission form (Form 3595, Rev. 1/2010) in the pouch of the biohazard bag. Allow blood specimen to clot and transport, or place in the refrigerator if not transporting at that time. Do not hold over seven days before transporting.

Requisition form

The Routine HIV-1 Ab Screen test request is included in the attached Virology submission form. Order the test by checking the box for routine HIV-1 Ab Screen. The Virology Submission Form #3595 rev. 11/22/2011 is found at the Georgia Public Health Laboratory website

<http://www.health.state.ga.us/pdfs/lab/manual/2011/appendices/Virology%20Form.pdf> and include the following:

1. Unique patient identifier (number not name).
2. Test(s) requested.
3. Date specimen collected.
4. Submitter's name, address, and code.
5. Any information submitter needs for patient identification, e.g., chart number, address, physician name, contact person and phone number.
6. Race, sex and age/DOB.

SHIPMENT OF SPECIMENS

Specimens may be mailed, shipped by common carrier, or delivered to the laboratory by courier. Place the biohazard bag with its contents inside the cardboard outer can. Place only one or two specimens in the cardboard can so that they can be removed without mishap. If a screw-cap mailer is shipped by the Postal Service, the cap must be secured by tape, or the Postal Service will return them for taping. Be sure to use the proper mailing label for the final specimen destination. Virology HIV outfits can be obtained from Laboratory Services and Supply, telephone number is (404) 327-7920.

REPORTING AND INTERPRETATION OF RESULTS

The following chart provides information regarding turn-around times (the time the specimen is received to the time the test is completed) and interpretations.

DESCRIPTION	TEST PROCEDURE	TURNAROUND TIME
HIV Antibody Screening	EIA	2-3 working days
HIV Antibody Confirmation	Western Blot	5-7 working days

When EIA results are negative, the test results will be reported as “Negative” and no further testing will be required.

The Georgia Public Health Laboratory uses the APHL/CDC criteria shown below for the interpretation of the Western Blot.

Interpretation	Criteria
<i>Negative</i>	The absence of any and all bands-not just viral bands.
Indeterminate	The presence of any viral or non-viral band or bands that fail to meet the positive criteria.
Positive	The presence of any two of the following bands: <ul style="list-style-type: none">• P24• Gp41• Gp120/gp160

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

The following recommendations are made regarding follow-up specimens:

1. If the result of a Western Blot is indeterminate, submit another specimen for testing within a month. If the second specimen is also indeterminate, the patient should be tested again at three and six months.
2. When a patient receives his/her first positive test result and has not identified a high risk behavior, collect a verification specimen at the time the patient is given the results of the first test.

Interpreting Routine HIV EIA Screening and WB Results

1. Specimens that are initially Non-Reactive by HIV-1/HIV-2 PLUS O EIA will be reported as negative for HIV-1 (M and O Groups) and HIV-2 antibodies.
2. Specimens that are initially Reactive by HIV-1/HIV-2 PLUS O EIA are retested in duplicate to validate the initial test results. If, after repeat testing, both duplicate specimens are Non-Reactive results will be reported as negative for HIV-1 (M and O Groups) and HIV-2 antibodies.
3. If, after repeat testing, either of the duplicates are Reactive the result is considered repeatedly reactive. Repeat-reactive specimens will be confirmed by HIV-1 WB. If HIV-1 Ab Screen and WB results are positive, the results will be reported as positive for HIV-1 (M and O Groups), and appropriate follow-up (e.g., referral for HIV-related medical evaluation/care) should be conducted with the patient.
4. If the HIV-1 WB is indeterminate or negative, the results will be reported as indeterminate or negative, and GPHL will submit a sample to CDC to test for HIV-2.

UNACCEPTABLE SPECIMENS

1. ID on form and specimen do not match (ID mismatch).
2. No ID on form.
3. No name on form or tube.
4. No ID on specimen.
5. Over 14 days old.
6. Broken in transit.
7. Insufficient quantity for testing (QNS).
8. No sample received with form.

HIV-1 VIRAL LOAD TESTING

404-327-7980

INTRODUCTION

The Versant HIV-1 RNA 3.0 Assay bDNA is an in-vitro signal amplification nucleic acid probe assay that is performed on plasma of confirmed HIV-1 positive patients to determine the level of infection and is not a diagnostic test for HIV infection. Currently, this test is offered to patients in the Ryan White Program in the management of individuals infected with HIV-1. Evaluation of HIV-1 RNA levels is valuable in the clinical assessment of disease progression prior to initiation of therapy, in monitoring the progression of infection and in assessing a response to anti-retroviral therapy. The Versant HIV-1 RNA 3.0 Assay uses the (bDNA) technology to achieve the quantitative detection of HIV-1 RNA. The results received in the Versant HIV-1 RNA 3.0 Assay test procedure are reported as HIV-1 RNA copies/ml and log copies/ml. The bDNA test has a linear range of 75-500,000 copies/ml.

SPECIMEN COLLECTION/LABELING/REQUISITION FORM

1. Follow all safety precautions when drawing blood from patients.
2. No special patient preparation is necessary before collection. Use proper size needle (large enough to prevent hemolysis of the red blood cells) for the vein location and age of the patient.
3. Please collect all specimens in a sterile collection tube with EDTA as the anticoagulant or in the Prepared Plasma Tube (PPT) provided by the Georgia Public Health Lab (GPHL).
4. Collect one PPT tube using standard venipuncture techniques. After collection of the whole blood in the PPT tube, gently invert the PPT tube 8-10 times.
5. Label the specimen with the patient's identification number, submitter code and the date.
6. Centrifuge the whole blood specimen for a minimum of 10 minutes at 1,100xg at room temperature within two hours of collection.
7. Place the specimen in a biohazard bag and store in a refrigerator at 4° C for no longer than 72 hours instead of freezing. If longer storage is required, the plasma should be transferred to a secondary tube prior to freezing with at least 2.5 ml plasma.

Requisition form

The Virology Submission Form #3595 rev. 11/22/2011 may be found at the Georgia Public Health Laboratory website

<http://www.health.state.ga.us/pdfs/lab/manual/2011/appendices/Virology%20Form.pdf> and include the following:

1. Unique patient identifier number.
2. Date specimen collected.
3. Submitter's name, address, and code, if applicable.
4. Check the "Viral Load bDNA" box.
5. Any information submitter needs for patient identification, e.g., chart number, or address.
6. The date of onset of illness, if applicable.
7. Race, ethnicity, sex and age.

Place the HIV form in the pouch of the biohazard bag along with the specimen so that it is ready for courier pickup.

SHIPMENT OF SPECIMENS

Specimens will be delivered to the laboratory by courier according to a schedule provided for your facility.

REPORTING AND INTERPRETATION OF RESULTS

The sensitivity of the VERSANT® HIV-1 RNA 3.0 Assay (bDNA) is 75 copies/ml with a linearity range of 75-500,000 copies/mL. The test results are reported as:

1. Samples with values less than 75 copies/ml are below the quantitative limit of the assay and are reported <75 copies/ml (<1.88 log copies/ml).
2. Samples with values equal to or greater than 75 copies/ml contain HIV-1 RNA in the quantity identified in the report.
3. Samples with values greater than 500,000 copies/ml are above the upper quantitative level of the assay and are reported >500,000 copies/ml (>5.70 log copies/ml).

Treatment evaluation

Viral load tests are used to help doctors determine which treatments are best for HIV positive individuals. This testing is also used to determine whether the chosen treatment is working effectively. If viral load drops more than three-fold during the treatment, the therapy is considered to be working. Healthcare providers should consider changing therapy if:

1. Viral load fails to drop at least three-fold.
2. Viral load does not fall below detectable levels (<75copies/mL) within five to seven months.
3. Viral load rises, or drops to undetectable levels and then rises, suggesting resistance to anti-HIV therapies.
4. CD4 count fails to rise.

5. Clinical deterioration exists.

Note: The aim of treatment is to reduce viral load to undetectable levels <75 copies/ml (<1.88 log copies/ml) for as long as possible. A result of 10,000 to 20,000 copies/ml and below is considered low, while 50,000 copies/ml and above is considered high.

UNACCEPTABLE SPECIMENS

1. No ID on form.
2. No name on form or tube.
3. No ID on specimen.
4. ID on specimen and form do not match.
5. Not approved for testing by the indicated method, e.g., blood for VDRL.
6. Serum instead of plasma.
7. Grossly hemolyzed, lipemic, turbid, or contaminated.
8. Broken in transit.
9. With an insufficient quantity for testing (QNS).

RABIES
404-327-7980

INTRODUCTION

The Virology Unit accepts animal heads submitted for testing rabies in accordance with the Georgia Rabies Animal Control Manual for testing at the Central Laboratory in Decatur. The goal is to report an accurate and reliable diagnosis so that rabies treatment can be initiated or terminated as necessary. The current methodology for rabies detection is the direct fluorescent antibody (DFA) test, which is the most accurate microscopic test available for the diagnosis of rabies. The key factor in obtaining quality results is the condition of the specimen received. Due to the importance of rabies diagnosis, the specimen must not be compromised.

SPECIMEN COLLECTION/LABELING/REQUISITION FORM

1. Only specimens received in good condition with at least two identifiable principal brain parts are approved for reporting test results. Brain parts must include the cerebellum and the brain stem.
2. In all cases, there must have been exposure of human or domestic animals to the suspected rabid animal.
3. The Virology Unit is not equipped to handle whole carcasses; therefore, only the **head** is accepted as a specimen, except bats and animals of similar size, which should be submitted whole. Whole carcasses of any larger animal will be returned to the sender for resubmission.
4. The following guidelines are recommended for the removal of animal heads: (Whenever possible, this procedure should be performed by a person who has received pre-exposure rabies vaccine).
 - a. Protective gloves and clothing as well as face and eye protection should be worn while the head is being removed and packaged.
 - b. Sever the head between the foramen magnum and the atlas so as not to damage the skull. Local veterinarians or trained animal control personnel can assist in this removal. Never advise clients to remove animal heads!
 - c. Allow fluids and blood to drain from the head. Keep the head as clean as possible and place the head in a double plastic bag for transport to the laboratory.
 - d. If fleas or ticks are present, spray insecticide into the plastic bag containing the head before closing. Do not send maggots.
 - e. Gloves should be cleaned and disinfected or discarded following use, and cutting surfaces and instruments should be thoroughly cleaned and disinfected.
5. Only brain material (not the entire head) of very large animals (e.g. cows/horses) will be accepted, as the laboratory is not equipped to handle these large heads due to limited hood and sterilizer space. Removal of the brain should only be attempted by a veterinarian. Whole heads of large animals received by the laboratory will be returned to the sender for resubmission of the brain only.

6. Rodents (rats, rabbits, mice, gerbils, hamsters, guinea pigs, chipmunks, squirrels, moles, etc.) are not usually involved in the rabies cycle and will not be accepted for testing without prior arrangements with the Epidemiology Branch (404-657-2588) or the Georgia Public Health Lab in Decatur (404-327-7980).
7. If specimens cannot be delivered to the laboratory immediately, refrigerate, but **do not** freeze. Frozen specimens cannot be tested until they thaw, which may cause a delay in reporting.
8. Do not send tissue in a preservative such as formalin, as rabies testing cannot be performed on such specimens.

Requisition Form

1. A Rabies Submission Form #3062 should accompany each specimen submitted for rabies examination. This form should be filled out completely and legibly, making sure to include accurate addresses and phone numbers for use in reporting results. If you do not have a GPLH submitter code, please call GPLH at 404-321-2240 to have one assigned to you prior to submission.
2. The Rabies Submission Form #3062 may be found on the Georgia Department of Public Health website at <http://www.health.state.ga.us/pdfs/lab/manual/2011/appendices/Rabies%20Form.pdf>. Fill out the form completely and legibly. Include accurate addresses and phone numbers for reporting.
3. A copy of the rabies report is forwarded to the Georgia Department of Public Health Office of Epidemiology for data collection and review. Rabies reports are kept for a period of three years.

SHIPMENT OF SPECIMENS

Containers for rabies shipment are available from the Decatur Central Lab Virology Unit (404-327-7980).

1. Properly package the specimen by placing the animal head in a double plastic bag and secure the bag by twisting and knotting. For bats or similar size animals, do not remove heads, but submit the whole animal. For large animals (e.g. cows, horses, bears, goats, etc.) submit the brain only.
2. Place the sealed bag containing the specimen on top of the cold packs in the shipper container. Seal the Styrofoam shipper. Place the completed submission form in the brown envelope, and tape to the lid of the sealed shipper. Place the shipper in a cardboard box and tape the address for shipment. Do not seal the box until shipment, so the animal control officer can inspect the container.
3. The package should be shipped prepaid to the Virology Unit. Use a method of shipment that will assure prompt delivery such as Greyhound, Federal Express (FedEx) or UPS next day.

4. Any bite case in which the case history reveals a strong probability of rabies, **particularly in a case of human exposure**, should be handled with utmost speed. Call the Virology Unit ahead of time and advise the laboratory of expected time of arrival if rabies detection test need to be done same day. Hand deliver specimens to the laboratory.
5. Avoid shipping specimens on weekends or holidays without prior approval. A better alternative is to refrigerate and ship on Monday, unless the test result is urgent.
6. Rabies outfits can be obtained from the GPLH Virology Unit; the telephone number is (404) 327-7980. The Virology Unit does not furnish cold packs.

REPORTING AND INTERPRETATION OF RESULTS/CONSULTATION

Rabies testing is available Monday through Friday. All results are called to the submitters and reports will be issued the next business day following the receipt of the specimen, provided the specimen is received by 10:00 a.m. Reporting will be delayed on specimens that are received frozen.

1. Specimens received on Friday or those involved in emergency situations such as severe human head or neck exposures or human exposure for which emergency testing has been approved by Epidemiology Branch at 404-657-2588) will be tested and reported the same day received.
2. If the brain is damaged or decomposed to the point that the laboratory is uncertain as to whether the specimen is, in fact, the appropriate brain tissue, testing will not be done. Report will read "Unsatisfactory" with the comment: "brain tissue is damaged or decomposed beyond recognition of at least two identifiable brain parts." Only in case of human or animal exposure will the specimen be tested. If the test is positive, we will report as such. If the test is negative, a report of "Unsatisfactory" will be made with the comment: "brain tissue is damaged or decomposed beyond recognition of at least two identifiable brain parts." In this situation, an unsatisfactory test result should be managed as if positive.
3. All positive, negative, and unsatisfactory rabies results are telephoned to the contact submitter listed on the Rabies Submission form with follow-up electronic reporting (if available) or hard copy of the report sent by mail. For human exposures, the Virology lab contacts the health district office as well. Copies of each rabies submission form and results are mailed to the Georgia Public Health Epidemiology Office. All specimens should be submitted through animal control/environmental/health department only (not from victims, veterinarians office, etc).
4. If you have any more specific questions, please refer to the Georgia Rabies Animal Control Manual at the following website:

<http://health.state.ga.us/pdfs/epi/zvbd/Rabies%20Manual%202007%20Final%20with%20Cover.pdf>.

If you need consultation for a rabies exposure call Poison Control statewide 1-800-282-5846, Atlanta (404) 589-4400, or if you have difficult or emergency cases, contact the Epidemiologist-On-Call, (404) 657-2588.

UNACCEPTABLE SPECIMENS

The Central Laboratory specimen acceptance policy requires that all specimens be received in good condition with at least two (2) identifiable brain parts and there must have been exposure of humans or domestic animals to the suspected rabid animal.

Specimens will be reported “**Unsatisfactory**” with comments for the following reasons:

1. No known exposure of humans or domestic animals.
2. Brain tissue is damaged or decomposed beyond recognition of at least two (2) identifiable brain parts.
3. Tissue in preservative such as formalin.

VIRUS ISOLATION AND IDENTIFICATION 404-327-7980

INTRODUCTION

Virus culture provides a mechanism for the detection and identification of many human viruses which cause a wide variety of common illnesses. Viruses are isolated in cell culture and confirmed by enzyme immunoassay tests, and indirect fluorescence antibody tests. Respiratory viruses and herpes zoster virus can be detected by a direct fluorescent antibody test. Respiratory virus panel can be detected by PCR. Norovirus from gastrointestinal viral outbreaks can be detected by electron microscopy and PCR.

SPECIMEN COLLECTION/LABELING/REQUISITION FORM

Refrigerate specimens promptly after collection at 2-6° C for no more than 3 days prior to transporting. Specimens that must be held for longer intervals before transporting should be promptly frozen to -70° C or below. Avoid freezing specimens to -20° C because infectivity of viruses is rapidly lost at this temperature and they cannot be recovered by culture. Any container used for viral culture specimens should be sterile.

Swab Collection Procedures:

1. Do not use calcium alginate swabs to collect specimen as it inactivates some viruses.
 2. Use only transport media supplied with outfit or approved by the Virology Laboratory.
 3. After collection, place swab in tube of transport medium, break off stem where handled and discard, and cap tube.
- **Nasal/Pharyngeal:**
Swab each nostril leaving the swab in nose for a few seconds to absorb secretions. Rub the walls of the posterior pharynx with either dry swab or swab wetted with transport media.
 - **Oral:**
Swab oral lesions.
 - **Eye:**
Use sterile swab to remove any exudates or pus present in eye and discard. Moisten second swab with transport medium/saline rubbing affected conjunctiva. An Ophthalmologist or trained physician should collect corneal specimens using a spatula.
 - **Cervical:**
Use sterile swab to remove mucus from cervix and discard. Inserts second swab about 1 cm into cervical canal, rotate, swabbing lesions and remove.
 - **Rectal:**
Swab rectum to collect stool specimen.
 - **Vesicle Fluids/Skin Scrapings:**
Do not prepare site with disinfectants such as alcohol or betadine as these may inactivate virus; use only after specimen collection. In the case of primary infections with herpes simplex, virus may be recovered up to 7-10 days after onset. Collect specimen from base of lesions. Aspirate vesicle fluid with 26/27-gauge tuberculin syringe or

capillary pipette. Promptly rinse fluids collected into small volume of transport medium to prevent clotting. Swab open lesions to obtain both fluid and cells from the lesion base.

Other Collection Procedures:

- **Throat Washings:**

Adults-Gargle with smallest convenient volume (10 to 20 ml) of cell culture medium or general-purpose bacteriological broth expectorates into paper cup. Pour contents of the cup into screw-cap vial. Pediatrics to be collected in similar manner, however, throat swabs is sufficient.

- **Stools:**

Collect stool in a sterile container, transfer small portion (1 to 4 grams) into empty screw cap vial.

- **Urine:**

Collect urine in a sterile container; refrigerate immediately at 2 to 6 degrees Celsius.

- **CSF:**

Adults-obtain at least 2 ml, infants-1 ml, place in sterile screw cap vial. Do not dilute, refrigerate immediately.

- **Serum/Blood:**

Although serum is rarely used to recover viruses it is a suitable specimen for isolation of enterovirus from infected infants.

- **Autopsy/Biopsy:**

Aseptically collect specimens as soon as possible after death. Use separate sterile instrument for each collection site. Collect fresh tissue (1-2 grams) from affected site/lesion. Place each specimen in separate sterile container containing small amount of transport medium or saline, clearly label, and refrigerate. Specimen should not be fixed or placed in any sort of preservative solution.

Requisition Form

The Virology Submission Form #3595 rev. 11/22/2011 is found at the Georgia Public Health Laboratory website

<http://www.health.state.ga.us/pdfs/lab/manual/2011/appendices/Virology%20Form.pdf> and must include the following:

1. Physician or contact person's name and phone number.
2. Patient's name, age and sex.
3. Date of illness onset, symptoms.
4. Submitter name/address ("send report to") box.
5. Type of specimen collected, date collected.
6. Test requested.

SHIPMENT OF SPECIMENS

For shipping instructions, refer to the transportation section of this manual

Wrap packing material around specimen container, secure cap to prevent leakage; place the wrapped specimen inside a biohazard bag and seal tightly. Place the completed Virology requisition form #3595 rev. 1/2010 in the pouch of the biohazard bag. Place the biohazard bag with specimen in a Styrofoam shipper with adequate ice/cold packs to keep cold until the specimen is received. Dry ice is not recommended. Mail, ship common carrier or deliver by courier to GPHL, Attn: Virology Unit, 1749 Clairmont Road, Decatur, GA 30033 with deliveries made between 8:00am-4:30pm Monday-Friday. Viral culture outfits are available from the Laboratory Services and Supply, 1749 Clairmont Road; Decatur, GA 30033-4050, telephone number is 404-327-7920.

REPORTING AND INTERPRETATION OF RESULTS

Turn-around time (time specimen is received to the time the test is completed) for cultures varies from two to five weeks. See Table 1, for summary. Cultures yielding virus isolates may require more or less 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 test sensitivity. Failure to isolate a virus should not rule out the virus as a cause of the illness. Conversely, since people may asymptotically carry a variety of viruses, viruses may be isolated which are unrelated to the current clinical illness.

UNACCEPTABLE SPECIMENS

1. Improperly identified specimens (name on tube/form do not match).
2. No identification on form or tube.
3. Specimens with insufficient quantity for testing (QNS).
4. Improper specimen type sent.

Table 1. Viral Isolation and Identification

AGENT	METHOD	SPECIMEN TYPE	TURN-AROUND TIME
Adenovirus	Cell Culture/ Fluorescent Antibody(FA)/PC R	Throat washing (TW) or throat swab (TS), Naso-Pharyngeal (NP) washing or swab, conjunctival swab, urine, feces	3 weeks for cell culture 1-2 days for direct FA 1 week for PCR
Coxsackievirus	Cell Culture/ FA	TS, feces, CSF, pericardial fluid	3 weeks for cell culture
Cytomegalovirus	Cell Culture/ FA	Urine, TS, buffy coat,	5 weeks for cell culture
Echovirus	Cell Culture/FA	TS, feces, CSF, pericardial fluid	3 weeks for cell culture
Enterovirus	Cell Culture/FA	TS, feces, CSF, pericardial fluid vesicle scraping	3 weeks for cell culture
Herpes simplex virus	Cell Culture/ELVIS	Vesicle scraping (lesion), brain biopsy	3 days for cell culture
Influenza Virus	Cell Culture/FA/ PCR	TW or TS, NP washing or swab	3 weeks for cell culture 1-2 days for direct FA 1 week for PCR
Measles Virus	Cell Culture/FA	TS, Urine, CSF	3 weeks for cell culture 1-2 days for direct FA
Mumps Virus	Cell Culture/FA	TS, Urine, CSF	3 weeks for cell culture 1-2 days for direct FA
Norovirus	Electron Microscopy (EM)	Feces/stool	2 days
Para influenza Virus	Cell Culture/FA/ PCR	TW or TS, NP washing	3 weeks for cell culture 1-2 days for direct FA 1 week for PCR
Poliovirus	Cell Culture/FA	TS, Urine, CSF	4 weeks for cell culture
Respiratory Syncytial Virus	Cell Culture/FA/ PCR	NP washing or swab, TS	3 weeks for cell culture 1-2 days for direct FA 1 week for PCR
Respiratory Panel	Cell Culture/FA/ PCR	TW or TS, NP washing or swab	3 weeks for cell culture 1-2 days for direct FA 1 week for PCR
Rotavirus	EM, EIA	Feces/stool	1-2 days
Varicella (Herpes) Zoster Virus	Cell Culture/FA	Vesicle Scraping	3 weeks for cell culture 1-2 days for direct FA