

Georgia Department of Public Health

Vaccine Preventable Disease Epidemiology Unit (VPDEU)

Pertussis: Specimen Collection and Shipping Instructions

Pertussis should be considered in the differential diagnosis of patients presenting with prolonged cough with cyanosis, vomiting or apnea, regardless of vaccination history. Nasopharyngeal secretions are the optimal specimens for isolating the pertussis bacterium and obtaining a lab confirmed diagnosis. Nasopharyngeal swabs for culture and polymerase chain reaction (PCR) should be collected as soon as possible after onset of symptoms and prior to antibiotic treatment. The time of collection will greatly impact the ability to detect *B. pertussis*. There is a greater likelihood of positive cultures and/or PCR in the first two weeks of symptomatic infection than during later weeks of illness. However, PCR may detect organisms for a prolonged period of time regardless of viability. Confirmatory laboratory testing should be performed on **all** suspect or probable cases.

The Georgia Department of Public Health strongly recommends the collection of a nasopharyngeal swab to confirm a pertussis case. The preferred methods for laboratory diagnosis of pertussis are culture and polymerase chain reaction (PCR) and it is recommended that BOTH tests be performed.

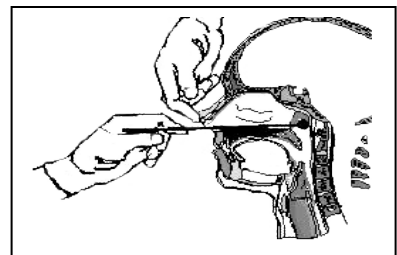
***Pertussis culture and PCR testing at the Georgia Public Health Laboratory is available by special arrangement only. To receive pre-approval and make arrangements for either pertussis culture or PCR testing, the District Health office or Georgia Department of Public Health VPDEU must be contacted at 404-657-2588.**

A. Specimen Collection Instructions

Nasopharyngeal Swab Collection

A video detailing how to collect a nasopharyngeal swab can be found at: <http://health.state.ga.us/epi/vpd/> in the Updates Section.

- Materials – Consult the District Epidemiologist or the Vaccine Preventable Disease Epidemiology Unit for the following:
 - Polyester (Dacron), rayon, or nylon-flocked swab (**Cotton-tipped or Calcium alginate swabs are not acceptable**)
 - Regan-Lowe transport media
 - ❖ Store at 2-8 degrees Celsius (or 36-46 degrees Fahrenheit) in a refrigerator
 - ❖ Remove from refrigerator and warm to room temperature
- Collection
 - Have patient sit with head against a wall or lie down, as patients have a tendency to pull away during this procedure
 - Insert swab into one nostril straight back (**not upwards**) and continue along the floor of the nasal passage for several centimeters until reaching the nasopharynx. The distance from the nose to the ear gives an estimate of the distance the swab should be inserted. Do not force swab, if obstruction is encountered before reaching the nasopharynx, remove swab and try the other side.
 - Rotate the swab gently for 5-10 seconds to loosen the epithelial cells.



Culture:

Remove swab and immediately inoculate Regan-Lowe transport media by inserting the swab at least ½ inch below the surface of the media. Bend or clip the wire swab handle to fit the transport medium tube and reattach the cap securely.

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PCR:

Remove swab and immediately place in a dry sterile container. Bend or clip the wire swab handle to fit the container. **NOTE: A dry swab, not in Regan Lowe media, is acceptable for PCR testing.**

- Specimen should be transported at refrigerator temperature and received by the laboratory as soon as possible and within one day from time of collection.

Laboratory Submission Instructions

- Notify District Public Health Office or the Vaccine Preventable Disease Epidemiology Unit **immediately** for coordination
- Label specimen transport tube with the patient name and date of specimen collection
- **UNAPPROVED OR UNLABELED SPECIMENS WILL NOT BE TESTED**

Culture

- Complete a Bacteriology lab form at:
<http://health.state.ga.us/pdfs/lab/manual/Bacteriology%20Form%203410.pdf>
with the following information:
 - Submitter code (if known), address, phone and fax number, and contact name
 - Patient name, address, phone number, date of birth, sex, race, and ethnicity (if available)
 - Date of specimen collection, source, type of specimen, clinical history and information
 - Check the box for Pertussis (Whooping) Cough, then check culture

PCR

Complete a Molecular Biology lab form at:

<http://health.state.ga.us/pdfs/lab/manual/2012/appendices/Molecular%20Form.pdf>

with the following information:

- Submitter code (if known), address, phone and fax number, and contact name
 - Patient name, address, phone number, date of birth, sex, race, and ethnicity (if available)
 - Date of specimen collection, source, type of specimen, clinical history and information
 - Check the box for Bordetella pertussis (PCR)
- **Ship specimens overnight** by courier or Federal Express on ice packs. If the shipment is delayed, refrigerate specimens at 2-8 degrees Celsius (or 36-46 degrees Fahrenheit) and transport the next day on ice packs by first class mail, common carrier, or courier.
 - Send specimens to the following address:
Georgia Public Health Laboratory
1749 Clairmont Road
Decatur, GA 30033-4050
ATTN: Bacteriology Laboratory

Contact Information

- For specimen outfit requests: call the Georgia Public Health Laboratory at 404-327-7921
- Additional lab forms available at <http://health.state.ga.us/programs/lab/manual.asp> in Appendix B
- For questions related to specimen collection and transport: contact local public health or the State VPD Epidemiology Unit, 404-657-2588

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Pertussis Laboratory Testing Procedures and Interpretation of Results

Culture

Cultures are held 7 days from the date of inoculation and read daily. Nasopharyngeal swabs received in transport medium tubes are inoculated immediately onto Regan-Lowe plates and incubated. After the final day of incubation, if no colonies typical of *B. pertussis* or *B. parapertussis* are present, the culture is reported as negative for these organisms. A positive culture report is based upon typical colony morphology, biochemicals and direct fluorescent antibody testing (DFA) 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 PCR are recommended for diagnosis of *B. pertussis* whenever possible. A positive culture is considered confirmatory and is the most reliable. "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.

PCR

The GPHL currently offers a **multitarget** PCR assay validated by the CDC, for qualitative detection of *Bordetella pertussis*, *B. parapertussis*, and *B. holmesii* DNA extracted from clinical specimens or culture isolates by real-time PCR. Most commercial laboratories use a **single target** PCR for IS481, which is present in multiple copies in *B. pertussis* and in lesser quantities in *B. holmesii* and *B. bronchiseptica*. Because this DNA sequence is present in multiple copies, IS481 is especially susceptible to falsely-positive results. Use of the GPHL multiple target assay improves specificity of PCR assays for pertussis. When requesting commercial PCR testing, clinicians are encouraged to inquire about which PCR target or targets are used by their laboratories. Interpretation of commercial PCR results, should be done in conjunction with an evaluation of signs and symptoms and available epidemiological information, as their specificity can vary greatly.