



## **Pertussis Specimen Collection and Shipping Instructions**

The Georgia Department of Public Health strongly recommends the **collection of a nasopharyngeal (NP) swab to confirm a pertussis case**. The preferred methods for a laboratory diagnosis of pertussis are culture and/or polymerase chain reaction (PCR) and it is recommended that BOTH tests be performed. To coordinate specimen collection and laboratory submission, call your [District Health Office](#) or the DPH Acute Disease Epidemiology Section at 404-657-2588 during business hours Monday through Friday, or 1-866-PUB-HLTH after-hours on evenings and weekends. **Please do not send specimens directly to the Georgia Public Health Laboratory (GPHL) or the Centers for Disease Control and Prevention (CDC).**

### **Specimen Collection Instructions:**

**Viral Testing:** This RT-PCR multiplex assay is intended for the qualitative detection of Bordetella pertussis, Bordetella parapertussis, and Bordetella holmesii in DNA extracted from clinical specimens or culture isolates. The presence of the virus DNA should be demonstrated in the following methods for the collection of specimens.

### **Specimen Collection:**

- Nasopharyngeal (NP) swab: (Culture and PCR)
  - Submit Dacron swabs with aluminum shafts from a dry sterile container placed immediately into in a Regan-Lowe semi-solid agar or Amies charcoal gel transport tube
- Nasopharyngeal (NP) swab: (PCR only)
  - Submit Dacron swabs with aluminum shafts from a dry sterile container placed immediately into a dry, sterile tube or in a tube of liquid universal transport medium (UTM).
- Nasopharyngeal aspirates:
  - Collect a minimum of 500 $\mu$  in a sterile leak-proof plastic container of physiological saline.
- Culture isolates
  - Submit a plate or slant exhibiting colony morphology and biochemical testing consistent with Bordetella spp on Regan-Lowe solid agar, Bordet Gengou solid agar, 5% sheep blood solid agar, Regan-Lowe semi-solid agar transport, or Amies charcoal gel transport.

### **Storage/Transport**

Consultation with district epidemiologist required prior to submission

- Nasopharyngeal swab (Culture and PCR) can be maintained at refrigerated temperature (2-8°C) within 30 minutes of collection and during transit for no more than 24-72 hours after collection
- Nasopharyngeal swab (PCR only) should be stored frozen (-20°C or colder) within 30 minutes of collection and shipped with dry ice
- Nasopharyngeal aspirates can be maintained at refrigerated temperature (2-8°C) within 30 minutes of collection and during transit for no more than 72 hours after collection. Nasopharyngeal aspirates can also be stored frozen (-20°C or colder) within 30 minutes of collection and shipped with dry ice.
- Cultures can be maintained at ambient temperature (15-30°C) or refrigerated temperature (2-8°C) during transit for no more than 24-48 hours after growth is present.

#### **Acceptable specimen type(s)**

- Nasopharyngeal (NP) swab
- Nasopharyngeal aspirates
- Culture isolates

**Culture Testing:** Bacterial culture for pertussis is most useful during the first 2 weeks of cough and prior to antibiotic use. Georgia Public Health Laboratory accepts cultures for *Bordetella pertussis* and *Bordetella parapertussis* in conjunction with PCR. Culture is done to identify *Bordetella pertussis* as well as Other *Bordetella* species and collection of specimens should be demonstrated in the following methods:

#### **Specimen Collection**

- Nasopharyngeal Swab
  - Polyester (Dacron), rayon, or nylon flocked swabs in Regan Lowe Transport medium
- Nasopharyngeal aspirates
  - Minimum volume of collections is 500µl in Regan Lowe Transport medium
- Culture Isolates
  - Inoculate a pure culture onto a slant or plate of charcoal agar with horse blood, such as Regan-Lowe medium, and send to GPHL with 24 hours or incubate at 35°C under high humidity. Schedule subculture so that the time between optimal growth and transport of culture is <24 hours.

#### **Storage/Transport**

- Swab
  - Regan Lowe semisolid transport medium tube is recommended. Ship specimen at room temperature (15-30°C). If a shipping delay is anticipated, use the refrigerated (2-8°C) shipping outfit
- Culture:
  - Isolates must be shipped using ambient (15-30°C) specimen outfit within 24 hours.

#### **Acceptable Specimen Type(s)**

- Nasopharyngeal swab or aspirate
- Culture

#### **Laboratory Submission Instructions**

- Notify County or District Public Health Office **immediately** for coordination

- Contact state VPD Surveillance Epidemiologist
- Label specimen containers with the patient's name, date of birth, and date of specimen collection.
- UNLABELED SPECIMENS WILL NOT BE TESTED
- Complete the GPHL Submission Form with the following information
  - Submitter code (if known), address, phone number, and contact name
  - Patient name, address, date of birth, sex, race, and ethnicity (if available)
  - Date of specimen collection, source, type of specimen, clinical history, and information
  - Test requested
    - If requesting a culture, under "Bacteriology" check the box labeled 6317-2 (*Order code 1050*)
    - If requesting a PCR, under "Molecular Biology" check the box labeled 414000 *Pertussis (RT-PCR)*
- **NOTE:** A separate submission form needs to be completed for EACH specimen submitted (i.e. if two specimens are collected – one for culture and one for PCR, two GPHL submission forms need to be completed).
- Ship specimens to the following address:

Georgia Public Health Laboratory  
 1749 Clairmont Road  
 Decatur, GA 30033-4050  
 ATTN: Molecular Laboratory

### **Interpretation of Results**

The timing of collection will greatly impact the ability to detect *Bordetella 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. Clinicians commonly use several types of laboratory tests to diagnose *Bordetella pertussis*. Scientists consider culture the gold standard because it is the only 100% specific method for identification. Other tests that can be performed include PCR (preferred) and serology.

### **Culture**

Culture is best used during the first 2 weeks following cough onset. 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 *Bordetella pertussis* or *Bordetella 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 culture 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 *Bordetella pertussis* whenever possible. A positive culture is considered confirmatory and is 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*, *Bordetella parapertussis*, and *Bordetella 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 PGHL 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.

## **Serology**

For the CDC single point serology test, the optimal timing for pertussis specimen collection is 2 to 8 weeks following cough onset, when the antibody titers are at their highest. However, serologic testing may be performed on specimens collected up to 12 weeks following cough onset as serologic tests are more useful for diagnosis in later phases of the disease. Commercially, there are several different serologic tests used in the United States with unproven or unknown clinical accuracy. Therefore, serology is not used as confirmatory or supportive laboratory evidence and should be used along with clinical signs and symptoms for investigative purposes. Please contact the state VPD Surveillance Epidemiologist with any questions or concerns regarding interpreting serologic results.