



California Department of Public Health - May 2008

Pertussis: Laboratory Testing



The preferred methods for the laboratory diagnosis of pertussis are culture and polymerase chain reaction. These tests are the basis for the CDC definition of a confirmed case of pertussis.

Culture isolation of *B. pertussis* is the gold standard and the preferred laboratory test for pertussis; however, the organism is difficult to isolate. Culture is less sensitive than PCR, but is 100% specific (no false positives). A negative culture result does not rule out pertussis infection. Confirm outbreaks with ≥ 1 culture-confirmed case. *B. pertussis* is most frequently recovered in the catarrhal or early paroxysmal stage of illness. Once cough has been present for ≥ 3 weeks, recovering the organism is unlikely.

B. pertussis usually grows after 3-4 days, however cultures cannot be considered negative for pertussis until after 10 days. The primary reasons for failure to isolate *B. pertussis* are bacterial or fungal contamination, lack of fresh media, and specimen collection too late in illness. Cultures can also be negative if taken from a previously immunized person or if antimicrobial therapy has been started.

Polymerase chain reaction (PCR) assay provides rapid results and is more sensitive than culture (accurately identifies more positive cases). However, false positive test results are a problem. A person with a positive PCR who does not have a cough is not a case.

PCR tests are less sensitive in previously immunized individuals, but are more sensitive than cultures in such patients. PCR tests are also more likely than cultures to be positive in patients who have received antimicrobial treatment. Delay in specimen collection is the main reason for a negative PCR test result in a patient with pertussis.

Alternative when culture or PCR is not available or when it has been ≥ 3 weeks since cough onset:

Serologic testing to detect IgG and IgA antibodies to pertussis toxin

Most commercial serological tests are imprecise and not recommended. The only currently acceptable serologic test is an enzyme-linked immunosorbent assay (ELISA) like test for detection of IgG and IgA antibodies to pertussis toxin.

Diagnosis of pertussis on the basis of a high single serum titer from this test is reasonably sensitive and specific in

persons >10 years of age if it has been > 2 years since the last dose of pertussis containing vaccine was received.

Tests that are not recommended:

Commercial ELISA tests that use whole *B. pertussis* or *B. pertussis* antigens rather than pertussis toxin (i.e., FHA tests) have high false positive rates and are not recommended. Testing for pertussis IgM antibody is also not recommended.

Direct fluorescent antibody (DFA) tests are not recommended for laboratory confirmation of pertussis, nor does a positive DFA test meet the CDC criteria for laboratory confirmation of a pertussis case. The sensitivity of these tests is low and they are performed reliably only by experienced technologists.

For testing questions, please contact the CDPH Microbial Diseases Laboratory at 510-412-3903.

Specimen collection

Specimens for culture or PCR must be obtained from a nasal wash or nasopharyngeal swab.

Materials:

- Gloves
- Mask for covering nose and mouth of health worker
- Eye protection/goggles for health worker to protect from coughs, sneezes, or splashes

Nasal wash collection

Additional materials for nasal wash collection:

- 0.9% saline: 6 ml sterile, non-bacteriostatic
- Sterile specimen container, tight sealing, leak-proof
- (such as a sterile sputum or urine cup)
- Sterile feeding tube #8 French, 16" length
- 5cc disposable syringe with disposable needle for drawing saline

Procedure:

1. Attach the needle to the syringe and draw 3 ml of sterile, non-bacteriostatic saline into the barrel of the syringe. Attach a soft feeding tube to the syringe tip. Slowly push saline through the tube and let a drop or two come out of the tip for lubrication.

2. Put on gloves and mask/goggles.

3. Instruct the patient not to swallow if possible. Tell the patient the procedure will not hurt, but may tickle or cause them to tear or even sneeze.

4. It is not necessary to tilt the patient's head back, but this may be helpful in small infants who are lying on their back. Keeping the head straight and upright will help avoid having the saline drain down the patient's throat.

5. Have the patient hold one nostril closed. For an infant/toddler, the person collecting the specimen or an assistant may need to hold the nostril closed.

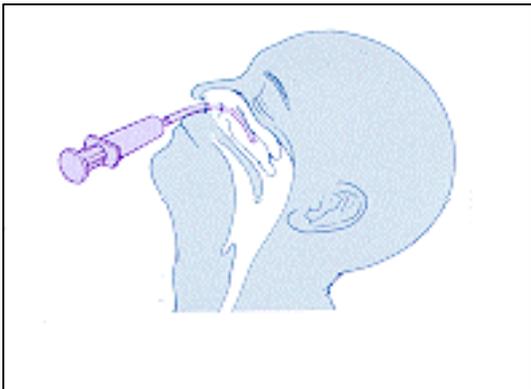
6. Insert the feeding tube about 3-4" (less for a child) **straight back** (not upwards) along floor of the nasal passage and inject the saline.

7. Immediately aspirate the saline. Inject the contents of the syringe into the specimen container. Look for mucous in the aspirate. If there is no mucous, repeat the procedure in the other nostril. You may use the same syringe, feeding tube, and specimen container, but instill new saline. A specimen with some mucous is a better specimen, but excessive mucous should be avoided.

8. You may hand the patient a tissue but the patient should not blow his/her nose until you have determined whether a second specimen from the other nostril is needed.

9. Specimen should be transported at refrigerator temperature and received by the laboratory <3 days of collection.

Note: Nasal wash is the preferred specimen; however, nasopharyngeal swab is acceptable.



Nasopharyngeal swab collection

Additional materials for NP swab collection:

- Dacron nasopharyngeal swab*
- Test tube with screw cap, plastic preferred
- Sterile saline, non-bacteriostatic, 3 ml

*Cotton-tipped or calcium alginate swabs are **not** acceptable. PCR assays may be inhibited by residues present in these materials

Procedure:

1. 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 (resistance will be met). The distance from the nose to the ear gives an estimate of the distance the swab should be inserted. Note: do not force swab - if an obstruction is encountered before reaching the nasopharynx, try the other side.

2. Rotate swab gently for 5-10 seconds to loosen the epithelial cells.

3. Remove swab and immediately place into Regan-Lowe transport media (the metal handle of the swab can be snipped to fit more easily into the transport container).

4. Specimen should be transported at refrigerator temperature and received by the laboratory <3 days of collection.

Tip: Have patient sit with head against a wall as patients have a tendency to pull away during these procedures.

