California Department of Public Health - August 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 of *B. pertussis* is the gold standard and the preferred laboratory test for pertussis; however, the organism can be 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. Length of PCR positivity is similar to that for cultures. 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
Currently available commercial serological tests have not been clinically validated; however, one serologic enzyme-linked immunosorbent assay (ELISA) like test* for detection of IgG and IgA antibodies to pertussis toxin may be useful for diagnosis.

*Focus Technologies, Cypress, CA

Diagnosis of pertussis on the basis of a high single serum titer from this test is expected to be 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 on smears made from nasopharyngeal specimens are not recommended for pertussis diagnosis, 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.

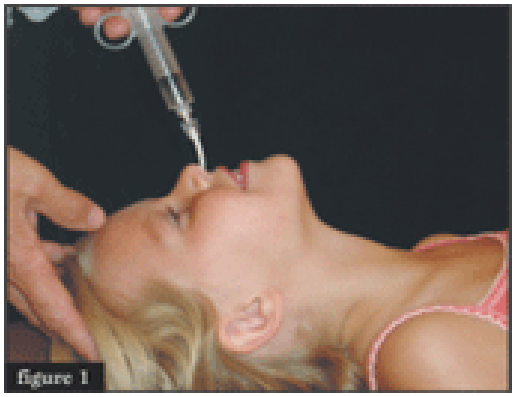
Specimen collection
Specimens for culture or PCR must be obtained from a nasal aspirate or nasopharyngeal swab. A nasal aspirate is the preferred specimen; however, a nasopharyngeal swab is acceptable.

Nasal aspiration (a video of this procedure is available at http://www.n-pak.com)

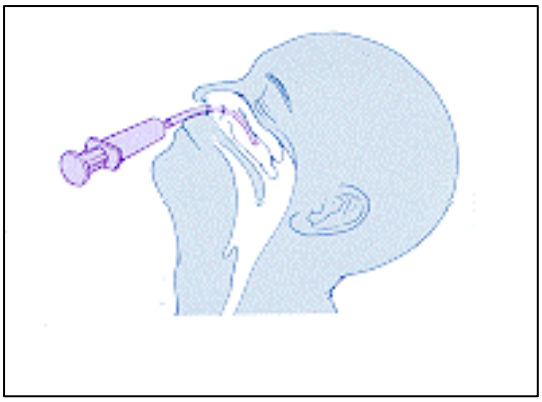
Materials:
- 0.9% saline: 6 ml sterile, non-bacteriostatic
- Sterile feeding tube #8 French, 16" length
- 5cc disposable syringe with disposable needle for drawing saline
- Sterile specimen container, tight sealing, leak-proof (such as a sterile sputum or urine cup)
- Mask and gloves

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 mask and gloves.

3. Have patient lie on their back with their neck extended. Neck extension is **very important** as it allows pooling of the aspirate in the nasopharynx.
4. Ask patient to **hold their breath**, if possible (age and cooperation dependent). Advance the tube along the floor of the nose about 3-4 inches (less for a child) until resistance is met at the nasopharynx.



5. Using a smooth motion and without moving the tube out of place, quickly push the syringe plunger to expel the saline and pull the plunger back to withdraw the aspirate (it helps to have fingers in place as the tube is inserted). All of the fluid should be instilled into the nasopharynx during the procedure. If the child is crying, try to time the aspiration with the exhalation of the cry since this should help prevent saline from leaving the nasopharynx. The recovered aspirate specimen should be approximately 2 ml in volume.
6. Carefully remove tube from nose and detach syringe.
7. Inject contents of syringe into specimen container.
8. Specimen should be transported at refrigerator temperature and received by laboratory as soon as possible and ≤3 days from time of collection.

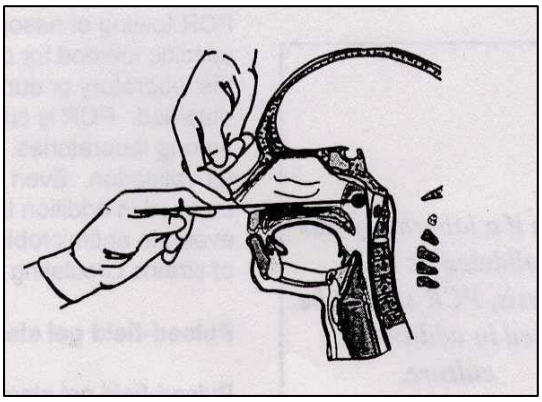


Nasopharyngeal swab collection

Materials:

- Dacron-tipped nasopharyngeal swab with flexible wire handle*
- Regan-Lowe transport media
- Mask and gloves

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

Procedure:

1. Put on mask and gloves.
2. Have patient sit with head against a wall as patients have a tendency to pull away during this procedure.
3. 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. Do not force swab, if obstruction is encountered before reaching the nasopharynx, remove swab and try the other side.
4. Rotate the swab gently for 5-10 seconds to loosen the epithelial cells.
5. 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.
6. Specimen should be transported at refrigerator temperature and received by laboratory as soon as possible and ≤3 days from time of collection.