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**
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 antibiotic therapy has been started.

**Polymerase chain reaction (PCR)**
PCR is a molecular test used to detect *B. pertussis* DNA. Results can be obtained more rapidly and unlike culture, live bacteria do not need to be present in the specimen. PCR is more sensitive (less likely to be falsely negative) than culture. However, false positive test results can be a problem. A person with a positive PCR who does not have a pertussis-like cough is not considered 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 can result in negative PCR test result in a patient with pertussis.

**Best practices for PCR testing**
- Only test patients with signs and symptoms of pertussis. Testing asymptomatic persons increases the likelihood of obtaining falsely-positive results.
- Only test patients during the first 3 weeks of cough when bacterial DNA is still present.
- Do not test patients who have had ≥5 days of antibiotics.

**Optimal specimen collection for PCR testing**
- Specimens for PCR testing should be obtained by aspiration or swabbing of the posterior nasopharynx.
- Specimens collected by nasopharyngeal (NP) aspiration, a saline flush of the posterior nasopharynx, are preferred over specimens collected by NP swab. A specimen collected by NP aspiration will contain a larger quantity of bacteria.
- Specimens collected by NP swab should be obtained using polyester (such as Dacron®), rayon, or nylon-flocked swabs. Cotton-tipped or calcium alginate swabs are not acceptable as residues present in these materials inhibit PCR assays.
- Throat swabs and anterior nasal swabs have unacceptably low rates of DNA recovery and should not be used for pertussis diagnosis.

**Bordetella pertussis DNA contamination of clinical specimens and falsely-positive PCR results**
- Some pertussis vaccines have been found to contain PCR-detectable *B. pertussis* DNA.
- Specimen contamination with vaccine DNA can best be avoided by:
  - Collecting specimens by nasal aspiration rather than by NP swab because the aspirate kit (syringe or bulb style) is a closed system.
  - Wearing clean gloves to collect specimens.
  - Avoid using liquid transport media, if possible. Semisolid or non-liquid (Regan Lowe) transport media for NP swabs are preferred; however, dry swabs may be used for PCR testing.
- Other methods to reduce the opportunity for cross contamination in the clinic setting are available at: [http://www.cdc.gov/pertussis/clinical/diagnostic-testing/diagnosis-PCR-bestpractices.html](http://www.cdc.gov/pertussis/clinical/diagnostic-testing/diagnosis-PCR-bestpractices.html)

*Falsely-positive PCR results from vaccine DNA contamination may occur since *B. pertussis* vaccine DNA found in Pentacel®, Daptacel®, and Adacel® can contaminate clinic environments and the hands of healthcare workers. If vaccine DNA contamination is present, this DNA can be transferred from healthcare worker hands to the handle of the NP swab. When the NP swab is placed into liquid transport media, *B. pertussis* DNA on the swab handle is washed off into the liquid medium. DNA for PCR testing is extracted from the liquid medium and vaccine DNA could cause falsely-positive PCR results.*
Understanding and interpreting PCR results

- There is no FDA approved PCR test for pertussis and PCR tests for pertussis are not standardized across clinical laboratories. Therefore, testing methods, DNA targets used and result interpretation criteria vary, and laboratories do not use the same cycle threshold (Ct) cutoffs for determining a positive result.
- High Ct values indicate low levels of DNA. Potential causes for low levels of detectable DNA include: poor specimen collection or DNA extraction, specimen type (NP swab versus aspirate), late testing and DNA contamination.
- Depending on the cutoffs used, laboratories may report PCR results with high Ct values as: positive, detected, indeterminate, equivocal or negative.
- Interpretation of PCR results, especially those with high Ct values, should be done in conjunction with an evaluation of signs and symptoms and available epidemiological information.

Serologic testing

CDC is in the process of validating serologic tests for pertussis and determining if such tests can reliably be used as laboratory confirmation of pertussis. These tests may be valuable, particularly when it has been ≥3 weeks since cough onset and culture or PCR are likely to be negative.

Until more is known about the validity of serologic testing, a commercially available serologic enzyme-linked immunosorbent assay (ELISA) like test (Focus Technologies, Cypress, CA) for detection of IgG and IgA antibodies to pertussis toxin may be useful for diagnosis.

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. Serologic results suggestive of pertussis infection cannot be used as laboratory confirmation for surveillance purposes.

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 result 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.

Specimen collection

Specimens for culture or PCR must be obtained from a nasal aspirate or NP swab. A nasal aspirate is the preferred specimen; however, an NP swab is acceptable. A CDC video demonstrating nasal aspiration and nasopharyngeal swab collection is available at: http://www.cdc.gov/pertussis/clinical/diagnostic-testing/specimen-collection.html

Nasal aspiration

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.

Procedure:
1. Put on mask and clean 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 place in semisolid or nonliquid (Regan Lowe) transport media by inserting the swab at least ½ inch below the surface of the media. Clip the wire swab handle to fit the transport tube or snap off the plastic handle and reattach the cap securely. A dry swab is acceptable for PCR testing.
6. 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 or plastic handle*
- Semisolid or nonliquid (Regan Lowe) transport media; a dry swab is acceptable for PCR testing
- Mask and gloves

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