Diagnosing pertussis in the early stages can be difficult, because early signs and symptoms are often nonspecific. Polymerase chain reaction (PCR) testing, a technique used to detect DNA sequences specific for *Bordetella pertussis*, is used for diagnosis; however, results should be cautiously interpreted, because they can often be false positive or false negative. The Centers for Disease Control and Prevention (CDC) has developed best practices to help optimize the use of PCR testing for the diagnosis of pertussis.

PCR testing in asymptomatic persons should be avoided because of the increased likelihood of false-positive results. Testing also should not be performed in asymptomatic close contacts of persons with confirmed pertussis and should not be used to guide the use of postexposure prophylaxis. PCR testing should be used only to confirm a diagnosis in persons with signs and symptoms consistent with pertussis.

Sensitivity of PCR testing is best in the first three weeks after the onset of cough, because bacterial DNA is still present in the nasopharynx. After four weeks, however, bacterial DNA is reduced, increasing the risk of false-negative results. PCR testing after treatment with antibiotics can also cause false-negative results. Therefore, testing after five days of antibiotic treatment is generally not recommended, because it is not likely to provide benefit.

Physicians should obtain specimens for PCR testing by aspiration or by swabbing the posterior nasopharynx with a polyester-tipped, rayon-tipped, or nylon-flocked swab. Residue found in cotton-tipped and calcium alginate swabs can inhibit PCR assays; therefore, these types of swabs should not be used. Nasopharyngeal aspirates that flush the posterior nasopharynx with a saline wash result in more bacterial DNA in the sample and are preferred whenever possible. For videos and images of specimen collection, go to http://www.cdc.gov/pertussis/clinical/diagnostic-testing/specimen-collection.html.

Some pertussis vaccines contain *B. pertussis* DNA that can be detected on PCR testing; accidentally transferring the DNA from a surface to a specimen can cause contamination and false-positive results. However, physicians do not need to switch vaccines if good practices are followed.

To help reduce cross contamination, vaccines should be prepared and administered in an area separate from where specimens are collected, and physicians should be cautious when preparing and administering the vaccine to avoid contaminating surfaces.

When using liquid transport media, DNA can be accidentally transferred from hands to the swab, which can then be washed off into the liquid medium. This liquid can then be extracted for PCR testing, causing a false-positive result. Use of a semisolid or nonliquid transport media, or a dry swab without media can prevent contamination. Nasopharyngeal aspiration uses an aspirate kit that is a closed system, which is less likely to be contaminated compared with a swab.

PCR assays are not standardized across laboratories. Most use a single target PCR for IS481; however, IS481 is especially prone to false-positive results because it can be found in multiple copies in *B. pertussis*. Specificity may be improved by using multiple targets. Physicians should be aware of which PCR targets are being used, and should interpret PCR testing results in combination with an assessment of signs, symptoms, and epidemiologic data.