



Best Practices for Health Care Professionals on the use of Polymerase Chain Reaction (PCR) for Diagnosing Pertussis

With the continuing resurgence of pertussis, health care professionals will see more patients with suspected pertussis. Polymerase Chain Reaction (PCR) is an important tool for timely diagnosis of pertussis and is increasingly available to clinicians. PCR is a molecular technique used to detect DNA sequences of the *Bordetella pertussis* bacterium and unlike culture, does not require viable (live) bacteria present in the specimen. Despite these advantages, PCR can give results that are falsely-negative or falsely-positive. The following compilation of best practices is intended to help health care professionals optimize the use of PCR testing for pertussis by avoiding some of the more common pitfalls leading to inaccurate results.

Testing Patients with Signs and Symptoms of Pertussis

Early signs and symptoms of pertussis are often non-specific, making it difficult to determine clinically who has pertussis in the earliest stages (<http://www.cdc.gov/pertussis/clinical/features.html>). However, only patients with signs and symptoms consistent with pertussis should be tested by PCR to confirm the diagnosis. Testing asymptomatic persons should be avoided as it increases the likelihood of obtaining falsely-positive results. Asymptomatic close contacts of confirmed cases should not be tested and testing of contacts should not be used for post-exposure prophylaxis decisions.

Optimal Timing for PCR Testing for Pertussis

PCR has optimal sensitivity during the first 3 weeks of cough when bacterial DNA is still present in the nasopharynx. After the fourth week of cough, the amount of bacterial DNA rapidly diminishes which increases the risk of obtaining falsely-negative results. For more information on diagnostic testing for pertussis, including the use of serology for late diagnosis, please reference: <http://www.cdc.gov/pertussis/clinical/diagnostic.html>.

PCR testing following antibiotic therapy also can result in falsely-negative findings. The exact duration of positivity following antibiotic use is not well understood, but PCR testing after 5 days of antibiotic use is unlikely to be of benefit and is generally not recommended.

Optimal Specimen Collection for PCR Testing for Pertussis

Specimens for PCR testing should be obtained by aspiration or swabbing the posterior nasopharynx (<http://www.cdc.gov/pertussis/clinical/diagnostic.html>). Throat swabs and anterior nasal swabs have unacceptably low rates of DNA recovery and should not be used for pertussis diagnosis. The swab tips may be polyester (such as Dacron®), rayon, or nylon-flocked. Cotton-tipped or calcium alginate swabs are not acceptable as residues present in these materials inhibit PCR assays. If feasible, nasopharyngeal (NP) aspirates that flush the posterior nasopharynx with a saline wash are preferred over swabs because this method results in a larger quantity of bacterial DNA in the sample.

Avoiding Contamination of Clinical Specimens with Pertussis DNA

Some pertussis vaccinesⁱ have been found to contain PCR-detectable *B. pertussis* DNA. Environmental sampling has identified *B. pertussis* DNA from these vaccines in clinic environments. While the presence of this DNA in the vaccines does not impact the safety or immunogenicity of these vaccines, accidental transfer of the DNA from environmental surfaces to a clinical specimen can result in specimen

ⁱ Vaccines shown to contain PCR-detectable DNA include Pentacel®, Daptacel®, and Adacel®. Leber A et al. Detection of *Bordetella pertussis* DNA in Acellular Vaccines and in Environmental Samples from Pediatric Physician Offices, in 2010 Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC): Boston, USA.

contamination and falsely-positive results. If health care professionals adhere to good practices, there is no need to switch vaccines.

Preparation and administration of vaccines in areas separate from pertussis specimen collection areas may reduce the opportunity for cross contamination of clinical specimens. Care should be taken when preparing and administering pertussis vaccines to avoid contamination of surfaces with vaccine. General adherence to basic infection-control measures may further prevent contamination of specimens:

- Wearing gloves immediately before and during specimen collection or vaccine preparation and administration with immediate disposal of gloves after the procedure, and
- Cleaning clinic surfaces using a 10% bleach solutionⁱⁱ to reduce the amount of nucleic acids in the clinic environment.

The use of liquid transport media likely also contributes to falsely-positive results from contaminant DNA. When using liquid transport media, DNA that is accidentally transferred from hands to the swab shaft can be washed off into the liquid medium which freely circulates around the transport tube; this liquid is later extracted to obtain DNA for PCR testing. Use of a semisolid or non-liquid transport media or transport of a dry swab without media should prevent contaminant DNA on the swab shaft from reaching the part of the specimen that is later extracted. If using liquid transport medium, the swab stick should be handled with care and only above the red line or indentation which marks where the shaft is snapped off after insertion into the medium. Performing NP aspiration rather than swabbing the NP may also prevent contamination from occurring as the aspirate kit (syringe or bulb style) is a closed system at the point of specimen collection.

Understanding and Interpreting Testing Results

PCR assays for pertussis are not standardized across clinical laboratories. Testing methods, DNA targets used and result interpretation criteria vary, and laboratories do not use the same cutoffs for determining a positive result. With PCR, high cycle threshold (Ct) values indicate low levels of amplified DNA; for pertussis, these values may still indicate infection but can also be the result of specimens contaminated with DNA from the environment at the time of specimen collection. Clinical laboratories might report high Ct values as any of the following: positive, detected, indeterminate, or equivocal. In addition, most clinical 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 multiple targets may improve specificity of PCR assays for pertussis. Clinicians are encouraged to inquire about which PCR target or targets are used by their laboratories. 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.

Summary

In summary, PCR is an important tool for diagnosis of pertussis especially in the setting of the current resurgence of pertussis disease. PCR can provide timely results with improved sensitivity over culture. Careful specimen collection and transport and a general understanding of the PCR assays performed will better ensure that clinicians obtain diagnostic test results that reliably inform patient diagnosis.

ⁱⁱ A 10% solution corresponds to 1 and a half cups of household bleach per gallon of water, or 1 part bleach to nine parts water.