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

Summary: *With the continuing resurgence of pertussis, health care professionals will likely see more patients with suspected pertussis. Proper testing criteria, timing of testing, specimen collection techniques, protocols for avoiding specimen contamination, and appropriate interpretation of test results are all necessary to ensure that Polymerase Chain Reaction (PCR) reliably informs patient diagnosis. 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 this advantage, 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.*

Recommendations for Testing

Whom should you test?

Only patients with signs and symptoms consistent with pertussis should be tested by PCR to confirm the diagnosis. For guidance in distinguishing signs and symptoms of pertussis from those of other conditions, see <http://www.cdc.gov/pertussis/clinical/features.html>. 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.

When should you test?

When possible, you should test patients for pertussis during the first 3 weeks of cough when bacterial DNA is still present in the nasopharynx, because after the fourth week of cough, the amount of bacterial DNA rapidly diminishes, increasing the risk of obtaining falsely-negative results by PCR. For more information on diagnostic testing, see <http://www.cdc.gov/pertussis/clinical/diagnostic-testing/diagnosis-confirmation.html>.

Also, PCR testing after 5 days of antibiotic use is unlikely to be of benefit, because PCR testing following antibiotic therapy also can result in falsely-negative findings, although the exact duration of positivity following antibiotic use is not well understood.

How should you obtain specimens?

You should obtain specimens for PCR by aspiration or swabbing the posterior nasopharynx, rather than by throat swabs or anterior nasal swabs which both have unacceptably low rates of DNA recovery and should therefore **not** be used for pertussis diagnosis. For more information, see <http://www.cdc.gov/pertussis/clinical/diagnostic-testing/specimen-collection.html>.

What should you do to avoid contamination of clinical specimens with pertussis DNA?

Some pertussis vaccines^[1] have been found to contain PCR-detectable *B. pertussis* DNA. Environmental sampling has identified *B. pertussis* DNA from these vaccines in clinic environments. While DNA in the vaccines

^[1] 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.

Please share this document with all physicians & staff in your facility/office.

does not impact the safety or immunogenicity, accidental transfer of the DNA from environmental surfaces to a clinical specimen can result in specimen contamination and falsely-positive results. If health care professionals adhere to good practices, there is no need to switch vaccines. Clinicians should adhere to the following vaccine preparation and administration best practices and basic infection-control measures, to prevent cross-contamination.

Best Practices for Preparing and Administering Vaccines

- Prepare and administer vaccines in areas separate from pertussis specimen collection because doing so may reduce the opportunity for cross contamination of clinical specimens.
- Take care to avoid contamination of surfaces when preparing and administering vaccines.

Adherence to Basic Infection-control Measures

- Wearing clean 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 solution 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.

Recommendations, Understanding and Interpreting PCR 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.**

For more information:

- For the entire guidance on PCR best practices in diagnosing pertussis, see <http://www.cdc.gov/pertussis/clinical/diagnostic-testing/diagnosis-pcr-bestpractices.html>
- For distinguishing clinical features of pertussis, see <http://www.cdc.gov/pertussis/clinical/features.html>.
- For more information on diagnostic testing, see <http://www.cdc.gov/pertussis/clinical/diagnostic-testing/index.html>.
- CDC's toll-free information line, 800-CDC-INFO (800-232-4636) TTY: (888) 232-6348, is available 24 hours a day, every day.

Categories of Health Alert messages:

Health Alert conveys the highest level of importance; warrants immediate action or attention.

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