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. Along with culture, Polymerase Chain Reaction (PCR) is an important tool for timely diagnosis of pertussis. PCR is a highly sensitive molecular technique used to detect DNA sequences of the *Bordetella pertussis, parapertussis, and holmesii* bacteria, and unlike culture, does not require viable (live) bacteria present in the specimen. The following compilation of best practices was written by the Centers for Disease Control and Prevention (CDC) (*http://www.cdc.gov/pertussis/clinical/diagnostic-testing/diagnosis-pcr-bestpractices.html*) and modified to fit the current practices at the Vermont Department of Health Laboratory (VDHL). For more information, please visit this website, or contact the VDHL at (802) 338-4724. This document 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 false-positive, false-negative, or inconclusive 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. However, only patients with signs and symptoms consistent with pertussis should be tested by PCR to confirm the diagnosis. These symptoms may include paroxysmal coughing, post-tussive vomiting, or multiple days of cough in someone with a pertussis exposure.

Testing asymptomatic persons should be avoided. 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 false-negative results.

PCR testing following antibiotic therapy also can result in false-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 at VDHL

Specimens for PCR testing should be obtained by swabbing the posterior nasopharynx. Please visit this website for a video showing proper collection technique:
http://www.cdc.gov/pertussis/clinical/diagnostic-testing/specimen-collection.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 (Dacron®), or nylon-flocked (Copan). Cotton-tipped or calcium alginate swabs are not acceptable as residues present in these materials inhibit PCR assays.

Avoiding Contamination of Clinical Specimens with Pertussis DNA During Specimen Collection

Some pertussis vaccines[i] have been found to contain PCR-detectable *B. pertussis* DNA. Environmental sampling has identified *B. pertussis* DNA from these vaccines in clinic environments. Accidental transfer of the DNA from environmental surfaces to a clinical specimen can result in specimen contamination and false-positive or inconclusive results. Preparation and administration of vaccines in areas separate from pertussis specimen collection areas will 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:

- Wear gloves before and during specimen collection with immediate disposal of gloves after the procedure.
- Routinely clean clinic surfaces using a 10% bleach solution[ii] to reduce the amount of nucleic acids (DNA) in the clinic environment.
- Handle the swab stick with care and only above the indentation which marks where the shaft is snapped off after insertion into the Regan Lowe transport tube.

[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.

[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. Prepare new bleach solution daily.