

# DPHHS HAN ADVISORY

**DATE:** February 16, 2011



**DPHHS Subject Matter Resource:**

For more information regarding this HAN, contact: the DPHHS Communicable Disease and Epidemiology Section at (406) 444-0273

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

**DPHHS Health Alert Hotline:**  
**1-800-701-5769**

**INSTRUCTIONS:**

**FORWARD** to local HAN contacts

**DPHHS HAN Website:**

[www.han.mt.gov](http://www.han.mt.gov)

**INFORMATION / RECOMMENDATIONS:**

Pertussis activity in Montana continues to climb for 2011, with several western counties experiencing higher cases than in previous years. For 2010, over 120 cases were reported compared to just 60 cases reported the previous year.

Pertussis is an endemic (common) disease in the United States, with periodic epidemics every 3 to 5 years and frequent outbreaks. In 2009, nearly 17,000 cases of pertussis were reported nationally - with many more cases going unreported.

Pertussis can be difficult to identify and manage. Other respiratory pathogens often cause clinical symptoms similar to pertussis, and co-circulation with other pathogens (bacterial and viral) does occur. In order to respond appropriately (e.g., provide appropriate antibiotic prophylaxis), it is important to confirm that *B. pertussis* is circulating in the community or institutional setting and to determine whether other pathogens are contributing to the increased activity. Polymerase chain reaction (PCR) tests vary in specificity, so obtaining culture confirmation of pertussis for at least one suspicious case within each cluster of cases is recommended any time there is suspicion of an increasing pertussis activity.

Recommendations for the PCR testing performed at the Montana Public Health Laboratory are attached.

**Categories of Health Alert Messages:**

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

**Health Advisory:** provides important information for a specific incident or situation; may not require immediate action.

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# This is an official CDC HAN Info Service Message

Distributed via the HAN Info Service  
February 16, 2011, 11:30 EST (11:30 AM EST)  
HANINFO-000319-2011-02-16-UPD-N

## 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<sup>1</sup> have been found to contain PCR-detectable *B. pertussis* DNA. Environmental sampling has identified *B. pertussis* DNA from these vaccines in clinic environments.**

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<sup>1</sup> Vaccines shown to contain PCR-detectable DNA include Pentacel<sup>®</sup>, Daptacel<sup>®</sup>, and Adacel<sup>®</sup>. 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.

**While DNA in the vaccines 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.

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|------------------------|--|
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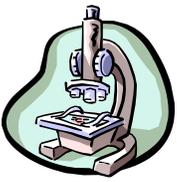
**HAN InfoService** provides general public health information; unlikely to require immediate action.

##This Message was distributed to **State and Local Health Officers, Public Information Officers, Epidemiologists and HAN Coordinators as well as Clinician organizations##**

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You have received this message based upon the information contained within our emergency notification data base. If you have a different or additional e-mail or fax address that you would like us to use please contact your State-based Health Alert Network program at your State or local health department.

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## Pertussis Real-time PCR Testing at the Montana Public Health Laboratory

### What is pertussis Real-time PCR testing?

Real-time pertussis PCR analysis detects specific nucleic acid amplification products as they accumulate in real-time. Real-time PCR uses a fluorescently labeled oligonucleotide probe, which eliminates the need for post-PCR processing. It is capable of screening genetic activity within hours using a minimal amount of sample material, and can detect a single molecule of DNA.

### What about culturing for pertussis?

Culturing for pertussis is not used as frequently with the advent of PCR testing **but should be requested on one or two suspected cases during an outbreak situation.**

### What about DFA testing?

DFA testing is no longer recommended, and no longer available at the MTPHL. PCR is preferred due to its improved specificity and sensitivity.

### What's the cost of pertussis real-time PCR?

The current cost is **\$95.00**. The suggested CPT code is **87798**.

### When is PCR testing indicated?

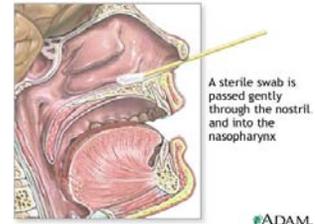
The patient **must be symptomatic** in order for the PCR to be meaningful. PCR testing on asymptomatic persons is not recommended and a positive PCR test on these individuals is not considered a case of pertussis. PCR testing may be able to detect pertussis 3-4 weeks after date of onset. PCR may also be able to detect pertussis after a patient has been started on antibiotic therapy.

### What's the turn around time?

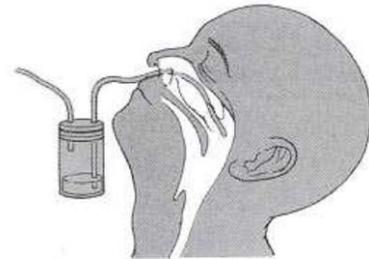
The PCR results can be available the same day as receipt in the laboratory, Monday - Friday. Special arrangements may be made for STAT testing.

**How do I collect a specimen for Pertussis PCR testing?** There are two options for obtaining the necessary columnar epithelial cells.

- ✓ **Nasopharyngeal swab:** **USE a flexible wire small dacron swab.** Bend the flexible wire in a small arc, and insert the swab into the nostril back to the nasopharyngeal cavity. Slowly rotate the swab as it is being withdrawn. Place swab in sterile test tube without transport media. If culture is requested, a second NP swab can be taken and placed in Regan Lowe Transport media for culture.



- ✓ **Nasopharyngeal Wash/Aspirate:** Introduce 1-2 ml in sterile saline into nasopharyngeal cavity, aspirate into a sterile vial. Store in a cold condition until transport.



**Note:** *Nasal or throat swabs are not recommended, and specimens submitted in viral transport media are not acceptable for PCR.*

### How do I transport the specimen to the laboratory?

Ensure specimens are labeled and complete a laboratory request form. Enclose specimen in a biohazard bag and appropriate shipping container. **NP swabs** in sterile tubes and **Regan Lowe Transport** media used for culture can be transported at ambient (room) temperature. **Nasal washes or aspirates** must be transported in a cold condition (blue ice packs in a Styrofoam cooler).

### Who should I contact for testing information?

The direct phone number for the Public Health Laboratory Molecular Department is: **444-5995**. The toll free number for the MTPHL is **1-800-821-7284**.