Standard Operating Procedure for Culture Identification and Differentiation of Bordetella species

Adria D. Lee,Pamela K. Cassiday,Lucia C. Pawloski,Kathleen M. Tatti,Monte D. Martin,Elizabeth C. Briere,M. Lucia Tondella,Stacey W. Martin,The Clinical Validation Study Group

Abstract

The appropriate use of clinically accurate diagnostic tests is essential for the detection of pertussis, a poorly controlled vaccine-preventable disease. The purpose of this study was to estimate the sensitivity and specificity of different diagnostic criteria including culture, multi-target polymerase chain reaction (PCR), anti-pertussis toxin IgG (IgG-PT) serology, and the use of a clinical case definition. An additional objective was to describe the optimal timing of specimen collection for the various tests.

Clinical specimens were collected from patients with cough illness at seven locations across the United States between 2007 and 2011. Nasopharyngeal and blood specimens were collected from each patient during the enrollment visit. Patients who had been coughing for ≤ 2 weeks were asked to return in 2-4 weeks for collection of a second, convalescent blood specimen. Sensitivity and specificity of each diagnostic test were estimated using three methods—pertussis culture as the “gold standard,” composite reference standard analysis (CRS), and latent class analysis (LCA).

Overall, 868 patients were enrolled and 13.6% were *B. pertussis* positive by at least one diagnostic test. In a sample of 545 participants with non-missing data on all four diagnostic criteria, culture was 64.0% sensitive, PCR was 90.6% sensitive, and both were 100% specific by LCA. CRS and LCA methods increased the sensitivity estimates for convalescent serology and the clinical case definition over the culture-based estimates. Culture and PCR were most sensitive when performed during the first two weeks of cough; serology was optimally sensitive after the second week of cough.

Timing of specimen collection in relation to onset of illness should be considered when ordering diagnostic tests for pertussis. Consideration should be given to including IgG-PT serology as a confirmatory test in the Council of State and Territorial Epidemiologists (CSTE) case definition for pertussis.

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Guidelines

Title: Standard Operating Procedure for Culture Identification and Differentiation of *Bordetella* species.

Purpose: To describe the procedures used to identify and distinguish between *B.pertussis, B. parapertussis, B. bronchiseptica,* and *B. holmesii.*

Principle: *Bordetella* species are tiny gram-negative coccobacilli occurring singly or in pairs and may exhibit a bipolar appearance. They are strict aerobes and some members of the genus are motile. *B. pertussis* and *B. parapertussis* are non-motile, and produce no acid from carbohydrates. *B. pertussis* will not grow well on common blood agar bases or chocolate agar, whereas *B. parapertussis* will grow on blood agar and sometimes chocolate. Media for primary isolation consists of charcoal-based medium such as Regal-Lowe (RL) supplemented with glycerol, peptones and horse or sheep blood. The antibiotic agent cephalexin is added to reduce the growth of normal flora. *B. pertussis* may be recovered from secretions collected from posterior nasopharynx, bronchoalveolar lavage, and transbronchial specimens. *Bordetella*-like organisms (BLO) isolated from clinical specimens undergo phenotypic and biochemical observation to determine correct identification. Suspected *Bordetella* species isolates can also be phenotypically and biochemically tested to confirm identification.

Before start

- **Disclaimer:** Names of vendors or manufacturers are provided as examples of suitable product sources; inclusion does not imply endorsement by the Centers for Disease Control and Prevention or the Department of Health and Human Services.

Reagents: Regan-Lowe agar plates with cephalexin (RL+C) and without cephalexin (RL-C); blood agar plates.

Specimen Criteria: Acceptable specimens include isolates and nasopharyngeal aspirates or swabs. Swab should be polyester (such as Dacron), rayon, or nylon. Calcium alginate and cotton swabs are not acceptable. Regan-Lowe transport medium is recommended for specimens. Amies Charcoal transports are acceptable, but may decrease the probability of isolation. If only one swab is collected for both culture and PCR, the swab should be sent in Regan-Lowe transport.

Protocol

Step 1.

Culturing the Specimen/Isolate: Plate the NP specimen/isolate on RL+C and RL-C and streak the plates for isolation. Incubate plates at 37°C for up to 10 days, checking daily.

Step 2.

Phenotypic Differentiation: All phenotypic tests should be run on isolates grown on Regan-Lowe without cephalexin or blood agar plates. Growth should be no more than 3 days old for *B. pertussis* and *B. holmesii* or 1-2 days for *B. parapertussis* and *B. bronchiseptica.*

Step 3.

Identify any BLO on primary culture plates. RL+C will be the plate that is most useful for isolating *B. pertussis*, but the RL-C plate will be useful for isolating *B. holmesii*.

Step 4.
If possible, plate a single BLO colony on RL-C and blood agar. Use the same loop for both plates. Streak the plates for isolation to make sure there are no contaminants present.

Step 5.

Appearance on Regan-Lowe:

• *B. pertussis* appears as small, pearly white colonies on Regan-Lowe agar. Pick colonies and plate them for isolation on a RL-C plate. On this second plate, *B. pertussis* will grow faster and there should be sufficient growth for phenotypic testing after 3 days.

• *B. parapertussis* initially appears similar to *B. pertussis* but grows more quickly, usually within 2-3 days on primary culture. *B. parapertussis* is not inhibited by cephalexin. The colonies will be larger than *B. pertussis* colonies and have a slight brown pigment after 3-4 days.

• *Bordetella bronchiseptica* grows very well on Regan-Lowe agar and blood agar after 1 day of incubation and is not inhibited by cephalexin. The colonies are much larger than *B. pertussis* and usually have a slight brown pigment on Regan-Lowe.

• *B. holmesii* most resembles *B. pertussis* on Regan-Lowe agar – small, white colonies. It usually comes up in 2-3 days. Unlike *B. pertussis* it does grow on blood agar but does not grow well on agar containing cephalexin.

Step 6.

On the primary culture if you see BLO growing on the RL-C plate but not on the RL+C plate the isolate may be *B. holmesii*.

Step 7.

If the BLO grows well on blood agar, *B. pertussis* is almost certainly ruled out.

Step 8.

Gram Stain: Gram stain growth from the blood agar and/or the secondary RL-C plate

• *Bordetella* species are very small Gram negative short rods

• Compare with a Gram stain of a known *B. pertussis* for reference

• If the isolate is Gram positive of any shape, or Gram negative cocci or long rods, discard the isolate

• If the Gram stain is correct, test the isolate by slide agglutination

Step 9.

Slide Agglutination: If the isolate is negative for *B. pertussis* and *B. parapertussis*, set up the biochemical tests (Oxidase, Nitrate reduction, Urease, Motility, HIT agar slant, MacConkey, and Citrate) on growth from the blood agar plate (BAP).

Step 10.
Incubate biochemical test at 37°C for 24 hours.

Step 11.

Typical biochemical results for the four species: *B. pertussis* (no BAP growth, Oxidase+, Nitrate−, Urease−, Motility−, HIT- nopigment, MacConkey- no growth or fermentation, Citrate- no growth or color change); *B. parapertussis* (BAP growth, Oxidase−, Nitrate−, Urease+, Motility−, HIT- brown, MacConkey- growth/no fermentation, Citrate- growth/no color change); *B. bronchiseptica* (BAP growth, Oxidase+, Nitrate+, Urease+, Motility+, HIT- no pigment, MacConkey- growth/no fermentation, Citrate- growth/blue); *B. holmesii* (BAP growth, Oxidase−, Nitrate−, Urease−, Motility−, HIT-brown, MacConkey- growth/no fermentation, Citrate- growth/no color change).

Step 12.
Reference Values: Healthy, unexposed population is expected to be “negative”.

Step 13.

Limitations: Only acceptable specimens and suspected isolates of *B. pertussis*, *B. parapertussis*, *B.holmesii* and *B. bronchiseptica* should be tested with these protocols. Prior antibiotic treatment and/or vaccination with an anti-pertussis vaccine may interfere with recovery of *B. pertussis* from properly collected specimens. Failure to culture an isolate of *B. pertussis* does not necessarily mean the patient does not have pertussis.

Step 14.

Results and Interpretation: *B. pertussis* identification is confirmed on the basis of *B.pertussis* positive slide agglutination, morphological, and biochemical results. *B. parapertussis* identification is confirmed on the basis of *B. parapertussis* positive slide agglutination, morphological, and biochemical results. *B. bronchiseptica* will be positive for oxidase, nitrate reduction, urease, motility and citrate, but does not produce a brown pigment on HIT agar. *B. holmesii* will be negative for oxidase, nitrate, urease, and motility but does produce a brown pigment on HIT agar.

Step 15.
Test Failure: If any phenotypic or biochemical test fails to create a definitive result, repeat that particular step or test. If you are still unable to get a definite result, consult supervisor for further action.