# Standard Operating Procedure for Culturing Bordetella species from Nasopharyngeal Specimens

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## 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.

**Citation:** 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 Standard Operating Procedure for Culturing Bordetella species from Nasopharyngeal Specimens. **protocols.io**

dx.doi.org/10.17504/protocols.io.kq9cvz6

**Published:** 20 Nov 2017

## Guidelines

Title: Standard Operating Procedure for Culturing *Bordetella* species from Nasopharyngeal Specimens.

Purpose: To describe the procedures used to culture *Bordetella* species from nasopharyngeal specimens including nasopharyngeal aspirates and swabs.

Principle: Regan-Lowe (RL-C) Charcoal Agar plates are used in clinical laboratories for the isolation of *Bordetella pertussis*, the etiologic agent of whooping cough, from nasopharyngeal swabs and other sources of pharyngeal exudate. This medium was developed by Regan and Lowe as a transport medium for whooping cough specimens, but proved useful as an enrichment medium for the selective isolation of *B. pertussis* and *B. parapertussis*. It consists of charcoal agar as a basal medium supplemented with cephalexin (RL+C) to inhibit bacteria indigenous to the nasopharynx and defibrinated horse blood to support the growth of *Bordetella* species. Use of the medium without cephalexin in parallel with Regan-Lowe Charcoal Agar is recommended, since a few strains (<10%) of *B. pertussis* will not grow on selective plates; also the nonselective medium is used for subcultures to obtain a larger amount of growth for additional testing, such as agglutination or biochemical testing.

## 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 with Cephalexin (RL+C) and without Cephalexin (RL-C).

Equipment: Biological Safety Cabinet (BSC), 37°C Incubator, Pipette

Supplies: Inoculating Loop

Quality Control: Quality control is performed on Regan-Lowe with Cephalexin (RL+C) and Regan-Lowe without Cephalexin (RL-C) prior to use of media for diagnostic testing. QC organisms consist of *Bordetella pertussis* ATCC 12742 (positive control) and *Staphyloccus aureus* ATCC 29213 (negative

control)**.**

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.**
Split Preparation Procedure (if only one swab is collected for both culture and PCR): Use sterilized tweezers to hold the shaft of the swab and remove the swab from the transport media. Submerge the swab tip in 500µl of 0.85% sterile saline and cut the shaft of the swab above the top of the centrifuge tube using sterilized scissors. Cap the micro-centrifuge tube.

**Step 2.**
Vortex tube and incubate 2 minutes at room temperature.

**Step 3.**
Quick spin tube.

**Step 4.**
Remove 100µl for culture plating onto Regan-Lowe with and without cephalexin agar (50µl per plate).

**Step 5.**
Remove 400µl for PCR testing (200µl for extraction) and store remaining volume at -80°C.

**Step 6.**
Using either a pipette, or, if the specimen is really viscous, a transfer pipette, inoculate both a RL+C and RL-C agar plate with 50ul of nasopharyngeal specimen.

**Step 7.**
Using a sterile loop streak the inoculum in the first quadrant of the plate until all the liquid has been absorbed.

**Step 8.**
Incubate at 37°C with high humidity for 7-10 days.

**Step 9.**
Examine plates daily with a dissecting microscope and oblique illumination. Small, compact, glistening colonies are characteristic of Bordetella pertussis. Use a quality control strain for visual comparison. Typical colonies rarely are visible before 48-72 hours incubation.

**Step 10.**
Pick isolated colonies of “Bordetella-like organisms” (BLO) and streak on RL-C and blood agar. Incubate 1-3 days until there is sufficient growth for phenotypic testing.