Standard Operating Procedure for the Detection of Human, IgG Anti-Pertussis Toxin

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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.

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Guidelines

Title: Standard Operating Procedure for the Detection of Human, IgG Anti-Pertussis Toxin.

Purpose: To describe the procedures used to estimate IgG antibodies against Pertussis Toxin (PT) in human serum by the Human, IgG Anti-Pertussis Toxin ELISA.

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: 96 well microtiter strip assembly; Pertussis toxin (PT) antigen, in 50% (v/v) glycerol; Coating buffer, (0.05 M Carbonate – bicarbonate buffer, pH 9.6); IgG anti-PT standards (Standard) (lyophilized) A – F (15, 30, 60, 120, 240 & 480 International Units (IU)/mL); IgG anti-PT controls I (negative), II (26-68 IU/mL) & III (56-134 IU/mL) (Kit controls) (lyophilized); Assay buffer, (Phosphate buffer saline containing 4% BSA & 0.05% Tween – 20); Standard & Kit control diluent (Deionized water containing 0.05% Tween–20); 10X Wash solution concentrate, (10X PBS containing 0.5% Tween–20); Peroxidase labeled mouse monoclonal antihuman IgG conjugate (Conjugate) (Provided as a 1:5,000 dilution in 50% glycerol); TMB substrate, (3,3’,5,5’ tetramethylbenzidine in mildly acidic buffer); and, Stop solution, (1 N Hydrochloric acid).

Equipment/Materials: Adhesive microtiter plate / strip sealers (plate sealer); Disposable reagent reservoirs; Decapper; Deionized water; Microtiter plate or strip washer; Microtiter reader equipped to read absorbance at 450 nm; Software with the capability to use the four-parameter logistic (4PL) function for the analysis of absorbance data from a 96-well plate layout; Single and multichannel micropipettes with appropriate tips; Repeater pipettes with appropriate tips; Class A glassware; graduated cylinders, and beakers; Opaque box, slightly larger than microtiter plate / strip assembly; Thermometer; Timer; Vortex mixer; 50mL Conical tubes; 5mL Test tubes; and, Reservoir basins.

Protocol

Step 1.
Day 1 Plate Preparation: Allow Coating Buffer and PT antigen to reach room temperature (18-26°C).

Step 2.
Prepare coating solution: Add PT antigen to Coating buffer and gently vortex to mix.

Step 3.
Dispense 100 µL of Coating solution into each well of the microtiter strip assembly.

Step 4.
Cover the microtiter strip assembly with a plate sealer, and incubate overnight (14 to 24 hours) at 2-8°C.

Step 5.
Day 2: Allow the Standards, Kit controls, samples, Assay buffer, Standard & Kit control diluent, Wash solution concentrate and PT coated microtiter strips to come to room temperature (18-26°C).

Step 6.

Dilute the Wash solution concentrate with de-ionized water in the microtiter plate / strip washer reservoir. Mix gently.

Step 7.
Prepare serum samples: Mix 20 µL of serum to 1980 µL of Assay buffer. Gently vortex each serum sample prior to use.

Step 8.
Reconstitute IgG anti-PT standards by adding 1 mL of Standard & Kit control diluent into each vial and gently vortexing.

Step 9.
Reconstitute IgG anti-PT controls by adding 1 mL of Standard & Kit control diluent to each vial and gently vortexing.

Step 10.
Wash the coated microtiter strips (coated on the previous day) three times with 300 µL of Washsolution per well.

Step 11.
Pipette, in triplicate, 100 µL of Standards, Kit controls and serum samples into wells. To minimize assay drift within the plate, all standards, controls, and test samples should be dispensed into the wells within 15 minutes.

Step 12.
Cover the microtiter plate with a plate sealer and incubate for 2 hours ± 5 minutes at room temperature (18-26°C).

Step 13.
Prepare conjugate by carefully diluting room temperature Peroxidase labeled mouse monoclonal anti-human IgG conjugate (1:5000 dilution in 50% glycerol) with Assay buffer. Gently vortex to mix.

Step 14.
After completion of incubation period, wash microtiter strips three times with 300 µL of Wash solution per well.

Step 15.
Pipette 100 µL of Conjugate solution into all coated wells.

Step 16.
Seal plate and incubate 2 hours ± 5 minutes at room temperature (18-26°C).

Step 17.
Bring TMB substrate and Stop solutions to room temperature (18-26°C).

Step 18.
After completion of incubation period, wash microtiter strips three times with 300 µL of Wash solution

per well.

Step 19.
Pipette 100 µL of TMB substrate solution into all coated wells.

Step 20.
Incubate 9 - 11 minutes at room temperature (18-26°C). Avoid direct exposure to light during incubation.

Step 21.
Pipette 100 µL of Stop Solution into all coated wells.

Step 22.
Measure the absorbance at 450 nm within 30 minutes of adding Stop solution.

Step 23.
Calculations: For quantitative estimation, average optical densities of each standard is plotted against itsConcentration. Any automatic data processing software having four-parameter logistic (4PL) function can be used. The reported sample value also known as concentration will be the average of the triplicate wells.

Step 24.
Assay Repeat Criteria: If average OD of the Assay buffer wells is greater than 0.200; If average OD of Standard F (480 IU/mL) is less than 1.200 or greater than 3.000; If average OD of Kit control I (negative control) is greater than 0.400; If calculated value of either Kit control II or III is outside the provisional 3SD range for that control; If CV% for the mean OD of Standards A-F is greater than 15%; If correlation coefficient [r2] is less than 0.990; If CV% for the reported value (IU/mL) for Kit controls II and III is greater than 20%.

Step 25.
Sample Repeat Criteria: A serum sample should be retested if CV% of mean reported value (IU/mL) is greater than 25%.

Step 26.
Interpretation/Results: The final concentration (IU/mL) that is interpreted for each test sample is based on the average of two valid values calculated from two valid assays run on two separate days.

Step 27.
For samples with values < 15 IU/mL, if the final concentration cannot be attained from the average of two valid values due to CV%s > 25%, after three attempts, calculate the average of the three values and make note of the calculation on the assay worksheets and results. Interpretation of the final concentration is based on putative diagnostic cut-off points (Baughman et al, 2004). If the calculated concentration of the test sample is less than 49 IU/mL, the result is reported as “negative”. If the concentration is 49-93 IU/mL, the result is reported as “indeterminate”. If the concentration is ≥ 94 IU/mL, the results is reported as “positive”.