PROVIDENCE
Sacred Heart
Medical Center &
Children's Hospital

Department of Microbiology
Bordetella pertussis
Fluorescent Antibody Stain Procedure

I. Principle and Clinical Significance

Fluorescent antibody (FA) procedures are useful in the diagnosis of whooping cough. FA staining methods shorten the length of time required for a laboratory diagnosis. A direct test is performed on smears made from nasopharyngeal swabs to make a presumptive identification of *Bordetella pertussis*. A negative result should not be considered conclusive since false negative results may occur if the number of organisms in the specimen is low. Testing for *B. parapertussis* is not performed at SHMC because no positive FA specimens were detected over a 15 year period.

II. Reagents

A. Difco™ *Bordetella pertussis* Antigen
B. Difco™ FA *Bordetella pertussis* (conjugate)
C. Gull Mounting Fluid

III. Reagent Preparation

A. Antigen control slides:
   1. Dilute the antigen stock 1:80 with sterile distilled H_2O
   2. Put one drop in a circumscribed circle (7-10 mm diameter) on a clean microscope slide or premade circled slide, and spread evenly.
   3. Air dry.
   4. Gently heat fix.
   5. Store in a slide box at -20°C in the almond freezer. Mark the lot number, date made and expiration date of the antigen on the slide box.

B. FA *Bordetella pertussis*
   1. Rehydrate the conjugate with 5 mL distilled water. Rotate the vial gently to dissolve the components completely.
   2. Titrate the FA *Bordetella pertussis* conjugate using the *Bordetella pertussis* antigen slides.

Example:

Dilution of Conjugate	Fluorescence
1:2	4+
1:4	4+
1:8	4+
1:16	4+
1:32	2+

In this example, the last 4+ fluorescence is in the 1:16 dilution. One two-fold dilution less is selected for the working dilution to insure optimal fluorescence. Therefore, the working dilution is 1:8 in this case.

3. Dilute the 5 mL rehydrated conjugate from step 2 to the optimal titrated working dilution with distilled water. This is now the working dilution.
4. Aliquot the working dilution of FA B*. pertussis* conjugate into 0.5 ml quantities in 1.0-mL cryotubes.
5. Write the lot #, date aliquoted, and the expiration date on the storage boxes.
6. Store at -20^{o}C.

C. Mounting Fluid:
1. Gull Mounting Fluid is a standardized reagent grade glycerin adjusted to pH 7.2.
2. Store at room temperature.
3. Shelf life: stable until expiration date.

IV. Methods

A. Specimen Collection:

A small dacron swab on a fine, flexible shaft is used to sample the nasopharynx. With the patient's head immobilized, the swab is gently inserted into the nostril until it reaches the posterior nasopharynx and is left in place for 15-30 sec. The tickling sensation of the swab usually induces a cough.

B. Specimen preparation:
1. Smear the nasopharyngeal swab specimen onto two slides within a circumscribed area approximately 1 cm in diameter. Duplicate slides are made in case repeat FA or Gram Stain or both are needed.
2. Allow the smears to air dry, and then fix them by heating gently.
3. Outpatient specimens usually include two slides. These slides only need gentle heat fixation prior to staining.

C. Controls: (Performed each day of testing)
1. Positive: *B. pertussis* antigen control slide with FA *B. pertussis* conjugate.
2. Negative: Slide containing *E. coli* organisms stained with FA *B. pertussis* conjugate.

D. Staining Procedure:
1. Remove a vial of the working FA conjugate from the freezer, and thaw.
2. Apply several drops of the FA Bordetella *pertussis* conjugate onto the prepared slides.
3. Spread the conjugate over the surface of the smears using a wooden applicator stick.
4. Place the slides in a moist chamber.
5. Incubate 30 min at room temperature in a moist chamber protected from light.
6. Gently rinse off the excess conjugate with distilled water.
7. Air dry.
8. Add a small drop of FA Mounting Fluid to the center of the stained area, and cover with a cover slip.
9. Examine at least 50 oil immersion fields in each smear using the

fluorescent microscope.

V. Results

A. Carefully examine the patient smears for very small coccobacilli occurring singly, in pairs or occasionally in small groups.
NOTE: With Difco conjugate, *B. pertussis* cells are a bright fluorescent yellow-green with a clear-cut periphery and a non-staining or faint staining center. They cells appear like “doughnuts”.

B. Note also the cellular elements present on the slide. Good quality specimens should contain columnar epithelial cells.

VI. Interpretation

A. Positive:
The specimen contains organisms that fluoresce brightly with the *B. pertussis* conjugate and have morphology consistent with *B. pertussis.*

B. Negative:
1. The specimen contains no fluorescent organisms.
2. The specimen contains organisms that fluoresce with the *B. pertussis* conjugate but do not have the correct morphology. Questionable morphology may be resolved by Gram staining one of the extra smears. Gram negative diplococci, gram positive cocci and diphtheroid-like rods may show nonspecific fluorescence. These slides should be reviewed if the results are questionable.

C. Controls:
1. *B. pertussis* Ag with *B. pertussis* conjugate must show 3-4+ fluorescence.
2. *E. coli* with *B. pertussis* conjugate must be negative for fluorescence.
3. If the controls do not perform as indicated, the test results must be repeated and cannot be reported.

D. Record all of the results in the FA notebook, and enter the QC results into the computer.

VII. Reports

A. Negative: Report "No *Bordetella pertussis* seen by direct fluorescent antibody stain".

B. Positive: Quantitate and report: "*Bordetella pertussis* seen by direct fluorescent antibody stain".

C. If there are rare to no cellular elements evident on the slide preparation, report the comment: "Poor quality specimen. Suggest recollection."

D. All positive smears should be called to the physician, epidemiology and the county health department.

E. Attach the comment code “PRTCOM” which states: THE CDC RECOGNIZES ONLY PCR AND CULTURE AS CASE-DEFINING LAB TESTS FOR PERTUSSIS, NOT THE DFA TEST. THE DFA FOR PERTUSSIS IS USEFUL TO IDENTIFY PROBABLE CASES.

VIII. Limitations of the Procedure

A. The fluorescent antibody technique provides only presumptive identification of *Bordetella pertussis*. A negative result does not rule out whooping cough if relatively few organisms are be present due to the stage of the disease or prior antibiotic therapy.
B. Since all outbreaks in the Northwest have been caused by *B. pertussis* and not B*. parapertussis*, this test only aids in the diagnosis of *B. pertussis* infections.
C. The FA method can produce false negative and false positive results. However, since clinical material for culture is often inadequate, local pediatricians have requested an alternative method to culture that might provide data relevant for the diagnosis of pertussis in cases with a poor clinical response.

IX. Summary

1. NP swab specimen should be used.
2. Smears are made on two to four microscope slides.
3. Air dry, and gently heat fix.
4. Add appropriate conjugate to each of the specimens and control smears:
   a. Pos. Cont: *B. pertussis* Ag + FA *B. pertussis* conjugate
   b. Neg. Cont: *E. coli* + FA *B. pertussis* conjugate
   c. Specimen: Add FA *B. pertussis* conjugate
5. Spread the conjugate over the smear.
6. Incubate for 30 min at room temperature in a moist chamber in the dark.
7. Rinse off excess conjugate with distilled water.
8. Air dry.
9. Coverslip with FA mounting fluid.
10. Examine using the 100X oil objective.

X. References

1. Fluorescent Antibody Techniques and Bacterial Applications, U.S. Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, Atlanta, Georgia. HEW Publication No. (CDC) No. 78-8364.
2. Murray, P.R., Baron, E.J., Pfaller, M.A., Tennover, F.C.,Yolken, R.H., Manual of Clinical Microbiology, Seventh Edition. 1999, pp. 615-616.

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calcium alginate to Dacron. 06/07/2011 Removed viral transport medium as acceptable.