INTENDED USE

Accu-Mab™ Plus Bordetella pertussis and parapertussis DFA test is a monoclonal antibody-based reagent intended for use in the identification and differentiation of Bordetella pertussis and Bordetella parapertussis by the direct fluorescent antibody (DFA) technique.

Bordetella pertussis DFA and Bordetella parapertussis DFA are supplied in two separate vials.

SUMMARY AND EXPLANATION

Pertussis, also known as whooping cough, is a highly contagious bacterial disease caused by Bordetella pertussis. Bordetella parapertussis is associated with a milder form of the disease. Worldwide, whooping cough affects nearly 295,000 deaths. Despite generally high coverage with DTP and DTaP vaccines, pertussis is one of the leading causes of vaccine-preventable deaths worldwide, with 90% of all cases occurring in developing countries. Pertussis is the only vaccine-preventable disease that is associated with increasing deaths in the U.S. Bordetella pertussis is a very small Gram-negative aerobic coccobacillus that appears singly or in pairs. Its metabolism is respiratory and never produces acid from carbohydrates. The bacteria are nutritionally fastidious and are usually cultivated in rich media supplemented with blood. The organism grows slowly and requires 3-6 days to form pinpoint colonies. Using DFA technique, the required to detect this organism can be greatly reduced.

The direct fluorescent antibody (DFA) technique has been used for many years for the rapid direct detection of B. pertussis and B. parapertussis. The use of polyclonal antibody-based DFA reagent has been considered lacking in specificity because of cross reactions with normal nasopharyngeal flora. The use of monoclonal antibody-based DFA, like Accu-Mab Plus, shows greater specificity.6,7

PRINCIPLE

Accu-Mab™ Plus Bordetella pertussis / parapertussis DFA reagents employ a fluorescein conjugated murine monoclonal antibody which is highly specific for the LOS of B. pertussis, and a rhodamine conjugated murine monoclonal antibody which is highly specific for the LPS of B. parapertussis. The specificity of the monoclonal antibodies is due in large part to the uniqueness of the respective polysaccharide structures found in the LOS of B. pertussis and the LPS of B. parapertussis.8-10 Both monoclonal antibodies are affinity purified to ensure high specificity and low background. The use of a fluorescein conjugated antibody is specifically designed for fluorescence microscopy. It is beyond the scope of this bulletin to try to identify all the organisms of interest.

REAGENTS SUPPLIED

1. Bordetella pertussis DFA (reagent A): 1 ml liquid (40 tests) in an amber glass vial. The reagent contains approximately 25 µg/ml of fluorescein-conjugated anti-pertussis monoclonal antibody with 0.1% (w/v) sodium azide as a preservative.

2. Bordetella parapertussis DFA (reagent B): 1 ml liquid (40 tests) in an amber glass vial. The reagent contains approximately 1.75 µg/ml of rhodamine-conjugated anti-parapertussis monoclonal antibody with 0.1% (w/v) sodium azide as a preservative.

ADDITIONAL MATERIALS AND REAGENTS REQUIRED BUT NOT SUPPLIED

The following are not included in the kit and will need to be prepared from standard laboratory grade chemicals (see “Preparing Additional Reagents”):

1. Wash Buffer: Phosphate buffered saline (10 mM sodium phosphate, 150 mM sodium chloride) pH 7.2 to 7.4

2. Mounting Fluid: Prepared by mixing 9 parts reagent grade glycerol (glycol) to 1 part wash buffer. A commercially available glycerol-based mounting fluid may be used.

3. Immersion Oil: Always use a high quality immersion of specifically designed for fluorescence microscopy. Standard immersion oils may exhibit their own fluorescence and interfere with viewing.


5. Negative Controls (optional): Known culture isolates of a Bordetella species other than B. pertussis, B. parapertussis, or B. bronchiseptica.

6. Staining Jars: To avoid cross contamination between positive control slides and clinical specimens during staining, separate staining jars must be used for:
   a. clinical specimen slides for Reagent A
   b. pertussis positive control slides
   c. clinical specimen slides for Reagent B
   d. parapertussis positive control slides

7. Miscellaneous: Glass microscope slides, cover slips, 25µl pipet, moisture chamber.

WARNING AND PRECAUTIONS

1. This product is FOR IN VITRO DIAGNOSTIC USE and must only be used by qualified and trained laboratory personnel.

2. The reagent contains 0.1% Sodium azide as an antimicrobial agent. Sodium azide is poisonous and is an irritant to skin, eyes and mucous membranes. Although not hazardous at this concentration, care should be taken to avoid contact with skin and eyes. Protective gloves and splash-proof glasses/goggles should be worn when using reagent containing sodium azide. If splashed on skin or in eyes, flush thoroughly with water and seek medical attention if irritation persists. The user of this reagent must be familiar with DFA techniques, the limitations of such techniques and be trained in the use of all equipment required including the use of a fluorescence microscope. It is beyond the scope of this bulletin to address all of the laboratory skills required to successfully use this reagent (see “Limitations of the DFA Technique”).

STORAGE AND REAGENT STABILITY

1. The reagent should be stored at 2°C to 8°C.

2. The reagent is stable to the expiry date printed on the vial label when stored properly at 2°C to 8°C.

3. A visual examination of the vial for turbidity (i.e. bacterial growth) and mold growth must be made prior to each use. The reagent is considered hazardous at this concentration, care should be taken to avoid contact with skin and eyes. Protective gloves must be worn by all laboratory personnel. The user of this reagent must be familiar with DFA techniques, the limitations of such techniques and be trained in the use of all equipment required including the use of a fluorescence microscope. It is beyond the scope of this bulletin to address all of the laboratory skills required to successfully use this reagent (see “Limitations of the DFA Technique”).

SPECIMEN PREPARATION

The DFA reagent is suitable for use on either direct nasopharyngeal smears or on smears prepared from cultures of B. pertussis or B. parapertussis.

Nasopharyngeal Smear Preparation

1. Obtain a nasopharyngeal sample using a dry swab. Store at 2°C to 8°C.

2. Using an inoculation loop pick a SMALL amount of a colony and place in the Wash Buffer. Spread the emulsified material on a clean glass microscope slide.

3. Using a coverslip, press gently to flatten the smear. Fix by flooding the smear with 95% ethanol for at least 1 minute. Drain the ethanol and let air dry at room temperature. Do not heat fix.

Culture Isolates

1. On a clean microscope slide place a large drop of Wash Buffer in the desired smear area.

2. Using an inoculation loop pick a SMALL amount of a colony from a culture plate and place in the Wash Buffer. Spread the emulsified material on a clean glass microscope slide.

3. Fix smears by flooding the slide with 95% ethanol for at least 1 minute. Drain the ethanol and let air dry at room temperature. Do not heat fix.

TEST PROCEDURE

For Bordetella pertussis identification:

1. Stain the clinical specimen slide and the positive control slide at the same time, but in separate staining jars.

2. Add 25 µl Bordetella pertussis DFA (reagent A) to the fixed specimen smear labeled "A." Spread the reagent evenly over the surface.

3. Place a slide in a moisture chamber for 30 minutes at room temperature (DO NOT ALLOW THE REAGENT TO DRY ON THE SLIDE OR RESULTS WILL NOT BE ACCURATE).

4. Place a slide in a staining jar containing Wash Buffer for 5 minutes (B. pertussis positive control slides should be washed in a separate staining jar). Repeat with 2 changes of fresh Wash Buffer. The two wash steps may be increased to 10 minutes each if background fluorescence is found to be too high.

5. Dip slide in fresh distilled or purified water for 1 minute.

6. Remove the slide and allow to drain and air dry. Excess water may be blotted from slide with a lint free tissue before air drying.

7. Add a small drop of Mounting Fluid to the centre of the smear and cover with a glass cover.

For Bordetella parapertussis identification:

1. Stain the clinical specimen slide and the positive control slide at the same time, but in separate staining jars.

2. Add 25 µl Bordetella parapertussis DFA (reagent B) to the fixed specimen smear labeled "B." Spread the reagent evenly over the surface.

3. Place a slide in a moisture chamber for 30 minutes at room temperature (DO NOT ALLOW THE REAGENT TO DRY ON THE SLIDE OR RESULTS WILL NOT BE ACCURATE).

4. Place a slide in a staining jar containing Wash Buffer for 5 minutes (B. parapertussis positive control slides should be washed in a separate staining jar). Repeat with 2 changes of fresh Wash Buffer. The two wash steps may be increased to 10 minutes each if background fluorescence is found to be too high.

5. Dip slide in fresh distilled or purified water for 1 minute.

6. Remove the slide and allow to drain and air dry. Excess water may be blotted from slide with a lint free tissue before air drying.
7. Add a small drop of Mounting Fluid to the centre of the smear and cover with a cover glass.

QUALITY CONTROL
At the time of use, test both positive and negative controls to check the performance of the reagent and technique.

Positive Control: Using known culture isolates of B. pertussis and B. parapertussis, prepare separate control smears of each according to the Specimen Preparation directions for cultures isolated above. Positive controls must be run with each set of samples. IMPORTANT: B. pertussis strain ATCC 12742 and B. parapertussis strain ATCC 15237 are both mutant strains lacking wild-type LOS/LPS structures and must NOT be used as positive controls.

Negative Control: If a negative control is desired, prepare a smear in the same manner as the positive control but using a Bordetella species OTHER THAN B. pertussis, B. parapertussis, or B. bronchiseptica. The DFA reagent may produce a positive result with some strains of the animal pathogen B. bronchiseptica (see Limitations of the DFA Technique).

INTERPRETATION OF RESULTS
Examination of the slides requires the use of a fluorescence microscope equipped with appropriate filters for viewing both fluorescent and rhodamine stained specimens (see “Microscope Requirements”). The user must also be familiar with the morphology of B. pertussis and B. parapertussis in order to be able to distinguish cells from possible fluorescent debris and background which may be visible on some smears.

Fluorescence should be graded in the following manner:

4+ Bright fluorescence with a clear-cut cell outline and sharply defined centre.
3+ Less brilliant fluorescence but still has a clear-cut cell outline and sharply defined centre.
2+ Definite but dim fluorescence with cell outline less well defined.
1+ Very dim fluorescence with cell outline indistinguishable from cell background.

Fluorescence should be graded when each field is first viewed since the fluorescent dyes, especially fluorescein, may fade rapidly after exposure to the excitation beam of the microscope.

Identification of B. pertussis
1. Examine the B. pertussis positive control smear with a fluorescence microscope using 1000x to 1500x magnification and an appropriate filter for fluorescein. The positive control should yield a green 4+ fluorescence for results to be considered valid.
2. Examine the negative control. No green fluorescent cells should be seen (maximum 1+).
3. Examine each specimen test slide (labelled as “A”) and grade the green fluorescence 1+ to 4+. Any cells with correct morphology and fluorescence of 2+ or greater may be considered a presumptive positive for B. pertussis.

Identification of B. parapertussis
1. Examine the B. parapertussis positive control smear with a fluorescence microscope using 1000x to 1500x magnification and an appropriate filter for rhodamine. The positive control should yield an orange-red 4+ fluorescence for results to be considered valid.
2. Examine the negative control. No orange-red fluorescent cells should be seen (maximum 1+).
3. Examine each test slide (labelled as “B”) and grade the orange-red fluorescence 1+ to 4+. Any cells with correct morphology and fluorescence of 2+ or greater may be considered a presumptive positive for B. parapertussis.

LIMITATIONS OF THE PROCEDURE
1. This DFA technique can provide only PRESUMPTIVE identification of B. pertussis and B. parapertussis. Culture, morphology, and serological characteristics must be considered in conjunction with the DFA results before a final identification can be made.
2. IMPORTANT: A NEGATIVE DFA result must NOT be considered conclusive since the presence of a very low number of organisms in the specimen may not be detected by routine microscopic examination.
3. This reagent is known to cross react with some strains of the animal pathogen Bordetella bronchiseptica. B. bronchiseptica may occasionally infect humans.

PREPARING ADDITIONAL REAGENTS
Wash Buffer
(Phosphate Buffered Saline pH 7.2 to 7.4)
All chemicals should be ACS grade or better. To make approximately 1.4 litres of PBS prepare the following:

1. Monobasic solution: In a 1 litre beaker dissolve 0.690g of sodium phosphate monobasic monohydrate (NaH2PO4·H2O) and 4.35g of sodium chloride (NaCl) in 500ml distilled water.
2. Dibasic solution: In a 2 litre beaker dissolve 1.420g of arabinose sodium phosphate dibasic (Na2HPO4·H2O) and 8.76g of sodium chloride (NaCl) in 1 litre of distilled or CAP/ASTM Type 1 water.
3. Place a pH meter probe into the dibasic solution. While gently stirring the dibasic solution slowly add the monobasic solution to the dibasic solution until a pH of 7.2 to 7.4 is reached. A small amount of monobasic solution will be left over and can be discarded.

Mounting Fluid
Mounting fluid can be prepared by mixing 0.5ml of Wash Buffer (PBS, pH 7.2 to 7.4) with 4.5ml reagent grade glycerin (glycerol).

Mounting fluid prepared in this manner can be stored at room temperature until needed. Do not use if visible signs (turbidity) of bacterial or mold growth are observed.

MICROSCOPE REQUIREMENTS
An epifluorescence microscope is required for the use of this DFA reagent. The correct selection of fluorescence filters and objective lenses along with proper epifluorescence illuminator alignment are all important in achieving adequate image intensity for fluorescent staining techniques. Due to the wide variety of microscopes and almost infinite combinations of filters and objectives, it is not possible to make specific hardware recommendations for the use of this DFA reagent. Critical microscope specifications are:

Fluorescence Filter Selection
Fluorescence filters are required for viewing both fluorescein and rhodamine stained specimens. The most common configuration consists of two separate filter sets which are used alternately to view slides for fluorescein and then rhodamine fluorescence.

An alternative configuration consists of a dual fluorescence filter set for simultaneous viewing of fluorescein and rhodamine stained specimens. Most dual fluorescence filters suffer from reduced image intensity compared with single filter sets.

IT IS CRITICAL that the filters selected are compatible with the fluorescein and rhodamine spectra shown below:

**Fluorescein (B. pertussis)**
Exciitation & Emission Spectra
Excitation $\lambda_{max} = 491nm$  
Emission $\lambda_{max} = 518nm$

**Rhodamine (B. parapertussis)**
Exciitation & Emission Spectra
Excitation $\lambda_{max} = 541nm$  
Emission $\lambda_{max} = 572nm$

**Objective Lens and Eyepie**
A 100x oil immersion objective and a 10x to 15x eyepiece are required for viewing stained smears (total magnification 1000x to 1500x). Optimum image quality and fluorescent intensity is best achieved using an objective specifically designed for fluorescence microscopy with the highest numerical aperture available.

**Illuminator**
Correct focus and alignment of the epifluorescent illuminator along with adequate bulb output (50W minimum mercury bulb recommended) are important in achieving optimum image quality. See your microscope manual for instructions.

REFERENCES