**INTRODUCTION**

**Accu-Mab™ Plus Bordetella pertussis / parapertussis DFA**

The reagent employs 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*.
- Both monoclonal antibodies are affinity purified to ensure specificity and low background.

Using the DFA technique, the reagent will impart a bright green fluorescence to *B. pertussis* and a brilliant orange-red fluorescence to *B. parapertussis*.

**REAGENT FORM AND STABILITY**

The reagent is supplied in liquid form in an amber glass serum vial. The formulation contains both antibody conjugates in a solution of phosphate buffered saline (50mM sodium phosphate, 150mM sodium chloride) pH 7.2 with 1% bovine serum albumin and 0.1% sodium azide as an antimicrobial agent. The reagent is stable to the expiry date indicated when stored at 2°C to 8°C.

**LIMITATIONS OF THE DFA TECHNIQUE**

The DFA technique can provide only PREDICTIVE identification of *B. pertussis* and *B. parapertussis*. Culture, morphological and serological characteristics must be considered in conjunction with the DFA results before final identification is made.

**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.

This DFA reagent is known to cross react with some strains of the animal pathogen *Bordetella bronchiseptica*. *B. bronchiseptica* may occasion ally infect humans.

**SAFETY AND USE PRECAUTIONS**

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

**CAUTION**

- 0.1% Sodium azide has been added to the reagent as an antimicrobial agent.
- Sodium azide is poisonous and is an irritant to skin, eyes and mucous membranes. Although not considered hazardous at this concentration, it 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.
- Flushed thoroughly with water and seek medical attention if irritation persists.

**PROCEDURE**

**Product**

The product is to be used by trained laboratory personnel only. 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 these instructions to address all of the laboratory skills required to successfully use this reagent (see “Limitations of the DFA Technique”).

**Materials Provided**

Each vial contains 1 ml of reagent which is sufficient for staining 40 smears. The reagent contains 25 µg/ml of the fluorescein conjugated anti-pertussis monoclonal antibody and 1.75 µg/ml of the rhodamine conjugated anti-parapertussis monoclonal antibody.

**Additional Materials Required**

- Wash Buffer: Phosphate buffered saline (10mM sodium phosphate, 150mM sodium chloride) pH 7.2 to 7.4
- Mounting Fluid: Prepared by mixing 9 parts reagent grade glycerol (gllycer) to 1 part wash buffer.
- Cover slips
- Microscope slide, 25µl pipet, moisture chamber

**Specimen Preparation**

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

**Cultural 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 emulsify thoroughly in the drop of Wash Buffer. Spread the emulsified material to form a very THIN uniform smear. It is VERY IMPORTANT that culture smears not be overloaded with organisms. An ideal smear will contain between 10 and 100 organisms per microscope field.
3. Allow smear to air dry and fix by flooding the smear with 95% ethanol and allowing the ethanol to evaporate at room temperature.

**Controls**

**Positive Control**

- Using known cultural isolates of *B. pertussis* and *B. parapertussis*, prepare separate control smears of each according to the Specimen Preparation directions for cultural isolates above. Positive controls must be run with each set of samples.

**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).

**DFA Reagent Handling**

**NOTE**

The reagent and stained slides must be handled in reduced light to minimize photodegradation of the fluorescent dyes.

**To open**

Carefully tear off the aluminum seal from the reagent vial (CAUTION: EDGES OF THE SEAL ARE SHARP). Remove the stopper using aseptic technique to minimize bacterial contamination. Always inspect the reagent vial for turbidity prior to use and discard any reagent which appears cloudy or turbid.

**Removing reagent**

- ALWAYS use a sterile pipet tip to remove reagent from the vial. Never introduce a pipet back into the vial after touching it to a microscope slide or other non-sterile surface.
- If a number of smears will be processed at once, sufficient reagent may be removed from the vial using a sterile pipet and placed into a small plastic microcentrifuge tube. Subsequent dispensing of the reagent may then be done from the aliquot in the plastic tube without the need for aseptic technique.

**Storage of DFA Reagent**

The reagent should be stored at 2°C to 8°C and is stable to the expiry date printed on the vial. ALWAYS USE ASEP TIC TECHNIQUE WHEN OPENING THE VIAL FOR USE. A visual examination of the vial for turbidity (i.e. bacterial growth) and mold growth must be made prior to each use. THE REAGENT MUST NOT BE USED IF ANY SIGNS OF TURBIDITY OR MOLD ARE PRESENT.

For convenience, the reagent may be aliquoted into small polypropylene microcentrifuge tubes and frozen at -20°C protected from light. DO NOT freeze in the original amber glass vial and DO NOT subject the reagent to more than one freeze-thaw cycle.

**Staining**

1. Add 25 µl of the DFA reagent to the fixed smear and spread evenly over the surface.
2. Place 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).
3. Place slide in a staining jar containing Wash Buffer for 5 minutes (B. pertussis and *B. parapertussis* positive control slides should be placed in separate staining jars). Repeat with fresh Wash Buffer. The two wash steps may be increased to 10 minutes each if background fluorescence is found to be too high.
4. Dip slide in fresh distilled or Type 1 water for 1 minute.
5. Gently air-dry slide.
to distinguish cells from possible fluorescent debris and background which may be visible on some smears. Fluorescence should be graded in the following manner:

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

Fluorescence should be graded when each field is first viewed with the fluorescein filter, then the rhodamine filter. A possible fluorescent cell may fade rapidly after exposure to the excitation beam of the microscope. Since fluorescein fades more rapidly than rhodamine upon exposure to light, it is recommended that slides be examined for B. pertussis before being examined for B. parapertussis.

Identification of B. pertussis

1. Examine the B. pertussis positive control smear with a fluorescence microscope using 1000x to 1000x 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 test slide 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 1000x 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 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.

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 4.265g of sodium chloride (NaCl) in 500ml distilled or CAP/ASTM Type 1 water.
2. Dibasic solution: In a 2 litre beaker dissolve 1.420g of anhydrous sodium phosphate dibasic (Na2HPO4) and 8.766g 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 lens along with proper epifluorescence illuminator alignment are all important in achieving adequate image intensity for fluorescent staining techniques. Since 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 fluorochrome filter set for simultaneous viewing of fluorescein and rhodamine stained specimens. Most dual fluorochrome filters suffer from reduced image integrity 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) Excitation & Emission Spectra

Excitation λmax = 491nm Emission λmax = 518nm

Rhodamine (B. parapertussis) Excitation & Emission Spectra

Excitation λmax = 541nm Emission λmax = 572nm

PREPARING ADDITIONAL REAGENTS

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REFERENCES