Department of Microbiology
Pneumocystis DFA Stain Procedure

I. INTRODUCTION AND CLINICAL SIGNIFICANCE

Pneumocystis carinii is a unicellular, eucaryotic organism that is present in the lungs of many mammalian species, including man. The organism is spread by airborne routes usually causing asymptomatic infection. Exposure during childhood may result in mild or subclinical cases with the organism existing in latent state through adulthood. In individuals with compromised immune systems, the organism becomes opportunistic and can cause diffuse, interstitial pneumonia.

II. PRINCIPLE

The Monofluo™ *Pneumocystis* carinii Staining Reagent contains monoclonal antibodies labeled with fluorescein isothiocyanate (FITC). The antibodies react with all *Pneumocystis carinii* forms (cysts, sporozoites and trophozoites) and also with the extracellular matrix present in infected specimens.

III. SPECIMEN PREPARATION AND HANDLING

1. Handle all specimens as potentially infectious. Procedures that create aerosols should be conducted in a biological safety hood.
2. Transtrachial aspirates, bronchial washes or bronchoalveolar lavages, and induced sputums are acceptable specimens. Mucoid specimens such as induced sputums require treatment to remove mucous prior to staining. See Procedure Notes, E.
3. Cytospin centrifugation is the preferred method of slide preparation, but specimen preparation is also possible by centrifugation at 1500 X for 5 min.
4. Place a drop of sediment on a slide.

IV. REAGENTS

1. MONOFLUO™ *Pneumocystis carinii* IFA Test Kit
2. Sputolysin
3. No preparation of the Test Kit reagents is necessary. They are ready to use as supplied.
4. Shelf life and storage. Do not use the kit or any of the components beyond the stated expiration date. Store staining reagent in the dark at 2-8^{o}C.

V. QUALITY CONTROL SLIDES:

Positive and negative control slides should be included each time the test is performed. Positive quality control slides are prepared from a bronchial washing or bronchoalveolar lavage specimen that has been confirmed as positive for *Pneumocystis*. A negative control slide is prepared from a bronch wash or bronchoalveolar lavage that has been confirmed as negative for *Pneumocystis*. These slides are heat fixed and stored at -18^{o}C to -20^{o}C in the almond freezer.

VI. FLUORESCENCE STAINING PROCEDURE

1. If the slides have been frozen, allow them to reach room temperature or use 37°C slide warmer before proceeding with the staining procedure.
2. Fix smears by flooding with acetone for 10 min at room temp.
3. Place the slides in a humidified chamber.
4. Add 1-2 drops of *Pneumocystis carinii* staining reagent to each well containing a dried smear. Spread the reagent to cover the entire well. Use a separate bulb to spread the drop for each patient.
5. Incubate for 30-35 min at 37^{o}C. Do not allow the *Pneumocystis carinii* staining reagent to dry on the slide during the procedure.
6. Using a wash bottle, remove excess *Peumocystis carinii* staining reagent with a gentle stream of water around the <u>outside</u> of the well.
7. Place the slides in a coplin jar containing distilled H_20. Make sure the control slides are washed separately form the patient slides. Dip gently, and do not agitate.
8. Air dry the slides on the 35 - 40^{o}C slide warmer.
9. Add 1-2 drops of mounting medium to the slide. Apply a coverslip.

VII. EXAMINATION AND EVALUATION OF TEST RESULTS

A. Scan each area using the 40 X objective. Use the edge of the well or waxed line to determine the plane of focus. Use 100 X oil immersion to confirm characteristic staining patterns.

B. Specimens containing *Pneumocystis* have large, round to elliptical shaped cysts (4-7 um in diameter) that are found both individually and in clusters that stain a bright apple green. Two or more well-defined cysts must be observed for the smear to be considered positive. Trophozoites will also stain apple green. They appear as small crescent shaped and pleomorphic structures 2-8 um in size. Brightly staining extracellular matrix may also be present. Specimens with only brightly staining amorphous material or typical trophozoitic forms should prompt a search for cysts.

C. Organisms other than *Pneumocystis* and cellular material from respiratory specimens should appear counterstained as red to red-orange or gold material.

D. A specimen is considered negative if characteristic fluorescence, as described above, is not observed.

VIII. LIMITATIONS OF THE PROCEDURE

A. A negative result does not exclude the possibility of *Pneumocystis* infection in the patient. Sample collection and preparation are critical steps in the testing procedure. Collection of the sample at an improper time during the course of the disease or misuse of the reagents provided can result in failure to detect *Pneumocystis.* Diagnosis should be made in conjunction with clinical symptoms.

B. In order for negative results to be considered valid, the presence of

specimen on the slide must be confirmed by light microscopy.

C. If the bronchial wash or bronchoalveolar lavage specimen is negative, but the patient continues to exhibit symptoms associated with a respiratory illness, other etiologic agents should be suspected, and appropriate diagnostic procedures (including culture) should be performed.

IX. NOTES

A. Excess mucus in specimens may prevent adequate staining. Thick sputum smears may prevent adequate staining. Care must be taken to make smears as thin as possible. Nonspecific trapping of the *Pneumocystis carinii* Staining Reagent may occur if the specimen is not adequately washed.

B. The staining reagent contains Evans Blue, which is a potential carcinogen. Avoid spilling or splashing the reagent on skin or clothing. Flush any areas that may have come in contact with the reagent thoroughly with water.

C. The mounting medium contains formaldehyde which is an irritant. Flush any areas that may have come in contact with this medium with water.

D. The staining reagent contains sodium azide. If the staining reagent is disposed of in the sink, flush with large amounts of water to prevent azide build-up.

E. Preparation of mucoid or induced sputa.
   1. Transfer 2-3 mL of sputum into a 15-mL centrifuge tube.
   2. Dilute sputolysin 1 part (1 mL) sputolysin to 9 parts (9 mL) H_2O. Make this up fresh as needed. Add an equal amount of diluted sputolysin to the specimen.
   3. Vortex the tube. Allow to stand for 15 min.
   4. Centrifuge for 5 min at 1500X, and discard the supernatant.
   5. Make the slides as previously stated in III.

X. REFERENCES

A. Genetic Systems Corporation. Seattle, WA, 98121. USA. 1997.

B. Stat-Pack Behring Diagnostics Inc. 17 Chubb Way, Somerville, NJ, 98876. 1985.

C. MONOFLUO Pneumocystis carinii IFA, Redmond WA, November 2000

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