Appendix XIX

PNEUMOCYSTIS CARINII DFA TEST

I. Introduction

The Merifluor-Pneumocystis DFA test is an in vitro test for the direct detection of Pneumocystis carini cysts and trophozoites in bronchoalveolar lavage (BAL), bronchial wash (BW); sputum or biopsy specimen.

II. Collection and Transport

BAL, wash and sputum should be collected using standard procedures. Biopsy specimens e.g. transbronchial, open lung or others must not be fixed and are transported to the lab on a saline moistened piece of gauze in a sterile container. Tissue should not be allowed to dry or become immersed in saline. All specimens should be transported as soon as possible to the laboratory. PCP testing can be done on the day after receipt except specimens received Friday or the day before a holiday must be stained and read that day.

III. Procedure

Reagents

FITC- *P. carinii* conjugate
Control slides
Distilled water
FA mounting fluid
Sputolysin: diluted 1:10 (i.e. 300 Ul sputolysin 3.0 mL distilled water)

Materials

Vortex
Sterile pipettes
10 - 100 uL Eppendorf pipette
Humidified chamber
Coplin jars
Fluorescent microscope

Preparation of Slides

BAL and BW:

1. Centrifuge the BAL or BW for 10 minutes at 1800 x g.

2. Remove and discard all but 0.5 mL of the supernatant. Thoroughly resuspend the pellet in the remaining 0.5 mL of fluid.

3. Make a thin smear twice the size of a cytospin spot and allow to air dry.

4. Fix in acetone for 5 minutes in a coplin jar, then air dry.

5. Slide must be stained within 8 hours or freeze at -20^{0}C.

Sputum .. See Sputolysin Procedures AppXXII

1. Combine equal volumes (3 mL each) of sputum and diluted sputolysin. Vortex mixture.

2. Incubate for 3 minutes at 35^{0}C.

3. Vortex the mixture briefly and add an equal volume of PBS and entrifuge at 1300 x g for 5 minutes.

4. Remove the supernatant, leaving 0.5 mL to resuspend the pellet.

5. Make a smear twice the size of a cytospin spot. Allow to air dry.

6. Fix in acetone for 5 minutes in a coplin jar, then air dry.

7. Slide must be stained within 8 hours or freeze at -20^{0}C.

Biopsy Specimen

1. Prepare a freshly cut surface on a fragment of tissue.

2. Touch the cut surface to a FA slide. Make several non-overlapping imprints within the well, avoiding smearing using several cuts.

3. While imprints are still moist on the slide, fix by adding 1 - 2 drops of acetone and allow to air dry.

4. Slide must be stained within 8 hours or freeze at -20^{0}C.

Staining - DFA

1. Cover the smear with 30 uL of *P. carinii* FITC-conjugate antibody.

2. Incubate in a humidified chamber for 30 minutes at 36^{0}C.

3. Wash slide twice with distilled water for 2 minutes in a coplin jar.

4. Allow the slide to dry.

5. Mount using coverslip and mounting fluid.

6. Read with fluorescence microscope with the FITC / Evans Blue filter and 40x objective.

Interpretation of Results

POSITIVE: Any specimen which contains two typical cysts exhibiting apple-green fluorescence of characteristic morphology. Generally cysts, 5 - 8 um diameter, are found together with trophozoites in clusters. Clusters can be variable in size and may appear with or without "honeycomb" like structure. Some cysts fluoresce evenly throughout their structure whereas other cysts may fluoresce mainly on their periphery and produce a "honeycomb" appearance within the clusters.

NEGATIVE: Red fluorescence without any characteristic apple-green fluorescence as described above.

IV. Reporting

POSITIVE: "*Pneumocystis carini* positive by immunofluorescence".

NEGATIVE: "*Pneumocystis carinii* negative by immunofluorescence".

Telephone all results.

V. Quality Control

Positive and negative control slides should be stained each time the staining procedure is performed. Refer to a senior technologist if controls do not work or for any other problems with staining, reading or reporting results.

VI. Reference

1. Merifluor Pneumocystis, Meridian Diagnostics, Inc. 3471 River Hills Drive, Cincinnati, Ohio, 45244. Tel. 513-271-3700.