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Section: Technical Manual	Subject Title: Fungi-fluor™ Stain	
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FUNGI-FLUOR™ STAIN

Principle

The Fungi-Fluor™ stain is used for the rapid identification of various fungal infections in fresh or frozen clinical specimens.

The active, fluorescing dye in the staining solution is Cellufluor which is the disodium salt of 4,4'-bis[4-anilino-6-bis-(2-hydroxyethyl) amino-s-triazin-2-ylamino]-2,2'-stilbenedisulfonic acid. Fungi-Fluor™ staining solution is a 0.05% solution of this dye in deionized water with potassium hydroxide added as a clearing agent. The Fungi-Fluor™ counter staining solution B is an aqueous solution of Evans Blue dye used to reduce background fluorescence. Cellufluor binds nonspecifically to beta-linked polysaccharides found in the cell walls of various organisms such as chitin and cellulose.

Materials

Staining Solution A
Counterstaining Solution B
Absolute alcohol
Water
Fluorescent Microscope (250-400 nm filter)

Precautions

1. Store in a dark or opaque bottle, tightly sealed, at room temperature.
2. Avoid eye or skin contact: use gloves and protective glasses.

Procedure

1. Prepare smear of specimen and allow to air dry.
2. Fix on the rack with absolute alcohol for 5 mins. until dry. Fixed smears can be held indefinitely until ready to stain and examine.
3. Add few drops of Fungi-Fluor solution A (Cellufluor) for 1 minute.
4. Rinse gently with tapwater.

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5. Apply coverslip to wetted slide and examine with the fluorescent microscope using the designated filter. If there is a delay, add distilled water to the coverslip just prior to examination. Use a fresh tube of water daily.
6. Optional for thicker smears. Add few drops of the counterstain Fungi-Fluor solution B. Rinse gently with tap water and then proceed as in step 5 above.

Quality Control

Stain a smear of *Candida albicans* daily.

Interpretation

Use 20x or 40x objective.

Fungal elements will appear yellow-green against a red-orange background when counterstain is used. Observe for characteristic morphology.

References

1. Manufacturers' Instructions (Data Sheet #316). Fungi-Fluor™ kit - Polysciences, Inc., July 1995
2. Clin. Micro. Newsletter 9:33-36, March 1, 1987.
K.L. McGowna. "Practical Approaches to Diagnosing Fungal Infections in Immunocompromised Patients".
3. J. Clin. Micro. 28:393-394, Feb. 1990. V.S. Baselski et al. "Rapid Detection of *Pneumocystis carinii* in Bronchoalveolar Lavage Samples by Using Cellofluor Staining".