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Section: Mycology Bench Manual	Subject Title: Fungi-Fluor™ Stain	
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FUNGI-FLUOR™ STAIN

Purpose

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

Principle

The active, colourless, 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 chitin and cellulose which are present in fungal cell walls.

When exposed to long wave UV light, fungal cell walls will fluoresce.

NB: Collagen, elastin, cotton fibres, plant material, some cells, cell inclusions and parasite cyst forms (eg. Acanthamoeba) may fluoresce.

Materials

Staining Solution A

Counterstaining Solution B

Absolute alcohol

Water

Fluorescent Microscope (Leitz Ortholux with G filter module exciting filter BP 350-460, suppression filter LP515 or equivalent)

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.

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Procedure

1. Prepare smear of specimen and allow to air dry.
2. Fix on the rack with absolute methanol for 5 minutes until dry. Fixed smears can be held indefinitely until ready to stain and examine.
3. Add a few drops of Fungi-Fluor solution A (Cellufluor) for 1 minute.
4. Rinse gently with tap water.
5. Apply coverslip to wetted slide and examine with the fluorescent microscope using the designated filter. If there is a delay, add fresh distilled water to the coverslip just prior to examination.
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.

NB: Gram stained smears can be overstained with Fungi-Fluor after removing immersion oil with alcohol. Similarly, Fungi-Fluor stained slides may be overstained with other stains such as GMS, PAS, Geimsa, etc.

Quality Control

Stain a smear of *Candida albicans* daily.

Interpretation

Use 25x or 40x objective.

Fungal cell walls will fluoresce apple-green. Observe for characteristic morphology to differentiate from artifacts and background. When the counterstain is used, fungi will appear yellow-green against a red-orange background.

Appearance of other structures / organisms:

- i) Fungal elements - intense peripheral staining with characteristic morphology.
- ii) *Pneumocystis carinii* - fainter staining cyst wall (5-7 µm diameter) and intensely staining internal "bean-shaped" or "double-parenthesis-like" structures with apposed sides flattened.

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- iii) *Acanthamoeba* sp. cysts - intensely staining double wall with wrinkled outer wall
(10-25 µm diameter)

References

1. Manufacturers' Instructions (Data Sheet #316). Fungi-Fluor™ kit - Polysciences, Inc., July 1995
2. V.S. Baselski et al. "Rapid Detection of *Pneumocystis carinii* in Bronchoalveolar Lavage Samples by Using Cellufluor Staining". J. Clin. Micro. 28:393-394, Feb. 1990.
3. D. H. Larone. Medically Important Fungi. A Guide to Identification. 3rd ed. 1995, ASM Press.