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Section: <b>Mycology Bench Manual</b>	Subject Title: <b>Calcofluor White Stain</b>	
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## **CALCOFLUOR WHITE STAIN**

### **Purpose**

Calcofluor White stain is useful for staining skin scrapings, hair, nail and thick tissue specimens. Calcofluor staining requires the addition of KOH which helps to dissolve keratinized particles and helps to emulsify solid, viscous material that may mask the fungal elements. Calcofluor method is **NOT** suitable for the detection of *Pneumocystis carinii*. Calcofluor stained smears are read under the UV microscope as for the Fungi-Fluor™ stain.

### **Procedure**

1. Place a portion of the specimen on the slide (select a purulent area if secretions).
2. Add 1 to 2 drops KOH and emulsify specimen. If it does not clear rapidly, place slide in petri dish and allow to stand about 10 minutes.
3. If tissue or scrapings, place the slide on 35<sup>0</sup>C bench top heating block for 15-20 minutes to speed clearing.
4. Add 1 or 2 drops of calcofluor white reagent and mix thoroughly. Calcofluor white may be added right after KOH.
5. Apply coverslip gently. Make sure specimen does not overflow.
6. Examine under fluorescent microscope (see Fungi-Fluor™ stain).
7. Always include a control slide positive for yeast or filamentous fungus.

**NB:** In the event when no Fungal Stain smear is available, Gram smear may be retrieved for over staining by Calcofluor White or KOH.

### **Interpretation**

Fungal cell walls fluoresce apple green (see Fungi-Fluor™ stain).

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### **References**

1. Manufacturers' Instructions:  
Calcofluor White Reagent - Difco.
2. Clin. Micro. Newsletter 9:33-36, Mar. 1, 1987. K.L. McGowan. "Practical Approaches to Diagnosing Fungal Infections in Immunocompromised Patients".