PROVIDENCE
Sacred Heart
Medical Center &
Children's Hospital

Fluorescent Fungus Stain Procedure

Department of Microbiology

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1.0 Purpose or Principle

Fluorescent brighteners, such as calcofluor white, will bind to β1-3 and β1-4 polysaccharides, specifically cellulose and chitin of fungal cell walls. When viewed with a fluorescent microscope, organisms impart a bluish fluorescence. Testing with calcofluor permits a more rapid turnaround time for fungal microscopy than traditional histological staining.

2.0 Clinical Significance

Microscopic examination of clinical specimens for the presence of fungi may provide a rapid indication of the cause of an infection, allowing for prompt initiation of appropriate antifungal therapy.

3.0 Scope

This procedure is classified under CLIA as Highly Complex. It should be carried out by technical personnel familiarized and trained on performing fluorescent microscopy and identifying fungal elements. Testing includes but is not limited to: microscope start up, shutdown, QC checks, record keeping of information vital to technical proficiency in accordance with the department SOP. Records are to be kept within the employee's record in the department of continued competence and proficiency on the procedure. Performance reviews of technical personnel are to be carried out annually.

4.0 Safety - Personal Protective Equipment

Performance of this procedure will expose testing personnel to biohazardous material and possible chemical hazards.

All specimens must be handled as potentially infectious material as outlined in the Microbiology Biohazards and Safety document.

The reagents and chemicals that are used in this procedure may be hazardous to your health if handled incorrectly. A brief listing of precautions for each chemical hazard is included in the reagent section of this procedure.

More extensive information concerning the safe handling of the reagents and/or chemicals used in this procedure, as well as other important safety information may be obtained by consulting the Material Safety Data Sheet (MSDS). Before performing any part of this procedure, the technologist must take any and all precautions and adhere to all prescribed policies.

This procedure may expose you to:

- Bloodborne pathogens
- Airborne pathogens
- Hazardous reagents

To perform this procedure, you must use:

- Gloves
- Laboratory Coat
- Biological safety cabinet

Disinfectant following procedure:

- Bleach dilution sprayers can be used for on demand disinfectant.

Reference for spill/decontamination

- MSDS
- Chemical hygiene plan

5.0 Specimen Requirements

Specimens submitted for fungal smear and/or culture.

6.0 Materials

6.1 Equipment

- Fluorescent microscope (Olympus BX41, with filter #2)

6.2 Consumables

- Glass microscope slides
- Cover slips (24 x 30 and 24 x 40)
- Disposable transfer pipettes

6.3 Reagents

- Fungi-Fluor® Kit (Polysciences, Inc.). Store protected from light at room temperature. Do not freeze. Fungi-Fluor® Solution is classified as an irritant. Consult MSDS for first aid measures.
    - Solution A – Fungi-Fluor® Stain (Potassium hydroxide 1% + Cellufluor)
    - Solution B – Fungi-Fluor® Counter Stain (Evans Blue dye)
- 20% Potassium Hydroxide

6.4 Control Materials

- **Positive Control:** *Candida albicans* ATCC 90028
- **Negative Control:** *E. coli* ATCC 25922

7.0 Procedure Instructions

7.1 Non-Keratinous Material Smear Preparation and Staining

1. Label a glass microscope slide with an accession barcode.
2. Apply specimen to the center area of a glass microscope slide. The specimen should be contained in an area no bigger than the size of a quarter.
3. Allow the smears to air dry. A slide warmer may be used to facilitate drying.
4. Place patient slides on a slide tray to be stained and read with the next batch.
5. Using a transfer pipette, place 1 drop of the Fungi-Fluor® Solution A onto each smear.
6. Using a separate transfer pipette, place 1 drop of the Fungi-Fluor® Solution B onto each smear.
7. Add a 24 x 40 coverslip to the slide.

7.2 Keratinous Material (Skin, Hair, Nails) Smear Preparation and Staining

1. Place portion of the specimen in a conical tube labeled with an accession label and add a few drops of 20% KOH.
2. Warm the material at 30°C for 20 min to accelerate digestion. Add a drop of the digested material to the center area of a glass microscope slide.
3. Using a transfer pipette, add 1 drop of the Fungi-Fluor® Solution A.
4. Add a 24 x 30 coverslip to the slide.

7.3 Smear Examination

1. Examine the smears on the Olympus BX41 fluorescent microscope, using filter #2. Verify that the control slides appear as expected before examining patient smears.
2. Scan each smear at x 100 magnification for yeast and hyphal elements and confirm morphology of suspicious items at x 400 magnification.

8.0 Interpretation and Reporting

8.1 Positive Smear Interpretation

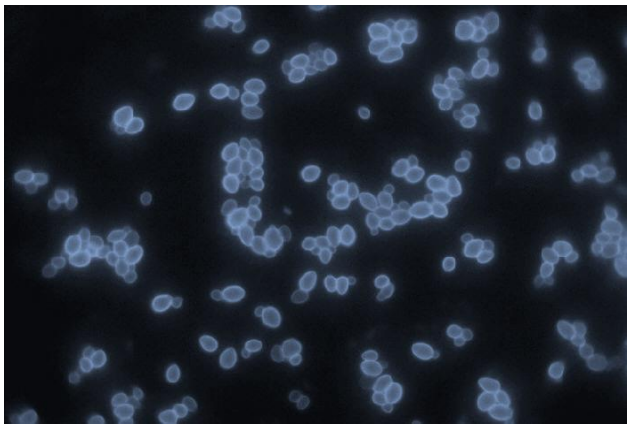
- Fungal elements should appear fluorescent bluish in color. Refer to the images below for examples.
- Care must be used when interpreting smears because nonspecific reactions may be observed. Cotton fibers fluoresce strongly and must be differentiated from fungal hyphae. Additionally, tissues such as brain biopsy specimens from patients with tumors may fluoresce and resemble hyphae suggestive of *Aspergillus* or other moulds with branching hyphae.
- Questionable items from normally sterile body sites should be reviewed by the microbiology director or supervisor. If the microbiology director and supervisor are unavailable, the smear should be reviewed by a second technologist.

8.2 Positive Smear Reporting

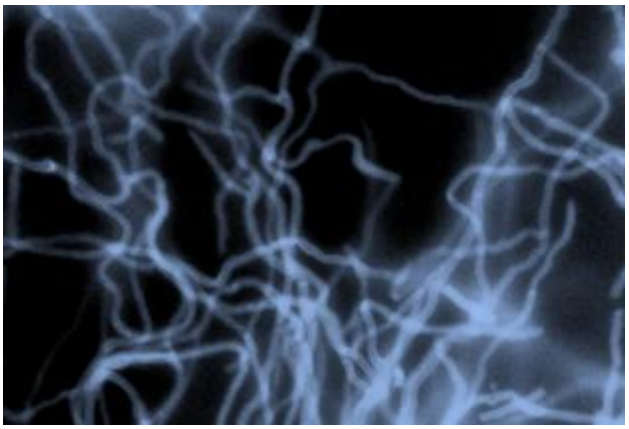
Smears that demonstrate fungal elements with typical fluorescence should be reported with the type of structures seen.

- **Budding yeast seen,**
- **Budding yeast with pseudohyphae seen,**
- **or Hyphal elements seen**

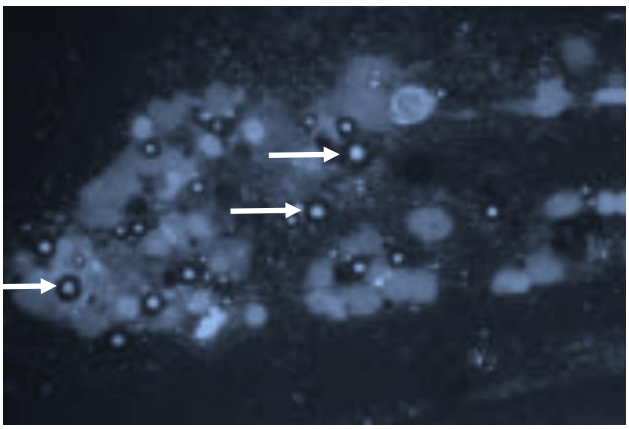
Budding Yeast x 400



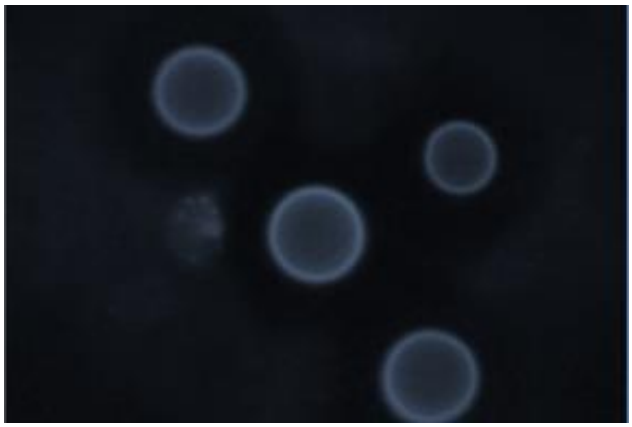
Hyphal elements of a dermatophyte x 400



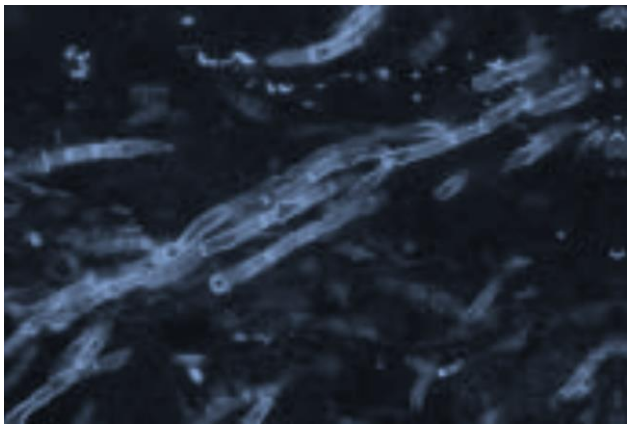
***Cryptococcus* x 400**
(note capsule depicted by the absence of stain)



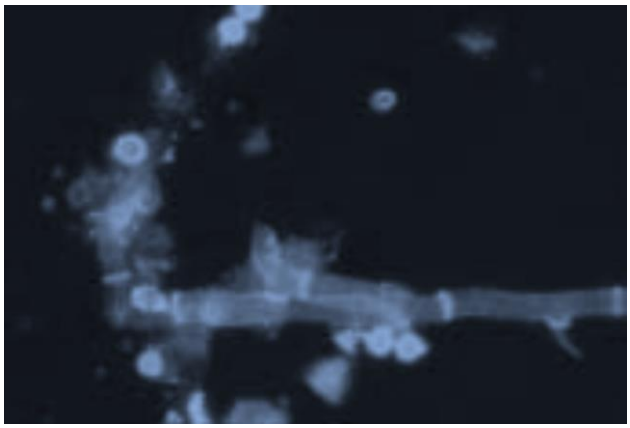
***Cryptococcus* x 1,000**
(note capsule depicted by the absence of stain)



***Aspergillus* in lung tissue**

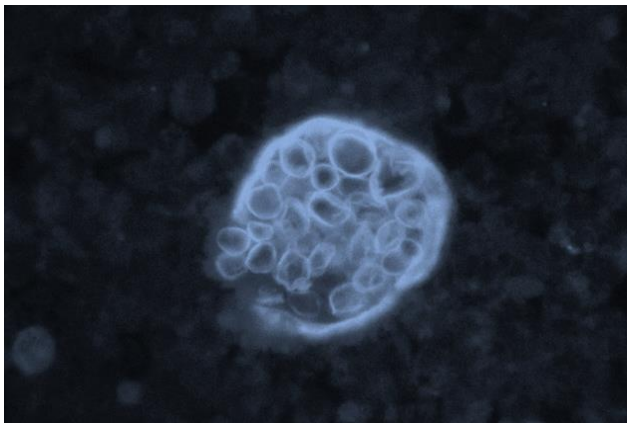


***Aspergillus niger* in ear discharge**

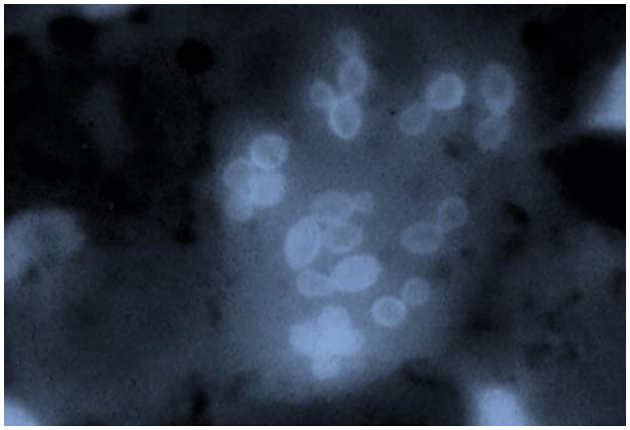


***Coccidioides immitis* spherules x 400**

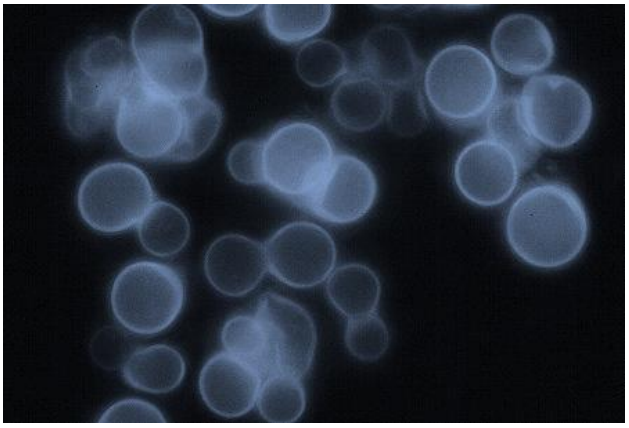
***Coccidioides immitis* spherules x 1,000**



***Histoplasma capsulatum* x 1,000**



***Blastomyces dermatitidus* x 1,000**



8.3 Negative Smear Interpretation and Reporting

If no fungal elements with typical fluorescence are seen, report **No fungal elements seen.**

9.0 Quality Control & Quality Assurance

9.1 Frequency

Positive and negative smears should be stained and examined with each batch of patient smears. Record QC results in LIS. If controls do not perform as expected, notify technical specialist, supervisor, and/or technical director.

9.2 Control Material

Smears of *Candida albicans* and *E. coli* serve as the positive and negative controls. The smears can be prepared with organism suspensions equivalent to a 0.5 McFarland. Place a drop of each control suspension on separate slides and dry using a slide warmer. Store slides in labeled boxes at room temperature.

9.3 Staining Control Slides

Follow the staining protocol outlined above for direct smears.

10.0 Limitations

Calcofluor is not a specific staining reagent. Interpretations must be based on both bright fluorescence and recognizable fungal structures.

11.0 References

- Package insert: Fungi-Fluor® Technical Data Sheet #316.
- Hageage, GJ, Harrington, BJ: Use of calcofluor white in clinical mycology. Lab. Med. 15:109-112, 1984.
- Hageage, GJ, Harrington, BJ: Calcofluor White: A Review of its Uses and Applications in Clinical Mycology and Parasitology Use of calcofluor white in clinical mycology. Lab. Med. 2003; 34:361-367
- Hoang, L.M.N, Phillips, P., Galanis, E. Clin. Micro. Newsletter Vol. 33, Issue 24, 12/15/2011, Pages 187-195.
- Versalovic, J, K. C. Carroll, G. Funke, J. H. Jorgensen, M. L. Landry, D. W. Warnock. 2011. Manual of Clinical Microbiology, 10th ed., Vol. 1, ASM Press, Washington, D.C.
- Clinical Microbiology Procedures Handbook, 3rd ed. and 2007 update, Vol. 2. Garcia, L.S., editor in chief. ASM Press, Washington, D.C.

12.0 Document Control

- Microbiology Director Approval: Dr. Ann Robinson 5/19/2000, Updates reviewed 2/9/2012 and 1/18/2013
- Medical Director Approval: Reviewed by Dr. Schappert 03/10/2010
- Reviews by Jerry Claridge: 06/14/2001, 03/04/2002, 03/2003, 04/2004, 07/2005, 01/2006, 05/31/2007, 05/2008, 07/2009, 04/01/2011, 02/09/12, 01/18/2013, 03/2013, Jason Ammons 07/2015
- Revisions & Updates: 2/9/2012 Updated procedure to include counterstain with dry smears. 1/18/2013 Updated to PPM format and added instructions for performing fluorescent microscopy on KOH preparations. 10/03/2014 Updated procedure for color fluorescence associated with the Olympus BX41.