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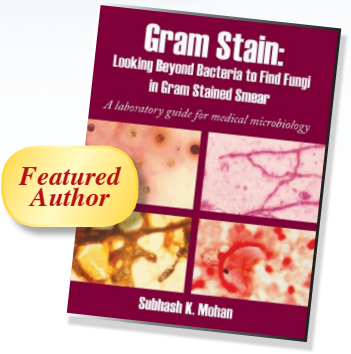
Polysciences, Inc.
Chemistry beyond the ordinary

News | Views | Insights

Usefulness of Fungi-Fluor®
In Medical Mycology & Microbiology

by Subhash K. Mohan



Featured Author

What are the advantages of Fungi-Fluor® by Polysciences?

Fungi-Fluor is a reagent that is used for staining purposes in microbiology and other diagnostic labs during direct examination of the clinical specimens to detect a variety of microorganisms. The reagent has several advantages over other routine procedures in medical microbiology. Fungi-Fluor helps to brighten the microscopic field to a greater degree by enhancing visual clarity and turning objects brighter against the darker background. This phenomenon allows the smear reader to observe microorganisms better with increased clarity. There are certain limitations with other nonspecific fluorescent reagents that bind to other materials present in the specimen smear causing confusion in the morphological structures of various objects other than the microorganisms. The key in correct smear interpretation is to know the morphology of the fungi in order to rule out the morphology of the objects in a non-fungal nature. Fungi-Fluor by Polysciences, Inc. not only detects fungal elements but also detects *Pneumocystis jiroveci* (formerly known as *Pneumocystis carinii*) that is not picked up by other similar reagents produced by other companies.

Technical Case 1

A middle aged male patient was scheduled to receive a lung transplant. Preliminary routine laboratory tests were requested including sputum for C&S. No fungal culture was requested on sputum specimen. Specimen was processed for culture and a Gram stain smear was prepared, examined and reported as NBS. Within 48 hours of incubation, one colony of mold was isolated and referred to the departmental mycology section for ID. The mold was categorized as Zygomycetes by examining wet prep lacto-phenol cotton blue (LPCB) pending identification. The entity of the fungus was such that it is either a frequently environmental contaminant or a true infectious agent. The recovery of Zygomycetes from immunocompromised hosts alerts the clinicians to treat the patient promptly since Zygomycetes have the tendency to invade the arterial blood system causing severe necrosis with fatal consequences if left unattended. The sputum specimen for the patient was repeated since there was no evidence from the direct smear but the C&S culture only. On repeat specimens, same results were produced and the Gram smear was still NBS. The clinician did not want to take the chance and the lung transplant was postponed until after the fungal infection had been cleared. A third BAL specimen from this patient was collected and submitted to microbiology. This time C&S and fungal culture were requested. Gram stain results were reported as negative for fungus, however, a fungal smear stained with Fungi-Fluor showed positive for *Aseptate hyphae* indicating the presence of Zygomycetes ***(image 1.1)***. Upon re-viewing the Gram smear, *Aseptate hyphae* ***(image 1.2)*** were also observed but did not attract the smear reader's eye at the time it

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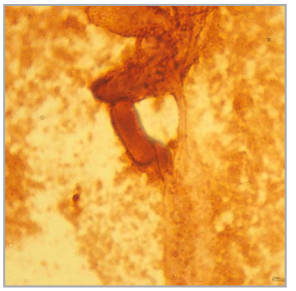
1.1 Fungi-Fluor® 400x *Aseptate Hyphae*



1.2 Gram Stain 1000x *Aseptate Hyphae*

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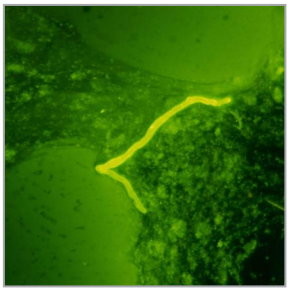
was first examined since the smear reader was focused on bacteria and not fungi. Such examples are explicitly described in my recently published book on Gram stain for finding fungi.

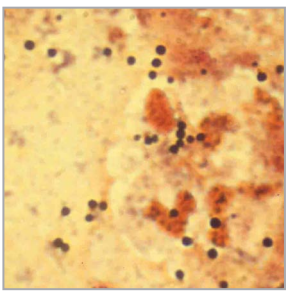
In clinical microbiology, Gram stain is a routine procedure done on clinical specimens submitted for C&S analysis. In routine bacteriology, Gram stain is usually read to detect bacteria only from clinical specimens. However, technologists reading Gram stain smears do not realize that there are other organisms besides bacteria that may be detectable from the Gram stain but are missed for the reason that the focus to observe bacteria being much smaller in size takes attention away from thinking about other organisms such as fungi. Although fungi are much larger in size and even if they are observable, they are frequently missed by the smear reader. When clinical specimens are sent to microbiology for C&S only, fungi present in the specimen are not specifically analyzed to recover them. As a result, laboratory proficiency and the patient care are compromised.

Technical Case 2

A lung aspirate specimen collected from a post lung transplant patient was submitted to the microbiology lab for C&S, fungal culture, TB and viral studies. A gram stain smear and fungal smear were both negative for bacteria and fungus. However one to two colonies of each were recovered from the C&S and fungal culture media and identified as *Aspergillus fumigatus* and reported to the clinician. Within 24 hours a repeat specimen from the lung aspirate was sent for C&S and fungal analysis that also grew one or two colonies of fungus similar to the one before, but the Gram stain smear and fungal smear were negative. The third lung aspirate was received soon after for C&S and fungal culture. The Gram stain smear showed no bacteria or fungi, however, the fungal smear stained by Fungi-Fluor (reagent produced by Polysciences, Inc.) showed some fungal elements ***(image 2.1)*** that did not appear straightforward and required additional help to correctly identify them in one of the three categories such as *Pseudohyphae, septate hyphae* or *Aseptate hyphae*. If *septate hyphae*, do they appear hyaline in nature or phaeomycotic, i.e. dark fungal elements. Categorizing each entity is very crucial in terms of making a diagnostic decision by the clinician. We decided to re-view the Gram stain smear. Upon looking at the Gram stained smear visually, it brought important irregularity to the author's attention about the production of the Gram stain smear.



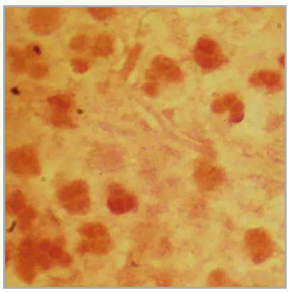
2.1 Fungi-Fluor® 400x *Septate Hyphae*



2.2 Under decolorized Gram Stain 1000x

The Gram stain smear prepared was under-decolorized. The author was surprised that the smear reader did not raise the issue of quality by rejecting the smear asking for a replacement. Upon re-examining the original Gram stain smear under the microscope, it was very confusing and tough to read the under-decolorized smear that indicated fungal like cells ***(image 2.2).***

All of the images taken from this poorly stained smear are uninterpretable. Therefore, the smear was restained using acetone-alcohol to decolorize the under-decolorized Gram stain smear followed by safranin. Although the smear was too thick taking more than 20 seconds to decolorize it was still difficult to spot cells and the organisms hidden between layers of specimen. The re-stained Gram smear however, had improved in quality to a greater degree and *septate hyphae* were clearly visible in the re-stained original Gram stain mear ***(image 2.3).***



2.3 Original Gram Stain 1000x

How does Fungi-Fluor® help during direct examination of clinical specimens?

Fungi-Fluor produced by Polysciences, Inc. (Calcofluor White) contains celluflour, a fabric brightener, that binds nonspecifically to polysaccharides and chitin (similar to cellulose) present in the cell wall of fungi. Producing apple-green fluorescence when exposed to long wave length UV light under fluorescent microscope. Fungi-Fluor produces a brightness and contrast of the microscopic field on the smear and increases visual clarity that is many times compromised in Gram staining and other direct staining procedures. Fungi-Fluor by Polysciences, Inc. not only detects fungal elements but also detects *Pneumocystis jiroveci* that is not picked up by other similar reagents produced by other companies.

The Gram Stain Book

This new textbook focuses on the detection and classification of fungal elements in Gram stains. Newly developed flowcharts, clues and key details regarding structural characteristics have been added to guide the reader in the right direction. Throughout the years, the author has accumulated many scenarios in which fungal elements were not detected on the original Gram stain evaluation, but were found to be positive upon review once the culture grew a fungus. Finally, the book contains a chapter with a practice examination including microscopic images representative of scenarios commonly encountered in the clinical microbiology laboratory.

The utility of the Gram stain in clinical microbiology may be enhanced by the expansion of its reported detection ability to include yeast and filamentous fungi. Filamentous fungi may not always be readily recognized in the Gram stain, but increased experience and a little imagination make the detection of fungal elements possible.

A Gram stain is not an excellent procedure to detect fungi. However, the benefit given upon finding fungi in the Gram stain surpasses any specific procedure suitable for the detection of fungi in direct clinical specimens.

The author found fungi in the NeatStain Gram Stain Kit *(Cat. #25036)* smears and helped to change the course of patient's diagnosis and the therapy in a timely fashion. The author claims that many cases

would have been left undiagnosed if the Gram-stained smears were not specifically checked out for microorganisms beyond bacteria during routine microscopic examination of the clinical specimens.

Recent cases where Fungi-Fluor® has played a role in resolving matters

- IFA reported +/- PC; however, the technologist reporting the positive PC was not convinced and asked to mycology for opinion. Fluorescent staining done on the smear showed no PC cysts but 1+ yeasts. It has been indicated by the IFA manufacturer that yeast can cause false positive. Once again Fungi-Fluor turned out to be the winner.

- An automated blood culture machine indicated a positive blood bottle with a very high index reading. The blood culture technologist prepared a Gram stain and found structures resembling fungi. Mycology reviewed the smear and noticed similarity of some of the many structures seen fitting into the morphology of fungi. To rule out false positive results, Fungi-Fluor on the positive blood culture was performed and found no fungal elements seen in the blood whose Gram smear depicted fungi. Once again, Fungi-Fluor was a winner because the huge number of structures present in the Gram stained smear suspected of fungi would have posed no problem to brighten up the entire microscopic field if these structures were true fungi.

- A sputum specimen and a BAL on two different patients received at different times were negative for fungal elements but displayed dull fluorescing branching bacillary forms that the mycology expert immediately suspected for *Nocardia.* Upon looking up Gram smear results on the specimens, it only mentioned the presence of pus cells and commensal flora without mentioning the branching bacilli that were noticed in the fungal smear stained by Fungi-Fluor. Upon review of the Gram smear, Gram positive beaded and branching bacilli resembling *Nocardia* were observed. The specimen was retrieved and processed for *Nocardia* culture on selective and differential medium (sodium pyruvate agar). The culture grew *Nocardia* from the corresponding cultures and was reported.

- Similarly microbiology technologists encountered objects resembling fungal elements in Gram smears but were unable to name or categorize them. Such organisms were seen in Fungi-Fluor smears and were correctly identified as non-fungal entities such as *Prototheca, Microsporidium* and other parasites.

Many more examples on all groups of fungi are explicitly described in the author's recently published book on Gram Stained smears for finding fungi. Some of them are also mentioned in the quiz section for practice.

About the Author of Gram Stain

Looking Beyond Bacteria to Find Fungi in Gram Stained Smear: A Laboratory Guide for Medical Microbiology

Subhash Kumar Mohan, was born in Punjab, India. He received a graduate degree in integrated medical systems in 1967 and worked in India for five years before emigrating to Vancouver, Canada in 1972. He lived briefly in the UK from 1974 to 1976, working in a laboratory performing bacteriological and chemical analyses of drinking water and studying microbiology at the Isleworth Polytechnic Institution in London. Subhash returned to Canada in 1976 to work in a private medical laboratory and study at the Michener Institute for Medical Technology, completing the Canadian Society for Medical Laboratory Science (CSMLS) national certification exam for registered technologists in clinical microbiology in 1977.

After acquiring so much experience on the bench, Subhash had a dream to publish a medical mycology book for clinical microbiology laboratorians. Subhash has taken Hans Gram's simple stain, which revolutionized the field of bacteriology, into the field of medical mycology for the detection of fungi in clinical specimens.

Subhash led the discovery of a new *Candida* species *(Candida subhashii),* which he isolated from the peritoneal fluid of a patient with end-stage renal failure. Experts decided to name the isolate in honor of **Subhash Mohan** for his extensive work in the field of mycology.

Cat. #	Description
25043	Gram Stain: Looking Beyond Bacteria to Find Fungi in Gram Stained Smear: A Laboratory Guide for Medical Microbiology

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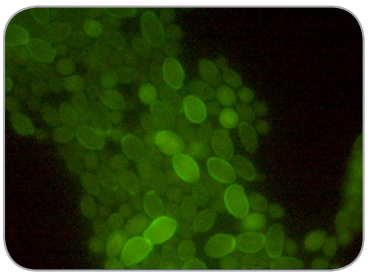
Fluorescent Staining Kits

Fungi-Fluor® Kit for Fungal Detection

Quick fluorescent stain and counterstain procedure for various fungal organisms. Kit can be used to screen a variety of specimen types from sputum to skin scrapings for fungal detection.

- Rapid - 3 minutes to stain and read, more accurate than KOH preps
- Greater morphologic detail than PAS or silver stains
- Counterstain greatly reduces background fluorescence

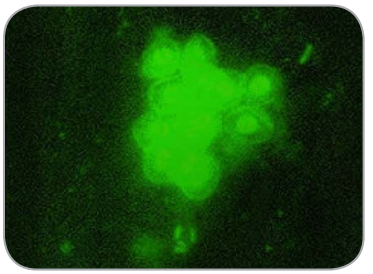


Candida Albicans, 100x

Fungi-Fluor® Pneumocystis Kit

Offers a fast, fluorescent staining procedure for *Pneumocystis jirovecii* in bronchial specimens. Early detection allows appropriate treatment and may improve the chances of patient survival.

- Direct *Pneumocystis jirovecii* cysts
- Rapid - 3 minutes to stain and read
- Slides can be reactivated if fluorescence dims
- Minimal prep and clean-up

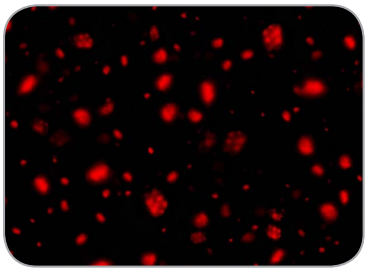


Pneumocystis jirovecii pneumonia

TB Fluorostain Kit

Allows fluorescent mycobacteria to stand out brightly on a darkened background. Smaller numbers of mycobacteria are easily identified with a fluorescent stain.

- Less than 12 minutes to perform staining
- Microwave method reduces time and enhances results
- Less Auramine O and Rhodamine B, reducing costs & hazards
- Faster counterstain



TB Fluorostain lung biopsy control, 40x

Fungi-White

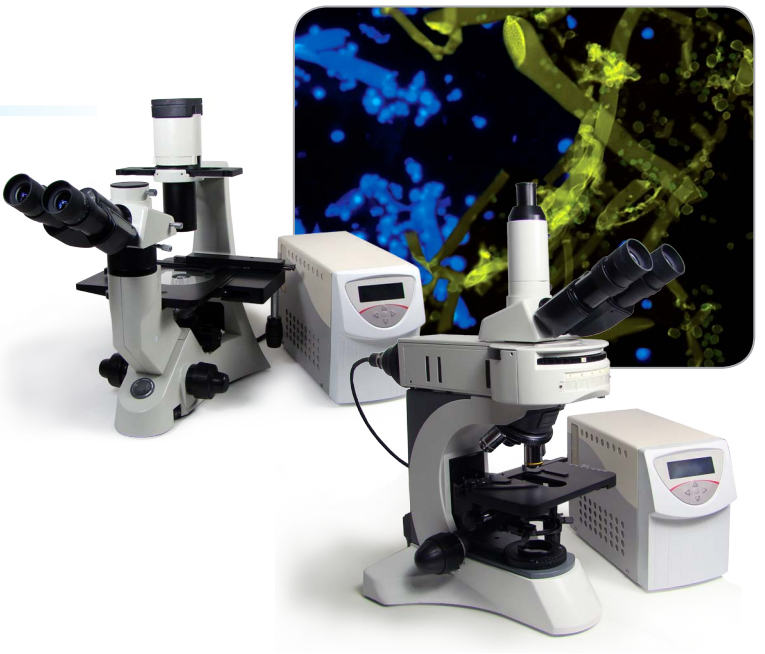
Suitable to use in the identification of fluorescent fungal elements and trichomonas detection. Rapid chemo-fluorescent method useful for the detection of Microsporidia and *Acanthamoeba spp.* Fungi-White dye fluoresces when exposed to ultraviolet light (below 400nm wavelength). The procedure is useful on fecal smears, cell cultures or other sample materials. Use of this method on plastic sections requires modifications to the standard protocol.

Cat. #	Description	
17442 17442E	Fungi-Fluor® Fungal Detection Kit *for Europe orders only*	1 Kit
22363 22363E	Fungi-Fluor® Pneumocystis Kit *for Europe orders only*	1 Kit
22422	TB Fluorostain Kit	1 Kit
24692	Fungi-White	1 Kit

We offer a complete line of fluorescent stain kits and reagents to learn more please visit **www.polysciences.com/fluoro**

Product Spotlight

Fluorescence Microscopes



Inverted & Upright Fluorescence Microscopes

Equipped with a turret condensor, EWF10x focusing eyepieces with eye guards, variable koehler 6 volt 30 watt illumination and FITC/GFP and DAPI with LP emission filter sets. Light source is a self-aligning 200w metal halide life of 1500 hours. Guaranteed to provide a high quality optical performance that will exceed your expectations.

Digital Camera and Imaging Software *(optional)*

Preview real-time video and instantly capture images without interrupting the display screen. CMOS Digital Camera 5.0 MP shows a full screen live display allowing you to store captured images in over 10 different file formats.

Cat. #	Description	
25039	Upright Fluorescence Microscope	1 Unit
25040	Inverted Fluorescence Microscope	1 Unit
25041	Digital Camera & Imaging Software	1 Unit

Neat Stain Staining Kits

Specifically designed for stat procedures and/or laboratories using manual staining methods. Neat Stain Staining Kits rapid technique staining procedure provides excellent results for routine examination.

We offer Neat Stain in three different kits a 3-step differential Hematology Stain, Gram Stain and Trichrome Stain Kits.

- **Neat** - Self-contained foil sealed, pre-filled multi-well containers
- **Fast** - Immerse slides sequentially in each reagent for specified time, air dry and read
- **Convenient** - Additional foils for re-sealing containers

Cat. #	Description	
25034	Neat Stain Hematology Stain Kit	1 Kit
25035	Neat Stain Trichrome Stain Kit	1 Kit
25036	Neat Stain Gram Stain Kit	1 Kit
25033	Neat Stain Slide Holder	1 Unit