

Fungi-Fluor[®] Kit Pneumocystis Kit

Fast Fluorescent Stain for in vitro Diagnostics

Introduction:

Pneumocystis carinii is a unicellular, eukaryotic organism which is present in the lungs of many mammalian species and can cause *Pneumocystis carinii* pneumonia (PCP) in severely immunocompromised individuals.^{1,2} It is suggested that most children experience mild or subclinical infection with *Pneumocystis carinii* and that the organism remains in a latent state until immunosuppression triggers reactivation.¹⁻⁵ Prior to the AIDS epidemic, those primarily at risk have been premature infants, patients with immunodeficiency disorders and patients receiving immunosuppressive treatment.^{1,2,4-11} Since 1981, the prevalence of PCP has significantly increased due to the susceptibility of individuals with AIDS. PCP develops in 60-85% of AIDS patients and is the most common opportunistic infection of the group.^{12,13} Rapid clinical diagnosis of *Pneumocystis carinii* is very important since the organism quickly infiltrates lung tissue causing dyspnea, fever, and cough.²

Advantages of Fungi-Fluor[®] Kit Pneumocystis Kit

- Faster and more definitive results than methenamine silver staining
- Less costly and time-consuming than immunofluorescent assays
- Positive results read in 3 minutes
- Unique staining pattern enables *Pneumocystis carinii* to be easily distinguished from other organisms
- FDA cleared for *in vitro* diagnostic use in the U.S.A.

Specimen Types

- Bronchoalveolar lavage (BAL)
- Bronchial wash
- Bronchial brush
- Lung Imprint

There are three life forms of *Pneumocystis carinii*: the cyst, the trophozoite, and the sproozoite.^{1,9} The Fungi-Fluor[®] Kit Pneumocystis Kit has been designed to detect cysts, which have these characteristics:

- 5-8 μ m in diameter, thick-walled
- Most easily recognized form of *Pneumocystis carinii*
- Cluster in masses within an extra cellular matrix
- Intense staining of thickenings in cell walls gives "double parenthesis" appearance.

Specimen Preparation:

The specimen or smear should be fixed in absolute methanol for 1 to 5 minutes. This may vary with the thickness of the smear. The fixation step will help adhere the specimen to the slide and prevent loss of material during staining. Frozen sections or specimens should be fixed in absolute methanol for 5 to 10 minutes depending on section thickness or laboratory protocol. Slides can be stained immediately after rinsing gently with distilled or deionized water.

Staining Procedure

Control: Use the positive control slide provided with the kit to assure staining results with the patient specimen. Control slides may be dipped in deionized water prior to staining to aid in penetration of the solution.

1. Place slide in a horizontal position and apply a few drops of Fungi-Fluor[®] Pneumocystis stain directly on the specimen. The entire specimen should be covered or flooded. Stain for 1 minute.
2. Drain the solution from the slide and rinse very gently with running distilled or deionized water. Slides can be gently dipped in two changes of distilled or deionized water.
3. Slides may be air dried or viewed directly as a wet mount.
4. Slides may also be dehydrated through 2 changes of 95% ethanol, absolute ethanol, and then xylene followed by coverslipping with Poly-Mount (Cat. # 08381) Coverslipping Media for a permanent slide.

Microscope Filter Requirements:

Fluorescence microscope filter descriptions can vary with the type of microscope used. We suggest you discuss filter options with your specific microscope manufacturer to assure proper excitation levels for viewing the specimen. The microscope manufacturer should have a list of filters with common descriptions to match your needs.

1. Ultra Violet, Calcofluor White Filter - Excitation 340nm to 380nm with Suppression Filter 430nm The color of the fluorescent material should be blue.
2. H3 Violet Plus Blue, Wide Band FITC Filter - Excitation 420nm to 490nm with Suppression Filter 515nm - The color of the fluorescent material should be yellow-green or apple-green.

The filters listed all give yellow-green or apple-green fluorescence.

- Leica/Lietz D, G, or H3 filters
- Olympus Blue Exciter Cube
- Nikon B-3A Filter Cube
- Riechert-Jung 713
- Ziess 05, 06, and 07 Filter Cubes

Positive Staining:

Specimens containing two typical cysts with either the apple-green or blue fluorescence can be considered positive as for *P. carinii*. *P. carinii* cysts appear round, uniform in size and non-budding with a diameter of 5-8µm. The highly characteristic staining pattern of the peripheral cyst wall with intense internal staining of the "double parenthesis" structure should be noted. Free "double parenthesis" forms can be seen and are presumably degenerating cysts. Cysts are found in clusters.

Comparative Techniques for Detection of *Pneumocystis carinii*

Technique	Comments
<ul style="list-style-type: none"> • Methenamine silver nitrate • Toluidine Blue O 	<ul style="list-style-type: none"> - Stain cyst wall only - Tedious and time consuming procedure - Requires high level of expertise for interpretation - May require noxious chemical use - Appearance: purple-brown to black, lavender to blue-violet
<ul style="list-style-type: none"> • Wright Giemsa Gram Staining 	<ul style="list-style-type: none"> - Stain trophozoites & sporozoites only - High level of expertise required - Extensive time necessary for interpretation - Appearance: deep blue to purple
<ul style="list-style-type: none"> • Direct/Indirect Fluorescent Immunoassay 	<ul style="list-style-type: none"> - Long staining times - up to 1 hour or more - High background with staining of extracellular matrix - Incubation steps required - Appearance: bright apple-green fluorescence
<ul style="list-style-type: none"> • Fungi-Fluor® <i>Pneumocystis</i> Kit 	<ul style="list-style-type: none"> - Minimal prep & clean-up - 3 Minutes to stain and read specimens - Distinct fluorescent staining pattern - Little technical expertise necessary to stain - Low cost per slide - Well documented - Appearance: bright apple-green fluorescence or blue depending on the filter used.

Precautions and Storage:

Fungi-Fluor® Solution is an irritant. Exercise normal care in handling.
 Fungi-Fluor® Solution should be stored protected from light at room temperature. Do not freeze.

References

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| 1. Thorax, 40:461-570(1985). | 10. Blood, 39:759-770 (1972). |
| 2. Ann. Intern. Med., 80:83-93 (1974). | 11. Pediatrics, 19:543-565 (1957). |
| 3. Pediatrics, 66:56-62 (1980). | 12. Am. Rev. Respir. Dis., 136:1199-1206 (1987). |
| 4. Rev. Infect. Dis., 8(6):1001-1011(1986) | 13. J. Respir. Dis., 8:83-93 (1987). |
| 5. Ann. Intern. Med., 100:663-671 (1984). | 14. JAMA, 258(23):3385 (1987). |
| 6. J. Pediat., 82:404-415 (1973). | 15. J. Clin. Microbiol., 28(2):393-394 (1990). |
| 7. J. Clin. Microbiol., 20:887-890 (1984). | 16. J. Clin. Microbiol., 29(3):645-647 (1991). |
| 8. J. Infect. Dis., 140:143-147 (1987). | 17. Clin. Micro. News, 13(1):3-5 (1991). |
| 9. J. Respir. Dis., June, 83-94 (1987). | |

Ordering Information

Cat. #	Description	Size
22363-1	Fungi-Fluor® <i>Pneumocystis</i> Kit (100 applications with 10 Control Slides)	1 kit
22251-1	<i>Pneumocystis carinii</i> Control Slides	10 slides/box
08381-120	Poly-Mount® Coverslipping Media	120ml
08381-940	Poly-Mount® Coverslipping Media	940ml

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