

## Use of the UV ParaLens Adapter as an Alternative to Conventional Fluorescence Microscopy for Detection of *Pneumocystis carinii* in Direct Immunofluorescent Monoclonal Antibody-Stained Pulmonary Specimens

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**The UV ParaLens light microscope adapter offers a useful and cost-effective alternative to conventional fluorescence microscopy for *Pneumocystis carinii* identification, particularly in AIDS patients. In a blinded study, in which 153 pulmonary specimens were examined for *P. carinii* by direct immunofluorescence, 40 of 42 specimens positive by fluorescence microscopy were also positive by ParaLens. No false positives were observed.**

*Pneumocystis carinii* is a major cause of life-threatening pneumonia (PCP) in both AIDS- and non-AIDS-immunosuppressed patients. PCP occurs in up to 80% of patients with AIDS in the United States, and with the spread of the human immunodeficiency virus (HIV) this diagnosis is being made more frequently (3, 5). The importance of rapid and accurate diagnosis of PCP is apparent in the face of such increasing numbers, given that early steroid therapy has a beneficial effect on outcome (6) and that drug toxicity and the need for invasive procedures for detection of other causes of pneumonia can be minimized (3, 5).

The classification of *P. carinii* is controversial, as it displays both fungal and protozoal characteristics (3, 12). Its life cycle is also uncertain; however, recognizable diagnostic stages include precysts, cysts, and trophozoites (8, 12). The laboratory diagnosis of PCP requires microscopic demonstration of organisms in appropriate pulmonary specimens obtained from sputum induction, bronchoscopy, tracheal aspiration, or biopsy (4, 11, 12). A wide variety of stains have been used to demonstrate all or some of the stages of *P. carinii*, including cyst wall stains such as methenamine-silver, organism stains such as Giemsa and its variants, and more recently available immunostaining methods (7, 11, 12). Some of the problems associated with the cyst wall and organism stains include technical difficulty, lack of specificity, and the time and expertise required to read slides (11). Fluorescent-antibody techniques have been shown to increase diagnostic sensitivity, most notably with induced sputum specimens (5, 12). A previous evaluation in our laboratory of a direct immunofluorescent-antibody (DFA) method revealed 99% sensitivity and negative predictive value (13); its simplicity and rapidity make it our method of choice and allow us to offer same-day results in most cases. A major drawback of fluorescent-antibody detection methods, especially for smaller laboratories, remains the requirement and expense of a fluorescence microscope plus a darkroom.

We compared the use of the UV ParaLens adapter (Becton Dickinson, Franklin Lakes, N.J.) to conventional epi-

fluorescence microscopy (FM) for reading DFA-stained pulmonary specimens for *P. carinii* in order to determine whether the ParaLens offers a useful alternative to FM for such examinations. The ParaLens adapter converts a standard light microscope to epiilluminated fluorescence and consists of a high-intensity halogen light source with a fiber optic cable connected to a specialized adapter that attaches to a spare lens port. The adapter contains a 470- to 490-nm excitation filter, a dichroic beam splitter at 505 nm, a barrier filter at 515 nm, and a 60× oil immersion lens. This system was originally developed for use with fluorescence techniques in malaria diagnosis (1, 2, 9) and has also been recently applied to fluorescent detection of rabies antibody (10). The list price of the ParaLens is approximately \$2,400.

Conventional FM was done with a Zeiss epifluorescence microscope (excitation filter, 450 to 490 nm; mirror, 510 nm; barrier filter, 520 nm; Carl Zeiss, Inc., New York, N.Y.) with a 40× dry objective. The price of such a scope generally ranges from \$10,000 to \$20,000.

In this study, specimens received in the laboratory were refrigerated and processed within 24 h. All induced sputum and selected other mucoid specimens were treated with a mucolytic agent (Sputolysin; Behring Diagnostics, Somerville, N.J.), followed by centrifugation (1,875 × *g*, 10 min), manual slide preparation, and fixation. DFA staining (developed by Genetic Systems, Inc., Seattle, Wash., and offered by Sanofi Diagnostics Pasteur, Inc., Chaska, Minn.) was performed as recommended by the manufacturer, except that specimens were fixed for 1 min in absolute methanol rather than for 10 min in acetone and Mayer's albumin (50% egg white albumin and 50% glycerin; Anderson Laboratories, Inc., Fort Worth, Tex.) was used on one well per slide; both modifications reduce specimen loss during staining (12, 13). After staining and incubation at 37°C for 30 min, the slides were read and scored as positive or negative. Although the monoclonal antibodies stain all forms of *P. carinii*, only the presence of three or more cysts was used to define a positive specimen. The entire procedure takes approximately 1.5 h to complete.

A total of 153 lower respiratory tract specimens (100 consecutive, followed by 53 random) obtained from 111 patients by sputum induction (*n* = 104), endotracheal suction

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( $n = 6$ ), or bronchoalveolar lavage ( $n = 43$ ) were prospectively examined in a blinded fashion by two full-time parasitology technologists (M.A.W. and L.S.S.). Each slide was examined with both FM and the ParaLens adapter. The patient group included 66 HIV-infected patients, 31 non-AIDS-immunocompromised patients (i.e., receiving immunosuppressive therapy for organ transplantation, malignant tumor, or other disease), and 14 patients without a specific immunosuppressed state. Of 42 specimens positive by FM, 40 were also positive by ParaLens (sensitivity 95.2%). All 111 FM-negative specimens were also negative by ParaLens (specificity 100%). Thirty-six of the 42 positive specimens (86%) were from AIDS patients, and the remaining positive specimens were from the non-AIDS-immunocompromised group; 34 of 42 positive specimens (81%) were induced sputum. The two specimens positive by FM but negative by ParaLens were reexamined by the two technologists by both techniques. Both specimens (one induced sputum specimen and one bronchoalveolar lavage specimen from non-AIDS-immunocompromised patients) were confirmed as positive by FM with only very rare single cysts or small cyst clusters which did not fluoresce brightly enough with the ParaLens to be considered positive. The morphology and number of organisms identified were comparable with both techniques, except for five specimens in which slightly fewer organisms were observed with the ParaLens; this did not hinder diagnosis.

Extraneous fluorescence, which often is noted on the slides under the conventional fluorescence microscope, appeared to be reduced by the ParaLens adapter, perhaps because of the slightly lower fluorescent intensity. The ParaLens was used in an active laboratory under normal room lighting conditions, and the technologists observed that dimming the lights occasionally made screening easier but was not necessary for proper specimen examination. The ParaLens magnification (60 $\times$  oil immersion lens), although higher than that used with conventional FM (40 $\times$  dry lens), seemed to compensate for the reduced fluorescent intensity.

The ParaLens offers sensitivity and specificity comparable to those of conventional FM for the detection of *P. carinii* in DFA-stained pulmonary specimens, particularly from HIV-infected patients, who tend to harbor large numbers of organisms (3, 13). The device may be a useful alternative for laboratories in which cost, space, and/or the unavailability of a darkroom preclude the use of a conventional fluorescence microscope. In addition, use of the ParaLens is likely to be applicable to a variety of other fluorescence detection techniques as well. More widespread use of sensitive and rapid

fluorescent-antibody techniques currently available should improve primary PCP diagnosis, particularly with the increasing reliance on induced sputum as a first-line diagnostic specimen.

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