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Use of UV ParaLens Adapter for Detection of Acid-Fast Organisms

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Auramine-stained mycobacterial smears from 136 clinical specimens were interpreted by using the UV ParaLens adapter (Beckton Dickinson), and results were compared with smear interpretations using a traditional fluorescent microscope and culture. The sensitivity and specificity of the ParaLens were 84 and 93%, respectively. Smears yielding discrepant results were overstained by the Kinyoun method. Overall, the sensitivity of auramine-stained smears interpreted with the UV ParaLens was comparable to that of Kinyoun-stained smears.

The recent increase in incidence of mycobacterial infection has emphasized the need for rapid, reliable, and cost-effective screening of acid-fast smears. Sensitivity and ease of interpretation make fluorescent microscopy (FM) the standard for detecting mycobacteria in smears; however, the high cost of fluorescent microscopes may prohibit some laboratories from using fluorescent diagnostic techniques.

Two systems (UV ParaLens [Becton Dickinson] [Fig. 1] and Makler Fluorescence Objective [Flow, Inc.]) which convert a traditional light microscope to a fluorescent microscope through the use of a halogen light source, fiber optic cable, and microscope adapter which attaches to a spare lens port have been developed. These epifluorescent illumination systems are lightweight and portable, may be operated with a battery-powered light source, and are significantly less expensive than traditional fluorescent microscopes.

Studies have demonstrated the successful use of the fluorescent adapter systems in detection of fluorescein-stained *Plasmodium* species, rabies virus antibody, and *Pneumocystis carinii* (2–4). While one reference notes the ability to detect fluorescein-stained mycobacteria with such adapters, no prior studies have fully evaluated the application of the fluorescent adapters to mycobacteriology. We compared the auramine-stained smear results for 136 clinical specimens obtained by using the UV ParaLens (PL) fluorescent adapter with the smear results obtained by using traditional FM and with culture results to determine the value of the PL as an alternative to FM for detection of mycobacteria.

The current study compared auramine-stained smear results obtained by using the UV PL (Becton Dickinson) to traditional FM and acid-fast bacillus culture. Smears were prepared from all patient specimens submitted to the laboratory for mycobacteriology over 14 consecutive weeks. An additional five, randomly selected positive smears from prior clinical specimens were added to each day's workload. Sputa accounted for 70% of all specimens; the remaining specimens were sterile body fluids (pleural, pericardial, peritoneal, synovial, and cerebrospinal) (15.5%), blood (5%), tissue (8%), and gastric aspirate (1.5%). In accordance with the standard processing protocol, contaminated specimens were concentrated and decontaminated by the *n*-acetylcystine–NaOH method, and normally sterile body fluids were concentrated only (5). Smears were heat fixed and stained with TB Auramine O (Remel Laboratories, Lenexa, Kans.). Mycobacterial cultures were performed on all specimens.

A microbiology technologist read all auramine-stained smears using a Nikon fluorescent microscope under 20× and 40× objectives. A pathologist and an Infectious Diseases fellow read the smears using first the UV PL 40× oil objective attached to an Olympus binocular microscope and then using the conventional fluorescent microscope. The PL reviewers were blinded to each others' results and to the technologist's FM results. Results were reported as 1+ (rare), 2+ (few), 3+ (moderate) or 4+ (many) in accordance with the standards of interpretation published by the Centers for Disease Control and Prevention (1).

Smears interpreted as negative by either PL reviewer and as positive by the technologist were overstained with the Kinyoun stain. These smears were mixed with other Kinyoun-stained smears and read by one blinded reviewer. Three hundred oil fields per slide were reviewed; smears were considered positive if more than one acid-fast bacillus was seen.

A total of 136 auramine-stained smears were reviewed (Table 1). The results of the three reviewers' by FM were concordant. Of 65 FM-positive smears, 53 and 56 were scored as positive and 12 and 9 were interpreted as negative by PL reviewers 1 and 2, respectively. Only six smears were falsely interpreted as negative by both PL reviewers, and all of these were quantified as 1+ or 2+ by FM interpretation. Of 71 FM-negative smears, 68 and 64 were scored as negative and 3 and 7 were interpreted as positive by PL reviewers 1 and 2, respectively. No smear was falsely interpreted as positive by both PL reviewers. Four of the 15 false-negative smears were interpreted as positive after the fluorescent smear was overstained by the Kinyoun method.

With FM as the standard, the sensitivity of the PL ranged from 81.5 to 85%, giving an overall sensitivity of 84%. The specificity ranged from 90 to 95.7%, for an overall specificity of 93%.

Table 2 shows the results of auramine-stained smear interpretation compared with culture results. Of 54 positive cultures, 46 and 47 smears were scored as positive by reviewers using the PL and 53 smears were scored positive by FM. False-negative smears were most commonly associated with growth of *Mycobacterium avium-M. intracellulare*. Of 80 nega-

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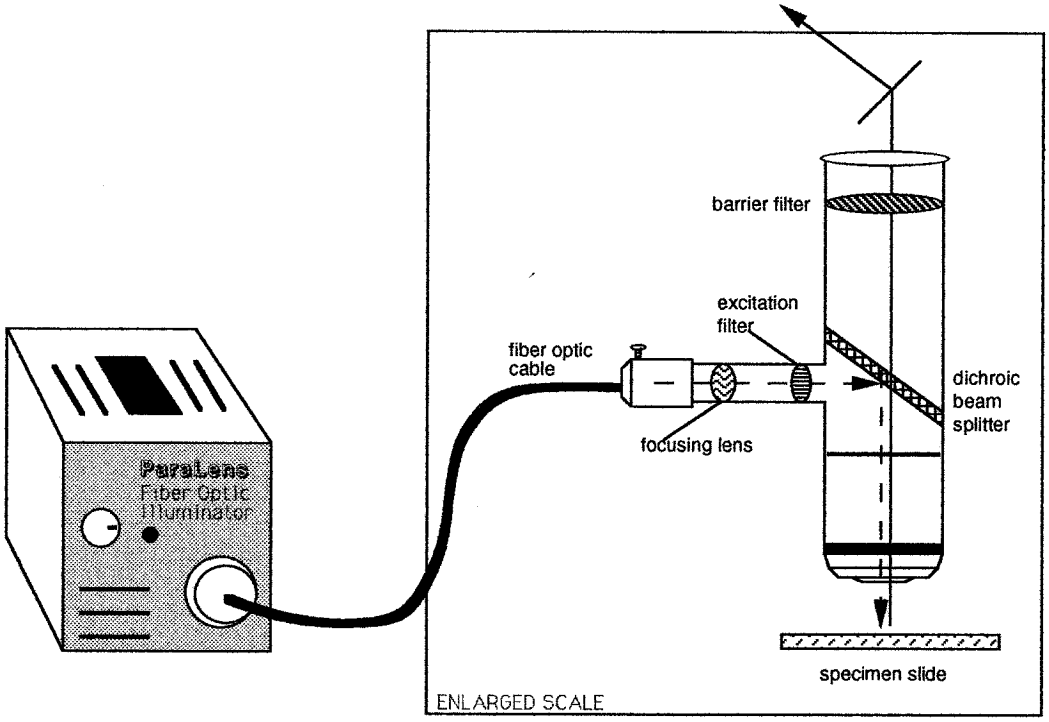

FIG. 1. The UV PL (Becton Dickinson) consists of a halogen light source and the PL objective. The halogen light is housed in a metal box (15 by 13 by 22 cm) and is powered by standard alternating current. A fiber-optic cable transmits UV light to a side port on the PL objective. Within the objective a focusing lens and excitation filter transmit light to a dichroic beam splitter, which then illuminates the specimen slide and reflects only safe visible light to the observer.

tive cultures, 70 and 64 smears were scored as negative by the PL reviewers and 68 were scored as negative by FM. False-positive smears were usually from repeat sputum samples from patients under therapy for *M. tuberculosis*. Compared with culture, the sensitivity of auramine-stained smears interpreted by the PL averaged 86.1% and that of traditional FM was 98%. The specificities for the PL and FM were 83.7% and 85%, respectively.

In the present study the detection of auramine-stained mycobacteria using the UV PL was demonstrated with a variety of specimen types and with several mycobacterial species. No difference in sensitivity of the UV PL was noted for the various specimen types. The adapter was simple to install and use, and its presence did not impede routine use of the other microscope objectives.

Specificity of the PL was excellent compared to FM and would likely be equivalent to FM in a clinical setting since questionable positives are usually confirmed by a Kinyoun stain or a second reviewer. Sensitivity of the PL was decreased compared with FM in part due to the decreased intensity of the PL adapter. This resulted in poor detection of small numbers of organisms. Similarly, compared with culture, the PL was shown to be less sensitive than FM. The sensitivity of the PL appeared nearly equivalent to that of Kinyoun-stained smears since only 4 of 15 false-negative smears were interpreted as positive after overstaining. However, this comparison was not ideally evaluated in the current study, since Kinyoun smears were not performed prospectively on all specimens. Because the level of sensitivity achieved with the PL was comparable to that achieved with the Kinyoun-stained smear and because screening of smears is more rapid with FM, the PL may be advantageous for laboratories currently performing only non-fluorescent stains.

On the basis of this study the PL adapter cannot be recommended as a replacement for the traditional FM in mycobacteriology but may be an acceptable, low-cost alternative in laboratories unable to afford a fluorescent microscope.

TABLE 1. Comparison of UV PL and FM auramine smear results

FM interpretation (*n*)	PL interpretation[a]			
	Reviewer 1		Reviewer 2	
	No. positive	No. negative	No. positive	No. negative
Positive (65)	53	12	56	9
Negative (71)	3	68	7	64

[a] Sensitivity, 84%; specificity, 93%.

TABLE 2. Comparison of FM and UV PL Smear Results with culture results

Culture result (*n*)	No. of smears scored correctly[a]		
	PL reviewer 1 (46)	PL reviewer 2 (47)	FM (53)
Positive (54)			
M. avium-M. intracellulare (25)	20	20	24
M. tuberculosis (24)	21	22	24
M. marinum (2)	2	2	2
M. kansasii (2)	2	2	2
M. scrofulaceum (1)	1	1	1
Negative (80)	70	64	68

[a] Sensitivities: FM, 98.1%; PL, 86.1%. Specificities: FM, 85%; PL, 83.7%. The total numbers of samples scored positive are given in parentheses.

Such laboratories might also benefit by using the PL adapter for interpretation of fluorescent smears for detection of larger organisms (such as *Giardia* and *Pneumocystis* spp.) which have inherently brighter intensity, making their detection less dependent on organism number.

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