Use of Chlorazol Black E Mounts of Corneal Scrapes for Diagnosis of Filamentous Fungal Keratitis

PHILIP ALOYSIUS THOMAS, JAYARAMAN KALIAMURTHY, CHRISTADOSS ARUL NELSON JESUDASAN, AND PITCHAIRAJ GERALDINE

• PURPOSE: **To determine whether chlorazol black E, a chitin-specific stain, can be used to detect fungal filaments in corneal scrapings and to compare its sensitivity as a diagnostic aid for fungal keratitis with that of gram and lactophenol cotton blue stains.**
• DESIGN: **Prospective study, laboratory investigation.**
• METHODS: **Between December 1, 2005 and July 31, 2006, corneal scrapes from 163 patients with ulcerative keratitis were used for culture and to prepare smears that were stained by lactophenol cotton blue, chlorazol black E, or gram stains. A diagnosis of fungal keratitis was established if fungal growth occurred on the inoculated areas of multiple culture plates.**
• RESULTS: **Fungi were isolated from corneal scrapes of 82 patients. Taking fungal culture positivity as the gold standard for diagnosis of fungal keratitis, direct microscopic examination of chlorazol black E mounts had a sensitivity of 82% and specificity of 98%; culture results and chlorazol black E results were identical in 89.6% of patients. Lactophenol cotton blue mounts and gram-stained smears had a sensitivity of 85%, specificity of 90% to 91%, and 88% agreement with culture results.**
• CONCLUSIONS: **Chlorazol black E can be used for detection of fungal filaments in corneal scrapings; however, it is less sensitive than lactophenol cotton blue and gram stains as a diagnostic aid for fungal keratitis. (Am J Ophthalmol 2008;145:971–976. © 2008 by Elsevier Inc. All rights reserved.)**

THIS STUDY DESCRIBES THE USE OF CHLORAZOL black E to detect fungal filaments in corneal scrapings in comparison with conventional staining techniques. Corneal scrapes from 163 patients with ulcerative keratitis were used. The chlorazol black E mounts had a sensitivity of 82% and specificity of 98%; culture results and chlorazol black E results were identical in 89.6%. The results suggest that chlorazol black E is a promising diagnostic aid for fungal keratitis.

Accepted for publication Jan 21, 2008.

From the Department of Microbiology, Institute of Ophthalmology, Joseph Eye Hospital, Tiruchirapalli, Tamil nadu, India (P.A.T., J.K.); the Institute of Ophthalmology, Joseph Eye Hospital, Tiruchirapalli, Tamilnadu, India (C.A.N.J.); and the Department of Animal Science, Bharathidasan University, Tiruchirapalli, Tamil nadu, India (P.G.).

Inquiries to Philip Aloysius Thomas, Department of Microbiology, Institute of Ophthalmology, Joseph Eye Hospital, Tiruchirapalli, 620 001,Tamil nadu, India; e-mail: philipthomas@satyam.net.in

Fungal keratitis (mycotic keratitis, keratomycosis, and fungal corneal ulceration) is a well-known ophthalmologic problem in developing countries such as China,[1] Ghana,[2] and India.[2–5] In 2006, contact lens-associated keratitis resulting from Fusarium species assumed epidemic proportions in several other countries, including the United States.[6] These observations underline the need for rapid diagnosis of fungal keratitis as a prelude to instituting specific antifungal therapy. The diagnosis of fungal keratitis can be made by isolating fungi in culture of corneal scrape or biopsy material, but this requires a minimum of 48 to 72 hours. However, a tentative diagnosis of the condition often can be made within a few minutes after direct microscopic detection of fungal hyphae or yeast cells in corneal material.[7] The sensitivities of the commonly used microscopy methods for fungal keratitis, such as the potassium hydroxide (KOH) wet mount, and gram-stained smear, have been found to vary widely, being very high in some centers,[1,3,4,8,9] but lower elsewhere[5,10,11]; moreover, these methods have drawbacks such as the frequent presence of background artifacts in KOH preparations and weak staining in gram-stained smears.[7] The calcofluor white (CFW) staining method allows a rapid, sensitive, and specific diagnosis of fungal keratitis,[3,7,9] but its use is limited by the need for an expensive fluorescence microscope; moreover, background fluorescence is prominent and certain tissue elements, such as collagen, elastin, and keratin, also strongly fluoresce, which may confuse an inexperienced observer.[12] Thus, there is a need for a staining technique for diagnosis of fungal keratitis that is rapid and easy to perform as well as highly sensitive and specific, and that requires only routine bright-field microscopy.

Chlorazol black E is a stain with a high affinity for chitin, a unique structural polysaccharide (a homopolymer of β-[1,4]-linked D-N-acetylglucosamine), which is found in fungal cell walls but not in vertebrate tissues; chlorazol black E stains the cell walls of filamentous fungi and of yeasts a blue-black color.[13] A chlorazol black E-stained wet mount of fingernail and toenail samples is a valuable diagnostic method for onychomycosis because it accentuates the presence of even small numbers of fungal hyphae without staining contaminants such as cotton or elastic fibers.[14] We reasoned that a chlorazol black E-stained wet film of corneal scrapes may allow a rapid, sensitive, and specific diagnosis of fungal keratitis. A search of the

0002-9394/08/$34.00
doi:10.1016/j.ajo.2008.01.020

PubMed database, using the keywords *chlorazol black* and *keratitis*, revealed that chlorazol black E hitherto has not been tried as a staining technique in diagnosis of fungal keratitis. We undertook this study with a two-part hypothesis: 1) chlorazol black E can be used to detect fungal hyphae in corneal scrapings, and 2) chlorazol black E is more sensitive than the gram and lactophenol cotton blue staining methods for diagnosis of fungal keratitis.

METHODS

• **PATIENTS AND TEST SAMPLES:** During the one-year period before the start of the study, microscopic examination of lactophenol cotton blue mounts and gram-stained smears of corneal scrape samples had a sensitivity of 75% to 78% in culture-proven fungal keratitis at our institution. Because the chlorazol black E mount hitherto has not been used as a diagnostic aid in fungal keratitis, its sensitivity as a diagnostic aid in onychomycosis[14] was taken as a guide to project a desired sensitivity and specificity of approximately 90% in fungal keratitis, for which we calculated[15] a sample size of 142 patients to yield a significance level of $\alpha = 0.05$. In fact, after obtaining approval from the institutional review board and informed consent from each patient, we enrolled 163 patients with suspected microbial (ulcerative) keratitis over an eight-month period (December 1, 2005 through July 31, 2006). These patients previously had not undergone microbiologic investigation of their condition.

Under topical anesthesia (4% lignocaine hydrochloride) and slit-lamp magnification, corneal scrapes were obtained by qualified cornea specialists from the base and edge of each ulcer using a sterile, blunt cataract knife. Corneal scrapes were collected first for microscopic evaluation and subsequently for culture procedures.

Gram, lactophenol cotton blue, and chlorazol black E stains were used for microscopic evaluation of corneal scrapes. There was no randomization of sampling order; however, over the eight-month study period, the order of the corneal scrapes for the three stains varied considerably. Corneal scrape material was smeared thinly within a marked area on glass slides for gram stain. To prepare a chlorazol black E wet mount, the material was placed within a marked area on a glass slide, covered with one drop of chlorazol black E (1% chlorazol black E [Sigma, St Louis, Missouri, USA] in 2-methoxy ethanol + 0.5% glycerol)[16] stain, followed by a cover slip. To prepare a lactophenol cotton blue wet mount, a similar procedure was followed as for the chlorazol black E mount, but using lactophenol cotton blue as the mounting fluid. Two trained ocular microbiologists (P.A.T. and J.K.), who were masked from clinical examination findings, examined all the slides by routine bright-field microscopy. The gram-stained smear was examined at ×100, ×400, and ×1000 magnification, whereas the lactophenol cotton blue and chlorazol black E wet mounts were examined at ×100 and ×400 magnification. The number of fields examined varied depending on the quantity of corneal scrape material on the slide. However, for lactophenol cotton blue and chlorazol black E wet mounts, at least 25 fields were examined at ×100 magnification and 100 fields at ×400 magnification, whereas for gram-stained smears, at least 25 fields were examined at ×100 magnification, 100 fields at ×400 magnification, and 250 fields at ×1000 magnification. A filamentous structure was deemed to be a fungal filament if it could be recognized under low power (×100) and confirmed by higher magnification (×400), exhibited branching and septation, and was found within the central area of the smear.[17] The stained smear or wet mount was considered positive for fungus if at least one fungal filament was noted in the entire slide and was considered negative if not even one fungal filament was noted in the entire slide. Independent results were recorded by each of the two observers to enable calculation of interobserver variability. Intraobserver variability was not determined.

After obtaining corneal material for microscopic evaluation, corneal scrapes were obtained for culture. The corneal scrapes were directly inoculated onto plates of sheep blood agar (SBA), neutral Sabouraud glucose neopeptone agar, Emmons modification (SDA), and if material remained, on cystine tryptone agar (CTA) as well, by making several rows of C-shaped streaks on the agar. The SBA and CTA plates were incubated at 37 C for five days (to exclude the presence of bacteria), whereas the SDA plates were kept at room temperature for up to two weeks. Fungal colonies appearing on the plates were identified by standard methods.[7] Fungal growth in culture was deemed to be significant (fungal culture positive and culture-proven fungal keratitis) by growth of the same fungus on the C streaks on more than one culture medium; in most of the fungal culture-positive cases, luxuriant growth was obtained within three to four days on the C streaks made on the inoculated plates.

RESULTS

A DIAGNOSIS OF FUNGAL KERATITIS WAS ESTABLISHED BY positive culture results in 82 of the 163 patients investigated. The principal fungi isolated were species of Fusarium, followed by Aspergillus, and phaeohyphomycetes (pigmented fungi).

Fungal filaments were detected in chlorazol black E mounts (Figure, Top left and right) of corneal scrape material (chlorazol black E positive) from 67 (82%) of 82 patients with culture-proven fungal keratitis; fungal filaments were not detected (chlorazol black E negative) in 79 (98%) of 81 patients in whom fungal culture results were negative (Table 1). Fungal filaments also were detected in gram-stained smears (gram-smear positive; Figure, Bottom left) and lactophenol cotton blue mounts (lactophenol

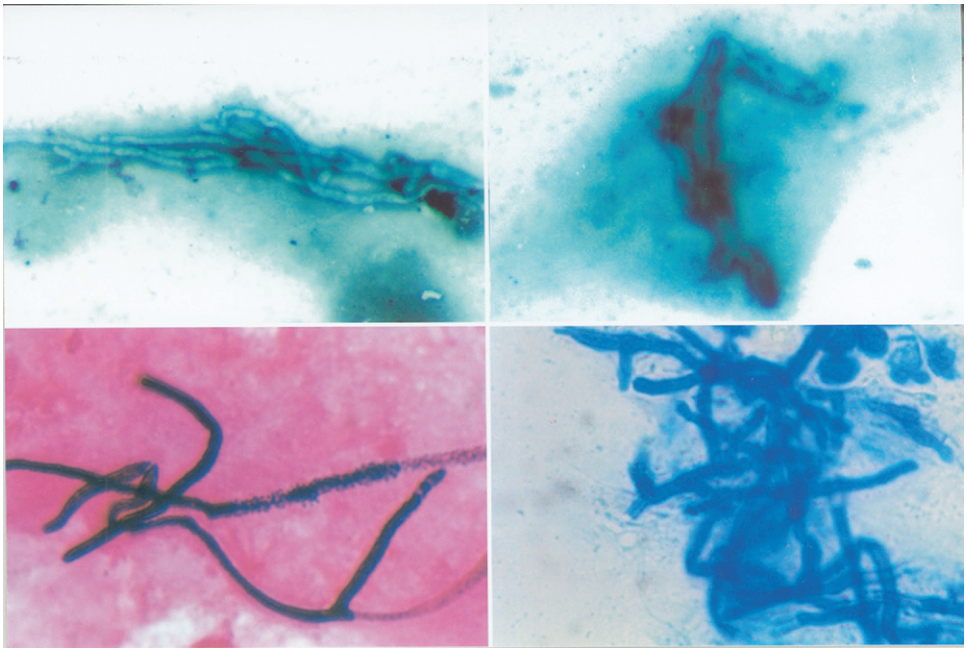

FIGURE. Fungal hyphae in corneal scrapes of patients with keratitis. (Top left) Chlorazol black E mount of corneal scrape material showing fungal filaments (hyphae) of Fusarium species (original magnification, ×400). (Top right) Chlorazol black E mount of corneal scrape material showing filaments (hyphae) of a pigmented fungus (original magnification, ×400). (Bottom left) Gram-stained smear of corneal scrape material showing fungal filaments (hyphae) of Aspergillus species (original magnification, ×1000). (Bottom right) Lactophenol cotton blue mount of corneal scrape material showing fungal filaments (hyphae) of Fusarium species (original magnification, ×400).

TABLE 1. Results of Microscopic Evaluation Using Different Staining Methods in 163 Patients with Keratitis

	Chlorazol Black E		Gram Stain		Lactophenol Cotton Blue	
Fungal Culture	Positive	Negative	Positive	Negative	Positive	Negative
Positive	67	15	70	12	70	12
Negative	2	79	8	73	7	74

Fungal culture results: positive = significant growth of fungi in culture; negative = fungi not grown or growth in culture not significant.
Microscopic evaluation results: positive = at least one fungal filament seen in the entire smear; negative = not even one fungal filament seen in the entire smear.

cotton blue positive; Figure, Bottom right) of corneal scrape material from 70 (85%) of 82 patients with culture-proven fungal keratitis; fungal filaments were not detected in gram-stained smears (gram-smear negative) from 73 (90%) of 81 patients nor in lactophenol cotton blue mounts (lactophenol cotton blue negative) from 74 (91%) of 81 patients in whom fungal culture results were negative (Table 1). Thus, as a diagnostic aid in fungal keratitis, the chlorazol black E mount had a sensitivity of 82%, which was significantly lower ($P < .001$) than the 85% sensitivity obtained with gram-stained smears and lactophenol cotton blue mounts; however, the specificity of chlorazol black E mounts (98%) was higher than that of the gram (90%) and lactophenol cotton blue (91%) preparations (Table 2).

The degree of agreement between results of microscopic evaluation and of culture (microscopic evaluation positive in culture-positives, microscopic evaluation negative in culture-negatives) was 89.6% for chlorazol black E mounts, 87.7% for gram-stained smears, and 88.3% for lactophenol cotton blue mounts (Table 2). The degree of agreement between the different staining methods used was 90.2% between chlorazol black E and gram, 93.9% between chlorazol black E and lactophenol cotton blue, and 94.5% between gram and lactophenol cotton blue (Table 2).

In the 81 patients whose fungal cultures yielded negative results, all three staining methods also produced negative results in 72 (88.9%) patients, but all three were positive in two (2.5%) patients; in the remaining seven patients,

TABLE 2. Salient Features of Chlorazol Black E Mount, Gram-Stained Smear, and Lactophenol Cotton Blue Mount as Diagnostic Aids for Fungal Keratitis

Feature	Chlorazol Black E Mount	Gram-Stained Smear	Lactophenol Cotton Blue Mount
Sensitivity (%)	82	85	85
Specificity (%)	98	90	91
Predictive value of positive test results (%)	97	90	91
Predictive value of negative test results (%)	84	86	86
Agreement with culture (%)	89.6	87.7	88.3
Agreement with results of chlorazol black E mount	—	90.2	93.9
Agreement with results of gram smear	90.2	—	94.5

gram and lactophenol cotton blue stains were positive and chlorazol black E stain was negative in four, Gram stain alone was positive, whereas lactophenol cotton blue and chlorazol black E stains were negative in two and lactophenol cotton blue stain alone was positive, whereas Gram and chlorazol black E stains were negative in one (Table 3). Thus, even in the patients whose fungal cultures showed negative results, the gram-stained smear and lactophenol cotton blue mount yielded more positive results (eight and seven, respectively) than did the chlorazol black E mount (only two).

No interobserver variability was noted in results of microscopic evaluation of gram-stained smears (Table 4). With reference to the lactophenol cotton blue and chlorazol black E mounts, the degree of interobserver variability was 0.6%.

In chlorazol black E mounts, fungal hyphae were delineated clearly because the cell walls and cross walls (septa) stained a distinctive, blue-black color; there was no staining of collagen fibers, necrotic tissue debris, or other structures (Figure, Top left and right). In gram-stained smears, the protoplasm of the fungal filaments was stained; cell walls and septa, which were not stained, were seen by negative staining and refractive properties (Figure, Bottom left). In lactophenol cotton blue mounts, fungal cell walls were stained in some samples, whereas the fungal protoplasm was stained in other samples (Figure, Bottom right).

The chlorazol black E mount was positive in 79% of patients from whose ulcers Aspergillus or Fusarium species were isolated. Similarly, the gram-stained smear was positive in 83% and 82%, whereas the lactophenol cotton blue mount was positive in only 62% and 74% of patients from whose ulcers Aspergillus or Fusarium species, respectively, were isolated. Interestingly, all the three staining methods yielded positive results in 100% of patients from whose ulcers phaeohyphomycetes (Curvularia species, *Bipolaris spicifera*, and Exserohilum species) were isolated. In 13 patients from whose ulcers fungi were isolated that could not be identified, both the chlorazol black E mount and the gram-stained smear showed positive results in 10 (77%), whereas the lactophenol cotton blue mount showed positive results in 12 (92%). This data is not shown in any table.

TABLE 3. Results of Microscopic Evaluation of Chlorazol Black E, Gram, and Lactophenol Cotton Blue Stains in Relation to Culture Results

Results of Microscopic Evaluation	Fungal Culture Results		Total
	Positive	Negative	
All three stains positive	63	2	65
All three stains negative	9	72	81
Chlorazol black E negative, gram positive, lactophenol cotton blue positive	4	4	8
Chlorazol black E negative, gram positive, lactophenol cotton blue negative	2	2	4
Chlorazol black E negative, gram negative, lactophenol cotton blue positive	0	1	1
Chlorazol black E positive, gram negative, lactophenol cotton blue positive	3	0	3
Chlorazol black E positive, gram positive, lactophenol cotton blue negative	1	0	1
Total	82	81	163

Chlorazol black E positive = at least one fungal filament detected in chlorazol black E mount.

Chlorazol black E negative = fungal filaments not detected in chlorazol black E mount.

Gram positive = at least one fungal filament detected in gram-stained smear.

Gram negative = fungal filaments not detected in gram-stained smear.

Lactophenol cotton blue positive = at least one fungal filament detected in lactophenol cotton blue mount.

Lactophenol cotton blue negative = fungal filaments not detected in lactophenol cotton blue mount.

TABLE 4. Interobserver Variability in Microscopic Evaluation of Chlorazol Black E and Lactophenol Cotton Blue Mounts and Gram-Stained Smears of Corneal Scrapings from Patients with Keratitis

Test Performed	No. of Positive Results (% Total of 163 Samples) Detected by	
	Observer 1	Observer 2
Chlorazol black E mount	69 (42.3%)	68 (41.7%)
Lactophenol cotton blue mount	76 (46.6%)	77 (47.2%)
Gram-stained smear	78 (47.9%)	78 (47.9%)

DISCUSSION

RAPID IDENTIFICATION OF AN INFECTING ORGANISM IS essential to ensure the successful medical therapy of corneal infections. This is particularly true for infections caused by fungi because, in addition to the need for special growth media, these organisms require at least 48 to 72 hours (sometimes longer) to grow out in culture. Hence, microscopic evaluation of corneal scrapings obtained from patients with suspected fungal keratitis is of utmost importance because rapid detection of fungal structures permits early institution of antifungal therapy.

Several techniques for microscopic detection of fungal structures in corneal scrapings have been described, such as the KOH mount, gram-stained smear, lactophenol cotton blue mount, methenamine silver (GMS)-stained smear, and CFW. In experienced hands, the KOH mount has been found to yield sensitivities of 88.7%,[1] 91%,[3] 99.3%,[4] and even 100%[9] in diagnosis of fungal keratitis. However, even in recent studies, lower sensitivities also have been reported: 62.3% in New Delhi,[5] 71.4% in Iran,[18] and 76 % in New York.[11] This disparity may arise because the KOH mount is unreliable if hyphae are rare[19]; moreover, KOH mounts cannot be explored as fully as stained slides, are not suitable for viewing under the oil immersion objective, and commonly exhibit false-positive artifacts, which may confuse an inexperienced observer.[7,19] The gram-stained smear also exhibits variable sensitivities in diagnosis of fungal keratitis, being as high as 98%,[8] 89.2%,[4] and 88.2%,[3] but also as low as 42.9%,[18] 60%,[5] and 65%[10] in recent studies. A drawback of the gram stain is that it is not specific enough to allow easy differentiation of stained organisms from background corneal and inflammatory cells. In addition, it stains the protoplasm of some, but not all, fungal hyphae, and the staining may not be uniform but only a stippling, whereas other fungi may stain only weakly; the gram-stained smear may yield false-positive results in evaluation of keratitis because of the precipitation of the crystal violet component, or because of staining of fibrous protein.[19] Lactophenol cotton blue mounts have been reported to yield sensitivities of 77%[9] to 78%[20] in diagnosis of fungal keratitis; drawbacks include nondigestion of corneal tissue and nondetection of unusual fungi. A method for staining fungal hyphae in corneal scrapings by GMS stain was reported in 1976,[19] with a sensitivity of 87%; however, a more recent study reported a sensitivity of just 56%.[21] Moreover, this staining technique takes one hour and requires nine steps.

Calcofluor white is a laundry brightener that binds to polymers of β-linked polysaccharides, particularly cellulose and chitin found in fungal cell walls, and this explains the high sensitivities of the CFW staining method, more than 90% in some series,[3,9] in establishing a diagnosis of fungal keratitis. Limitations of this technique include the high cost of fluorescence microscopy, the possibility of excessive background fluorescence, and nonspecific staining of collagen or elastic fibers and keratin.[12] However, the efficacy of the CFW stain for diagnosis of fungal keratitis highlights the relevance of using a stain that binds to the chitin of fungal cell walls to establish a diagnosis of fungal keratitis. We chose chlorazol black E for our study because it is a chitin-specific stain that is already in use for the diagnosis of a human fungal infection, namely onychomycosis[13,14]; moreover, the stain requires the use of an ordinary bright-field microscope, which is available in every diagnostic clinical laboratory.

Preparation of chlorazol black E mounts was a simple procedure, requiring spreading of corneal scrape material on a glass slide, application of a drop of chlorazol black E stain, and covering the same with a cover slip, the entire process taking about 30 seconds. The chlorazol black E mount was ready for viewing under the microscope within two minutes of its preparation. Thus, the chlorazol black E mount could be considered as a simple, rapid method for microscopic evaluation of corneal scrapes in fungal keratitis.

The present study had a two-part hypothesis: one, that chlorazol black E could be used to demonstrate fungal hyphae in corneal scrape material, and two, that the chlorazol black E mount of corneal scrapes would be more sensitive than the gram-stained smear and the lactophenol cotton blue mount for diagnosis of fungal keratitis. The first part of the hypothesis was validated, because the chlorazol black E mount demonstrated fungal hyphae in corneal scrape material from 69 patients with keratitis. However, the second part of the hypothesis was not validated, because the chlorazol black E mount turned out to be significantly less sensitive (82%) than the gram-stained smear (85%) and lactophenol cotton blue mount (85%) in diagnosis of fungal keratitis. Despite this shortcoming, we believe that the chlorazol black E mount holds promise as a diagnostic aid in fungal keratitis for several reasons, outlined below.

The chlorazol black E mount was more specific (98%) than the gram-stained smear (90%) and lactophenol cotton blue mount (91%). We suggest, therefore, that the gram-stained smear and lactophenol cotton blue mount, being more sensitive, could be used as screening microscopic evaluation tests for fungal keratitis, whereas the chlorazol black E mount, being more specific, could be used as a confirmatory test. Such a confirmatory test would be especially valuable in instances where only a single screening test is positive, because there may be hesitation in starting antifungal therapy while waiting for the culture results. For example, in nine patients in the present study, either the gram-stained smear or the lactophenol cotton blue mount (but not both) produced positive results (Table 3). The chlorazol black E mount demonstrated positive results and culture also produced positive results in four of the nine patients, the chlorazol black E mount produced negative results and culture also produced negative results in three patients, whereas chlorazol black E produced negative results but positive culture results in two patients. If culture is considered to be the gold standard for diagnosis of fungal keratitis, then the results of the chlorazol black E mount had a higher degree of agreement with culture results than did the results of the lactophenol cotton blue mount and gram-stained smear (Table 2). If microscopic evaluation is

considered to be the gold standard for diagnosis of fungal keratitis, then the results of the chlorazol black E mount had a very high degree of agreement with those of the gram-stained smear (90.2%) and lactophenol cotton blue mount (93.9%).

The chlorazol black E stain is chitin specific; hence, it stains the cell walls of fungi and does not stain potential contaminants. A limitation of the chlorazol black E mount in the present study was that it yielded negative results in 15 patients with culture-proven fungal keratitis; however, the gram-stained smear and lactophenol cotton blue mount also produced negative results in nine of these 15 patients. The chlorazol black E mount also produced negative results in another seven patients whose culture results were negative but in whom the lactophenol cotton blue or gram stains (or both) yielded positive results. A possible explanation for this is that it is unlikely that every scraping from a fungal corneal lesion will contain hyphal fragments that are visible on a smear or are viable in culture media.[17] Additional studies on the chlorazol black E mount in different centers may improve the sensitivity of this staining method in diagnosis of fungal keratitis.

In conclusion, the present study has shown that chlorazol black E, a chitin-specific stain, can be used to stain fungal filaments in corneal scrapings. The chlorazol black E mount is simple to prepare and can be used as a rapid technique for microscopic evaluation of corneal scrape material in suspected fungal keratitis. Although the chlorazol black E mount was less sensitive than the gram-stained smear and lactophenol cotton blue mount for diagnosis of fungal keratitis, the results of chlorazol black E were in agreement with those of culture and those of the other stains in a high percentage of patients. Chlorazol black E may be evaluated further as a diagnostic aid for fungal keratitis.

THE AUTHORS INDICATE NO FINANCIAL SUPPORT OR FINANCIAL CONFLICT OF INTEREST. INVOLVED IN DESIGN AND conduct of study (P.A.T., J.K., C.A.N.J., P.G.); collection (C.A.N.J.), management, analysis, and interpretation of data (P.A.T., J.K., P.G.); and preparation, review, and approval of the manuscript (P.A.T., J.K.). The approval of the Institutional Review Board of the Institute of Ophthalmology, Joseph Eye Hospital, was obtained for the conduct of this study. Written informed consent for the use and disclosure of protected health information was obtained from all subjects enrolled in the study.

REFERENCES

1. Xie L, Zhong W, Shi W, Sun S. Spectrum of fungal keratitis in North China. Ophthalmology 2006;113:1943–1948.
2. Leck AK, Thomas PA, Hagan M, et al. Aetiology of suppurative corneal ulcers in Ghana and South India, and epidemiology of fungal keratitis. Br J Ophthalmol 2002;86:1211–1215.
3. Gopinathan U, Garg P, Fernandes M, Sharma S, Athmanathan S, Rao GN. The epidemiological features and laboratory results of fungal keratitis. A 10-year review at a referral eye care centre in South India. Cornea 2002;21:555–559.
4. Bharathi MJ, Ramakrishnan R, Vasu S, Meenakshi R, Palaniappan R. Epidemiological characteristics and laboratory diagnosis of fungal keratitis. A three-year study. Indian J Ophthalmol 2003; 51:315–321.
5. Chowdhary A, Singh K. Spectrum of fungal keratitis in North India. Cornea 2005; 24:8–15.
6. Chang DC, Grant GB, O'Donnell K. et al. Multistate outbreak of Fusarium keratitis associated with use of a contact lens solution. JAMA 2006;296:953–963.
7. Thomas PA. Current perspectives on ophthalmic mycoses. Clin Microbiol Rev 2003;16:730–797.
8. Dunlop AA, Wright ED, Howlader SA, et al. Suppurative corneal ulceration in Bangladesh: a study of 142 cases, examining the microbiological diagnosis, clinical and epidemiological features of bacterial and fungal keratitis. Aust N Z J Ophthalmol 1994;22:105–110.
9. Sharma S, Silverberg M, Mehta P, Gopinathan U, Agrawal V, Naduvilath TJ. Early diagnosis of mycotic keratitis: predictive value of potassium hydroxide preparation. Indian J Ophthalmol 1998;46:31–35.
10. Tanure MA, Cohen EJ, Grewal S, Rapuano CJ, Laibson PR. Spectrum of fungal keratitis at Wills Eye Hospital, Philadelphia, Pennsylvania. Cornea 2000;19:307–312.
11. Ritterband DC, Seedor JA, Shah MK, Koplin RS, McCormick SA. Fungal keratitis at the New York eye and ear infirmary. Cornea 2006;25:264–267.
12. Monheit JE, Cowan DF, Moore DG. Rapid detection of fungi in tissues using calcofluor white and fluorescence microscopy. Arch Pathol Lab Med 1984;108:616–618.
13. Pierard GE, Arrese JE, De Doncker P, et al. Present and potential diagnostic techniques in onychomycosis. J Am Acad Dermatol 1996;34:273–277.
14. Elewski BE. Onychomycosis: pathogenesis, diagnosis, and management. Clin Microbiol Rev 1998;11:415–429.
15. Sommer A. Epidemiology and statistics for the ophthalmologist. New York, New York: Oxford University Press, 1980: 47–73.
16. Robards AW, Purvis MJ. Chlorazole black E as a stain for tension wood. Stain Technol 1964;39:309–315.
17. Jones DB, Wilson L, Sexton R, Rebell G. Early diagnosis of mycotic keratitis. Trans Ophthalmol Soc U K 1970;89:781–797.
18. Shokohi T, Nowroozpoor-Dailami K, Moaddel-Haghighi T. Fungal keratitis in patients with corneal ulcer in Sari, Northern Iran. Arch Iran Med 2006;9:222–227.
19. Forster RK, Wirta MG, Solis M, Rebell G. Methenamine-silver-stained corneal scrapings in keratomycosis. Am J Ophthalmol 1976;82:261–265.
20. Thomas PA, Kuriakose T, Kirupashankar MP, Senthil Maharajan V. Use of lactophenol cotton blue mounts of corneal scrapings as an aid to the diagnosis of mycotic keratitis. Diagn Microbiol Infect Dis 1991;14:219–224.
21. Upadhyay MP, Karmacharya PCD, Koirala S, et al. Epidemiological characteristics, predisposing factors and aetiological diagnosis of corneal ulceration in Nepal. Am J Ophthalmol 1991;111:92–99.

Biosketch

Philip Aloysius Thomas, MD, PhD, is a Professor and Head, Department of Ocular Microbiology, Institute of Ophthalmology, Joseph Eye Hospital, Tiruchirapalli, Tamil nadu, India. He has written a book on clinical microbiology, contributed chapters to several books and published around 90 research papers on ocular infections. He is a recipient of Tamil nadu Scientist Award in Medical Sciences of Tamil Nadu State Council for Science and Technology, India for contributions to studies on ocular infection.

Biosketch

Jayaraman Kaliamurthy, MSc, PhD, is an Assistant Professor, Department of Ocular Microbiology, Institute of Ophthalmology, Joseph Eye Hospital, Tiruchirapalli, Tamil nadu, India. His special interest is ocular infections and disseminated Aspergillus infections. He has contributed one chapter to a book and published 15 research papers in Journals.