Comparison of Fluorescence Microscopy and Different Growth Media Culture Methods for *Acanthamoeba* Keratitis Diagnosis

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Abstract. *Acanthamoeba* keratitis (AK), a potentially blinding infection of the cornea, is caused by a free-living protozoan. Culture and microscopic examination of corneal scraping tissue material is the conventional method for identifying *Acanthamoeba*. In this article, we compared several methods for AK diagnosis of 32 patients: microscopic examination using fluorescent dye, specific culture on growth media—non-nutrient agar (NNA), culture on liquid growth media—peptone yeast glucose (PYG), and TYI-S-33. AK was found in 14 patients. Thirteen of the specimens were found AK positive by fluorescence microscopic examination, 11 specimens were found AK positive on PYG growth media, and 9 specimens were found AK positive on TYI-S-33 growth media. Only five specimens were found AK positive on NNA growth media. Therefore, we recommend using fluorescence microscopy technique and culture method, especially PYG liquid media.

INTRODUCTION

Acanthamoeba keratitis (AK), a potentially blinding infection of the cornea, is caused by a free-living protozoan that is ubiquitous in nature, found commonly in water; soil; air; cooling towers; heating, ventilating, and air-conditioning (HVAC) systems; and sewage systems.¹ The *Acanthamoeba* life cycle includes the motile protozoan (15–45 mm in diameter) and dormant cyst (10–25 mm diameter) forms. Cysts are double walled and resistant to environmental stressors or intentional killing by freezing, desiccation, and chlorine in water supplies, swimming pools, and hot tubs. The trophozoite has an amoeboid shape with pseudopodia, and feeds on small algae, bacteria, and other protozoans.² In the cornea, they are thought to feed on keratocytes. *Acanthamoeba* mostly manifest in young healthy adults; up to 70% of reported cases have been associated with contact lens use. Essentially, any event that disrupts the corneal epithelium is a potential risk factor for AK. Patients suffering from AK typically present with a unilateral, red, and painful eye. Early stages examination may reveal a nonspecific epitheliopathy, which can progress to ulceration surrounded by infiltration. Other findings associated are limbitis, perineuritis, pseudodendritic keratitis, anterior uveitis, granulomatous stromal reaction, and ring infiltrate. Treatment of AK is challenging because of the organism's ability to encyst as a response to commonly used topical antibiotics.^{3,4} Drugs that are considered effective include polyhexamethylene biguanide (PHMB), propamidine isethionate (Brolene), chlorhexidine digluconate 0.02%, polymixin B, neomycin, and clotrimazole 1%.^{5,6}

The culture of corneal scraping tissue material is the conventional method for identifying *Acanthamoeba*. Nevertheless, molecular tests such as DNA polymerase chain reaction (PCR) appear to have a sensitivity of 84% and specificity of 100%.^{7–9} However, culture and microscopic examination by visualizing the pathogen on stained smears still remain the most common diagnostic tools in use.^{10,11}

In this article, we compared several methods for AK diagnosis: microscopic examination using fluorescent dye, specific culture on growth media—non-nutrient agar (NNA), culture on liquid growth media—peptone yeast glucose (PYG), and TYI-S-33.

MATERIALS AND METHODS

Patient characteristics. Thirty-two corneal scrapings that were suspected as AK infected were obtained from 32 patients from three medical centers in the north of Israel; they included 11 female and 21 male patients (mean age 26.7). Patients selected for the above diagnostic procedure presented with clinical features applicable to *Acanthamoeba* infection, namely severe tenacious corneal infection, perineuritis in the presence of excruciating pain additional to other characteristic signs and symptoms suggesting the disease. Moreover, excluding one male who suffered a minor corneal injury contaminated with soil, all patients were admitted for poor contact lens hygiene.

Specimen acquisition and transport to the laboratory. After a comprehensive slit-lamp examination, topical anesthesia using oxybuprocaine hydrochloride 0.4% eye drops was administered into the eye of a patient suspected of having AK infection. A specimen was collected by scraping the corneal lesion at its peripheral borders using a 25G needle. The collected material was applied to a circumscribed area on a glass microscope slide. An additional sample in a similar volume was taken from the remaining unscraped peripheral border of the lesions and collected into a sterile Eppendorf tube (Miniplast, Ein-Shemer, Israel) that contained 1 mL sterile physiological solution (NaCl 0.9%).

All specimens, both glass slides and Eppendorf tubes, were transported in a protective tank to the Clinical Microbiology Laboratory at Poriya Medical Center (Tiberias, Israel) with an interval of less than 2 hours following corneal scraping.

Microscopic examination. Specimens were stained by Calcofluor White dye (BD Diagnostics, Sparks, MD). Microscopic examination was performed using a fluorescence microscope under ×400 magnification.

Calcofluor White is a chemofluorescent dye with an affinity for the polysaccharide polymers of amoebic cysts. The

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double walls of AK cysts stained bright greenish white and glow in contrast to the black background of the supporting tissues.

Culture. The content of the Eppendorf tubes was resuspended using a vortex shaker for 1 minute for tissue release from the needle and to form a homogeneous suspension. Tube content was divided equally (300 mL) between growth media: 1 mL PYG, 1 mL TYI-S-33, and NNA. *Escherichia coli* were seeded onto agar media for amoeba nourishment. All the growth media used were produced in our laboratory in accordance with relevant protocols.

Growth media were incubated for 5 days at 28°C. At the end of the 5-day incubation period the supernatant liquid media was discarded and the pellet was examined by microscope in $\times 400$ magnification. Agar growth media (NNA) were examined by binocular microscope in $\times 100$ magnification for detection of amoebas and trophozoites on a seeded layer of *E. coli*. Assuring positive quality control, an *Acanthamoeba polyphaga* ATCC 30461 was separately applied to the above growth media. As negative control, unmanipulated growth media kept under the same conditions described ultimately proved barren. Each specimen was evaluated twice by each of two experienced laboratory staff members; the same results were obtained on all occasions.

RESULTS

Specimens were obtained from 32 patients who were suspected to be AK positive. AK was found in 14 patients (44% of the whole group). Thirteen of the specimens were found AK positive by fluorescence microscopic examination, 11 specimens were found AK positive on PYG growth media, and 9 specimens were found AK positive on TYI-S-33 growth media. Only five specimens were found AK positive on NNA growth media. Five specimens were positive in all methods; three specimens were positive by fluorescence microscopy, PYG, and TYI-S-33 growth media; two specimens were positive by fluorescence microscopy and PYG growth media; one specimen was positive by fluorescence microscopy and TYI-S-33 growth media; two specimens were positive only by fluorescence microscopy; and one specimen was positive only by PYG growth media (Table 1).

DISCUSSION

AK is a rare disease in which amoebae invade the cornea of the eye. This may result in permanent visual impairment or blindness. *A. castellanii* and *A. polyphaga* are the most commonly reported subspecies to cause keratitis.^{12,13} The *Acanthamoeba* life cycle includes the motile protozoan and the dormant cyst. Both forms are found in infected tissue, but only the trophozoite form is infectious. AK diagnosis is difficult; occasionally, it is mistaken for other corneal infections. A high level of suspicion is crucial. AK infection should be considered in cases of corneal trauma associated with soil or contaminated water as well as in patients who wear contact

lenses, especially if improper contact lens hygiene is suspected. It should also be suspected in cases of persistent corneal infection that does not respond properly to treatment.^{1,2}

Considering infection severity and treatment complexity, rapid and efficient laboratory diagnosis is necessary. Consequently, it is crucial to promote awareness among clinicians and laboratory technicians to select the best detection method. Apart from molecular biology techniques, microscopic examination and culture methods are most commonly used for AK detection; therefore, it is important to choose appropriate growth media. This study demonstrates that fluorescence microscopy method with Calcofluor White stain is the most appropriate for AK detection in terms of simplicity, sensitivity, and rapid diagnosis. Furthermore, taking into account the similar laboratory findings obtained by the two staff members reinforces the reliability of the above diagnostic approach. Nevertheless, a fluorescence microscope is required—apparatus that is frequently not found in laboratories because of its high cost. Of all growth media we have examined, PYG appears to be most efficient. As a whole, liquid media enables contents centrifugation and thereby pellet examination, which allows specified parasite screening and raising AK detection probability, contrary to screening action of the solid media with binocular microscope, which may be a complex and longer procedure.

The main disadvantage of the culture method compared with microscopic examination is the excessive time necessary in the classical culture procedure because of the long incubation needed for amoeba growth. Moreover, since the first sample taken during corneal scraping might occasionally contain a greater number of cysts compared with the second taken for culture, one might expect a lower yield for the second specimen. On the other hand, considering that not all laboratories are equipped with a fluorescence microscope needed for the suggested procedure, there is a necessity to further extend the variety of methods tested in a single study. This study is limited by the lack of results recovered by other alternative common diagnostic procedures such as Giemsa staining or KOH. However, previous studies^{14,15} comparing different laboratory methods for the diagnosis of AK reported satisfactory results using direct smear. Despite the abundant previous reviews reporting the yield of the overall procedures used to diagnose AK, not many compared direct smear examined under Calcofluor White to self-produced PYG media. Hence, we find this report as adding significant data.¹⁶

In summary, we recommend using fluorescence microscopy technique and culture method, especially PYG liquid media; these ensure a cost-effective, simple, and efficient method for AK detection and diagnosis.

Received March 11, 2015. Accepted for publication April 7, 2015.

Published online May 11, 2015.

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TABLE 1
Number of positive specimens

All methods	FM + PYG + TYI-S-33	FM + PYG	FM + TYI-S-33	FM	PYG
5	3	2	1	2	1

FM = fluorescence microscopy; PYG = peptone yeast glucose.

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