Improved Method For Direct Examination Of Specimens For Fungi

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Summary

For routine direct diagnosis of fungal infections KOH preparations are commonly used, which have the advantage of rapid diagnosis leading to early treatment of the infections; but they also have the disadvantage that the fungal elements sometimes may not be clearly demonstrable, thus giving misleading results.

In order to improve upon this method, brightener "Tinopal" was added to the KOH solution; this lead to fungal elements to appear clearer and brighter, thus causing improvement in the demonstration of fungi in clinical specimens.

Introduction

In the diagnosis of superficial mycotic infections direct demonstration of fungal elements is an important diagnostic procedure. With Pityriasis versicolor and a few other mycoses, caused by organisms which cannot be easily cultured, this is the sole method of confirming the clinical diagnosis.

For ordinary routine use Potassium hydroxide (KOH) prepartion is the widely used direct preparation for demonstration of fungal elements in clinical specimens. Although this preparation is technically simple and inexpensive to perform, it requires training in distinguishing background materials and artefacts from fungal elements which may not be prominant and clear[2].

Recently it has been shown that the fluorescent brightener, Calcofluor white M2R will rapidly bind to fungi in forozen or paraffin embeded tissue and cause the fungal elements to fluoresce intensely after staining, when viewed under a fluorescent microscope[3].

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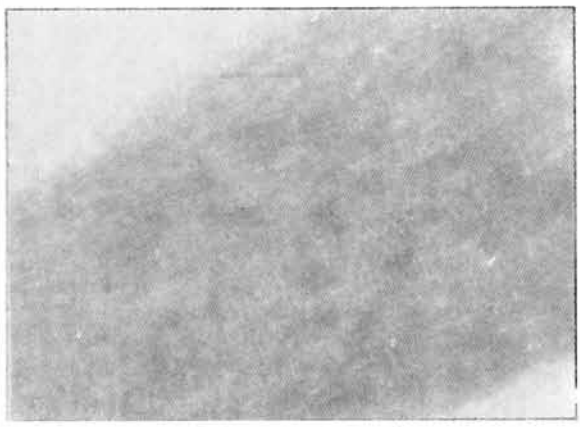
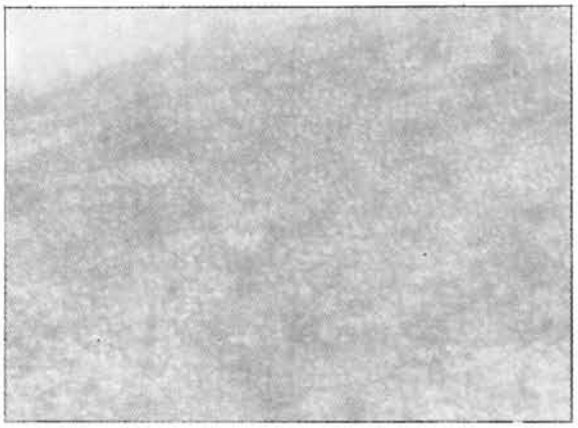
Photograph 1

Endothrix infection of hair, with spores in shaft



KOH treated, 400X

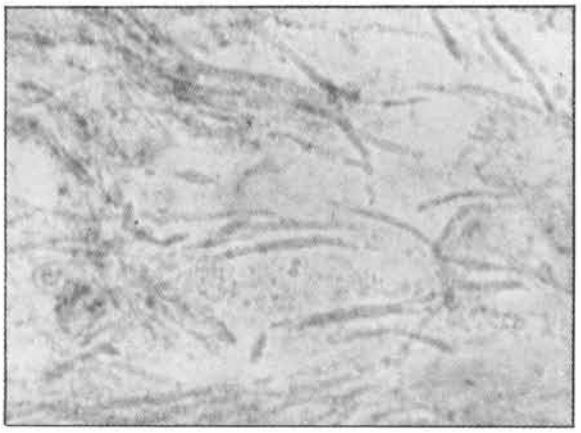


KOH - Tinopal treated, 400X

Photograph 2

Endothrix infection of hair, hyphae in hair shaft

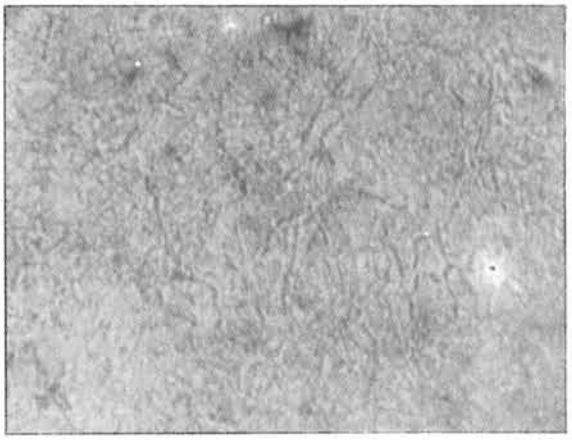
KOH treated, 400X



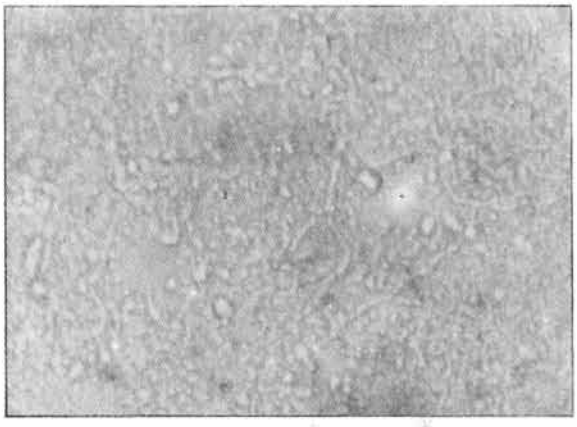
KOH - Tinopal treated, 400X

Photograph 3

Fungal hyphae in scales



KOH treated, 400X



KOH - Tinopal treated, 400X

The purpose of this study was to examine other brighteners to make fungal elements appear more prominant in KOH prepartions instead of in-stained tissue preparations.

Material & Methods

Specimens: Culture positive samples of hair and skin scales of different patients, from which dermatophytes had already been cultivated, except those of Malasezia furfur which had been diagnosed on direct examination, were tested.

Solutions: The brightener used for study was "Tinopal" (diamino-stilbene disulphonic acid cyanuric chloride). The solutions used were 1% Tinopal in 20% KOH and 20% KOH solution alone.

Preparation of Specimens: The specimen from the same patient was divided into two portions, one portion was mounted in Tinopal-KOH and the other in KOH alone.

The preparations were gently warmed and left for 15 minutes for the clearing process to take place, and were than examined at 400X, using a binocular microscope.

Results

Fifteen samples of skin scrapings and hair clippings, the details of which are given in Table-I, were examined after clearing with Tinopal - KOH solution and the results compared with those obtained after clearing with KOH alone.

In each case it was found that the clearing effect was much better with Tinopal-KOH and the fungal elements appeared clearer and brighter than with KOH solution alone.

In specimens in which no fungal elements were found on direct examination after treatment with KOH, they were not found even after treatment with Tinopal-KOH solution except in specimen number 11 which no hyphae were found in KOH preparation but short clear hyphae were seen in KOH-Tinopal preparation.

TABLE - I
COMPARISON OF RESULTS OF EXAMINATION BETWEEN SPECIMENS TREATED WITH KOH AND KOH-TINOPAL SOLUTIONS

	Specimen examined	Culture result	KOH mount result	KOH-Tinopal mount result
1.	Skin scraping, groin	Epidermophyton flocosum	Long fungus hyphae seen	Long fungus hyphae seen, clearer
2.	Hair, scalp (Favus)	Tricho. schoenleini	Endothrix infection of hair with spores and hyphae	Endothrix infection of hair with spores and hyphae, clearer
3.	Hair, scalp	Tricho. schoenleini	Endothrix infection of hair with spores	Endothrix infection of hair with spores, clearer
4.	Hair, scalp	Tricho. violaceum	Endothrix infection	Endothrix infection, clearer
5.	Skin scales	Microsporum ferrugineum	No fungus spores or hyphae seen	No fungus spores or hyphae seen
6.	Skin scrapings, arms		Hyphae and blastospores seen, Malasezia furfur	Hyphae and blastospores seen, clearer, Malasezia furfur
7.	Skin scrapings, chest		Hyphae and blastospores seen, Malasezia furfur	Hyphae and blastspores seen, clearer, Malasezia furfur
8.	Hair, scalp	Tricho. violaceum	No fungal elements seen	No fungal elements seen
9.	Hair, scalp	Tricho. schoenleini	Fungus hyphae and arthrospores	Fungus hyphae and arthrospors, clearer

	Specimen examined	Culture result	KOH mount result	KOH-Tinopal mount result
10.	Hair, scalp	Tricho. schoenleini	Fungus hyphae	Fungus hyphae, clearer
11.	Skin	Tricho. mentagrophytes	No fungus spores or hyphae seen	Short clear hyphae seen
12.	Skin, groin	Epidermophyton floccosum	Long fungus hyphae seen	Long fungus hyphae, clearer
13.	Hair, scalp	Tricho. schoenleini	Endothrix infection, with long fungus hyphae and arthrospores	Endothrix infection, with long fungus hyphae and arthrospores, clearer
14.	Skin	Tricho. verrucosum	Long hyphae	Long clear hyphae
15.	Skin, groin	Epidermo. floccosum	No fungal elements	No fungal elements

Discussion

Clear diagnosis of fungal etiology of a lesion is essential and unless the fungal elements are clearly demonstrated, the results will be misleading and the patient generally will not be treated with the required antifungal agents. The cultures take a long time and in some cases, the fungi are not cultivable or may not grow. Under such circumstances clear demonstration of fungal elements by direct examination of material is an essential diagnostic procedure.

The KOH preparations have been widely used for direct examination, but as the fungal elements sometimes may not be clear and prominant, therefore, the sensitivity of the direct examination is lower as compared to cultural procedure and it varies with different laboratories depending upon the xperience of the observer. Bergman and colleagues[1] have reported a sensitivity of only 19 percent for KOH detection of Candida albicans in vaginal specimens, when compared with culture on Sabouraud's medium; in contrast Elder[2] reported a sensitivity of 70.6 percent of KOH preparation as compared with culture.

A modification of KOH preparation by adding Tinopal (which is a cheap and easily available substance) would be a step towards improved detection of fungal elements, by making them more prominant for easy recognition.

Acknowledgement

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References

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