Comparison of diagnostic methods in the diagnosis of dermatomycoses and onychomycoses

V. Panasiti,¹ R. G. Borroni,¹ V. Devirgiliis,¹ M. Rossi,¹ L. Fabbrizio,¹ R. Masciiangelo,² U. Bottoni³ and S. Calvieri¹

¹Department of Dermatology and Plastic Surgery, University of Rome 'La Sapienza', Rome, Italy, ²Department of Biostatistics, University of Rome 'La Sapienza', Rome, Italy and ³Department of Dermatology and Oncology 'Magna Graecia', University of Catanzaro, Catanzaro, Italy

Summary

Direct microscopic examination of potassium hydroxide (KOH)-prepared specimens is the simplest, cheapest method used for the diagnosis of mycotic infections of the skin. However, KOH preparations have been reported to have 5–15% of false-negative results, possibly because of the low visibility of scant, scattered fungal material of the nail scrapings and because the detection of fungal elements depends on the skill of the observer [Arch Dermatol 133 (1997) 1317; Clin Microbiol Rev 8 (1995) 240]. We compared two different KOH-based staining methods in order to obtain reliable results in shorter time than expected for cultures. A total of 124 patients with suspect diagnosis of dermatomycosis or onychomycosis were enrolled. Two scrapings from the same lesion of each patient were stained with KOH-Chlorazole and KOH-Acridine Orange (AO), respectively; cultural examination of the same specimen was considered as diagnostic gold standard. The two methods showed neither significantly different sensitivity nor specificity; however, for onychomycoses, we observed a slightly higher sensitivity for KOH-Chlorazole and a higher specificity for KOH-AO. We suggest the use of both techniques in order to improve detection of fungal infection, especially for onychomycoses.

Key words: dermatomycosis, onychomycosis, fluorescence.

Introduction

Potassium hydroxide (KOH) 10–20% is the routine procedure for staining mycological specimens in our Department. However, sometimes this method can show false positive and, more frequently, false-negative results. We compared the sensitivity and the specificity of KOH-Chlorazole with those of fluorescent staining by Acridine Orange (AO). This substance (2,8-bis-dimethylamino acridine) binds to fungal mucopolysaccharides, producing fluorescence when exposed to UV radiation.¹ It also makes a good fluorescent Schiff reagent. In 1960, Pickett et al. [1] using this method demonstrated fungal elements in tissue sections. The first description of a fluorescent staining for the diagnosis of superficial mycosis was made by Chick & Behar [2]. Williams [3] used AO for staining of mycetes, because in an acid medium, it selectively stains RNA (red) and DNA (green) identifying the nuclei in hyphae and spores. More recently, the usefulness of this technique in diagnosing dermatomycosis and mycotic keratitis has been reported.⁴ ⁵

Materials and methods

Scrapings from 124 patients with suspect diagnosis of dermatomycosis or onychomycosis were analysed according to the following procedure: two scrapings from the same lesion (skin or nail) were put on two new glass slides. Subungual curettage was used to obtain as much subungual debris as possible without discomfort to the patient. Scraping of the scales at the periphery of
skin lesions was performed to obtain material for examination.

One of the scrapings was treated with a solution containing: 40 ml of dimethyl sulphoxide (CH$_3$SOCH$_3$), 20 g of KOH, 200 mg of Chlorazole and 60 ml of distilled water. The other scraping was stained with a 1 : 1000 solution of AO prepared according to the technique as described by Chick et al. [2]: 1 ml of this solution was added to 9 ml of KOH 20% solution. The slide was stained with this compound (AO final dilution 1 : 10 000). The slides were inspected after moderate heating. The microscope used for the observation was a Leitz Orthoplan (Leitz, Wetzlar, Germany) (equipped with a mercury vapour arc lamp at 50 W, filter block I 2/3 for excited light in the wavelength band of 450–490 nm, bright-field condenser completely open).

A third scraping of the same lesion was put in Saboraud’s Dextrose Agar (SDA; dextrose 40 g, agar 20 g, peptone 10 g, distilled water adjusted to pH 5.5–1000 ml with addition of cycloheximide 0.5 g/l and chloramphenicol 0.05 g/l) and incubated at 27 °C for 15 days. After 15 days, the results of the stainings were compared with those of the cultures obtained from each of the 124 specimens.

Cultural examination was considered the diagnostic gold standard. Sensitivity was defined as the proportion of patients with positive cultural examination who had a positive staining result, whereas specificity referred to the proportion of individuals without positive cultural examination who had a negative staining result. We considered 95% confidence interval (CI) for sensitivity and specificity (binomial approximation).

**Results**

The KOH-Chlorazole staining showed presence of hyphae in the examined specimens because they acquired a brown–green colour (Fig. 1). The AO staining allowed to demonstrate mycotic elements, when present in skin or nail scraping, because they produced green or red fluorescence of fungal hyphae (Fig. 2). Results of stainings obtained by KOH-Chlorazole method and those obtained by KOH-AO were compared with the culture results. The sensitivity of KOH-Chlorazole method was 60.0% (CI 46.54; 72.44), the specificity was 71.9% (CI 59.87; 81.41). Accuracy was 66.16%.

The sensitivity of KOH-AO method was 61.7% (CI 48.21; 73.93), the specificity was 67.2% (CI 54.31; 78.41). Accuracy was 64.52%.

When we compared the KOH-Chlorazole technique to cultural examination for skin scrapings, the sensitivity was 65.5% (CI 45.67; 82.06) and specificity was 70.3%.

![Figure 1 KOH-Chlorazole staining: presence of hyphae.](image1)

![Figure 2 KOH- Acridine orange staining: evidence of septate hyphae.](image2)

### Table 1 Results of stainings of skin scrapings.

<table>
<thead>
<tr>
<th>KOH + Chlorazole</th>
<th>KOH + Acridine Orange</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture+/stain+</td>
<td>19</td>
</tr>
<tr>
<td>Culture-/stain+</td>
<td>11</td>
</tr>
<tr>
<td>Culture+/stain-</td>
<td>10</td>
</tr>
<tr>
<td>Culture-/stain-</td>
<td>26</td>
</tr>
<tr>
<td>Total</td>
<td>66</td>
</tr>
</tbody>
</table>

### Table 2 Results of stainings of nail scrapings.

<table>
<thead>
<tr>
<th>KOH + Chlorazole</th>
<th>KOH + Acridine Orange</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture+/stain+</td>
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</tr>
<tr>
<td>Culture-/stain+</td>
<td>7</td>
</tr>
<tr>
<td>Culture+/stain-</td>
<td>14</td>
</tr>
<tr>
<td>Culture-/stain-</td>
<td>20</td>
</tr>
<tr>
<td>Total</td>
<td>58</td>
</tr>
</tbody>
</table>
(CI 53.02, 84.13). Accuracy was 68.18%. For nail scrapings, the sensitivity of KOH-Chlorazole was 54.8% (CI 36.03; 72.69) and specificity was 74.1% (CI 53.71; 88.89). Accuracy was 63.79%. KOH-AO compared with cultural examination in skin scrapings showed a sensitivity of 86.2% (CI 68.33; 96.11) and a specificity of 54.1% (CI 36.92; 70.52). Accuracy was 68.18%. For nail scrapings, sensitivity of KOH-AO was 38.7% (CI 21.85, 57.82) and specificity was 85.2% (CI 66.26; 95.81). Accuracy was 58%. The frequencies of the results of the staining of skin and nail scrapings are shown in Tables 1 and 2 respectively. The fungal species identified with cultural examination are shown in Fig. 3.

Discussion

Methods traditionally used for the diagnosis of dermatomycosis are fungal culture on Sabouraud’s dextrose agar and KOH staining of skin and/or nail scrapings.6 Fungal culture is more specific than the KOH staining, therefore, we used culture on Saboraud’s dextrose agar as diagnostic gold standard in this study. In fact, even if it is known that cultural examination, especially from nail scrapings, might be successful in 60–70% of cases only (for instance, because nonviable fungal elements are neglected), this remains the diagnostic method of reference in the mycology laboratory of our Department. Direct microscopic examination of KOH-prepared material is a simple and cheap method used in the diagnosis of mycotic infections. However, KOH preparations have been reported to have 5–15% of false-negative results, possibly because of the low visibility of scant, scattered fungal material obtained from the nail scraping and because the detection of fungal elements depends on the skill of the observer.7, 8

We compared two different KOH-based methods in order to obtain reliable results in shorter time than expected for cultures. KOH-AO was simple, rapid, and easy to read. In fact, the fluorescent stain allows even a non-experienced eye to easily identify the fungal structures, which leap out of a dark field. Furthermore, it was possible to identify the fungal hyphae as thin septate or broad aseptate filaments. The presence of septa and branches clearly differentiated the fungi from artefacts, allowing a reliable interpretation of stainings. However, the KOH-Chlorazole staining did not require a microscope equipped with fluorescent bulb.

KOH-AO results are as useful as those obtained with KOH-Chlorazole. We did not find high sensitivity and specificity for the two methods when results of skin and nail scrapings were considered together. However, when we focused only on the data from skin and nail material, respectively, our results showed that KOH-AO staining was more sensitive than KOH-Chlorazole for skin, and KOH-AO was more specific than KOH-Chlorazole for nail material. Therefore, we conclude that for practical purposes, when dealing with nail scrapings, the use of both techniques can improve the detection of fungal infection, without waiting for the results of the culture. Moreover, because of its high specificity, KOH-AO could be used as an exclusion test in cases of negative results on nail scrapings.

Last, it would be interesting to compare our results with those obtained with other techniques, for instance calcofluor white fluorescence staining.9–11 This method has been proven to give good results, especially for the diagnosis of superficial skin mycotic infection.11

References

1 Pickett JP, Bishop C, Chick EW. A simple fluorescent stain for fungi: selective staining of fungi by means of a fluor-