



## Note

# Comparison of direct microscopy, culture and calcofluor white for the diagnosis of onychomycosis

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## ABSTRACT

**Background:** Mycological diagnosis of onychomycosis can be performed by direct microscopy (KOH), cultures and calcofluor white.

**Aims:** To compare the percentage of positivity and the degree of correlation of KOH, cultures and calcofluor white for the diagnosis of onychomycosis.

**Methods:** Descriptive, transversal and comparative study. Samples of toenails with onychomycosis were used for KOH, cultures and calcofluor white under fluorescence. The percentage of positivity of the different techniques was calculated and the degree of correlation between them was determined (Epi Info v 3.4.3<sup>®</sup>).

**Results:** KOH was positive in 66.67% of the cases, cultures in 33.33% and calcofluor white in 57.58%. KOH and calcofluor white had a higher percentage of positivity than culture ( $p < 0.01$  and  $p < 0.05$  respectively). The degree of correlation between KOH and calcofluor white was excellent ( $\kappa = 0.8085$ ;  $p < 0.0001$ ); however, the degree of correlation between KOH and culture and between calcofluor white and culture was poor.

**Conclusions:** The use of calcofluor white is not recommended in routine laboratories because it does not seem to bring any additional benefits when comparing with KOH. This is especially important when funding is a great problem.

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## Comparación del examen directo, el cultivo y el blanco de calcoflúor para el diagnóstico de onicomicosis

## RESUMEN

**Antecedentes:** El diagnóstico micológico de onicomicosis puede establecerse mediante el examen microscópico directo (KOH) de una muestra clínica, el cultivo y la adición de blanco de calcoflúor.

**Objetivos:** Comparar el porcentaje de positividad y el grado de correlación entre la adición de KOH, el cultivo y la tinción con blanco de calcoflúor en el diagnóstico de onicomicosis.

**Métodos:** Estudio descriptivo, transversal y comparativo. Se utilizaron muestras de fragmentos ungueales de los pies para la adición de KOH, cultivo y adición de blanco de calcoflúor para examen bajo fluorescencia. Se calculó el porcentaje de positividad de las diferentes técnicas y se determinó el grado de correlación entre ellas (Programa Epi Info v 3.4.3<sup>®</sup>).

**Resultados:** La adición de KOH dio un resultado positivo en el 66,67% de los casos, el cultivo en el 33,33% y la tinción con blanco de calcoflúor en el 57,58%. El KOH y el blanco de calcoflúor se asociaron a un mayor porcentaje de positividad que el cultivo ( $p < 0,01$  y  $p < 0,05$ , respectivamente). El grado de correlación entre el KOH y el blanco de calcoflúor fue excelente ( $\kappa = 0,8085$ ;  $p < 0,0001$ ), mientras que fue débil entre el KOH y el cultivo y entre el blanco de calcoflúor y el cultivo.

**Conclusiones:** No se recomienda el uso sistemático de blanco de calcoflúor en los laboratorios porque no parece conferir beneficios adicionales cuando se compara con el KOH. Esto reviste especial importancia cuando los recursos son limitados.

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### Palabras clave:

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Onychomycosis is a growing global health problem. The prevalence of the disease is rising worldwide and ranges from 2.1% to 9.1%.<sup>1,4</sup> Dermatophytes, yeasts and non-dermatophyte molds are the main etiological agents.<sup>1,2,4,6,11,13,14,19–21</sup> The mycological diagnosis of onychomycosis can be performed by direct microscopy (KOH), cultures and calcofluor white under fluorescence (CW).<sup>1,11</sup> KOH is very simple, fast and the most cost-effective mycological technique but it does not allow species identification.<sup>11,12</sup> CW is very sensitive and specific, especially for the detection of dermatophytes, but it is an expensive technique that requires purchasing a fluorescence microscope.<sup>8,9,22</sup> Cultures are more specific than KOH but have a higher percentage of false negatives.<sup>18,22</sup>

Because the confirmation of the diagnosis of onychomycosis is difficult and there are several diagnostic techniques available, we designed a descriptive, transversal and comparative study to determine the percentage of positivity and the degree of correlation of KOH, culture and CW for the diagnosis of onychomycosis. We included 33 patients with clinical characteristics of distal or lateral subungual onychomycosis or total dystrophic onychomycosis of the toenails that were attended at the Dermatology Services of General Hospital of Mexico and General Hospital “Dr. Manuel Gea Gonzalez” between October and December of 2011. We excluded all the patients that did not give their consent to participate in the study, as well as patients with psoriasis, lichen planus, alopecia areata, pityriasis rubra pilaris, autoimmune bullous dermatoses, genodermatoses or trauma and all the patients that had received topical or systemic antifungal treatment in the past 12 months. The studied variables were sex, age, KOH result (positive or negative), culture result (positive or negative), CW result (positive or negative) and etiological agent isolated.

The samples were obtained by curettage of the subungual hyperkeratosis as proximal as possible and were used for KOH, culture and CW. Direct examination with KOH was performed as previously described.<sup>18</sup> KOH was considered positive when hyphae and/or conidia were observed. Cultures in Sabouraud dextrose agar and Mycosel<sup>®</sup> and the identification of the isolated fungi were done as previously described.<sup>18</sup> For CW, we prepared a working solution with 9 ml of PBS (pH 7.2) and 1 ml of CW 0.1% solution (in Tris HCl 0.1 M; pH 7.2). The sample was placed on a slide, fixated with polyvinylpyrrolidone and covered for 10 min with a mixture of the working solution and 10% potassium hydroxide (1:1). Then the slide was washed with distilled water, dried at room temperature and observed between 380 and 410 nm on a fluorescent light microscope (Zeiss Axioskop with a HB050 light source; Carl Zeiss, Jena, Germany). The test was considered positive when the sample contained round or filamentous forms with bright blue peripheral fluorescence.

Statistical analysis was performed with Epi Info v. 3.4.3<sup>®</sup> and absolute and relative (percentage) frequencies were calculated for each variable. The difference in the percentage of positivity of the tests was calculated and the statistical significance was determined by the Chi-square test. The degree of correlation between the three diagnostic techniques was determined with the Kappa index and it was classified as excellent (above 0.75), fair to good (0.40–0.75) or poor (below 0.40).<sup>5</sup> Every patient was asked to sign a written consent. All the procedures performed were approved by the Review Board and Ethics Committee of the General Hospital “Dr. Manuel Gea Gonzalez”. We followed the principles of the Helsinki Declaration and The Mexican General Health Law.

In our study, 63.64% of the patients were female and the mean age was 51.33 ± 16.99 years. KOH was positive in 22 (66.67%) cases. This percentage was very similar to the 59.3%,<sup>7</sup> the 61.7%<sup>16</sup> and the 63.4%<sup>17</sup> obtained by other studies. However, our result was much higher than the 40.31% obtained by Reisberger et al.<sup>15</sup>

We found 11 (33.33%) positive cultures. This percentage was lower than the 44%,<sup>3</sup> the 52.9%,<sup>7</sup> the 58.3%<sup>16</sup> and the 61.8%<sup>17</sup>

obtained by other studies. The result of our study was higher than the 25.84% obtained by Reisberger et al.<sup>15</sup>

In our study, CW was positive in 19 (57.58%) cases. This contrasts with the results of the study of Haldane et al., where CW had a sensitivity of 92% and a specificity of 95% for detecting dermatophytes in 207 skin scrapings.<sup>10</sup> However, Gupta et al. found a lower percentage of positivity than our study (31.3%) probably because they worked with patients who were undergoing antifungal therapy.<sup>8</sup>

KOH had a higher percentage of positivity than culture ( $p < 0.01$ ). This is very similar to the results of Weinber et al. who found that the sensitivity of KOH was 80% compared to the 59% of culture ( $p = 0.00002$ ).<sup>22</sup> In our study, CW also had a higher percentage of positivity than culture ( $p < 0.05$ ), but it was not more positive than KOH ( $p = 0.4465$ ). Haldane et al., when comparing CW with KOH, found very similar sensitivities (92% and 88% respectively) and specificities (95% for both).<sup>10</sup> It is important to highlight that the expertise of the examiner is a key factor for interpreting the results of KOH and since our laboratories are specialized in mycological diagnosis, the level of positive responses with KOH is likely to be high when compared to others. In the present study, KOH and CW had an excellent degree of correlation ( $\kappa = 0.8085$ ;  $p < 0.0001$ ); however the degree of correlation between KOH and culture ( $\kappa = 0.1818$ ;  $p = 0.1917$ ) and between CW and culture ( $\kappa = 0.1923$ ;  $p = 0.213$ ) was poor.

Three fungal agents were isolated: *Trichophyton rubrum* (63.64%), *Candida albicans* (27.27%) and *Scopulariopsis* spp. (9.09%). *T. rubrum* is usually the most frequent etiological agent isolated worldwide, followed by yeasts.<sup>1,6,11</sup>

The main limitation of this study is the small number of patients that could be included because of the cost of the CW test. However, our data clearly showed in Mexican population that the use of CW in clinical laboratories for routine diagnosis of onychomycosis is not recommended. This is a valid assumption, at least in our setting where funding is always a problem, because we did not find additional benefits with the use of CW after KOH was negative. We recommend additional studies with larger sample sizes.

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