Methods for Diagnosing Onychomycosis

A Comparative Study and Review of the Literature

Monica A. Lawry, MD; Eckart Haneke, MD, PhD; Katherine Strobeck, MD; Sandra Martin, DPM; Barbara Zimmer, PhD; Patrick S. Romano, MD, MPH

Objective: To identify the sensitivity of several readily available diagnostic tests for onychomycosis.

Design: Cross-sectional study.

Setting: Dermatology and podiatry departments at a teaching hospital.

Patients: Sixty-three adult men and women with a clinical examination highly suggestive of onychomycosis.

Main Outcome Measures: Sensitivity of each test and of several test combinations.

Results: Nail samples underwent 6 diagnostic tests. Routine histopathologic examination with periodic acid–Schiff stain (PAS) (PATHPAS) was 85% sensitive. Sensitivities for potassium hydroxide dissolution and centrifugation combined with PAS, fluorescent brightener, or chlorazol black E were 57%, 53%, and 53%, respectively. Culture on Sabouraud agar with chloramphenicol and cycloheximide (Mycosel agar) was 32% sensitive; on Litman-oxgall agar, 23% sensitive. The most sensitive combination of tests, both culture methods plus PATHPAS, was 94% sensitive (not statistically different from the sensitivity of PATHPAS alone [P=.26]).

Conclusions: When onychomycosis is suspected clinically, PATHPAS of the nail is the single most sensitive of the diagnostic tests we evaluated. Because it is quickly performed and relatively operator independent, PATHPAS is practical for clinical and research purposes. Further study is needed to determine if sensitivity may be enhanced by combining PATHPAS with cultures obtained by several collection methods (clipping, curettage, and shaving). Such combinations may serve as sensitive and efficient strategies for diagnosing onychomycosis.

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PATIENTS AND METHODS

Adults with nail changes highly suggestive of onychomycosis were entered into this cross-sectional study. Patients were examined by dermatologists (M.A.L. and E.H.), dermatology residents, and a podiatrist (S.M.) in inpatient and outpatient departments at a teaching hospital. The criterion for enrollment was nail dystrophy that could be categorized clearly into 1 of the following 3 types: full-thickness onychodystrophy (FTO) with thickened and discolored nail; early distal-lateral subungual onychomycosis–type changes (DLSO) with onycholysis and subungual debris; and superficial chalky leukonychia as can be seen in superficial white onychomycosis (SWO). We excluded patients with changes suggestive of proximal subungual onychomycosis, patients with psoriasis, and patients who did not consent to have a sample clipped from their distal nail.

Basic demographic information was recorded for each patient. Any potentially pertinent history was recorded. The sample site (fingernails or toenails) was recorded, along with the category of nail changes (FTO, DLSO, or SWO) as already described.

Samples were obtained with the use of standard nail clippers in a manner described by Hull et al.10 The distal free edge of the nail plate, along with any attached subungual debris, was clipped just distal to its attachment to the nail bed, resulting in no discomfort to the patient. No curettage or special attempt was made to obtain further subungual debris. Each patient underwent sampling of at least 1 nail, but more were sampled if available to provide sufficient material. A note was made if the sample was less than 3 mm in its greatest dimension. Specimens were placed in sterile urine containers and labeled for identification.

Specimens were divided into approximately equal-sized pieces, with 1 piece reserved for additional testing if necessary. All specimens were subjected to each of the following tests: culture on Sabouraud agar with chloramphenicol and cyclocitremide (Mycosel agar; BBL, Franklin Lakes, NJ); culture on Littman-oxgall agar; routine histopathologic examination with periodic acid–Schiff stain (PAS) (PATHPAS); potassium hydroxide (KOH) dissolution of nail and centrifugation of nail (KONC) with PAS (KONCPA); KONC with fluorescent stain (KONCFLU); and KONC with chlorazol black E stain (KONCBE). The specimens submitted for PATHPAS were placed in standard skin biopsy specimen containers with 4% formaldehyde. No special procedures were used to soften the specimens before processing. The results were reported by one of us (M.A.L.) who was unaware of the other test results for each specimen.

The KONCPA method was performed as described by Liu et al.15 Specimens were warmed to 56°C with 3 mL of 20% KOH for 30 minutes, then washed with isotonic sodium chloride solution. Specimens were then centrifuged at 3000 rpm for 5 minutes. The pellet was divided equally among 3 slides, crushed with another slide, and allowed to air dry. One slide was stained with PAS (KONCPA) according to standard procedures, the second slide was stained with 2 drops of chlorazol black E solution (KONCBE), and the last slide was stained with fluorescent brightener (Blankophor; Bayer, Wuppertal, Germany) (KONCFLU). The KONCPA and KONCBE slides were examined under light microscopy at×10 magnification. The KONCFLU slides were examined under a fluorescent microscope (Zeiss, Thornwood, NY) at×10 magnification. The entire surface of the specimen was scanned in less than 1 minute, and ×40 magnification was used to confirm organisms. A test result was considered positive when the cell wall of the organism and/or septations could be identified easily.

The investigator (M.A.L.) was unaware of all other test results for a particular specimen at the time of interpretation of each slide.

All specimens were planted into Mycosel agar and Littman-oxgall agars and incubated using standard mycologic technique. The results were interpreted by one of us (B.Z.) who was unaware of the results of the other tests. Cultures were considered positive if a dermatophyte grew. Culture yields of nondermatophyte molds or yeasts were considered positive only if the same organism grew on a second culture obtained from the portion of the original specimen reserved in the sterile urine container.16 All cultures were held at least 4 weeks before findings were considered negative. The species of each dermatophyte, nondermatophyte mold, or yeast was determined by means of light microscopy and a subsulture, if required.

The data on each patient were entered into a desktop computer for analysis with the use of Epi Info, version 6.04b (Centers for Disease Control and Prevention, Atlanta, Ga). The frequency of positive results for each test was calculated. To analyze the data, a criterion standard for the diagnosis of onychomycosis was defined as is generally accepted in clinical practice: clinical morphologic findings suggestive of onychomycosis plus at least 1 positive test result. The sensitivity of each test was calculated as the proportion of nails with a positive result, among those with a confirmed diagnosis of onychomycosis. The negative predictive value of each test was calculated as the proportion of nails without a confirmed diagnosis of onychomycosis, among those with a negative test result.17

Because it is standard technique to use a combination of direct microscopy and culture techniques to enhance sensitivity, combinations of each staining method with cultures were also analyzed.18 We used the McNemar statistic to assess the statistical significance of sensitivity differences between paired tests. P values were calculated by means of the χ² test.

<table>
<thead>
<tr>
<th>Samples</th>
<th>No. of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td></td>
</tr>
<tr>
<td>Fingernails</td>
<td>4</td>
</tr>
<tr>
<td>Toenails</td>
<td>59</td>
</tr>
<tr>
<td>Morphologic feature</td>
<td></td>
</tr>
<tr>
<td>FTO</td>
<td>59</td>
</tr>
<tr>
<td>DLSO</td>
<td>1</td>
</tr>
<tr>
<td>SWO</td>
<td>3</td>
</tr>
</tbody>
</table>

Forty-seven (75%) of our 63 patients received a diagnosis of onychomycosis according to our criterion standard. Sixteen patients (25%) had clinical nail changes suggestive of onychomycosis, but had negative results of all tests. The sensitivities and negative predictive values for individual tests are reported in Table 1 and similar results for combinations of tests are reported in Table 2.

As shown in Table 1, PATHPAS was significantly more sensitive in detecting onychomycosis than any of the other single tests (P≤.05). All of the KONC methods were significantly more sensitive than the culture tests. Table 2 shows the combination of Mycosel and Litt-
The standard for establishing the diagnosis of onychomycosis long has been accepted as clinical impression plus at least 1 positive result of a laboratory test (usually fungal culture or direct microscopy with KOH).19 These tests appear to lack sufficient sensitivity to be relied on independently, although there are a lack of data to confirm this.9,13 Often, these tests are combined in an effort to enhance sensitivity.16 Onychomycosis is common, affecting 2% to 18% of the general population.20 Increasing public awareness and the number of new treatments available make an efficient diagnostic strategy with good sensitivity even more necessary.21

We evaluated the sensitivity of diagnostic tests that can be used in a typical outpatient setting. We evaluated some methods previously described by others as more sensitive than KOH examination and culture (KONCPA).19 The KONCPA technique results in a more homogenized and concentrated specimen than routine KOH testing. The sensitivity of KONCPA is further enhanced by staining with PAS to highlight fungal elements. We combined other fungus-enhancing stains (fluorescent stain and chlorazol black E) with the basic KONC technique to compare sensitivity of methods using these more easily applied stains (KONCFLU and KONCBE).3,12

Patients were recruited based on a clinical examination highly suggestive of onychomycosis that was clearly categorizable into the most common clinical types. In fact, most patients had FTO changes with thickened and discolored nails and subungual debris. Methods for collection were designed to be relatively operator independent, quickly performed, and well tolerated by the patient, making the results and conclusions of the study clinically applicable. Although our sample collection technique (nail plate clipping) is likely not optimal for fungal cultures, it has been demonstrated that, when performed alone, the frequency of positive culture results obtained individually from distal nail plate clipping, subungual curettage, and nail plate shaving is not statistically different. This may result from the fact that onychomycosis begins as a disease of the distal nail bed. A portion of the distal nail bed is adherent to the ventral nail plate and is therefore included in nail plate clippings.10,20 Another factor that may have resulted in our low frequency of positive fungal culture results is the high proportion (94%) of patients with FTO-type changes. Although organisms in the distal nail plate and attached subungual debris may be visible with direct microscopy in these nails, they may not be visible on culture. Thus, staining methods have a higher yield than culture techniques in these patients.23

Our results showed that PATHPAS had the highest frequency of positive results (40 patients [63%]) in this sample population. This is lower than one would expect based on the clinical appearance of the nails in our patients. In fact, 16 (25%) of our patients had no test result that was positive for fungus. However, in a literature review, Bigby9 found that the pretest probability for a combination of KOH examination and fungal culture ranged from 46% to 53% when performed on nails with dystrophy highly suggestive of onychomycosis. Together with our results, this suggests that other causes of nail dystrophy probably account for a significant portion of negative results. Further study of potential associations of nail dystrophy with diabetes, arterial and/or venous insufficiency, osteoarthritis, trauma, aging, and other factors is needed. The fact that nononychomycosis origins may be a significant cause of nail dystrophy further underscores the need for establishing a diagnosis before treatment with oral antifungal drugs.

The sensitivity of PATHPAS is clearly superior (P ≤ .05) to the other individual methods we evaluated.

### Table 1. Analysis of Individual Diagnostic Tests for Onychomycosis*

<table>
<thead>
<tr>
<th>Test</th>
<th>No. With Positive Result (N=63)</th>
<th>Frequency, %</th>
<th>Sensitivity, %</th>
<th>NPV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>PATHPAS</td>
<td>40</td>
<td>63</td>
<td>85</td>
<td>70</td>
</tr>
<tr>
<td>KONCPA†</td>
<td>27</td>
<td>43</td>
<td>57</td>
<td>44</td>
</tr>
<tr>
<td>KONCFLU†</td>
<td>25</td>
<td>40</td>
<td>53</td>
<td>42</td>
</tr>
<tr>
<td>KONCBE†</td>
<td>25</td>
<td>40</td>
<td>53</td>
<td>42</td>
</tr>
<tr>
<td>Mycosel†</td>
<td>15</td>
<td>24</td>
<td>32</td>
<td>33</td>
</tr>
<tr>
<td>Littman-oxgall agar†</td>
<td>15</td>
<td>24</td>
<td>32</td>
<td>33</td>
</tr>
</tbody>
</table>

*Specificity, likelihood ratio for a positive test result, and positive predictive value could not be calculated because all tests were included in the criterion standard. NPV indicates negative predictive value. Test names are explained in the “Patients and Methods” section.

† The sensitivity of these tests is significantly (P = .002 to P < .001) worse than the sensitivity of PATHPAS by McNemar test.

### Table 2. Analysis of Combinations of Diagnostic Tests for Onychomycosis*

<table>
<thead>
<tr>
<th>Combination</th>
<th>Sensitivity, %</th>
<th>NPV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycosel + Littman-oxgall agar†</td>
<td>45</td>
<td>38</td>
</tr>
<tr>
<td>Mycosel + PATHPAS‡</td>
<td>91</td>
<td>80</td>
</tr>
<tr>
<td>Cultures + PATHPAS‡</td>
<td>94</td>
<td>84</td>
</tr>
<tr>
<td>Cultures + KONCPA</td>
<td>77</td>
<td>59</td>
</tr>
<tr>
<td>Cultures + KONCFLU</td>
<td>74</td>
<td>57</td>
</tr>
<tr>
<td>Cultures + KONCBE</td>
<td>72</td>
<td>55</td>
</tr>
</tbody>
</table>

*N = 63. NPV indicates negative predictive value. Test names are explained in the “Patients and Methods” section.

† The combination of Mycosel and Littman-oxgall agar constituted cultures for subsequent tests.

‡ The sensitivities of these combinations of tests were not significantly better than that of PATHPAS alone (P = .26 and P = .13) by McNemar test.
Combining PATHPAS with culture techniques results in a small increase in sensitivity that is not statistically significant \((P=.13)\). Our sampling technique should have had little impact on the staining test results because the homogenized pellet was divided equally before staining. The sensitivities of the staining methods are slightly operator dependent. However, the criteria for a positive result of a staining test (histological identification of fungal elements) is widely accepted by dermatologists and pathologists and therefore should be applicable to clinical practice. The spectrum of clinical disease in our study (most patients had FTO-type changes) may have led to a higher frequency of positive test results. However, based on what is understood about early onychomycosis (DLSO type), sensitivity of PATHPAS would depend on the degree to which subungual debris (hyperplastic nail bed) remains adherent to the distal nail plate clipping. Certainly, patients with proximal white subungual–type changes would be expected to have low frequency of positive results among our panel of tests.

The specificity of each test could not be calculated because our criterion standard included each test. The clinical relevance of specificity is unclear, since these tests would not be used to screen clinically normal nails for onychomycosis. The main focus of such testing is to minimize the frequency of false-negative test results in achieving the diagnosis (sensitivity). However, similar calculations (data not shown) were performed using only the combined KONC methods as the criterion standard. The specificity of fungal cultures was 94% to 100% under these conditions. The possibility of false-positive test results is likely greater with fungal cultures than with the KONC methods; therefore, all nondermatophyte organisms were required to grow on a second culture before being considered true pathogens. False-positive results for the KONC tests were likely a rare event because of the specific criteria for visualizing the cell wall and/or septa of the organism. The only commonly encountered finding that simulated fungus enough to lead to examinations of the organism. The only commonly encountered finding that simulated fungus enough to lead to examinations of the organism.

The PATHPAS test fails to identify the species of the fungal organism. This is probably not clinically significant unless the organism is a nondermatophyte pathogen that may be resistant to certain drugs. Ellis et al determined the frequency of nondermatophyte onychomycosis as 2.5% in their study of 118 patients with onychomycosis. These authors concluded that yeasts and molds are found most often as contaminants in dermatophyte onychomycosis and that their presence has no influence on the outcome of therapy. Our results showed a significantly larger proportion of nondermatophyte pathogens among cultures with positive results. Most were also positive for at least 1 staining test (Table 3). Our results may have been skewed because of our low frequency of positive culture results.

Some clinical situations, such as immunosuppression and paronychia, increase the likelihood that a yeast is a true pathogen. In these situations, it is important to identify the organism before treatment. In our study, morphologic features of yeast were easily differentiated from those of true hyphae on PATHPAS and KONC tests, though this aspect was not studied specifically. In addition, histological examination of the nail plate and attached subungual debris allows one to determine the precise location of the organism, which may serve to clarify its role as a true pathogen. The PATHPAS method also provides a permanent record that can easily be used in consultation if necessary.

Because of the potential nonspecific results of fungal cultures, other methods for identifying the pathogenic organism in onychomycosis have been evaluated. Immunoperoxidase staining and polymerase chain reaction restriction enzyme analysis have been described as methods for identifying the species of the organism.

The literature regarding evaluation of diagnostic tests for onychomycosis dates back to 1968. Davies examined the frequency of positive results of KOH examination and culture for Trichophyton rubrum in 3955 patients for whom cultures had previously been positive for \(T\) rubrum onychomycosis. In that study, 46% of specimens showed positive culture or KOH examination. Recently, Bigby reviewed the deficiencies of the work by Davies, but considers it to be the best available source of information about sensitivity and specificity of diagnostic tests for onychomycosis.

Since the work by Davies, there have been several descriptive and prevalence studies of diagnostic methods for onychomycosis. However, study design and small sample size limit the applicability of their results. Suarez et al compared results of PATHPAS of nail clippings with fungal culture findings of the same nail. They reported the frequency of PATHPAS and culture findings in patients with proven severe recalcitrant onychomycosis (100% and 94%, respectively) and in patients with nail dystrophy of unknown causes (33% and 23%, respectively). Although their study compared frequencies of positive test results, no criterion standard was defined to allow for calculation of sensitivity.

Mehregan et al examined 20 nail specimens from patients with suspected onychomycosis based on results of clinical examination. In this small sample, they reported 60% frequency of positive culture results and 75% frequency of positive PATHPAS results (no sensitivities were calculated). Malcher et al examined 23 distal nail plate clippings and concluded that PATHPAS was as reliable as culture and superior to KOH examination. However, their control group with “onychodystrophy of

Table 3. Organisms Identified on 21 Positive Culture Results

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. With Positive Result</th>
<th>Frequency, %</th>
<th>No. With Positive Stain Test Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trichophyton rubrum</td>
<td>11</td>
<td>52</td>
<td>10</td>
</tr>
<tr>
<td>Aspergillus species</td>
<td>3</td>
<td>14</td>
<td>1</td>
</tr>
<tr>
<td>Acremonium species</td>
<td>2</td>
<td>9.5</td>
<td>1</td>
</tr>
<tr>
<td>Fusarium species</td>
<td>2</td>
<td>9.5</td>
<td>2</td>
</tr>
<tr>
<td>Epidermophyton floccosum</td>
<td>1</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Trichophyton mentagrophytes</td>
<td>1</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Trichosporon species</td>
<td>1</td>
<td>5</td>
<td>1</td>
</tr>
</tbody>
</table>

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known cause” was not clearly defined, and no sensitivities were calculated.

Scher and Ackerman31 examined biopsy specimens of the nail unit from patients with clinically suspected onychomycosis who had negative results of KOH and fungal cultures. In their descriptive study, they demonstrated hyphae in the cornified nail bed and ventral nail plate most often, suggesting these are the most common locations of hyphae in onychomycosis. Nail unit biopsy is certainly useful in many clinical situations, but it is unlikely to be widely accepted as a test for onychomycosis if other sensitive techniques have less associated morbidity.

CONCLUSIONS

The PATHPAS method appears to be the single most sensitive of those we evaluated for diagnosing onychomycosis. It is easily performed and atraumatic, and results are available relatively quickly. Although PATHPAS appears to be the best single method for diagnosing onychomycosis, it is clearly not acceptable as a criterion standard. Further study is needed to determine the sensitivity of PATHPAS plus fungal cultures obtained by combined sampling methods (clipping, subungual curettage, and nail plate shaving). This may allow for the development of a highly sensitive and efficient strategy for diagnosing onychomycosis.

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Reprints: Monica A. Lawry, MD, Department of Dermatology, Kaiser Permanente Medical Group–South Sacramento, 6600 Brucelive Rd, Sacramento, CA 95823 (e-mail: Monica.A.Lawry@kp.org).

REFERENCES