# Use of Cutting Edge Diagnostic Methods for Onychomycosis

Curtis T. Thompson, M.D.
Clinical Professor of Dermatology and Pathology
Oregon Health and Sciences University
Portland, Oregon, USA

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## No conflict of interest

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Arch Dermatol. 2007 Jun;143(6):799-800.

# Direct identification of dermatophyte DNA from clinical specimens by a nested polymerase chain reaction assay.

Yang CY, Lin TL, Tzung TY, Cheng LC, Wang JT, Jee SH.

Mikrobiyol Bul. 2011 Jan;45(1):150-8.

# [DNA extraction and identification of Trichophyton rubrum by real-time polymerase chain reaction from direct nail scraping specimens of patients with onycomycosis].

[Article in Turkish]

Berk E[1], Kuştimur S, Kalkancı A, Oztaş OM.

Author information

**Abstract**

Trichophyton rubrum is the most frequently encountered dermatophyte species causing onichomycosis. The routine diagnosis of dermatophytes depends on the direct microscopic examination (DME) and culture methods, however due to the phenotypic identification problems related to those agents, the molecular methods come into question. The aim of this study was performance of real-time polymerase chain reaction (RT-PCR) for the identification of T.rubrum by compa from nail samples of patients with the complaints of onychomycosis. A total of 90 patients of whom 58 w dermatology outpatients clinics of our hospital with the complaints of color/shape changes in the nails ar included in the study, together with the 20 healthy volunteer subjects as controls. The nail scraping samp controls were examined with direct microscopy using 15% potassium hydroxide, dimethyl sulphoxide an cultivated onto Sabouraud dextrose agar with and without cycloheximide. For DNA isolation, after the dis tool, phenol-chloroform-isoamyl alcohol purification method were used. The amplification and demonstra performed by using specific primers and probes following TaqMan protocol of RT-PCR (LightCycler-Roc patients yielded positive and 18 yielded negative results with DME. Growth of molds was detected in the DME positive patients and all of the isolates were identified as T.rubrum. No fungal growth was seen in t DME negative. In DME positive group, 67 (93%) patients were found to be positive in RT-PCR, while 8 ( positive in DME negative group. All of the culture positive samples (n= 20) were also found positive in R control group with healthy nails yielded negative results in DME, culture and RT-PCR methods. The perf compared to direct microscopy that had higher sensitivity than culture and the sensitivity, specificity, posi RTPCR assay were estimated as 93%, 56%, 89% and 67%, respectively. In conclusion RT-PCR was th assay in the diagnosis of onichomycosis. Although RT-PCR seems more expensive than culture, for the for the molecular methods, the difference in total cost doesn't count much. In conclusion, by the use of successfully done from a relatively difficult clinical specimen, namely nail scraping, a protocole that coul laboratory was established and species-level identification in a short time was accomplished in this stud

J Dermatol. 2009 Apr;36(4):202-8. doi: 10.1111/j.1346-8138.2009.00624.x.

# Comparative study of direct polymerase chain reaction, microscopic examination and culture-based morphological methods for detection and identification of dermatophytes in nail and skin samples.

Uchida T[1], Makimura K, Ishihara K, Goto H, Tajiri Y, Okuma M, Fujisaki R, Uchida K, Abe S, Iijima M.

Author information

**Abstract**

The positive rates of dermatophytes isolated and identified by conventional methods are rather low. Moreover, clinical isolates sometimes show atypical morphology, and in such cases microscopic methods are not applicable for identification. The present study was performed to assess the utility of specific polymerase chain reaction (PCR)-based methods for Trichophyton rubrum and Trichophyton mentagrophytes as diagnostic tools for dermatophytoses. Both conventional morphological identification and specific PCR methods based on the nuclear ribosomal internal transcribed spacer (ITS)1 DNA sequence were performed to identify dermatophyte species from clinical specimens of patients who visited Kawasaki Social Insurance Hospital between 16 May and 17 August 2005. Specific PCR methods were also directly applied to clinical specimens, and the results of the two methods were compared. The clinical samples examined consisted of 126 skin scale specimens and 80 nail specimens. The positive rates of culture isolation from clinical specimens were 67% and 33% for skin scale and nail specimens, respectively. In contrast, PCR analysis yielded a positive rate of 100% for clinical isolates from both skin scales and nails, and rates of 95% and 99% were obtained by direct application to clinical specimens. The results of the present study indicated that specific PCR is highly advantageous as a diagnostic tool for detection and identification of dermatophytes on direct application to skin scale or nail specimens.

Test Guide

# Onychomycosis

## Diagnostic Tests

Onychomycosis is a common disorder that typically entails long-term systemic therapy with potential side-effects. Moreover, optimal therapy varies with the pathogen identified. Thus, it is important to diagnose the infection accurately before beginning therapy. Tables 1 and 2 provide a comparison of available diagnostic methods. A combination of KOH and culture appears to be the current standard of practice; however, there is considerable variability in the diagnostic sensitivity obtained by various investigators (Table 2). PAS and molecular methods appear to have more consistent sensitivity among the investigators and are highly sensitive.

**Table 1. Methods for Diagnosing Onychomycosis**

| Method | Advantages | Disadvantages |
|---|---|---|
| Biopsy and PAS (ie, histopathology) | Rapid and simple to perform; relatively high sensitivity | Does not determine viability of fungi; does not determine causative pathogen |
| KOH and microscopy | Visualizes and differentiates living and dead hyphae; rapid turnaround time | Does not differentiate dermatophytes from nondermatophytes; ie, does not determine genus, species |
| Culture | Allows species identification, confirms viability | Subject to growth of nonpathogenic contaminants; requires 7 to 28 days for results |
| DTM | In-office culture method; relatively specific to dermatophytes, but nondermatophytes may cause false-positive results; 7 to 14 days for results, less costly than in-lab culture | Does not determine genus, species; does not identify nondermatophyte infections; moderate complexity test that requires CLIA certificate of compliance —may not be suitable for all physician offices |
| Molecular | Differentiates dermatophytes, nondermatophytes, and yeast; can determine species, strain, and subtype (PCR/RFLP); sensitivity not affected by antifungal therapy; <5 days for results | Costly, requires highly-skilled personnel; limited availability |

PAS, periodic acid–Schiff; KOH, potassium hydroxide; DTM, dermatophyte test medium.

http://www.questdiagnostics.com/testcenter/testguide.action?dc=TG_Onychomycosis

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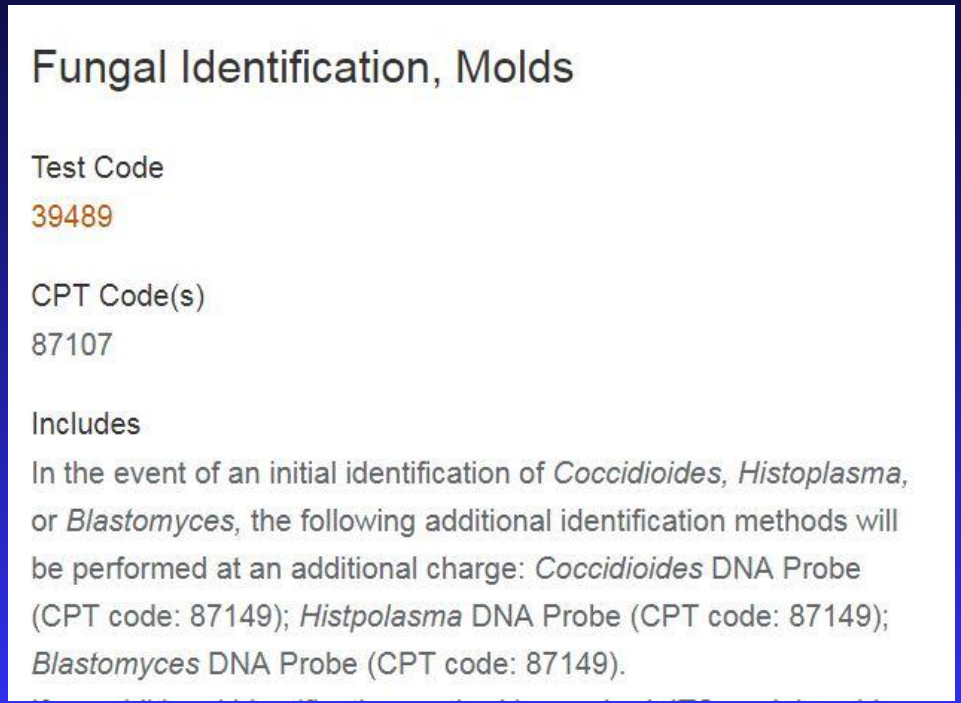
# Molecular not widely available for dermatophytes



## Fungal Identification, Molds

Test Code

39489

CPT Code(s)

87107

Includes

In the event of an initial identification of *Coccidioides, Histoplasma,* or *Blastomyces,* the following additional identification methods will be performed at an additional charge: *Coccidioides* DNA Probe (CPT code: 87149); *Histpolasma* DNA Probe (CPT code: 87149); *Blastomyces* DNA Probe (CPT code: 87149).

http://www.questdiagnostics.com/testcenter/testguide.action?dc=TG_Onychomycosis

# PCR won’t work if there is . . .

- . . . a sampling is an issue

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- . . . a sampling is an issue

Subungal debris is better than nail plate for sampling

What can the clinician do to submit the best specimen for nail fungal diagnostics?

# Nail Fungus Diagnostics

- Challenges
  - Nail plate-difficult to stick to a slide
  - Subungal debris cannot be embedded in block

# Submit specimen dry in a small envelope

# Test nail plate first with PAS

## Periodic Acid-Schiffs Stain (P.A.S.) Stain for Nails

**Procedure:**

1. Place a small amount of Gelatin in water bath.
2. Using positively charged slide, pick up desired sections.
3. Place in 60 deg. C oven for 30-60 minutes.(More time for "difficult" specimens)
4. Deparaffinize slides using Xylene or Xylene substitutes and hydrate through alcohols.
5. Rinse slide in running tap water.
6. Rinse slide in distilled water.
7. DO NOT "DIGEST" SLIDES!!!
8. Place slide in 1% Periodic Acid for 12 minutes.
9. Rinse slide quickly in distilled water.
10. Place slide in Schiffs Solution for 15 minutes.
11. Rinse slides in warm tap water for 5 minutes.
12. Place slide in Light Green Stain as needed to reach desired background intensity. (Approx. 30 seconds)
13. Dehydrate slide through 3 changes of Absolute Alcohol.
14. Clear slide through 3 changes of Xylene or Xylene substitute.
15. Coverslip using permanent mounting media.

**Results:**

Basement membrane, Fungi, Glycogen and Mucin: PINK TO RED

Other tissue: Green

**Reference:**

American Master Tech Scientific, Inc. "PAS Kit Procedure."

https://www.cta-lab.com/nail_resources.html

# Test nail plate first with PAS

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Important for nail adherence to slide

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# PAS on embedded nail is difficult

- Make sure amount of tissue on PAS slide matches the amount that was grossed
  - Lab may need to repeat
  - The more gentle the washes, the less tissue washes off

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Gentle movement important

https://www.cta-lab.com/nail_resources.html

# Subungal debris only preserved if sample is submitted dry.

JAAD
Journal of the American Academy of Dermatology



# Subungual debris cytopathology increases sensitivity of fungus detection in onychomycosis

Christian S. Jordan, MD, PhD, Brandon Stokes, CHT, Curtis T. Thompson, MD

# Thin Prep on Subungal Debris

# Centrifuge with slide

# Centrifuge with slide

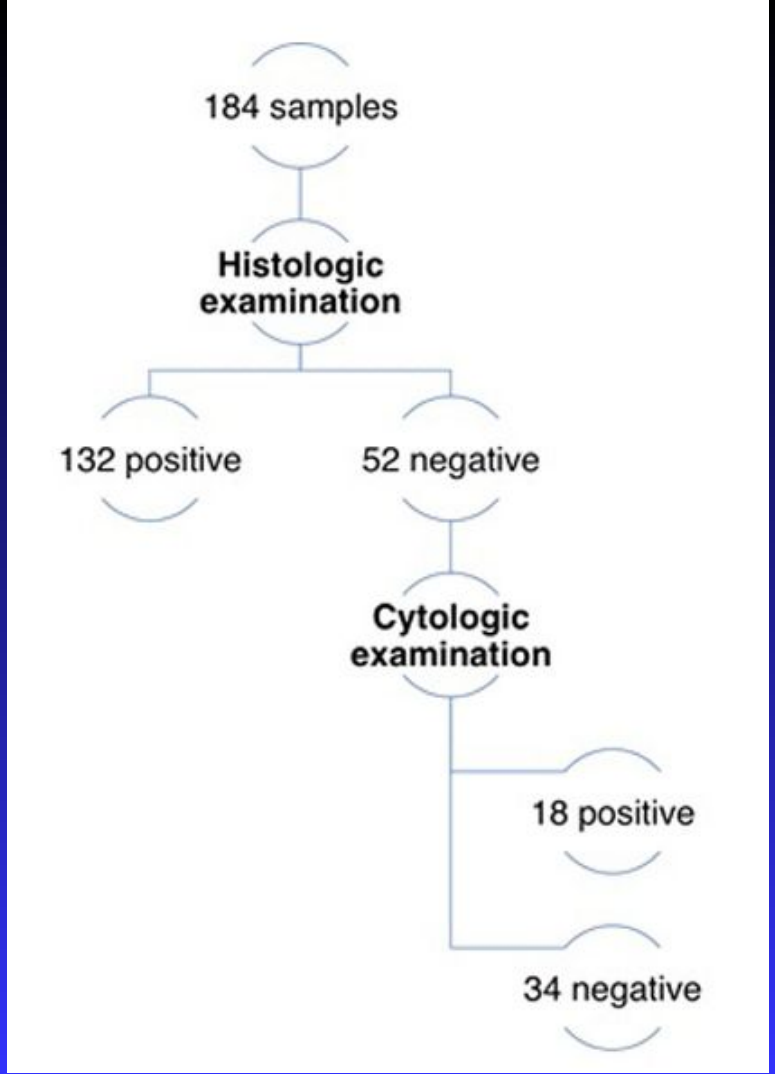
184 samples
Histologic examination
132 positive
52 negative
Cytologic examination
18 positive
34 negative

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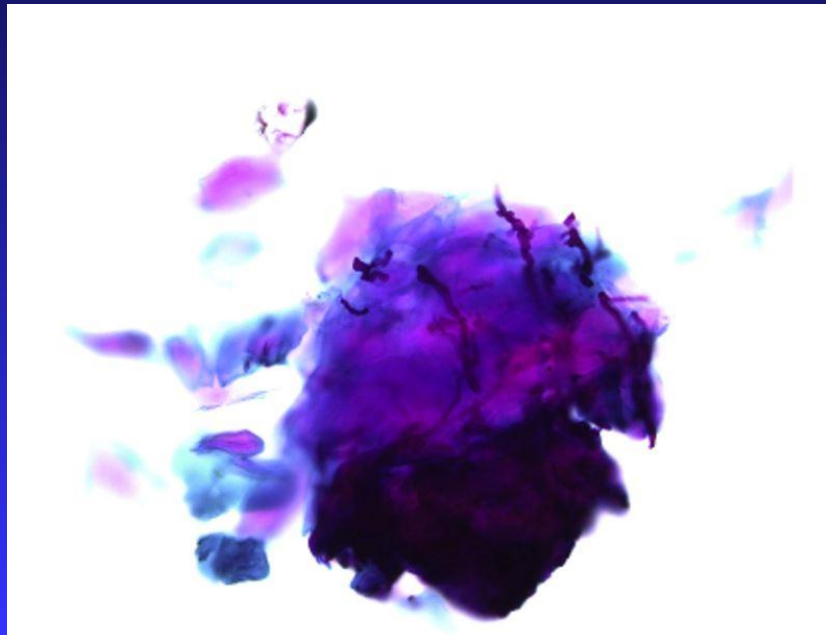
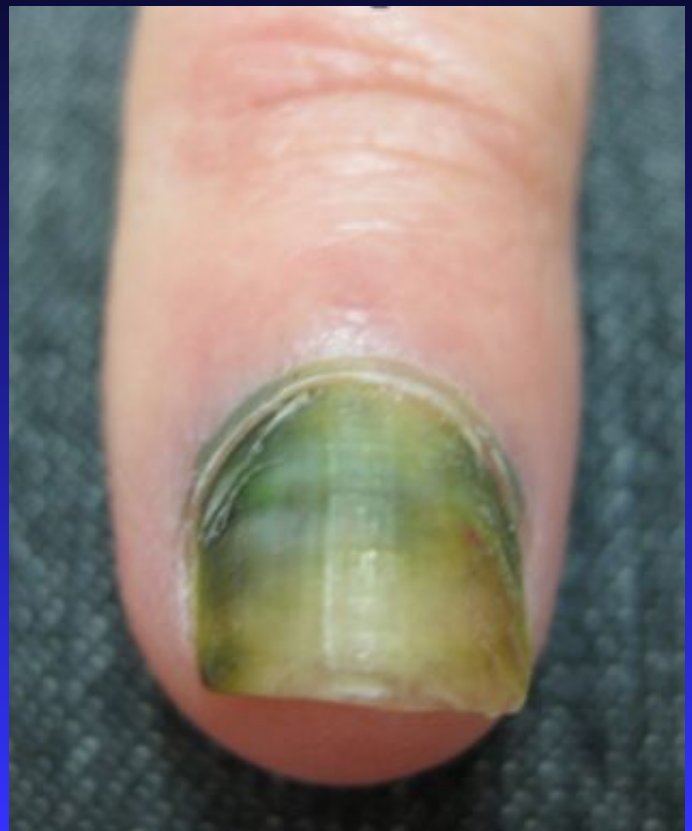
**Fig 1.** Onychomycosis. Microscopic examination of PAS-stained subungual debris. (Original magnification: ×400.) Subungual debris was collected by centrifugation of the formalin in which nail clipping specimens were submitted. Microscopic examination of a thin-layer preparation of PAS-stained subungual debris reveals multiple darkly staining fungal forms associated with a single keratin aggregate.

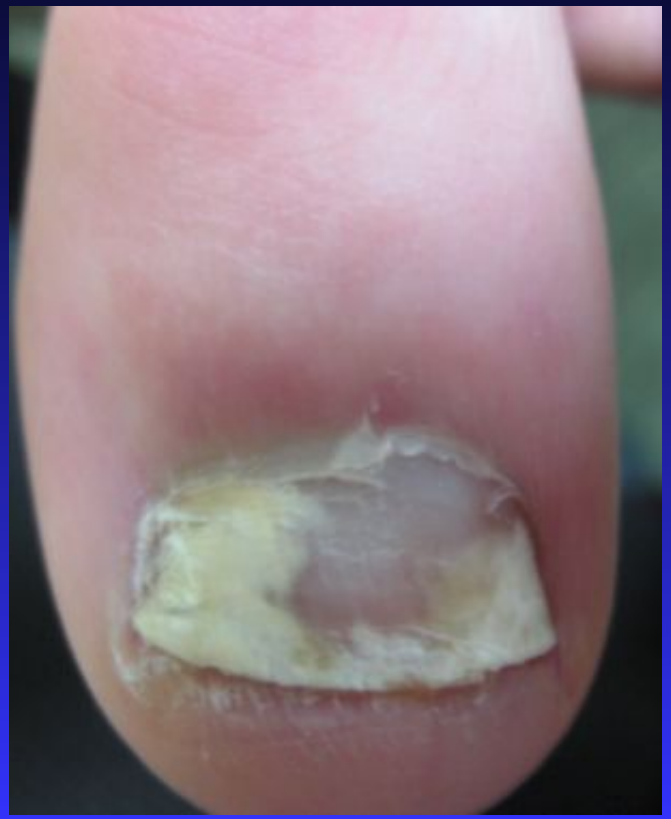
# Thin prep PAS if plate negative

- Subungal debris suspended in small amount of liquid



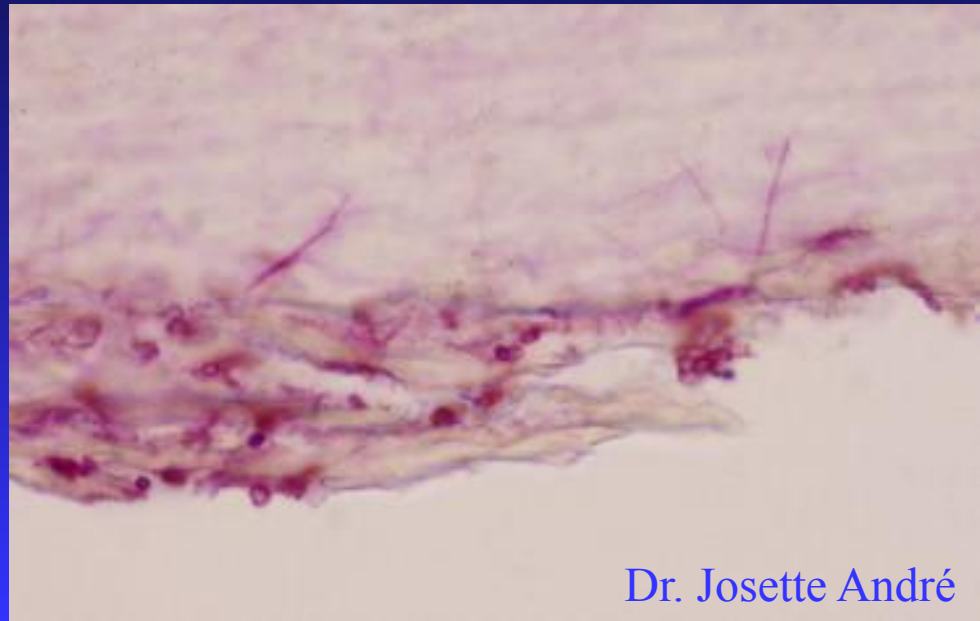
# Mold





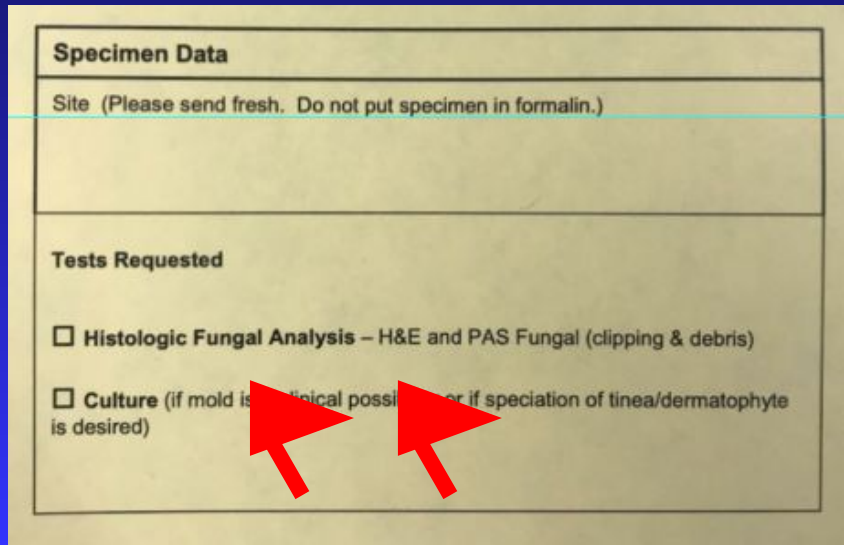
# Mold vs Dermatophyte

- Cannot tell histologically
- Invades vertical to nail plate.



# Mold

- Clinical suspicion
- Culture with cycloheximide-free media
  - Notify lab of possibility
  - Culture not as sensitive as PAS



# Acknowledgements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- Brandon Stokes--Portland

# Thanks!

curtisinportland@gmail.com