

Onychomycosis Testing

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I. Policy Description

Onychomycosis, also known as tinea unguium,¹ is a fungal infection of the nail typically caused by pathogenic fungal dermatophytes, such as *Trichophyton rubrum*, *Trichophyton mentagrophytes*, and *Epidermophyton floccosum*; onychomycosis may also be caused by yeasts, including *Candida parapsilosis* and *Candida guilliermondii*, or non-dermatophyte molds, including *Neoscytalidium dimidiatum*, *Onychocola canadensis*, the *Aspergillus* species, *Scopulariopsis* species, *Alternaria* species, *Acremonium* species, and *Fusarium* species.¹⁻³

II. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Medical Policy Statements do not ensure an authorization or payment of services. Please refer to the plan contract (often referred to as the Evidence of Coverage) for the service(s) referenced in the Medical Policy Statement. If there is a conflict between the Medical Policy Statement and the plan contract (i.e., Evidence of Coverage), then the plan contract (i.e., Evidence of Coverage) will be the controlling document used to make the determination. Specifications pertaining to Medicare and Medicaid can be found in Section VII of this policy document.

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. If there is a conflict between this Policy and any relevant, applicable government policy [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit their search website <https://www.cms.gov/medicare-coverage-database/search.aspx> or [the manual website](#).

- 1) For individuals with signs of onychomycosis, direct microscopic examination with potassium hydroxide, fungal culture of desquamated subungual material, or fungal stain of a nail clipping(s) **MEETS COVERAGE CRITERIA**.
- 2) For individuals with onychomycosis and for whom anti-fungal therapy has failed to resolve infection, nucleic acid amplification testing (NAAT) **MEETS COVERAGE CRITERIA**.

- 3) To screen for, diagnose, or confirm onychomycosis, NAAT (see Note 1) **DOES NOT MEET COVERAGE CRITERIA.**

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

- 4) To screen for, diagnose, or confirm onychomycosis, attenuated total-reflectance fourier transform infrared (ATR-FTIR) spectroscopy **DOES NOT MEET COVERAGE CRITERIA.**
- 5) Testing for the presence of fungal-derived sterols (e.g., ergosterol) **DOES NOT MEET COVERAGE CRITERIA.**

NOTES:

Note 1: Nucleic acid testing (e.g., PCR, PCR-RFLP, and next-generation sequencing [NGS]) of the following microorganisms: *Candida* species, *Aspergillus* species, *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Epidermophyton floccosum*, *Neoscytalidium dimidiatum*, *Onychocola canadensis*, *Scopulariopsis* species, *Alternaria* species, *Acremonium* species, and *Fusarium* species.¹⁻³

III. Table of Terminology

Term	Definition
AAFP	American Academy of Family Physicians
AAP	American Academy of Pediatrics
ATR-FTIR	Attenuated total-reflectance fourier transform infrared
BAD	British Association of Dermatologists
CDC	Centers of Disease Control and Prevention
CLIA '88	Clinical Laboratory Improvement Amendments Of 1988
CMS	Centers For Medicare and Medicaid Services
CPS	Canadian Paediatric Society
DLSOM	Distolateral subungual onychomycosis
DNA	Deoxyribonucleic acid
FDA	Food and Drug Administration
GC	Gas chromatography
GC/MS	Gas chromatography–mass spectrometry
HIV	Human immunodeficiency virus
HPLC	High performance liquid chromatography
HPLC/MS	High performance liquid chromatography/mass spectrometry
ITS	Internal transcribed spacer
KOH	Potassium hydroxide
LC	Liquid chromatography

LC/MS	Liquid chromatography with tandem mass spectrometry
LDT	Laboratory-developed test
NAAT	Nucleic acid amplification testing
NDM	Non-dermatophyte mould
NGS	Next-generation sequencing
OSI	Onychomycosis severity index
PAS	Periodic acid-schiff
PCR	Polymerase chain reaction
PCR-RFLP	Polymerase chain reaction-restriction fragment length polymorphism
rDNA	Ribosomal DNA
RNA	Ribonucleic acid
SSI	<i>Streptomyces subtilisin</i> inhibitor
TDOM	Total dystrophic onychomycosis

IV. Scientific Background

Onychomycosis is a fungal infection of the nail that causes approximately 50% of nail disease cases⁴ and is considered the most common nail disorder based on clinical statistics.⁵ Onychomycosis infections can be obtained through several sources, including hotel carpets, bathtubs, saunas, pool decks, and public showers, and may be generated by dermatophytes, yeast, or mold. Data show that toenails are impacted 25 times more often than fingernails,² and the first and fifth toe nail are more likely to be infected owing to the fact that footwear more frequently damages these nails.³

Dermatophytes are pathogenic fungi that can infect the skin, hair, and/or nails,⁶ and they are estimated to cause 90% of onychomycosis toenail cases and 50% of fingernail cases.⁷ These fungi attach to a surface such as an epithelial cell, extract nutrients, and grow as hyphae or filaments forming molds; this process allows the dermatophyte to seed several conditions, including onychomycosis (tinea unguium), athlete's foot (tinea pedis), and scalp ringworm (tinea capitis).⁸ Wollina, et al. (2016) suggest that an estimated 68% of onychomycosis cases are due to dermatophytes, 29% of cases due to yeasts, and 3% due to molds; further, mixed flora was identified in 5% to 15% of cases. Several types of dermatophytes may produce an onychomycosis infection, including *Trichophyton rubrum*, *Trichophyton mentagrophytes*, and *Epidermophyton floccosum*.⁷ In the United Kingdom, 85-90% of nail infections are due to dermatophytes,³ while non-dermatophyte molds are estimated to cause between 2% to 25% of all onychomycosis cases.² Non-dermatophyte mold onychomycosis causative agents include the *Aspergillus* species; incidence rates with this species vary between 1% to 35% of all cases and almost 71% in the elderly population.²

A mature nail is comprised of the nail bed, nail plate, nail matrix, and nail fold.¹ Onychomycosis-causing pathogens live on the keratin of dead corneocytes and primarily infect the nail bed; after the nail bed thickens or becomes hyperkeratotic, the nail matrix is damaged.⁷ The nail plate may also be invaded during the infection, eventually becoming detached or warped, allowing the affliction to intensify.⁷ If a toenail case is not treated, the fungi, mold, or yeast could spread to

the foot, causing tinea pedis in appropriate conditions; infections may also spread to the hands or groin area.³ If skin is externally disrupted, allowing bacteria entry into the body, the infection may also cause foot ulcers, cellulitis, osteomyelitis, and gangrene in diabetic patients.³ While an official diagnosis requires lab results, typical visual cues for an onychomycosis infection include a jagged edge of the infected area of the nail “with spikes directed to the proximal fold, white-yellow longitudinal striae in the onycholytic nail plate, and colored parallel bands.”⁹ Subungual short spikes are also indicative of onychomycosis.⁷

Several types of onychomycosis have been identified and include distolateral subungual (DLSOM), superficial white, proximal subungual, endonyx, and total dystrophic (TDOM) onychomycosis.⁹ Superficial white onychomycosis is rare, develops only in toenails, and occurs when the pathogens invade the nail through the nail plate; in proximal subungual onychomycosis, the infection occurs through the cuticle and typically develops in patients with a suppressed immune system.¹ Endonyx onychomycosis, which is caused by *T. soudanense*, occurs when the nail plate thickens; finally, the most advanced stage of onychomycosis is TDOM which may take up to 10 or 15 years to develop and can mature from any of the four main onychomycosis types mentioned above.¹

The global prevalence of onychomycosis is estimated at 5.5% of the total population.^{4,10} Ameen, et al. (2014) estimate the onychomycosis prevalence in the United Kingdom at 3% of the adult population, while Wollina, et al. (2016) estimate the prevalence in both the United States and Europe at 4.3% of the total population. Further, studies with a hospital-based population report an incidence at 8.9%.¹ Both lifestyle and general climate can impact infection rates.

As onychomycosis causes approximately 50% of nail disease cases, an estimated 15% of nail disorders can be contributed to metabolic conditions or inflammatory disorders, and five percent due to malignancies or pigment ailments.¹ Non-infectious nail diseases may include lichen ruber, yellow nail syndrome, psoriasis unguium, and tumors.¹ Onychomycosis may be stimulated by other nail disorders such as psoriasis.¹¹ When compared to nail psoriasis, onychomycosis infections tend to have more layers of parakeratosis, a greater amount of neutrophils and serous lakes, and a more blurred and/or irregular nail transition zone than psoriasis-based infections.¹²

Several ailments or conditions increase the risk of an onychomycosis infection, including diabetes, obesity, old age, immunosuppression, smoking, human immunodeficiency virus (HIV),⁴ and cancer; further, patients who receive dialysis or who have previously received a transplant also experience a greater risk of developing an onychomycosis infection.¹ Diabetics are almost three times more likely to develop onychomycosis than non-diabetics; current data suggests that an estimated 34% of all diabetics have been diagnosed with the ailment.³ Patients with HIV typically experience a more severe infection with all fingernails and toes infected due to a compromised immune system.³ Onychomycosis is rare in pediatric populations, except in children with Down syndrome or immunodeficiencies (Solis-Arias & Garcia-Romero, 2017). Adults are more likely to develop onychomycosis compared to young adults, which may be contributed to the fact that older adults are more likely to exhibit reduced peripheral circulation, larger and potentially abnormal nail surfaces, difficulty grooming and maintaining efficient hygiene levels, and may have a greater chance of exposure to pathogenic fungi.³ Athletes also experience onychomycosis infections at a greater incidence, with data suggesting that athletes

are 2.5 times more likely to develop an infection than the general population, with infections seven times more prevalent in toenails than fingernails.¹³ This is likely due to the warm and moist environment in the shoe and sock, close quarters with other athletes, and/or trauma to the foot during sporting activities.

Proprietary Testing

An onychomycosis diagnosis should be given based on both clinical results and mycological lab results.¹ Several types of tests have been developed to diagnose onychomycosis. The current diagnostic gold standard includes direct microscopy with potassium hydroxide (KOH) and fungal culture, as these methods can identify the pathogenic species and fungal viability; additional tests include polymerase chain reaction (PCR) testing, fluorescent staining and periodic acid-Schiff (PAS) staining.^{4,14} It has been reported that KOH testing is only 60% sensitive and cannot identify the species, but it can differentiate between dermatophytes and saprophytes based on a positive result; “Currently, the most sensitive test (95%) is a pathologist interpreted nail clip biopsy that has been stained with periodic acid-Schiff (PAS) plus Grocott methenamine silver.”⁷ Mycologic culture may be used for suspected onychomycosis cases with negative KOH results if spores, hyphae, or other fungal structures were seen via microscopy; histologic evaluation of a nail clipping using PAS stain may assist in an onychomycosis diagnosis with more sensitive results than those given by mycologic culture.¹⁵ An *Aspergillus* species causative agent may be suspected with a negative culture result but a positive KOH test.² Fungal cultures must be interpreted by a mycologist and, while they are specific, they are only about 60% sensitive and take several weeks to grow.⁷ When utilized together, fungal culture and PCR can determine the source of the infection; the addition of PCR can improve species detection by 20% and will assist in differentiating between onychomycosis and nail dystrophy. PCR, when used with fungal culture, allows for a “much faster, highly sensitive, and very specific diagnosis.”¹ Multiplex qPCR assays have shown to be reliable for onychomycosis diagnostics with a shorter response time than traditional culture methods.⁶

Many commercial tests are available.

For example, a multi-component test developed by Ipsum Diagnostics uses PCR to quickly identify the disease-causing agent in an onychomycosis infection alongside additional histology testing methods to provide same day results and evidence-based treatment options for both bacterial and fungal species.¹⁶

SSI Diagnostica has developed a commercial Dermatophyte Real Time PCR Kit which allows for the diagnostic detection of dermatophytes in nail samples, particularly *T. rubrum*.¹⁷

LabCorp has developed a fungus (mycology) culture test which analyzes a nail sample for an onychomycosis infection and delivers results in 30-42 days.¹⁸

MicroGenDX offers a next-generation sequencing test to identify both bacterial and fungal species for nail infections. The test also provides a corresponding antibiotic list, based on antibiotic resistance genes detected. The test also prioritizes 16 items for 24-hour rapid results, which are as follows: “Methicillin resistance, Vancomycin resistance, Beta-lactam [resistance], Carbapenem [resistance], Macrolide [resistance], Aminoglycoside [resistance], Tetracycline

[resistance], *Enterococcus faecalis* *Streptococcus agalactiae* (group B), *Streptococcus pyogenes* (group A), *Enterococcus faecium*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Candida albicans*, *Trichophyton rubrum*". MicroGenDX reports 99.2% accuracy of MicroGenDX qPCR with NGS.¹⁹

Vikor Scientific has developed the Nail-ID™ test which uses advanced molecular PCR technology to deliver rapid results "through a value-based technology platform, ABXAssist™, which incorporates regional sensitivity and susceptibility patterns, medication costs, antibiotic spectrum of activity, and FDA guidance."²⁰ The Nail-ID™ is able to deliver results in 24 hours after the sample is received, can detect polymicrobial infections simultaneously, and may identify as many as 49 antibiotic resistance genes to assist with treatment regimens.²⁰

EuroImmun launched EuroArray Dermatomycosis, a PCR-based test that detects 56 fungi species causing skin, hair, and nail infections. This test detects 23 dermatophytes, three yeasts, and three molds in one reaction.²¹

Finally, BakoDx launched a Terbinafine resistance PCR test for Onychomycosis that detects 12 fungal mutations and terbinafine resistance in *Trichophyton rubrum* and *Trichophyton mentagrophytes*. This assay quickly detects resistance-associated mutations so that prescribing physicians can limit the use of ineffective medications and save patients time and costs. The assay has 99.9% specificity and 86% sensitivity.²²

Current onychomycosis treatments encompass antifungal medications (i.e. tavaborole and efinaconazole) and laser therapy; other treatments in the pipeline include iontophoresis and photodynamic therapy.⁴ Dermatophyte infections may be treated with fluconazole, terbinafin, or itraconazole, while *Candida spp.* infections respond best to fluconazole.¹ Oral antifungal treatments are effective, but typically cause several unwanted side effects; on the other hand, topical antifungal treatments are less effective due to difficulties penetrating the nail but cause minimal side effects.²³ If the nail matrix is involved, which can typically be identified by yellow streaks tarnishing the nail, both a systemic and topical antimycotic drug are recommended.¹ Treatments may occur over a period of months or years before an improvement is noticed; further, a toenail onychomycosis infection is reportedly more difficult to treat than a fingernail infection, and a recurrence rate is estimated between five percent to 50%.⁷ An article by Gupta, et al. (2019) report that a relapse is likely to occur within the first 2.5 years after the infection has been cured; moreover, they state that to maximize cure rates, biofilms should be disrupted, drugs with more than one route of delivery should be utilized, and non-traditional treatments should be used in a timely manner if initial treatments are not efficient. Preventive strategies include retaining clean footwear, keeping toenails short and using topical antifungal agents.

Other fungal infections, such as dermatophytoma, may occur with onychomycosis infections, making these infections harder to treat; dermatophytoma can typically be identified "as a dense concentration of fungal hyphae within or under the nail plate and is generally white or yellow/brown in color, and linear (streaks) or round (patches) in shape."²⁵ A classification system has been developed to categorize the severity of an onychomycosis infection, termed the Onychomycosis Severity Index (OSI).²⁶ This score is determined by "multiplying the score for the area of involvement (range, 0-5) by the score for the proximity of disease to the matrix (range,

1-5). Ten points are added for the presence of a longitudinal streak or a patch (dermatophytoma) or for greater than 2 mm of subungual hyperkeratosis. Mild onychomycosis corresponds to a score of 1 through 5; moderate, 6 through 15; and severe, 16 through 35.”²⁶

Analytical Validity

Fungal fluorescent staining and internal transcribed spacer (ITS) ribosomal DNA (rDNA) PCR sequencing methods were compared to traditional direct microscopy with KOH detection methods for onychomycosis diagnostics; data from a total of 204 patients was used.²⁷ Fungal fluorescent staining was found to have a sensitivity of 97% and a specificity of 89%, while ITS rDNA PCR had a sensitivity of 78% and a specificity of 90%; the researchers concluded that the “Use of fluorescence enhanced the sensitivity of direct examination by 12% compared with KOH. PCR-based sequencing increased the sensitivity by 6% compared with culturing.”²⁷

Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) is a PCR technique that can be used to diagnose onychomycosis developed by Lubis, et al. (2018); this method was compared against the fungal culture gold standard. Samples were collected from 35 patients; this PCR-RFLP method was found to have a specificity of 28.57% and a sensitivity of 85.71%.²⁸ While the sensitivity is high, a low specificity may suggest that this technique be used alongside the gold standard for onychomycosis testing to further improve sensitivity instead of replacing the traditional diagnostic method altogether.

Joyce, et al. (2019) measured the effectiveness of quantitative PCR and next-generation sequencing instead of traditional, but expensive, KOH and culture techniques in diagnosing 8,816 “clinically suspicious” toenail samples; approximately 50% of the toenail samples were found to contain fungi and bacteria. The authors stated that these “Molecular methods were successful in efficiently quantifying microbial and mycologic presence in the nail. Contributions from dermatophytes were lower than expected, whereas the opposite was true for nondermatophyte molds.”²⁹

Gustafson, et al. (2019) used a real-time PCR assay on 425 clinical samples of suspected onychomycosis; results were compared to traditional KOH microscopy results. “Of 425 clinical samples suspected of onychomycosis analyzed by fungal culture and PCR, 219 samples were positive for both (52% agreement). Of the 206 discordant samples, 95% were resolved in favor of PCR by DNA sequencing.”³⁰ These researchers also analyzed a larger data set of 2,452 samples. It was identified that histopathology has a positivity rate of 85%, PCR had a positivity rate of 73% and culture had a positivity rate of 54%; “PCR outperformed culture compared to histopathology for sensitivity (80% versus 49%), specificity (92% versus 79%), positive predictive value (94% versus 77%), and negative predictive value (76% versus 52%).”³⁰

De Bruyne, et al. (2019) used attenuated total-reflectance Fourier transform infrared (ATR-FTIR) spectroscopy as an alternative method to diagnose onychomycosis; spectral differences were used for dermatophytes (1692-1606 and 1044-1004 cm^{-1}) as well as for nondermatophytes and yeasts (973-937 cm^{-1}). An accuracy rating of 96.9% was given when identifying between uninfected nails, and nails infected with either dermatophytes, yeasts, or nondermatophytes;

further, when discriminating between dermatophytes, yeasts, and nondermatophytes, classification rates were given of 91.0%, 98.6% and 97.7% respectively.³¹

Liquid chromatography-tandem mass spectrometry has been used by Ho, et al. (2019) to identify ergosterol, a sterol that most fungi cannot survive without, as a new diagnostic tool for fungal infected nails. Samples from 20 participants were collected and analyzed, which is a relatively small sample size. However, the researchers determined that this mass spectrometry diagnostic method “seemed to be better at detecting combinations of nail conditions” than current techniques, but further studies need to be completed to determine the sensitivity and specificity of this method.³²

Mourad, et al. (2019) compared Chicago sky blue staining and Calcofluor white staining to traditional KOH wet mount and culture techniques; samples from 50 patients with dermatophytosis of the hair or nail were used. Both Chicago sky blue staining and Calcofluor white staining of the hair and nail were found to be more specific and sensitive for the diagnosis of fungal infections when compared to traditional diagnostic methods because the KOH wet mount technique is reportedly a “simple, rapid, and inexpensive test but lacks color contrast and gave more false positive (artifacts) and false-negative results as compared to these new stain methods.”³³

Caldwell, et al. (2020) compared commercial multiplex PCR versus Periodic Acid–Schiff (PAF) testing for the diagnosis of Onychomycosis. A total of 209 Onychomycosis patients were recruited for the study and two toenail samples from each patient were sent for PCR and PAS testing. Of the 203 patients, “109 (53.7%) tested positive with PAS, 77 (37.9%) tested positive with PCR. Forty-one patients tested positive with PAS but negative with PCR, and nine tested positive with PCR but negative with PAS.” The authors conclude that the clinical practice of PAS biopsy staining should continue for confirmation of a fungal toenail infection before treatment. PCR test may be added optionally as it allows for species identification.³⁴

Clinical Utility and Validity

The frequency of onychomycosis infections was measured in patients with psoriasis compared to controls by Romaszkievicz, et al. (2018); data from a total of 2527 patients was used, with 2325 patients presenting with nail abnormalities and onychomycosis suspicion with no previous history of psoriasis, 102 psoriatic patients with onychomycosis suspicion, and 100 controls. The researchers used direct microscopy and culture to identify fungal infections, and found that “The prevalence of onychomycosis did not differ significantly between psoriatic patients and non-psoriatic patients with nail alterations.”³⁵ However, it was identified that the characteristics of the fungi isolated from the patients “differed significantly between psoriatic and non-psoriatic patients,” which is important to note regarding treatment regimens.³⁵ Another study, completed by Gallo, et al. (2019), also measured onychomycosis prevalence between psoriatic and non-psoriatic patients; similar results were found. This study analyzed data from a total of 9281 patients and found similar infection rates between psoriatic and non-psoriatic groups; however, once again, the “spectrum of fungal species isolated was different,” with patients in the non-psoriatic group more likely to be infected with yeasts than patients in the psoriatic group.³⁶

A meta-analysis was completed by Velasquez-Agudelo and Cardona-Arias (2017) to determine the utility, validity and performance of culture, nail clippings with PAS staining, and KOH testing for onychomycosis diagnostic purposes; this meta-analysis search utilized “5 databases and 21 search strategies.” Results showed that “The diagnostic tests evaluated in this meta-analysis independently showed acceptable validity, performance, and efficiency, with nail clipping with PAS staining outperforming the other two tests.”³⁷ Another study by Gupta, et al. (2018) measured several types of onychomycosis confirmatory testing methods such as KOH, culture, and PAS. It was determined that PAS was once again “the most sensitive confirmatory test and KOH the least expensive”; incorrect diagnoses made without confirmatory tests led to the unnecessary spending of several hundred Canadian dollars, suggesting that confirmatory lab diagnostics are preferred before treatment.³⁸

Martinez-Herrera, et al. (2015) measured the number of onychomycosis cases due to opportunistic molds; this retrospective study analyzed data from 4220 onychomycosis cases and found that only 32 cases (0.76%) were caused by opportunistic molds. This study also found that the age group most affected was between 41 and 65 years old.³⁹ Further, the authors also reported that “The most frequent isolated etiological agents were: *Aspergillus sp.* and *Scopulariopsis brevicaulis*.”³⁹

Haghani, et al. (2019) examined the species distribution of “causative agents” of onychomycosis. A total of 257 patients contributed samples, and the agents in these samples were identified through PCR. Onychomycosis was identified in 180 cases, and “51.1% of these cases were caused by non-dermatophyte moulds (NDMs), 35% by yeast and 10.6% by dermatophytes.” The authors also found that novel triazoles and imidazoles such as “efinaconazole, luliconazole and lanconazole” showed “potent” activity compared to other antifungal agents. The authors concluded that “that obtained data will be useful to improve the knowledge of researchers, clinicians and dermatologists about onychomycosis distribution, species diversity and adoption of appropriate treatment.”⁴⁰

Trave, et al. (2021) studied the clinical utility of the EuroArray dermatomycosis kit, a PCR-based microarray to detect species involved in skin and nail infections. The researchers identified 100 patients suspected of onychomycosis who were evaluated based on three diagnostic methods: KOH preparation, culture, and EuroArray. Onychomycosis was diagnosed in 47 of 100 patients who were positive on at least one of three diagnostic tests and in 49 of 100 patients who were PCR-positive. Combining microscopy and PCR had better sensitivity than fungal culture, microscopy, and PCR alone. Culture rather than PCR resulted in more frequently positive results in molds, while dermatophytes were more frequently positive in both culture and PCR. *Trichophyton interdigitale* was the most frequent pathogen. The authors conclude that the EUROArray increased the sensitivity of microscopy and yields more rapid results than culture.⁴¹

Gupta, et al. (2024) completed a retrospective cohort study to assess confirmatory testing results of onychomycosis and compare results with their matching clinical diagnosis. A total of 96293 nail specimens from a nine-month period between 2022 and 2023 were included in the study. Specimens were tested with fungal culture, histopathology, and/or PCR. Clinical diagnosis was determined using International Classification of Diseases 10th Revision codes. “For clinically diagnosed onychomycosis patients, the overall positivity rate was 59.4%; a similar positivity rate

(59.5%) was found in patients with clinically diagnosed non-fungal nail dystrophy.” Additionally, “performing a histopathologic examination with PCR was more likely to provide pathogen identification results than using fungal culture.” The authors concluded that the results “support the use of confirmatory laboratory testing when there is a clinical diagnosis of onychomycosis.”⁴²

V. Guidelines and Recommendations

Centers of Disease Control and Prevention (CDC)

The CDC remarks that an onychomycosis infection may be diagnosed through visual inspection, questioning the patient on their symptoms, or a fungal culture. The CDC states “healthcare providers may take a small skin scraping or nail sample for testing.”⁴³

The CDC also notes that the term “onychomycosis” is the technical term for a “fungal nail infection.” “It can be caused by ringworm or by infection with other types of fungi such as yeasts. Onychomycosis can affect the fingernails or toenails, but onychomycosis of the toenails is more common.”⁴⁴

American Academy of Pediatrics

Within the AAP’s Red Book, recommendations include the following concerning diagnostic testing for onychomycosis: “Fungal infection of the nail (tinea unguium or onychomycosis) can be verified by direct microscopic examination with potassium hydroxide, fungal culture of desquamated subungual material, or fungal stain of a nail clippings fixed in formalin.”⁴⁵

The AAP also notes that confirmatory diagnostic tests are similar to those for tinea corporis. According to the AAP Red Book, fungal culture to diagnose tinea corporis can be used, but that “polymerase chain reaction and periodic acid-Schiff stain evaluation of specimens are available but are expensive and generally are not necessary.”⁴⁶

British Association of Dermatologists (BAD)

The BAD have published guidelines for the management of onychomycosis stating that “The clinical characteristics of dystrophic nails must alert the clinician to the possibility of onychomycosis. Laboratory confirmation of a clinical diagnosis of tinea unguium should be obtained before starting treatment. This is important for several reasons: to eliminate nonfungal dermatological conditions from the diagnosis; to detect mixed infections; and to diagnose patients with less responsive forms of onychomycosis, such as toenail infections due to *T. rubrum*. Good nail specimens are difficult to obtain but are crucial for maximizing laboratory diagnosis. Material should be taken from any discoloured, dystrophic, or brittle parts of the nail.”³

Further, the BAD also stated that “Traditionally, laboratory detection and identification of dermatophytes consists of culture and microscopy, which yields results within approximately two to six weeks. Calcofluor white is exceedingly useful for direct microscopic examination of nail specimens, as the fungal elements are seen much more easily than with potassium hydroxide, thereby increasing sensitivity.”³

More recent molecular genetic tools were also highlighted as a newer diagnostic technique for the detection of dermatophytes. Regarding PCR testing, the BAD has stated that “Real-time polymerase chain reaction (PCR) assays have been developed, which simultaneously detect and identify the most prevalent dermatophytes directly in nail, skin and hair samples and have a turnaround time of < two days. It appears that real-time PCR significantly increased the detection rate of dermatophytes compared with culture. However, PCR may detect nonpathogenic or dead fungus, which could limit its use in identifying the true pathogen. Restriction fragment length polymorphism analysis, which identifies fungal ribosomal DNA, is very helpful for defining whether the disease is caused by repeat infection or another fungal strain when there is a lack of response to treatment. However, this technique has not been implemented into routine clinical practice.”³

Finally, the BAD also stated that “histopathological analysis using periodic acid–Schiff staining is more sensitive than direct microscopy or culture. However, this technique is not currently available in the majority of dermatology clinics or mycology laboratories. Other diagnostic techniques under investigation include flow cytometry and confocal and scanning electron microscopy.”³

Canadian Paediatric Society (CPS)

The CPS notes that treatment effectiveness will differ depending on the type of fungal or mold infection, and therefore highlights the importance of sending nail clippings for culture to “allow differentiation between dermatophyte and non-dermatophytic fungal nail infections.” The CPS also remarks that “Terbinafine has excellent action against dermatophytes, but is less effective for *Candida* onychomycosis, and these cases are best treated with azoles.”⁴⁷ Reaffirmed in 2019.

The American Academy of Family Physicians (AAFP)

The AAFP published guidelines in 2013 regarding current trends in the diagnosis and treatment of onychomycosis. These guidelines suggested C evidence ratings for the following statements:

“Periodic acid–Schiff staining should be ordered to confirm infection in patients with suspected onychomycosis.”

When preparing a nail specimen to test for onychomycosis, the nail should be cleaned with 70% isopropyl alcohol, then samples of the subungual debris and eight to 10 nail clippings should be obtained.”⁴⁸

The AAFP also stated that an “Accurate diagnosis is crucial for successful treatment and requires identification of physical changes and positive laboratory analysis.”⁴⁸ Further, a diagnosis flowchart was given and states that if a nail is discolored or gives reason to suspect onychomycosis, nail clippings should be obtained and looked at under a microscope; if the microscopic viewing suggests a positive onychomycosis diagnosis, treatment should begin to identify the organism (treatment includes culture and/or histologic evaluations with periodic acid-Schiff staining).⁴⁸

Ely, et al. (2014) gave a C evidence rating when examining both “Tinea corporis, tinea cruris, and tinea pedis can often be diagnosed based on appearance, but a potassium hydroxide preparation or culture should be performed when the appearance is atypical” and “The diagnosis of onychomycosis should generally be confirmed with a test such as potassium hydroxide preparation, culture, or periodic acid–Schiff stain before initiating treatment.”

In 2021, AAFP released a “rapid evidence review” of onychomycosis, and states that “laboratory confirmation of nail infection is important for accurate diagnosis.” AAFP lists the following recommendations:

- “A potassium hydroxide (KOH) preparation with direct microscopy is the preferred diagnostic method because it is highly specific, has rapid results, and is cost-effective. Diagnosis by KOH preparation alone is sufficient for treatment initiation. However, if KOH results are negative and there is high clinical suspicion for onychomycosis, other testing may be performed to confirm the diagnosis.
- Fungal culture of nail clippings or subungual debris allows for species differentiation but is limited by cost and the time it takes to get results. Biopsy and periodic acid–Schiff stain of nail clippings can help assess the degree of nail plate involvement. Polymerase chain reaction can also confirm the diagnosis but is more expensive than other tests.
- Because samples should be taken from the most proximal area of onycholysis, the nail plate may need to be trimmed to reveal this area.
- Diagnostic testing is generally recommended before initiating treatment, but empiric treatment with terbinafine can be considered if testing is cost prohibitive.”⁵⁰

The AAFP also lists the accuracy of diagnostic testing. Potassium hydroxide preparation has a pretest probability of 62%, sensitivity of 55.9% to 80%, and specificity of 95%. Fungal culture has a pretest probability of 56%, sensitivity of 23% to 84.6%, and specificity of 99%. Biopsy plus periodic acid-Schiff stain has a pretest probability of 65%, sensitivity of 81% to 91.6%, and specificity of 89%. Polymerase chain reaction has a pretest probability of 32%, a sensitivity of 83%, and a specificity of 84%.⁵⁰

The Journal of Drugs in Dermatology (JDD)

The Journal of Drugs in Dermatology released guidelines for diagnosis and treatment of toenail Onychomycosis in the US. For diagnosis and testing, JDD recommends that: confirmatory laboratory testing should be performed using one or more of the following: microscopic examination (eg, potassium hydroxide [KOH], periodic acid-Schiff test [PAS]), or fungal culture. While polymerase chain reaction (PCR) techniques were considered useful for confirming diagnosis, they were deemed not cost effective enough for general use.”⁵¹

VI. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare

policies and coverage, please visit the Medicare search <https://www.cms.gov/medicare-coverage-database/search.aspx>. For the most up-to-date Medicaid policies and coverage, please visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). As an LDT, the U. S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use.

VII. Applicable CPT/HCPCS Procedure Codes

Procedure codes appearing in medical policy documents are only included as a general reference. This list may not be all inclusive and is subject to updates. In addition, codes listed are not a guarantee of payment.

CPT	Code Description
82542	Column chromatography, includes mass spectrometry, if performed (eg, HPLC, LC, LC/MS, LC/MS-MS, GC, GC/MS-MS, GC/MS, HPLC/MS), non-drug analyte(s) not elsewhere specified, qualitative or quantitative, each specimen
87101	Culture, fungi (mold or yeast) isolation, with presumptive identification of isolates; skin, hair, or nail
87149	Culture, typing; identification by nucleic acid (DNA or RNA) probe, direct probe technique, per culture or isolate, each organism probed
87150	Culture, typing; identification by nucleic acid (DNA or RNA) probe, amplified probe technique, per culture or isolate, each organism probed
87153	Culture, typing; identification by nucleic acid sequencing method, each isolate (eg, sequencing of the 16S rRNA gene)
87205	Smear, primary source with interpretation; Gram or Giemsa stain for bacteria, fungi, or cell types
87206	Smear, primary source with interpretation; fluorescent and/or acid fast stain for bacteria, fungi, parasites, viruses or cell types
87220	Tissue examination by KOH slide of samples from skin, hair, or nails for fungi or ectoparasite ova or mites (eg, scabies)
87480	Infectious agent detection by nucleic acid (DNA or RNA); Candida species, direct probe technique
87481	Infectious agent detection by nucleic acid (DNA or RNA); Candida species, amplified probe technique
87482	Infectious agent detection by nucleic acid (DNA or RNA); Candida species,
87798	Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; amplified probe technique, each organism
87800	Infectious agent detection by nucleic acid (DNA or RNA), multiple organisms; direct probe(s) technique

87801	Infectious agent detection by nucleic acid (DNA or RNA), multiple organisms; amplified probe(s) technique
88312	Special stain including interpretation and report; Group I for microorganisms (eg, acid fast, methenamine silver)
88749	Unlisted in vivo (eg, transcutaneous) laboratory service

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VIII. Evidence-based Scientific References

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IX. Revision History

Revision Date	Summary of Changes
01/01/2022	Initial Effective Date
07/19/2022	Updated background, guidelines, and evidence-based scientific references. Literature review did not necessitate any modifications to the coverage criteria. Revised code disclaimer statement
06/28/2023	Updated background, guidelines, and evidence-based scientific references. Literature review did not necessitate changes to coverage criteria. Policy edited for clarity and consistency. Committee approved: 06/28/2023
05/14/2024	Reviewed and Updated: Updated background, guidelines, and evidence-based scientific references. Literature review necessitated the following changes in coverage criteria: Added new CC2 to allow for NAAT testing in situations where treatment has been ineffective and anti-fungal resistance is suspected. CC2 reads: “2) For individuals with onychomycosis and for whom anti-fungal therapy has failed to resolve infection, nucleic acid amplification testing (NAAT) MEETS COVERAGE CRITERIA.” Inclusion of defining the NAAT initialism in CC2 results changing “nucleic acid testing” to “NAAT” in CC3. Committee approved: 05/14/2024
05/21/2025	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria. The following edits were made for clarity: For clarity, added “signs of” to CC1, now reads: “1) For individuals with signs of onychomycosis, direct microscopic examination with potassium hydroxide, fungal culture of desquamated subungual material, or fungal stain of a nail clipping(s) MEETS COVERAGE CRITERIA.” Committee approved: 05/21/2025

