ISSN 2664-4169 (Print) & ISSN 2664-7923 (Online)
South Asian Research Journal of Biology and Applied Biosciences
Abbreviated Key Title: South Asian Res J Bio Appl Biosci

| Volume-8 | Issue-2 | Mar-Apr -2026 | DOI: https://doi.org/10.36346/sarjbab.2026.v08i02.012

Original Research Article

Diagnostic Accuracy of Direct PCR for Dermatophyte Detection: A Comparative Study with Conventional Methods

Noor Adnan Abdullah[1*]
[1]Department of Biology, College of Science, University of Tikrit, Tikrit, Iraq

***Corresponding Author:** Noor Adnan Abdullah
Department of Biology, College of Science, University of Tikrit, Tikrit, Iraq

Article History
Received: 12.02.2026
Accepted: 03.04.2026
Published: 08.04.2026

Abstract: ***Background*:** Dermatophytosis is common superficial fungal infection worldwide which involve keratinized tissues including skin, hair and nails. Direct diagnostic approaches based on potassium hydroxide (KOH) microscopy and fungal culture have many limitations, mainly sensitivity and longer turnaround time. Direct polymerase chain reaction (PCR) recently became a rapid and sensitive diagnostic tool. ***Objective***: To assess the diagnostic performance of Direct PCR in identifying dermatophytes and compare it with conventional methods. ***Methods***: A Cross-sectional analytical study conducted from clinical samples of 100 patients suspected with dermatophytosis. All samples received KOH examination, fungal cultures and Direct PCR. Direct PCR have been evaluated against the fungal culture as a golden standard. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and 95% confidence intervals were calculated. Statistical significance was determined by McNemar's test. ***Results***: Direct PCR showed a detection rate of 70% compared to the fungal culture and KOH microscopy (40% and 25% respectively). The sensitivity and specificity of Direct PCR was 87.5% (95% confidence interval:73.2–95.8) and 41.7% (95% confidence interval:30.0–54.2), respectively. The PPV and NPV were 50.0% and 83.3%, respectively. Surgery for cardiovascular or hematologic indications to restore physiological functions in patients. There was a statistically significant difference between Direct PCR and fungal culture ($p < 0.001$). ROC curve analysis showed AUC approximately 0.65. The highest infection rate was observed in children aged less than 10 years, with the scalp being the most commonly affected site. ***Conclusions*:** that direct PCR is a highly sensitive diagnostic method which allows rapid detection compared to conventional methods and improves the early diagnosis of dermatophytosis. Despite a relatively low specificity as compared with culture of fungi, this could be due to the shortcomings of culture as a reference standard. Hence, can be useful to screen the patients effectively. Diagnostic algorithms are potentially useful tools and warrant additional refinement and implementation into standard clinical practice to increase diagnostic accuracy and optimize patient care.

Keywords: Dermatophytosis, Direct PCR, Dermatophytes, Diagnostic Accuracy, Fungal Culture, Molecular Diagnosis.

INTRODUCTION

Dermatophytes are among the most common pathogens responsible for superficial fungal infections in keratinized tissues such as skin, hair, and nails (dermatophytosis or tinea) [3]. These infections currently impact approximately 20–25% of the global population, representing a significant public health concern worldwide (Nenoff *et al*., 2020; Gupta & Foley, 2024). Dermatophytes are mainly divided into three genera: Trichophyton, Microsporum, and Epidermophyton. Their distribution varies based on environmental, geographical, and host-related factors. While dermatophytosis is rarely life-threatening, it leads to chronicity, recurrence, and significant psychosocial burden (Havlickova *et al*., 2008; Gupta & Foley, 2024; Monod & Méhul, 2019).

Conventional laboratory diagnostics rely on direct microscopy with potassium hydroxide (KOH) and fungal cultures. However, these methods have limitations, including low sensitivity and long turnaround times; fungal cultures may require weeks to produce results (Leung *et al.,* 2020; Gupta & Foley, 2024).

Copyright © 2026 The Author(s): This is an open-access article distributed under the terms of the Creative Commons Attribution **4.0 International License (CC BY-NC 4.0)** which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited.

Recent advances in molecular diagnostic methods, especially polymerase chain reaction (PCR), have greatly improved the detection of dermatophytes. PCR-based techniques target the internal transcribed spacer (ITS) regions of ribosomal DNA, allowing for precise and quick identification with high sensitivity, and results can be obtained within 24–48 hours (Schoch *et al*., 2012; Borman *et al*., 2024). However, most previous studies have used PCR after receiving fungal cultures, which significantly diminishes the advantage of reducing diagnostic time. Recently, a widely promising direct diagnostic approach for fungal pathogens has emerged as Direct PCR amplification (DPA) of pathogen DNA directly from clinical samples without the need for culture. This method can greatly decrease turnaround time and enhance detection, particularly in cases with low fungal load or prior antifungal treatment (Brillowska-Dąbrowska *et al*., 2007; Bergmans *et al*., 2007). However, studies assessing the diagnostic accuracy of Direct PCR compared to traditional methods are still limited, especially in clinical environments. Therefore, additional research is necessary to evaluate its diagnostic effectiveness and suitability for everyday clinical use (Buitrago *et al*., 2016; Gupta & Foley, 2024). Consequently, this study seeks to determine the diagnostic accuracy of Direct PCR for the quick identification of dermatophytes and to compare its performance with standard techniques, such as KOH microscopy and fungal culture.

METHODOLOGY

Study Design

This study implemented a cross-sectional analytical approach to it mat was aimed to comparative performance of Direct PCR with conventional techniques in dermatophytes diagnosis.

Study Population and Sample Collection

A total of 100 clinical samples were obtained from patients visiting the dermatology outpatient department with clinically suspected dermatophytosis.

The collected samples included:

- Skin scrapings
- Hair samples
- Nail clippings

To reduce contamination, all samples were taken under sterile conditions for each patient by a qualified physician after 70% ethanol was used to disinfect the area of interest.

From Each Sample, An Aliquot Was Divided into Three Parts:

Direct Microscopic Examination (KOH)

- Fungal culture
- Direct PCR

Direct Microscopic Examination (KOH)

A portion of each sample was examined using potassium hydroxide (KOH) at a concentration of 20%.

The specimen was placed on a clean glass slide, treated with KOH, gently heated, and examined under a light microscope using 10× and 40× magnifications to detect fungal elements such as hyphae and spores.

Fungal Culture

Samples were inoculated onto:

- Sabouraud Dextrose Agar (SDA)
- Dermatophyte Test Medium (DTM)

The plates were incubated at 28°C for 2–4 weeks and examined regularly for fungal growth.

Identification of Dermatophytes was Based On:

- Colony morphology
- Microscopic characteristics

DNA Extraction

It involved performing the recognized assay “directly” from various clinical samples, without prior culture step, using a method of thermal lysis. A pellet of all samples was resuspended in sterile distilled water and transferred to sterile mini-centrifuge tubes. This solution was incubated at 95–100°C for 10 min to lyse the cells, followed by centrifugation to isolate the supernatant containing tubular DNA. The DNA was kept at −20°C until required for further analyses.

PCR Amplification

The Internal Transcribed Spacer (ITS) region was targeted using universal primers in the previous process. Each 20 µL PCR reaction contained polymerase, forward and reverse primers, DNA template, and nuclease-free water. Amplification was performed on a thermal cycler (Bio-Rad, Inc. Hercules, CA) with the initial step of denaturation at 95°C for 10 minutes followed by 40 cycles of denaturation at 95°C for 10 seconds, annealing at 56°C for 5 seconds, and extension at 72°C for period of 24 seconds with final extension step at temperature of 72° C.

The PCR products were analyzed by agarose gel electrophoresis and visualized under ultraviolet (UV) light.

Table 1: Presents the primer sequences applied for each target gene investigated in this study

Primer		Primer sequence 5' → 3'	Product size (bp)	TM	Reference
ITS	F	TCCGTAGGTGAACCTGCGG	600bp	56	White *et al.*, 1990
	R	TCCTCCGCTTATTGATATGC			

Statistical Analysis

Version of SPSS was used to conduct the analysis. Categorical variables were reported as frequencies and percentages. Direct PCR performance was evaluated against fungal culture, which acted as the reference standard. Sensitivity, specificity, PPV and NPV were calculated with 95% confidence intervals (CI).

McNemar's test was used to assess differences Direct PCR compared with fungal culture, and a p-value of <0.05 was considered statistically significant. Overall diagnostic performance is analyzed by the receiver operating characteristic (ROC) curve.

RESULTS

A total of 100 clinical samples from suspected dermatophytosis patient receiving treatment at the Hospital in India were used for this study. The demographic showed variances among age, sex and infection site.

Table 2: Depicts the demographic characteristics of the studied patients.

Variable	No. of cases	Percentage (%)
Gender		
Male	60	60%
Female	40	40%
Age group		
<10 years	45	45%
10–20 years	30	30%
>20 years	25	25%
Site of infection		
Scalp	45	45%
Skin	35	35%
Nail	20	20%

The results showed that males were more affected than females. The highest infection rate was observed in children under 10 years of age. The scalp was the most common site of infection.

Conventional Diagnostic Methods

The results of conventional diagnostic methods are presented in Table (2).

Table 3: Results of Conventional Diagnostic Methods

Method	Positive	Negative	Percentage (%)
KOH	**25**	**75**	**25%**
Culture	**40**	**60**	**40%**

The results showed that fungal culture had a higher detection rate compared to KOH examination. Culture detected a greater number of positive cases, while KOH showed a lower detection rate.

This may be due to the fact that culture is capable of demonstrating the presence of viable organisms, whereas KOH examination requires maturity of fungus. The following table (3) shows the distribution of dermatophytes isolated by culture.

Table 4: Distribution of Isolated Fungal Species in Culture-Positive Cases (n=40)

Type of Isolated Fungus	Number of Positive Samples	Percentage (%)
Trichophyton violaceum	10	25.0%
Microsporum canis	8	20.0%
Trichophyton tunsurans	4	10.0%
Trichophyton rubrum	3	7.5%
Trichophyton verrucosum	3	7.5%
Epidermophyton floccosum	3	7.5%
Trichophyton soudanense	2	5.0%
Trichophyton schoenleinii	2	5.0%
Microsporum audounii	2	5.0%
Trichophyton mentagrophytes	1	2.5%
Microsporum gypseum	1	2.5%
Candida albicans	1	2.5%
Total	40	100%

Results of PCR Analysis

The results of PCR analysis are presented in Table (5)

Table 5: Results of PCR Analysis

Method	Positive	Negative	Percentage (%)
PCR	70	30	70%

The results demonstrated a higher detection rate for the PCR technique in comparison with conventional methods as a greater number of positive cases were revealed when it was employed (most of the authors showing a greater tendency for PCR to detect dermatophytes).

This may be attributed to the ability of PCR to detect fungal DNA even in samples with low fungal load or non-viable organisms.

Comparison between Direct PCR and Culture

The comparison between Direct PCR and fungal culture is presented in Table (6)

Table 6: Comparison of Direct PCR Results with Fungal Culture Results

	Culture Positive	Culture Negative
PCR Positive	35	35
PCR Negative	5	25

The results showed that Direct PCR detected a higher number of positive cases compared to culture.

PCR identified additional positive cases that were not detected by culture, which may be attributed to its ability to detect fungal DNA even in samples with low fungal load or non-viable organisms.

Diagnostic Accuracy of Direct PCR

The diagnostic accuracy of Direct PCR in comparison with fungal culture is presented in Table (7).

Table 7: Diagnostic Accuracy Parameters of Direct PCR Compared to Fungal Culture

Parameter	Value (%)
Sensitivity	87.5
Specificity	41.7
Positive Predictive Value (PPV)	50.0
Negative Predictive Value (NPV)	83.3

The sensitivity, specificity, PPV, and NPV for Direct PCR were 87.5% (95% CI: 73.2–95.8), 41.7% (95% CI: 30.0–54.2), 50.0% (95% CI: 38.7–61.3), and 83.3% (95% CI: 66.4–92.7) respectively. There was a statistically significant difference between Direct PCR and fungal culture using McNemar’s test ($p < 0.001$), with Direct PCR detecting a more positive cases.

ROC curve analysis showed an area under the curve (AUC) of approximately 0.65, indicating fair diagnostic performance.

DISCUSSION

The results of our study showed a greater detection rate by culture compared to KOH microscopy which reflects previously reported findings that culture is more sensitive than direct microscopy (Nenoff *et al*., 2020; Leung *et al*., 2020; Gupta & Versteeg, 2017) and is likely a reflection of the dependence of direct microscopy on sample quality, burden of fungi and operator expertise (Havlickova *et al*., 2008).

KOH examination has lower sensitivity than culture for early or mild infections (Leung *et al.,* 2020; Gupta & Versteeg, 2017; Monod, 2008). Direct PCR outperformed traditional methodologies in general and that was believed to be due, to a large extent, to the direct detection of fungal DNA from clinical specimens without having dependence on viable fungi (Borman *et al*., 2024; Gits-Muselli *et al*., 2024).

Unlike culture, where the fungal load may be very low or previous treatment with antifungals has made recovery impossible, with molecular techniques even small quantities of DNA can still be detected (Arabatzis *et al*., 2007; Schoch *et al*., 2012). This is comparable with the studies mentioned above that have reported an increased sensitivity using PCR based methods for dermatophyte detection (Brillowska-Dąbrowska *et al.,* 2007; Bergmans *et al*., 2007; Lindsley *et al*., 2001). Another great advantage is that Direct PCR lead to greatly decreased turnaround times, with the results being produced in 24–48 hours; this period stands in sharp contrast to 2–4 weeks' waiting when a specimen needs to be submitted for culture (Gits-Muselli *et al*., 2024; Arabatzis *et al*., 2007).

This quick diagnosis is essential for effective treatment and prevention of further disease spread, especially in the case of highly contagious diseases (Gupta & Versteeg, 2017; Havlickova *et al*., 2008). The prevalence of Trichophyton violaceum and Microsporum canis is further supported by trends seen in other areas worldwide (Nenoff *et al*., 2020; Summerbell, 2010). Furthermore, the increased rate of infections in children and typical scalp lesions are also consistent with the dermatophytosis epidemiology as per earlier reports (Havlickova *et al*., 2008; Gupta & Versteeg, 2017). While Direct PCR has many advantages, there are several limitations associated with it. It has specialist laboratory requirements and trained personnel, which limits the implementation to centres able to provide such diagnostic services (Borman *et al*., 2024).

Additionally, PCR can identify DNA from non-23viable organisms, which may result in false21positive responses (10–12; 53).

Such a relatively low specificity (41.7%) needs to be interpreted with caution. The difference in Direct PCR sensitivity is likely species specific. Some T species (e.g., T. rubrum, T.violaceum have resistance to thermal lysisdue to thicker cell walls, leading to false-negative results. In contrast, species like M. canis are more easily detected. This may explain why sensitivity did not reach 100%. This may not solely reflect false-positive PCR results, but could also be attributed to the limited sensitivity of fungal culture, which is traditionally used as the reference standard despite its known limitations (Borman *et al.,* 2024; Nenoff *et al*., 2020). Therefore, some PCR-positive/culture-negative cases may represent true infections that were not detected by culture.

To further improve diagnostic accuracy, viability PCR methods such as Propidium Monoazide (PMA)-PCR could be used in future studies to differentiate between viable and non-viable fungal organisms. This approach may enhance specificity and reduce false-positive results associated with conventional PCR techniques (Arabatzis *et al.,* 2007; Schoch *et al*., 2012).Future studies should also consider the use of composite reference standards that combine clinical findings, microscopy, culture, and molecularmethods to provide a more accurate assessment of diagnostic performance (Gits-Muselli *et al.,* 2024; Borman *et al.,* 2024).Overall, Direct PCR represents a significant advancement in dermatophyte diagnosis due to its high sensitivity and rapid detection. Its integration into routine clinical practice has the potential to improve diagnostic accuracy, facilitate early targeted treatment, and ultimately enhance patient outcomes (Gupta & Versteeg, 2017; Nenoff *et al*., 2020).

REFERENCES

- Arabatzis M, et al. Molecular detection and identification of dermatophytes using PCR-based methods. Med Mycol. 2007;45(7):657–662.Doi:10.1080/13693780701449473
- Bergmans AM, Schinkel J, van Ketel RJ, Westerveld D, Kooistra-Smid M, et al. Development of a PCR assay for detection of dermatophytes in nail samples. J Clin Microbiol. 2007;45(11):3790–3794. Doi:10.1128/JCM.01303-07
- Borman AM, Fraser M, Johnson EM. Molecular detection of dermatophytes. J Clin Microbiol. 2024.Doi:10.1128/jcm.01234-23

- Brillowska-Dąbrowska A, Saunte DM, Arendrup MC. PCR-based detection of dermatophytes directly from clinical samples. J Clin Microbiol. 2007;45(4):1204–1207.Doi:10.1128/JCM.02220-06
- Buitrago MJ, Bernal-Martínez L, Castelli MV, Rodríguez-Tudela JL, Cuenca-Estrella M. Performance of PCR assays for diagnosis of invasive fungal infections. Clin Microbiol Infect. 2016;22(10):884–891.Doi:10.1016/j.cmi.2016.07.017
- Gräser Y, Scott J, Summerbell R. The new species concept in dermatophytes—a polyphasic approach. Med Mycol. 2008;46(1):1–9. Doi:10.1080/13693780701753861
- Gupta AK, Foley KA. Antifungal treatment for dermatophytosis. J Fungi (Basel). 2024;10(2):123.Doi:10.3390/jof10020123
- Gupta AK, Versteeg SG. Diagnostic testing for onychomycosis: From microscopy to molecular techniques. J Am Acad Dermatol. 2017;76(5):802–810. Doi:10.1016/j.jaad.2016.12.001
- Havlickova B, Czaika VA, Friedrich M. Epidemiological trends in skin mycoses worldwide. Mycoses. 2008;51(Suppl 4):2–15.Doi:10.1111/j.1439-0507.2008.01606.x
- Kardjeva V, Summerbell R. Molecular diagnosis of dermatophyte infections. Mycopathologia. 2006;162(3):197–203.Doi:10.1007/s11046-006-0045-1
- Leung AKC, Lam JM, Leong KF, Hon KL. Dermatophytosis: An updated review. Drugs Context. 2020;9:2020-5-2.Doi:10.7573/dic.2020-5-2
- Lindsley MD, Hurst SF, Iqbal NJ, Morrison CJ. Rapid identification of dermatophytes using PCR. J Clin Microbiol. 2001;39(9):3459–3463.Doi:10.1128/JCM.39.9.3459-3463.2001
- Monod M, Méhul B. Recent advances in dermatophyte research. J Fungi (Basel). 2019;5(3):87.Doi:10.3390/jof5030087
- Monod M. Secreted proteases from dermatophytes. Mycopathologia. 2008;166(5-6):285–294.Doi:10.1007/s11046-008-9105-4
- Nenoff P, Krüger C, Schaller J, Ginter-Hanselmayer G, Tietz HJ. Mycology – an update part 2: dermatomycoses. J Dtsch Dermatol Ges. 2014;12(9):749–777.Doi:10.1111/ddg.12418
- Nenoff P, Verma SB, Ebert A, Süß A, Fischer E, Auerswald E, et al. Spread of terbinafine-resistant Trichophyton mentagrophytes type VIII in Europe. J Fungi (Basel). 2020;6(4):207.Doi:10.3390/jof6040207
- Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, et al. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for fungi. Proc Natl Acad Sci U S A. 2012;109(16):6241–6246.Doi:10.1073/pnas.1117018109
- Summerbell RC. Epidemiology and ecology of dermatophyte infections. Clin Dermatol. 2010;28(2):210–214.Doi:10.1016/j.clindermatol.2009.12.005.