

Research Article

A Comparative Study of Sabouraud Dextrose Agar and Dermatophyte Test Medium for the Diagnosis of Dermatophytosis

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A B S T R A C T

Background: Dermatophytosis is a common superficial fungal infection affecting keratinised tissues. Direct potassium hydroxide (KOH) microscopy is rapid and inexpensive, whereas fungal culture provides confirmation and permits morphological identification. This study compared Sabouraud Dextrose Agar (SDA) and Dermatophyte Test Medium (DTM), together with KOH microscopy, for the laboratory diagnosis of dermatophytosis.

Methods: A prospective cross-sectional study was conducted over six months in a tertiary-care microbiology department and included 44 patients with clinically suspected dermatophytosis. Clinical specimens were examined by 10% KOH microscopy and inoculated onto SDA and DTM. Cultures were incubated at 25–28°C and examined for up to four weeks. Dermatophyte identification was based on macroscopic and microscopic morphological characteristics. A culture-based reference method was used to assess the diagnostic performance of KOH microscopy.

Results: Of the 44 specimens, 30 (68.18%) were classified as culture-positive according to the study reference criteria. KOH microscopy was positive in 25 (56.82%) specimens. Using culture as the reference method, KOH microscopy showed a sensitivity of 70.0%, specificity of 71.43%, positive predictive value of 84.0%, and negative predictive value of 52.63%. DTM showed presumptive growth or colour change in 21 (47.73%) specimens, whereas SDA showed growth in 28 (63.64%). SDA permitted morphological species identification in 28 isolates; *Trichophyton rubrum* was the predominant species. DTM colour change was interpreted as presumptive and was not considered definitive confirmation without appropriate morphological assessment.

Conclusion: KOH microscopy remains a useful rapid screening method

but may miss culture-confirmed infections. SDA provided the higher culture yield in the present dataset and enabled morphological identification, whereas DTM provided presumptive information but required confirmation because colour change is not specific for dermatophytes. An integrated diagnostic approach using direct microscopy and appropriately interpreted culture may improve diagnostic accuracy.

Keywords: Dermatophytosis, Sabouraud Dextrose Agar, Dermatophyte Test Medium, KOH Microscopy, Trichophyton, Fungal Culture, Superficial Fungal Infection, Diagnostic Microbiology

Introduction

Dermatophytosis is one of the most common superficial fungal infections worldwide and primarily affects keratinised tissues, including the skin, hair, and nails. The infection is caused predominantly by species belonging to the genera *Trichophyton*, *Microsporum*, and *Epidermophyton*. Warm and humid climatic conditions in tropical and subtropical regions favour transmission and persistence of dermatophyte infections. In India, dermatophytosis represents an important clinical and public health problem, with changes in the distribution of causative species and increasing concerns regarding treatment response and antifungal resistance.¹

Although dermatophytosis can often be suspected clinically, clinical features alone may not reliably distinguish dermatophyte infection from other superficial fungal or non-fungal dermatoses. Laboratory confirmation is therefore important, particularly in patients with recurrent, extensive, atypical, or treatment-resistant disease. Direct potassium hydroxide (KOH) microscopy is a simple, rapid, and inexpensive method for demonstrating fungal elements in clinical specimens. However, its diagnostic yield may be influenced by specimen quality, fungal burden, adequacy of sampling, and observer interpretation.²

Fungal culture remains an important method for confirming dermatophyte infection and permits subsequent morphological identification of isolates. Sabouraud Dextrose Agar (SDA) is a routinely used fungal culture medium that supports dermatophyte growth and facilitates assessment of colony morphology and microscopic structures required for species identification.³ However, fungal growth on SDA may require several days to weeks, which can delay definitive laboratory diagnosis.

Dermatophyte Test Medium (DTM) was developed as a selective and differential medium for the presumptive detection of dermatophytes. It contains phenol red as a pH indicator, producing a characteristic colour change associated with alkalinisation of the medium. Selective

agents help suppress bacterial and some saprophytic fungal contaminants. DTM may therefore provide an earlier presumptive indication of dermatophyte growth than conventional fungal culture media.^{4,5} However, colour change on DTM is not by itself sufficient for species identification, and suspected dermatophyte growth requires appropriate morphological confirmation.

The choice of culture medium may therefore influence the speed and yield of laboratory diagnosis. While SDA provides valuable information for morphological and species-level identification, DTM may offer an advantage in early presumptive detection. A comparative evaluation of these media, together with direct KOH microscopy, may help establish a practical diagnostic approach for routine laboratories.

The present study was undertaken to compare SDA and DTM for the isolation of dermatophytes from clinically suspected cases of dermatophytosis and to evaluate the diagnostic utility of KOH microscopy using culture findings as the reference method. The study also assessed the time required for presumptive detection and the ability of SDA to facilitate morphological identification of dermatophyte species.

Materials and Methods

Study Design and Setting

This prospective cross-sectional study was conducted over six months in the Department of Microbiology, Sri Sathya Sai Medical College and Research Institute, Tamil Nadu, India. The study was approved by the Institutional Ethics Committee, and informed consent was obtained from all participants before sample collection. 06.04.2026 + IEC No.: 1002/24 dated 22.04.2024

Study Population

A total of 44 patients with clinically suspected dermatophytosis were enrolled. Patients presented with clinical features suggestive of superficial fungal infection, including erythematous annular plaques, scaling, pruritus, and patchy hair loss. Newly diagnosed, treatment-naïve individuals were included. Patients receiving antifungal therapy or those with underlying immunosuppressive conditions were excluded.

Sample Collection and Processing

Specimens were collected according to the anatomical site involved. Skin scrapings, nail clippings, hair specimens, and other clinically indicated specimens were obtained using standard techniques. The affected area was cleaned with 70% ethanol before collection, and skin specimens were obtained from the active advancing margin of the lesion. All specimens were transported to the microbiology laboratory

in sterile containers and processed on the day of collection.

For direct microscopy, a portion of each specimen was used to prepare a 10% KOH mount. The preparation was allowed adequate time for clearing of keratinous material, particularly in nail and other heavily keratinised specimens, and was then examined microscopically. The exact interval between preparation and microscopic examination was 30 minutes for skin and hair.

Direct Microscopy with KOH

Each specimen was examined using a 10% KOH preparation. The specimen was placed on a clean glass slide, 10% KOH was added, and a coverslip was applied. The preparation was gently warmed when required to facilitate clearing. After adequate clearing, the preparation was examined by light microscopy using 10× and 40× objectives for fungal elements, including hyaline, septate hyphae and spores. KOH microscopy was considered a direct screening test and was not used as the reference method for culture.

Culture Techniques

All specimens were inoculated onto SDA and DTM. SDA supplemented with chloramphenicol (50 µg/mL) and cycloheximide (500 µg/mL) was used to suppress bacterial and saprophytic fungal growth. DTM containing phenol red, chloramphenicol, and cycloheximide was used for presumptive detection of dermatophyte growth. The inoculated media were incubated at 25–28°C and examined regularly for up to four weeks.

A colour change on DTM from yellow to red was considered a presumptive indication of dermatophyte growth and was not regarded as definitive confirmation by itself. Suspected DTM-positive cultures were interpreted in conjunction with fungal growth and morphological characteristics.

Identification of Dermatophytes

Fungal growth on SDA was assessed for colony morphology, including texture, pigmentation of the obverse and reverse surfaces, and rate of growth. Microscopic examination was performed using Lactophenol Cotton Blue preparations to identify characteristic structures such as macroconidia, microconidia, and hyphal arrangements. Dermatophyte isolates were identified on the basis of characteristic macroscopic and microscopic morphological features according to standard clinical mycology identification criteria.⁶

For the purpose of diagnostic analysis, culture-confirmed dermatophyte infection was used as the culture-based reference method. DTM colour change alone was not considered a definitive reference standard. Because molecular identification was not performed, the term 'culture-based reference method' is used rather than implying an absolute gold standard.

Diagnostic Performance and Statistical Analysis

Sensitivity and specificity of KOH microscopy were calculated against the culture-based reference method. Sensitivity was defined as the proportion of culture-positive specimens that were KOH-positive, whereas specificity was defined as the proportion of culture-negative specimens that were KOH-negative.

$$\text{Sensitivity} = \text{TP} / (\text{TP} + \text{FN}) \times 100$$

$$\text{Specificity} = \text{TN} / (\text{TN} + \text{FP}) \times 100$$

$$\text{Positive predictive value (PPV)} = \text{TP} / (\text{TP} + \text{FP}) \times 100$$

$$\text{Negative predictive value (NPV)} = \text{TN} / (\text{TN} + \text{FN}) \times 100$$

Descriptive statistics were used to report frequencies and percentages. Data were compiled and analysed using Microsoft Excel.

Results

Demographic and Clinical Distribution

Among the 44 patients included in the study, the most frequent clinical presentation (Table 1) was tinea corporis in 14 cases (31.82%), followed by tinea cruris in 9 (20.45%) and tinea capitis in 7 (15.91%). Other or mixed clinical presentations accounted for 14 cases (31.82%).

The 44 clinical specimens comprised 34 skin scrapings, 4 nail clippings, 4 hair specimens, and 2 other/mixed specimens. Based on the specimen-wise dataset, KOH microscopy was positive in 25 specimens, DTM was positive in 21, and SDA showed fungal growth in 28. The category 'other/mixed specimens' comprised two specimens recorded as such in the study dataset. (Table 2)

The specimen-wise KOH positivity was 61.76% (21/34) for skin scrapings, 50.00% (2/4) for nail clippings, 25.00% (1/4) for hair specimens, and 50.00% (1/2) for other/mixed specimens. DTM was positive in 20/34 (58.82%) skin scrapings and 1/4 (25.00%) nail specimens. SDA showed growth in 24/34 (70.59%) skin scrapings, 2/4 (50.00%) nail

Table 1. Demographic and Clinical Distribution

Clinical type	Number of cases	Percentage (%)
Tinea corporis	14	31.82
Tinea cruris	9	20.45
Tinea capitis	7	15.91

Other/mixed	14	31.82
Total	44	100.00

Table 2. Distribution and Results According to Specimen Type

Specimen type	Total	KOH positive	KOH negative	DTM positive	SDA positive
Skin scrapings	34	21	13	20	24
Nail clippings	4	2	2	1	2
Hair specimens	4	1	3	0	1
Other/mixed specimens	2	1	1	0	1
Total	44	25	19	21	28

specimens, 1/4 (25.00%) hair specimens, and 1/2 (50.00%) other/mixed specimens.

Direct KOH Microscopy

Overall, 25 of 44 specimens were positive by KOH microscopy, giving a positivity rate of 56.82%. Hyaline, septate fungal hyphae and/or other fungal elements were observed in KOH-positive preparations.

Culture Findings

DTM demonstrated presumptive positive growth or colour change in 21 of 44 specimens (47.73%), whereas SDA demonstrated fungal growth in 28 of 44 specimens (63.64%). The two media were not considered interchangeable because DTM colour change was treated as presumptive and SDA provided the principal medium for morphological species identification.

The study dataset classified 30 of 44 specimens (68.18%) as culture-positive for the purpose of the KOH reference analysis. This figure represents the culture-based reference

classification and should not be interpreted as equivalent to the number of DTM-positive specimens or the number of SDA-positive specimens. (Table 3)

Using the culture-based reference classification, KOH microscopy showed a sensitivity of 70.0% (21/30), specificity of 71.43% (10/14), PPV of 84.0% (21/25), and NPV of 52.63% (10/19).

These calculations are internally consistent with the 2 × 2 table 3 above. The specimen-wise dataset was reconciled so that the total number of KOH-positive specimens is 25 rather than 26.

Time to Detection

DTM provided presumptive detection within approximately 5–10 days in most positive cultures, whereas SDA generally required longer incubation before visible growth and adequate morphological assessment. The DTM colour reaction was interpreted as presumptive and was not considered definitive in isolation.

Species Distribution from SDA Cultures

Table 3. Diagnostic Performance of KOH Microscopy

	Culture positive	Culture negative	Total
KOH positive	21	4	25
KOH negative	9	10	19
Total	30	14	44

Table 4. Species Distribution from SDA Cultures

Dermatophyte species	Number of isolates	Percentage (%)
Trichophyton rubrum	14	50.00
Trichophyton mentagrophytes	10	35.71
Microsporum gypseum	4	14.29
Total	28	100.00

Among the 28 isolates recovered on SDA, species-level morphological identification demonstrated *Trichophyton rubrum* in 14 (50.00%), *T. mentagrophytes* in 10 (35.71%), and *Microsporum gypseum* in 4 (14.29%). (Table 4)

Discussion

Accurate and timely diagnosis of dermatophytosis is important for appropriate patient management. In the present study, direct KOH microscopy and fungal culture were evaluated, with particular emphasis on the comparative utility of DTM and SDA.

The relatively lower positivity observed with KOH microscopy may be explained by several technical and biological factors. Direct KOH examination depends substantially on specimen quality and fungal burden. Inadequate sampling, collection from a non-representative area of the lesion, prior antifungal exposure, incomplete clearing of keratinised material, and difficulty in recognising sparse or fragmented fungal elements may result in negative microscopy despite culture-confirmed infection. These factors are particularly relevant to nail and other heavily keratinised specimens.

KOH microscopy may produce both false-negative and false-positive interpretations when compared with culture-based methods. False-negative results may occur because of inadequate or superficial sampling, low numbers of fungal elements, prior antifungal treatment, suboptimal specimen preparation, incomplete clearing, or difficulty in recognising sparse fungal structures. False-positive interpretation may occur when non-fungal artefacts, keratin debris, textile fibres, air bubbles, or other refractile structures are mistaken for fungal elements. Therefore, KOH should be regarded as a rapid screening method rather than a definitive species-identification test [2, 7].

In the present study, KOH microscopy showed a sensitivity of 70.0% and specificity of 71.43% against the culture-based reference classification. These findings support the usefulness of KOH as a rapid initial investigation while also demonstrating that a negative KOH preparation does not exclude dermatophytosis when clinical suspicion remains high.

DTM demonstrated presumptive positive findings in 21 of 44 specimens (47.73%), whereas SDA showed growth in 28 of 44 (63.64%). DTM was useful for providing an early presumptive indication of dermatophyte growth; however, its colour reaction should not be interpreted as definitive confirmation. DTM contains phenol red, and alkalinisation of the medium may produce a yellow-to-red colour change during growth of dermatophytes, but some non-dermatophytic fungi can also produce alkaline reactions and false-positive colour changes. Consequently, colour change alone cannot rule out false positivity [8].

In this study, suspected DTM-positive cultures were therefore interpreted in conjunction with fungal growth and morphological characteristics. DTM was used primarily as a presumptive medium, whereas SDA was used preferentially for morphological assessment and species identification. This distinction is important because the 21 DTM-positive specimens should not automatically be described as 21 definitively confirmed dermatophyte infections solely on the basis of colour change.

SDA remains valuable because it permits assessment of colony morphology and microscopic structures required for species identification. In the present dataset, 28 isolates grew on SDA and were identified morphologically. *Trichophyton rubrum* was the predominant species, followed by *T. mentagrophytes* and *Microsporum gypseum*. These findings are consistent with the continuing importance of *Trichophyton* species in dermatophytosis in India [9].

Overall, the findings indicate complementary roles for the three approaches. KOH microscopy provides rapid, low-cost direct screening; DTM may provide rapid presumptive culture information; and SDA permits more detailed morphological assessment and species identification. Rather than considering one method universally superior, the results support an integrated diagnostic strategy in which the clinical context, direct microscopy, and appropriately interpreted culture findings are considered together.

Conclusion

KOH microscopy remains a rapid and inexpensive screening method for dermatophytosis but may yield false-negative results when fungal elements are sparse or specimens are inadequate. In the present dataset, SDA demonstrated a higher overall culture yield than DTM and provided the principal medium for morphological species identification. DTM offered presumptive information, but its colour reaction was not sufficiently specific to serve as a standalone confirmation of dermatophytosis. An integrated approach combining direct microscopy with appropriately interpreted fungal culture is therefore preferable for routine diagnosis.

Limitations

- **Sample size:** The study included only 44 clinically suspected cases, which may limit the precision and generalisability of the findings.
- **Single-centre design:** The study was conducted at a single tertiary-care institution, and the results may not be generalisable to other populations or geographical settings.
- **No molecular confirmation:** Species identification relied on conventional morphological methods. Molecular identification could provide additional confirmation where available.
- **No antifungal susceptibility testing:** The study did not

assess antifungal susceptibility or resistance patterns.

- **Reference-method limitation:** Culture-based classification was used as the reference method for KOH diagnostic performance; no independent molecular reference standard was available.

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