

# Evaluation of Chicago sky blue stain against Potassium Hydroxide-Dimethyl Sulfoxide wet mount in the identification of dermatophytes

Majid Rauf Ahmad<sup>1</sup>, Iffat Javed<sup>2</sup>, Sohaila Mushtaq<sup>3</sup>

<sup>1</sup>Assistant Professor, Department of Pathology, Avicenna Medical College, Lahore, <sup>2</sup>Professor of Pathology, Department of Pathology, Amina Inayat Medical College, Lahore, <sup>3</sup>Professor of Pathology, Azra Naheed Medical College, Lahore

**Correspondence to:** Dr. Majid Rauf Ahmad, E-mail: majidrauf177@yahoo.com

## ABSTRACT

**Background:** Dermatophytoses infections are widespread in the developing world. The laboratory diagnosis of dermatophytes has been a challenge as it involves microscopy and trained personnel. Potassium hydroxide wet mount with dimethyl sulfoxide added is routinely used in direct microscopy. But it lacks color contrast and the hyphae may be missed on routine microscopy. The study aimed to evaluate the effectiveness of the Chicago sky blue stain against routine potassium hydroxide-dimethyl sulfoxide (KOH/DMSO) wet mount in direct microscopy.

**Patients and methods:** The study was carried out at the Department of Microbiology, Postgraduate Medical Institute, Lahore over a period of nine months from July 2013 till March 2014. Patients of either gender regardless of age, clinically diagnosed as having dermatophytoses by dermatologists were selected for this study. Specimens from 100 patients were collected from the dermatology outdoor of a tertiary care hospital for this study. They were evaluated microscopically with routine potassium hydroxide-dimethyl sulfoxide (KOH-DMSO) wet mount and Chicago sky blue (CSB) stain. Data were collected and entered in Statistical Package for the Social Sciences (SPSS) version 20.0.

**Results:** Out of a total of 100 samples collected from skin, hair and nails, 59% were positive on direct microscopy with KOH/DMSO wet mount. Whereas direct microscopy using CSB stain revealed dermatophytes in 62% of cases.

**Conclusion:** Chicago sky blue staining is a better technique for the detection of dermatophytes as compared to potassium hydroxide wet mount examination. It is simple, rapid, and easy to interpret. We recommend the use of this technique to improve the detection of dermatophytes without awaiting the results of the culture.

## Keywords:

Dermatophytes; Chicago sky blue; Potassium Hydroxide-Dimethyl Sulfoxide

## INTRODUCTION

Dermatomycoses are among the most prevalent infections in the world. They are common due to factors like cleanliness, financial status, humidity, heat, and crowded conditions.<sup>1</sup> Dermatophytes are difficult to diagnose solely based on clinical presentation. Routine laboratory diagnosis of dermatophytes is performed by direct microscopic examination of a clinical specimen followed by culture.<sup>2</sup> Microscopic examination of specimens of skin, hair and nail is a rapid and inexpensive diagnostic method but gives false-negative results in 15% of the cases and does not help in the differentiation of the causative species.<sup>3</sup>

The most common method of microscopy in the laboratory is a wet mount containing 20% potassium hydroxide (KOH).<sup>4</sup> It is a rapid and cheap method

involving gentle heating of slide for 5-10 minutes in case of a hair sample, 20-30 minutes for skin crusts and two hours for nail sample. However, this method requires experience to interpret the smears.<sup>5</sup> Addition of 36% dimethylsulphoxide (DMSO) to 20% potassium hydroxide (KOH) wet mount enables specimen to be examined at once without heating. It enables the clearing of keratin rapidly and prevents the rapid drying of the fluid.<sup>6</sup>

An alternative contrast stain is a Chicago sky blue stain having 1% Chicago sky blue used along with 10% KOH (potassium hydroxide) as a clearing agent. It stains the fungal hyphae blue against a pink background making it easier to identify them.<sup>7</sup> Chicago sky blue (CSB) stain is a quick and reliable diagnostic method with the potential to substitute potassium hydroxide (KOH) wet mount as a routine procedure for rapid dermatophytoses diagnosis. It is cheap and does not require a fluorescent microscope and qualified staff to examine the slide, as is the case with calcofluor white stain.<sup>8</sup>

**Competing interests:** The author declares no competing interests exist.

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The present study was conducted to devise a simpler and time-saving approach to diagnose dermatophyte infections on direct microscopy comparing Chicago sky blue (CSB) stain and potassium hydroxide-dimethylsulphoxide (KOH-DMSO) wet mount in clinically suspected dermatophytoses specimens.

### PATIENTS AND METHODS

Patients diagnosed clinically by the dermatologist to have dermatophytoses were included in the study. Patients who had used topical antifungal drugs or were being treated for dermatophytoses were excluded from the study. Specimens from one hundred patients of any age and either gender were collected from the dermatology outdoor of a tertiary care hospital in Lahore. Microscopy was done in the microbiology laboratory of Post Graduate medical institute. The data from the patient was noted on a proforma which included the patient's name, age, sex, address, presenting complaint and duration of illness. Family history, contact with animals, sharing of combs, brushes, towels, caps, shoes and history of taking antifungal medication were also recorded in the proforma. Clinical examination of the patient included noting the site, size, number and appearance of the lesion. Clinical diagnosis made by the dermatologist was noted and recorded in proforma.

In the case of the skin sample, the skin was cleaned with 70% alcohol to remove surface bacterial contamination. The skin scrapings were taken from the peripheral inflamed margins of the lesion with the help of a sterile scalpel blade. Nail clippings were collected with the help of sterile scissors. Hair was plucked from the area of scaling with the help of epilating forceps. Samples of skin crusts, hair and nail clippings were collected by the author on a clean piece of black paper 5cm in size which was neatly folded, labeled and then sent to the laboratory where they were evaluated on direct microscopy by two different techniques.

Two slides of each specimen were prepared. On one of the slides, a drop of 95-100% ethanol was added to fix the slide. The slide was left to dry completely. A drop of Chicago sky blue (CSB) stain was added to the slide. It was mixed with a small wooden stick and the slide was then placed in a glass Petri dish lined with a moist paper towel to prevent it from drying.

Thirty to sixty minutes were given to the slides of skin crusts and hair specimen while 2 hours were given to the nail specimen. The slide was then examined under 10X to identify fungal elements. Dermatophytes

were identified as blue staining fungal hyphae against a purple to a pink background of cellular debris. The magnification was increased to 40X to visualize the morphology of fungal hyphae. Blue filamentous, septate hyphae with or without arthrospores were confirmed as a dermatophyte.

On the other slide, a drop of KOH/ DMSO (20% KOH was dissolved in 40% aqueous dimethylsulphoxide) was added and a coverslip was applied. About 20 minutes were given for maceration and clearing. Using a light microscope, the slide was examined first under low power 10X and then under 40X. Dermatophytes were recognized by their typical narrow, regular, hyaline septate hyphae. In some cases, instead of hyphae, rectangular arthrospores were seen in chains or scattered in various places. This procedure was adopted for both skin and nail specimens.

In cases of hair specimen, the dermatophytes were confirmed by observing the arrangement of arthrospores with respect to the hair shaft. In case they were present within the hair shaft, they were noted as endothrix while their presence outside the hair shaft confirmed that infection to be ectothrix. SPSS version 20.0 was used for data entry and analysis. Pearson Chi-square test and Fischer exact test were used to determine the association of positive detected cases with CSB and KOH/DMSO. A p-value of  $\leq 0.05$  was considered significant.

### RESULTS

Out of a total of 100 specimens taken from patients, 61 samples were taken from skin except for hand, 18 from feet, 17 from nails, 13 samples were taken from hair, 5 samples from hand and 1 from the groin region. On direct microscopy, 62 specimens were positive with Chicago sky blue stain as compared to 59 which were positive with KOH/ DMSO wet mount. Table 1 describes the comparison between the Chicago sky blue stain and potassium hydroxide/dimethylsulfoxide wet mount. It can be seen that a total of 57 specimens were positive both on Chicago sky blue stain and KOH/DMSO wet mount and 36 specimens were negative by both methods. However, 5 specimens were appreciated on the Chicago sky blue stain but could not be detected on the KOH/DMSO wet mount. On the other hand, only 2 specimens could not be appreciated on the Chicago sky blue stain but were detected on KOH/DMSO wet mount. A significantly higher positivity ( $p < 0.05$ ) was observed by the Chicago sky blue stain as compared to KOH/DMSO wet mount preparation.

Table 1. Comparison between KOH/DMSO wet mount and CSB stain microscopic techniques for identification of dermatophytes

Microscopic technique	CSB positive	CSB negative	Total
KOH/DMSO positive	57	2	59
KOH/DMSO negative	*5	36	41
Total	62	38	100

\*p<0.05 by Fischer exact test

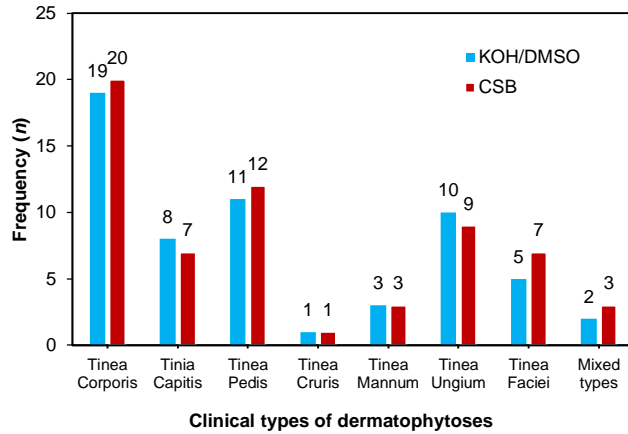


Figure 1. Results of microscopic examination with KOH/ DMSO wet mount and CSB stain in relation to the clinical types of dermatophytoses (n=100)

Figure 1 shows a comparison of microscopic examination with KOH/DMSO wet mount and CSB stain in relation to clinical types of dermatophytoses. CSB showed better results in skin samples, foot, and face while KOH/DMSO wet mount showed better results in head and nail specimens.

Figure 2A shows branching hyphae on direct microscopy on Potassium hydroxide/ Dimethylsulfoxide wet mount. Figure 2B shows the septate hyphae in blue colored which are much easier to identify as compared to the hyphae seen by potassium hydroxide/ dimethylsulfoxide wet mount.

**DISCUSSION**

Dermatophytes are a group of fungi that infect tissues rich in keratin (skin, hair, and nails), resulting in dermatophytoses.<sup>9</sup> Laboratory diagnosis of dermatophyte infections is based on observing hyaline septate hyphae by direct microscopic examination of the specimen. Direct microscopic examination is easier and cheaper but does not provide us with identification up to species level. Results of direct microscopy are false negative in 5% to 15% of cases as compared to cultures.<sup>10</sup> Species identification is usually based on cultures that require longer incubation periods of up to 4 weeks. However, it allows speciation of the fungal

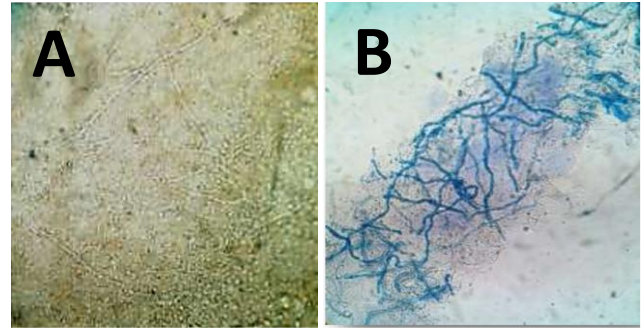


Figure 2. Microscopy of skin scrapings. (A) Potassium Hydroxide/Dimethylsulphoxide wet mount showing branching hyphae. (40X). (B) Chicago Sky Blue stain of showing blue septate hyphae (40X).

pathogen based on morphological features.<sup>11</sup> The challenges faced in the diagnosis of dermatophytoses is due to lack of standardization of specimen collection. Most reagents are also not commercially available.<sup>12</sup>

In the present study, 36% dimethylsulphoxide (DMSO) was added to 20% potassium hydroxide (KOH) wet mount. The addition of DMSO allows rapid clearance of keratin and prevents drying of the fluid which is an issue encountered in KOH wet mount. Adding dimethylsulphoxide to KOH helps in achieving quicker results as compared to without it.<sup>6</sup>

In this study, out of a hundred patients, 59% were positive on direct microscopy using KOH/DMSO wet mount. Dermatophyte detection was slightly higher in cases of tinea capitis (61.5%) as compared to tinea corporis (61.2%). The results are comparable to the previous report in which the detection rate of dermatophyte was 58.88% by using KOH/DMSO wet mount.<sup>13</sup>

However, one study was able to demonstrate dermatophytes in 73.3% of cases on microscopy.<sup>14</sup> Whereas a study performed in Rawalpindi on one hundred patients of tinea pedis showed only 34 % cases being positive on KOH microscopy.<sup>15</sup>

There have been earlier efforts to increase the sensitivity of direct microscopy for dermatophytes detection. Since KOH lacks color contrast, therefore various contrast stains have been used which stain fungal elements. Parker ink has been used but it does not provide suitable color contrast. Similarly, fluorescent dyes like calcofluor have been used but they require a fluorescent microscope with proper filters. These are not available in smaller laboratories.<sup>16</sup>

Chicago sky blue stain is a relatively new and alternative contrast stain that contains 1% Chicago sky blue and is used together with 10% KOH as a clearing agent. In this study, dermatophytes were detected in

62% of cases on direct microscopy using CSB stain. Dermatophyte detection was significantly higher in cases of tinea faciei (70%) as compared to that of tinea pedis (66.67%) and tinea corporis (64.52%). These results are similar to a previous report in which 55% of cases were detected using a Chicago sky blue (CSB) stain.<sup>8</sup> However, another study reported 75 out of 100 (75%) samples of dermatophytoses were positive using Chicago sky blue stain which is higher than our study.<sup>17</sup>

In this study, a comparison of two different potassium hydroxide (KOH) based techniques was done to obtain reliable results in a shorter period as compared to cultures. CSB stain was rapid and easier to interpret. Besides, fungal hyphae could easily be categorized as thin septate or broad aseptate filaments. Fungi can easily be differentiated from artifacts, based on the presence of branching septa allowing a reliable interpretation of staining. Moreover, CSB staining did not require a fluorescent microscope as is the case of calcofluor white. Similarly, CSB staining was more significant in the detection of dermatophytes in cases of tinea corporis (65.71% as compared to 58.57%) as compared to KOH-DMSO.

Overall, the comparison between the microscopic examination of the clinical specimens by KOH/DMSO wet mount and CSB stain showed a significantly higher positivity ( $p < 0.05$ ) of detecting dermatophytes by CSB as compared to KOH/DMSO wet mount preparation.

## CONCLUSION

The results of this study conclude that the Chicago sky blue (CSB) stain showed a significantly higher positivity ( $p < 0.05$ ) of detecting dermatophytes as compared to potassium hydroxide/dimethyl sulfoxide (KOH/DMSO) wet mount preparation on direct microscopy. Since CSB stain provides a color contrast as compared to KOH/DMSO, it becomes easier to detect hyphae of dermatophytes on a simple laboratory microscope. This study concludes that CSB stain can improve the detection of fungal infection, without waiting for the results of the culture.

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