



## RESEARCH ARTICLE

### COMPARISON OF CHICAGO SKY BLUE (NOVEL STAIN) WITH CALCOFLUOR WHITE AND POTASSIUM HYDROXIDE MOUNT FOR RAPID DIAGNOSIS OF DERMATOMYCOSIS AND ONYCHOMYCOSIS IN A TERTIARY CARE CENTRE

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#### ABSTRACT

**Purpose:** Dermatormycosis, the most common of mycotic infections, occur worldwide. In an epidemiological survey done in India, the prevalence of superficial fungal infection was 27.6%, out of which dermatormycosis was 75.6% and non-dermatormycosis was 24.4%. Rapid confirmation of dermatormycosis and onychomycosis is desirable because it allows the clinician to initiate appropriate therapy without delay. The existing modalities such as Calcofluor White stain requires fluorescent microscope whereas KOH is less sensitive compared to Calcofluor White stain.

**Aim of the study:** To compare the novel contrast stain Chicago Sky Blue with the established methods such as Calcofluor White stain and KOH for the rapid diagnosis of dermatormycosis and onychomycosis.

**Materials and Methods:** The infected nail clippings and skin scrapping were subjected to Chicago sky blue stain, Calcofluor White Stain and KOH mount. The microscopic findings were noted for the positive and negative results. Based on the results obtained, sensitivity and specificity of Chicago sky blue stain was assessed against Calcofluor White stain as reference method. Simultaneously, the diagnostic efficacy and cost effectiveness of Chicago sky blue, Calcofluor white stain and KOH mount was assessed.

**Results:** Chicago Sky Blue stain is 100% sensitive in detecting dermatormycosis and 83.4 % in detecting onychomycosis whereas KOH mount is only 93% sensitive in detecting dermatormycosis and 66.7% in onychomycosis. Chicago Sky Blue stain is 100% specific in detecting dermatormycosis and onychomycosis whereas KOH mount is only 77% specific.

**Conclusion:** Chicago Sky Blue stain was found to be highly efficacious, easy to perform and interpret as well cost-effective for detection of dermatormycosis and onychomycosis.

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## INTRODUCTION

Dermatormycoses, the most common of mycotic infections, occur worldwide. (Rippon, 1988) In an epidemiological survey done in India, the prevalence of superficial fungal infection was 27.6% out of which dermatormycosis was 75.6% and non-dermatormycosis was 24.4%. (Lakshmanan et al., 2015) Dermatormycoses are infections of the skin, hair and nail caused as a result of colonization of the keratinized layers of the body. This colonization is brought about by the organisms belonging to the three genera namely Trichophyton, Microsporum and Epidermophyton (Emmons et al., 1977; Luilma et al., 2005). Infection may also be caused rarely by the members of the genus Candida and by non-dermatormycotic

moulds belonging to the genera Fusarium, Scopulariopsis and Aspergillus (Pinto et al., 2006; Naveed et al., 2009). Interestingly dermatormycotic infections are predominant in the tropical and subtropical countries; especially in the developing countries like India where the hot climate and humid weather is favorable to the acquisition and maintenance of the disease. (Rippon, 1988; Rao, 1959) Based on their host specificity dermatormycoses are classified into three ecological groups namely geophiles (soil), anthropophiles (man) and zoophiles (animals). (Rippon, 1982)

### Need for the Study

The Potassium hydroxide mount (KOH) preparation is inexpensive but does not produce a colour contrast and requires considerable skill to interpret. Even in experienced hands, KOH has been reported to have a false-negative rate of

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5% to 15%. (Karmakar *et al.*, 1995) Calcofluor white (CW) with KOH, which is known to be very sensitive and specific for skin scrapings, has been shown to be 87% sensitive and 89% specific for the diagnosis of onychomycosis, but requires a fluorescent microscope. (Haldane and Robart, 1991) Chicago Sky Blue (CSB) stain, a newer contrast stain contains Chicago Sky Blue as one of its constituents. It can be added to KOH to highlight fungal elements and provide a colour contrast that makes reading and interpretation simple. Few studies have shown the utility of CSB stain in the diagnosis of dermatomycosis and onychomycosis. (Lim and Lim, 2011; Lim and Lim, 2008; Fonseka *et al.*, 2011; Tambosis and Lim, 2012; Lodha and Poojary, 2015) The cost of treatment is much more than the cost of laboratory testing. (Lakshmi pathy and Kannabiran, 2007) Hence, laboratory diagnosis has been reported to be a must before starting therapy. Rapid confirmation of dermatomycosis and onychomycosis is desirable because it allows the clinician to initiate appropriate therapy without delay.

### Aims and Objectives

The objectives of the present study are,

- To assess the sensitivity and specificity of novel stain, CSB stain against CW stain as reference method for the identification of dermatomycosis and onychomycosis.
- To assess the diagnostic efficacy and cost effectiveness of CSB stain, CW stain, KOH mount in the identification of dermatomycosis and onychomycosis.

## MATERIALS AND METHODS

This prospective study was carried out for a duration of 2 months in 2016 at a tertiary care hospital attached to a Medical College. The cases clinically suspected as dermatomycosis and onychomycosis were evaluated under this study after obtaining the informed consent from the participants. Detailed history and the findings of clinical examination were collected using a Proforma. Ethical clearance was obtained from the institutional ethics committee before conducting the study.

### Sample size

30 cases of clinically diagnosed dermatomycosis or onychomycosis were evaluated. Patients who had taken treatment with oral or topical anti-fungals in the past one month or patients with other nail dystrophies or skin lesions clinically diagnosed as other than dermatomycosis and onychomycosis were excluded from the study.

### Sample Collection

In cases of clinically suspected onychomycosis, affected nails were cleaned to remove contaminants and clipped short with nail clippers, the nail surfaces were curetted with a blunt scalpel and subungual debris was obtained with an eye curette. Nail clippings were taken to include subungual debris as far proximal as was tolerable and scrapings taken from their undersurface were placed on sterile black paper, wrapped and sent to laboratory immediately. (Rippon, 1988) In cases of clinically suspected dermatomycosis, areas of skin to be scraped were first cleaned with 70% isopropyl alcohol swab to remove traces of creams and reduce surface bacteria. Scraping of the scales at the periphery of skin lesions was performed to

obtain material for fungal examination and it was sent in sterile container or between sterile slides secured to the laboratory immediately. (Rippon, 1988) The nail clippings and skin scrapings were subjected to CSB stain, CW Stain and KOH mount as follows:

### Chicago Sky Blue stain

Preparation of the CSB stain:

CSB stain was prepared by dissolving 1 gm of the CSB dye in 100 ml of distilled water forming 1% solution. The skin or nail specimen was placed on clean glass slide. A drop of 10% or 20% KOH was added to the slide followed by a drop of 1% CSB stain. A Coverslip was gently pressed and excess of stain was blotted with the help of blotting paper. After waiting for approximately 20 min at room temperature, a microscopic examination was done under scanner view, low (10×) and high power (40×) objectives of the compound microscope. (Lim and Lim, 2011)

### Calcofluor White stain

A portion of the specimen (skin scraping or nail clipping) on the slide was placed. One to two drops of 10% or 20% KOH was added to specimen, slide was then placed in a Petri dish and allowed to stand about 30 minutes. 1 or 2 drops of Calcofluor white reagent was added and mixed thoroughly. Coverslip was applied gently and examined under fluorescent microscope. Control slide positive for yeast or filamentous fungus was included. (Haldane and Robart, 1991)

### Potassium hydroxide mount

Specimen was placed on a slide, and a drop of 10% /40% KOH (skin /nail) was added. A cover slip was applied with gentle pressure to drain away excess KOH. Incubation was done for 2 hours or more (up to 48 hours) until softening or digestion of the specimen occurred. Slides were microscopically evaluated for the presence of branching thread-like structures (hyphae) or yeast cells. When they were present, it was considered to be a positive test. (Rippon, 1988)

### Assessment

## LIGHT MICROSCOPY

### 1) Chicago Sky Blue Stain

The fungal cell walls stained blue with this stain against the purplish background of cellular debris and were easily discerned. (Lim and Lim, 2011; Lim and Lim, 2008; Fonseka *et al.*, 2011; Tambosis and Lim, 2012; Lodha and Poojary, 2015)

### 2) KOH Mount: (Rippon, 1988)

From diagnostic point of view, fungi causing dermatomycosis and onychomycosis may be seen as,

- Yeast-like: Spherical or oval forms, filamentous structures (pseudohyphae) may be seen. (E.g- *Candida albicans*)
- Filamentous fungi or moulds: thread-like filamentous hyphae which may be aseptate or septate. (E.g- *Aspergillus Sp.*, *Tinea Sp.*)

Certain organism like *Tinea versicolor* (Spaghetti-Meatball Appearance) may have characteristic appearance.

**Fluorescent microscopy: (Haldane and Robart, 1991)**

Calcofluor White Stain: This Stain selectively binds to the fungal cell wall made of Chitin. The dye fluoresces when exposed to ultraviolet light.

**Statistical analysis**

Sensitivity, Specificity, Positive predictive value, Negative predictive value and Accuracy of Chicago sky blue and KOH mount (using Calcofluor white stain as gold standard) as well as the diagnostic efficacy and cost effectiveness for the diagnosis of dermatomycosis and onychomycosis was assessed.

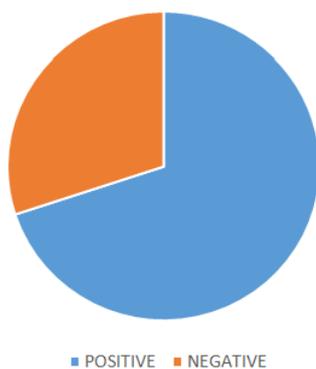
**RESULTS**

- 1)**Prevalance:** Out of 30 clinically diagnosed cases, 22 (73.3%) were dermatomycosis and 8 (26.7%) were onychomycosis. 17(56.6%) cases were seen in males and 13(43.3%) cases in females, mean age was 31.5 years.
- 2)**Reference Test:** Using Calcofluor White (CW) Stain as Gold Standard CW stain showed positive in 21 (70%) and 9(30%) negative cases. (Table 1, Chart 1)

**Table 1. Calcofluor White Stain as Gold Standard**

Total number of Clinically Diagnosed Cases (30)	Positive (21)	Negative (9)
Calcofluor White Stain	21	9
Dermatomycosis- 22	15	7
Onychomycosis- 8	6	2

**Chart 1- CW STAIN AS GOLD STANDARD**



**3) Comparison of CSB Stain and CW Stain (Gold Standard):**

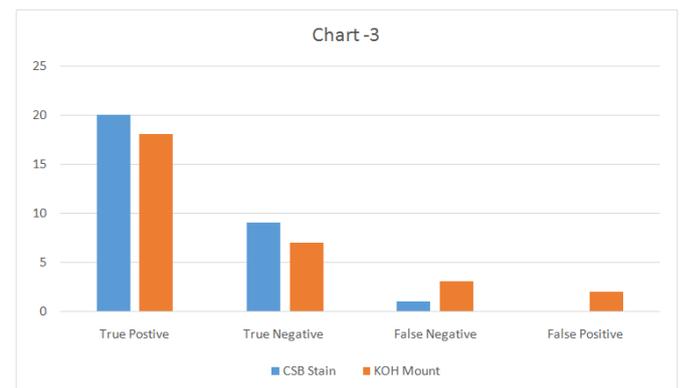
CSB stain showed 20 (95.2%) true positives and 9 (100%) true negatives. (Chart 2)

**4) Comparison of CSB Stain and KOH Mount**

CSB stain has showed 20 (95.2%) true positive cases whereas KOH mount has shown 18(85.7%). CSB stain has showed 9 (100%) true negative cases whereas KOH mount has shown 7 (77.7%). CSB stain has shown no false positive cases whereas KOH mount has shown 2 (10%) false positive cases. (Chart -3)



**Comparison of CSB Stain and CW Stain (Gold Standard)**



**Comparison of CSB Stain and KOH Mount**

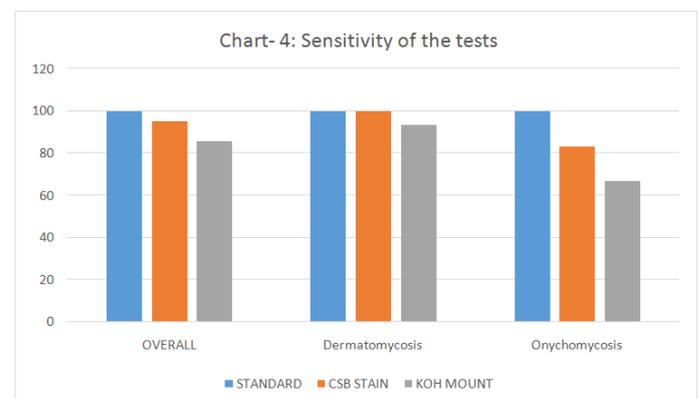
**5) Comparison of Sensitivity:**

Sensitivity =  $\frac{[TP]}{[TP+FN]} * 100$

CSB stain is 100% sensitive in detecting dermatomycosis and 83.4% in detecting Onychomycosis whereas KOH mount is only 93% sensitive in detecting dermatomycosis and 66.7% in Onychomycosis. (Table 2 and Chart 4)

**Table 2. Comparison of sensitivity**

Sensitivity data	CW stain (Gold Standard)	CSB stain	KOH mount
OVERALL	100%	95.23%	85.71%
Dermatomycosis	100%	100%	93.34%
Onychomycosis	100%	83.34%	66.67%



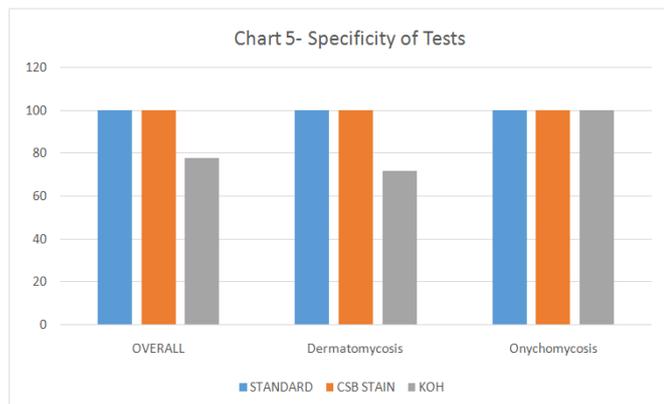
**6) Comparison of Specificity:**

Specificity =  $\frac{[TN]}{[FP+TN]} * 100$

CSB stain 100% specific in detecting dermatomycosis and onychomycosis whereas KOH mount is only 77% specific. (Table 3, Chart 5)

**Table 3. Comparison of specificity**

Specificity data	CW stain (Gold Standard)	CSB stain	KOH mount
OVERALL	100%	100%	77.78%
Dermatomycosis	100%	100%	71.43%
Onychomycosis	100%	100%	100%

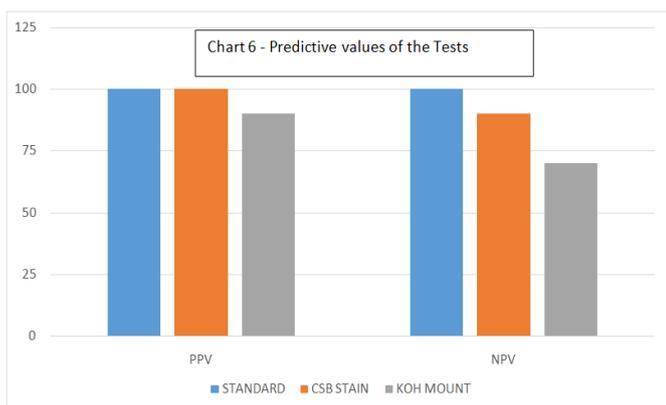


7) Comparison of Positive and Negative Predictive Values (Table 4, Chart 6)

Positive Predictive Value (PPV) =  $\{[TP] / [TP+FP]\} * 100$   
 Negative Predictive Value (NPV) =  $\{[TN] / [TN+FN]\} * 100$

**Table 4. Comparison of Positive and Negative Predictive Values**

Predictive Values	CW Stain (Gold Standard)	CSB STAIN	KOH MOUNT
PPV	100%	100%	90.0%
NPV	100%	90.0%	70.0%



8) Comparison of Efficacy (Table 5, Chart 7)

**Accuracy**, or efficacy =  $(\text{true positives} + \text{true negatives}) / \text{total test results} = (TP+TN) / \text{TOTAL} = (TP+TN+FP+FN)$

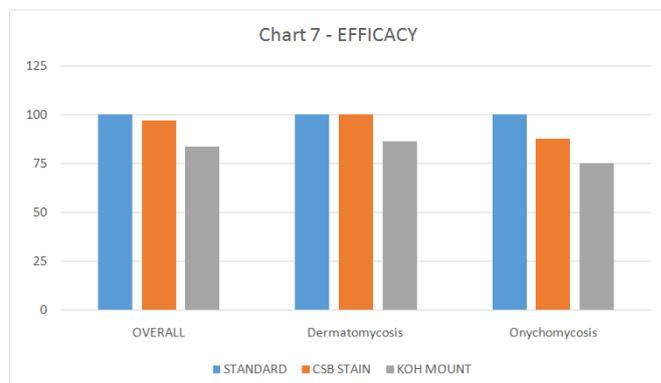
**Table 5. Comparison of efficacy**

EFFICACY	CW Stain (Gold Standard)	CSB STAIN	KOH MOUNT
OVERALL	100%	96.67%	83.34%
Dermatomycosis	100%	100%	86.36%
Onychomycosis	100%	87.5%	75%

9) Cost Effectiveness (Table 6):

**Table 6. Cost Effectiveness of Various tests**

Cost Effectiveness	CSB STAIN	CW STAIN	KOH MOUNT
Cost per 100 Samples	Rs 1200	Rs. 2200	Rs. 150



## DISCUSSION

In the present study, that was conducted on patients with dermatomycosis and onychomycosis for a duration of 2 months was found to have more cases of dermatomycosis than onychomycosis, affecting both males and females of all ages (Mean age=31.5). CSB Stain was compared with KOH using Calcofluor White Stain as Gold Standard in aspects of Sensitivity, Specificity, Positive and Negative Predictive Values, Diagnostic efficacy as well as cost effectiveness. In a study, for detection of onychomycosis, overall sensitivities were 63% for CSB stain and 39% for the KOH wet mount. Overall specificities were 96% and 92%, respectively. Positive and negative predictive values were 96% and 63%, respectively, for CSB stain and 88% and 49%, respectively, for the KOH preparation. (Lim and Lim, 2008) In a study, they compared CSB stain with the KOH wet mount, which was used routinely in their laboratory, and found a low level of agreement between the 2 (Kappa score of 0.352). They opined that the results were not surprising since the KOH wet mount, although used routinely in many laboratories, is actually an imperfect standard. They concluded that Chicago sky blue stain provides a color contrast, making interpretation easy and very affordable. (Fonseka et al., 2011) Lodha et al, compared CSB with KOH for detection of *Pityriasis.versicolor*. Direct microscopy with CSB stain, KOH mount and mycological culture showed positive results in 98 (98%), 92 (92%) and 56 (56%) patients, respectively. (Lodha and Poojary, 2015) In the present study, overall sensitivity of CSB Stain and KOH were found to have 95.23% and 85.71% sensitivity respectively, out of which for Dermatomycosis, 100% and 93.34% respectively and for Onychomycosis, 83.34% and 66.67% respectively. In the aspect of specificity, CSB Stain and KOH were found to have 100% and 77.78% overall specificity respectively, for Dermatomycosis, 100% and 71.43% respectively and for Onychomycosis, both were 100%. The data is comparable with the previous studies. (Lim and Lim, 2008; Fonseka et al., 2011; Tambosis and Lim, 2012; Lodha and Poojary, 2015) Sensitivity and specificity are intrinsic properties of a given test, Higher the values, better is the test, Therefore CSB stain is better than KOH mount in aspects of sensitivity and specificity. Also False negatives were around 10% for KOH mount which is in compliance with previous research data of 5-15% rate. (Karmakar et al., 1995) The Positive and Negative

predictive values are not intrinsic of the test but depend upon the prevalence of the superficial fungal infections in the population. Diagnostic Efficacy of CSB was found to be 96.67% and that of KOH mount was 83.34%. Based on cost effectiveness, the cost per 100 samples for CSB stain, CFW stain and KOH mount are Rs 1200, Rs 2200 and Rs 150 respectively. Though KOH mount is cheaper than CSB stain, its efficacy is lesser than that of CSB stain. CFW stain, despite its efficacy is not very feasible, due to the high cost of the stain and the equipments. The novel stain, CSB stain is best feasible due to its cost-effectiveness, diagnostic efficacy and easy methodology.

### Conclusion

In conclusion, CSB stain was found to be highly efficacious, easy to perform and interpret and cost-effective. The KOH stain requires expertise to interpret, and though it is cheaper, it has high false negative rates. The CSB stain overcomes the disadvantage of KOH stain by making the mount easier to interpret due to the colour contrast. The CSB stain was as sensitive as CW for detection of dermatomycosis. CFW stain is highly efficacious but the drawback is the cost of the fluorescent microscope required making its applicability in a developing country very less. CSB stain being equally efficacious, overcomes the cost burden of the CFW stain.

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