

THE SENSITIVITY AND SPECIFICITY OF CHICAGO SKY BLUE (CSB) DYE IN COMPARISON WITH POTASSIUM HYDROXIDE (KOH) METHOD FOR SUPERFICIAL DERMATOMYCOSIS

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Abstract: *Superficial dermatomycosis is a skin, nail and hair infection caused by fungal pathogen. Based on the pathogen, this infection can be divided into dermatophytosis, pityriasis versicolor and superficial candidiasis. The rapid and proper diagnosis is necessary to determine the initial therapy and prevent the treatment delay. Superficial dermatomycosis diagnosis can be performed using anamnesis, physical examination or supporting investigation. The routine investigation method commonly use Potassium Hydroxide (KOH) because the KOH method is easy to be performed, rapid, simple and affordable. Chicago Sky Blue (CSB) is a dye to give a better color contrast to the fungi so the fungi would be easier to be detected. Objectives of this research is to observe the sensitivity and specificity difference of Chicago Sky Blue (CSB) dye and Potassium Hydroxide (KOH) methods for Superficial Dermatomycosis. The research was performed using cross sectional design analytical obsevation with 30 research subjects. The subjects consist of 15 superficial dermatomycosis patients and 15 non-superficial dermatomycosis patients. The samples were taken from the patients lesion swabs. The samples were checked using KOH and CSB, then observed by the medical analyst. The superficial dermatomycosis samples consist of mostly dermatophytosis (53.33%), then pityriasis versicolor (26.67%) and superficial candidiasis (20%). The sensitivity and specificity of KOH were 86.67% and 100%, respectively. The sensitivity and specificity of CSB were 93.33% and 100%, respectively. The CSB dye method has a higher sensitivity than KOH. The fungal elements are nicely dyed and more easily detected using CSB dye.*

Keywords: KOH, Chicago Sky Blue, sensitivity, specificity, superficial dermatomycosis

INTRODUCTION

Superficial dermatomycosis or superficial mycosis is an infectious disease of the skin, hair and nail caused by pathogenic fungi. This infection is only restricted to the *stratum corneum* layer of skin epidermis. Superficial dermatomycosis can be divided into dermatophytosis, pityriasis versicolor and superficial candidiasis.¹

Dermatophytosis is an infectious superficial mycosis caused by the genus of *Trichophyton*, *Microsporum* and *Epidermophyton*. These fungi can invade keratin-containing tissues, like *stratum corneum*, hair and nail. Pityriasis versicolor is a chronic fungal infection of skin caused by *Malassezia furfur*. Superficial candidiasis is a skin and mucosal infection caused by the genus of *Candida* with the most common species of *Candida albicans*.²

The prevalence of superficial dermatomycosis is estimated to be 20-25% of world population and becomes one of the most common infection in human. Based on Rosida and Evy (2017) research, superficial dermatomycosis had the most number of case in the "Divisi Mikologi URJ Kesehatan Kulit dan Kelamin RSUD Dr. Soetomo Surabaya". The research demonstrated that the number of the case for superficial mycosis in 2011, 2012 and 2013 were 86.6%, 90.1% and 89.2%, respectively.

The proper diagnosis is necessary to determine the initial therapy and to prevent the treatment delay. Superficial dermatomycosis diagnosis can be performed using anamnesis, physical examination or supporting investigation. Several supporting investigations can be performed using microscopic observation, like KOH and Chicago Sky Blue method, fungal culture observation and Wood Lamp observation.

In fact, the investigation using KOH (potassium hydroxide) is a direct observation and the most common method used. Microscopic observation using KOH

10-20% could identify the structure of fungi. This technique is widely used as the initial screening test. The most possible reasons for using this technique is due to the rapidity, easiness, simplicity and affordability of this method.³

The other method used as alternative ways beside KOH observation is Chicago Sky Blue dye method. This dyeing technique could give better color contrast, so the fungal elements would be easily detected by the observants, especially for the beginner observants. The observation using Wood Lamp could only be used to diagnose dermatophytosis and pityriasis versicolor. This technique is not suitable for superficial candidiasis. The observation using fungal culture needs longer time to be interpreted, so this technique is less effective for a rapid interpretation diagnosis.⁴

A diagnostic test should have the sensitivity and specificity value to measure the test validity/accuracy in detecting or diagnosing a disease.⁵ Therefore, this research has aimed to observed the difference of the sensitivity and specificity value between the Chicago Sky Blue (CSB) observation and the potassium hydroxyde (KOH) observation to diagnose the superficial dermatomycosis.

RESEARCH METHODS

This research was performed using cross sectional design analitical observation method and was conducted in "Poliklinik Kulit dan Kelamin RS Siti Khodijah Muhammadiyah Cabang Sepanjang and Klinik dr. Makmuri Surabaya" during September-December 2019. The research population was all newly-admitted patients that were divided into superficial dermatomycosis and non-superficial dermatomycosis groups and met the research criteria. The reseach criteria consisted of several points; (1) The newly-admitted patients in all-ages range and was not receiving antifungal therapy (topical or oral); (2) The patients agreed to be respondents; and (3) The patients were not

in risk of any complications during the time of sampling for the research.

The samples were taken using the purposive sampling method. The number of samples needed for this research were originally 38 patients, yet due to the limitation of research duration (3 months), only 30 subjects that met the patients criteria were taken as samples.

The samples were taken from the patients body parts that were suspected as fungal lesions. The skin and nail area were scrapped off or swabbed, and the hair were plucked. Later, the scrapes and the hair were used as samples. The skin scrapes samples were taken using the superficial skin scrapping technique by scrapping skin or swabbing on the edge of the lesions (restricted to epidermis layer). The hair samples were taken using trichogram technique (hair pluck) by plucking a few strands of hair around the edge of the lesions using tweezers or needle holder. The scrapes and hairs samples were kept in the sample plastics and sent to the laboratory using *ice box*. For the KOH method, the samples were observed using 1 drop of KOH 20%, covered using cover glass and set aside for 10-15 minutes. For the Chicago Blue Sky (CSB) method, the samples were observed using 1 drop of KOH 20% + 1 drop of CSB, covered using cover glass and set aside for 10-30 minutes. The results were checked under the light microscope with 40-100x magnification. The samples revealed the fungal elements such as hyphae/arthroconidia, short hyphae “spaghetti and meatballs”, spores/blastospores and budding yeast were considered as positive samples, otherwise

would be considered as negative. The sensitivity and specificity of the KOH and CSB methods were also determined from the data analysis. The results were documented, recorded on the data sheets, evaluated and concluded.

RESULTS AND DISCUSSION

The research data was obtained from the anamnesis (medical records) and the supporting observation using potassium hydroxide (KOH) and Chicago Sky Blue (CSB) dye. The gender-wise distribution of the respondents resulted that from the total of 30 respondents, 12 patients were male (40%) and 18 patients were female (60%).

Table 1. The age-wise distribution of the respondents

Age (years)	Number(s)	Percentage (%)
11-20	5	16.67
21-30	5	16.67
31-40	10	33.33
41-50	8	26.67
51-60	2	6.66
Total	30	100

Table 1 showed that from the total of 30 respondents, the most number of age distribution were 31-40 years with the amount of 10 people (33.33%), and the lowest number of respondents were 21-30 years respondents with the amount of 2 people (6.66%). The lowest age of respondents was 12 years and the highest age of respondents was 60 years.

Table 2. The predilection sites and itch distribution of the respondents

Clinical Symptom	Predilection Sites	N	%
Itch	Body	8	26.67
	Extremity	11	36.67
	Crease	1	3.33
	Head	2	6.67
	Face	2	6.67
	Body+ extremity	3	10
	Body+crease	1	3.33
	Extremity+ face	1	3.33
	Extremity+ crease	1	3.33
Total		30	100

Table 2 shows the itching symptom was felt by all of the 30 respondents (100%). The highest number of predilection site was located in the extremity (11 respondents; 36.67%), followed by the body (8 respondents; 26.27%).

From The itchy patch colors of the respondents resulted that 17 respondents (56.67%) had the red-colored itchy patch, followed by 13 respondents (43.33%) that were observed to have the white-colored itchy patch.

Table 3. Fungal elements distribution of KOH and CSB observation

Fungal elements	KOH	CSB
Hyphae / Arthroconidia	6 (37.5%)	8 (50%)
Short hyphae/ "Spagettie and Meatballs"	4 (25%)	4 (25%)
Spore / blastospore	3 (18.75%)	2 (12.5%)
Budding yeast cell	3 (18.75%)	2 (12.5%)

Table 3 shows the most fungal elements found in the samples was hyphae/arthroconidia, in KOH observation (37.5%) as well as in CSB observation (50%), followed by short

hyphae/"Spagettie and Meatballs" in KOH observation (25%) as well as in CSB observation (25%), spore/blastospore and budding yeast in KOH observation (18.75%) and CSB observation (12.5%).

Table 4. The respondents examination results of KOH and CSB observation

Variables	KOH	CSB
Dermatophytosis (n=8)		
Positive	6 (75%)	8 (100%)
Negative	2 (25%)	0 (0%)
Pityriasis versicolor (n=4)		
Positive	4 (100%)	4 (100%)
Negative	0 (0%)	0 (0%)
Superficial candidiasis (n=3)		
Positive	3 (100%)	2 (66.67%)
Negative	0 (100%)	1 (33.33%)
Superficial Dermatomycosis (n=15)		
Positive	13 (86.67)	14 (93.33%)
Negative	2 (13.33%)	1 (6.67%)
Non-superficial dermatomycosis (n=15)		

Positive	0 (0%)	0 (0%)
Negative	15 (100%)	15 (100%)

Table 4 showed the number of the case for both superficial dermatomycosis and non-superficial dermatomycosis were each 15 respondents. The superficial dermatomycosis type of case was mostly

dermatophytosis (8 respondents; 53.33%), followed by pityriasis versicolor (4 respondents; 26.67%) and candidiasis (3 respondents; 20%).

Table 5. The sensitivity and specificity of KOH observation

Observation	Superficial dermatomycosis (+)	Superficial dermatomycosis (-)	Total
KOH (+)	13	0	13
KOH (-)	2	15	17
Total	15	15	30

$$\text{Sensitivity} = \frac{13}{15} \times 100\% = 86.67\%$$

$$\text{Specificity} = \frac{15}{15} \times 100\% = 100\%$$

Table 5 showed the positive and negative results of the samples from the superficial and non-superficial dermatomycosis cases using KOH

observation. The sensitivity and specificity were calculated to be 86.67% and 100%, respectively.

Table 6. The sensitivity and specificity of CSB observation

Observation	Superficial dermatomycosis (+)	Superficial dermatomycosis (-)	Total
CSB (+)	14	0	13
CSB (-)	1	15	17
Total	15	15	30

$$\text{Sensitivity} = \frac{14}{15} \times 100\% = 93.33\%$$

$$\text{Specificity} = \frac{15}{15} \times 100\% = 100\%$$

Table 6 showed the positive and negative results of the samples from the superficial and non-superficial dermatomycosis cases using CSB observation. The sensitivity and specificity were calculated to be 93.33% and 100%, respectively.

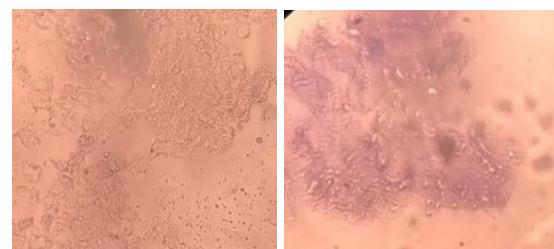


Figure 1. The observation of dermatophytosis using KOH (A) and CSB (B)

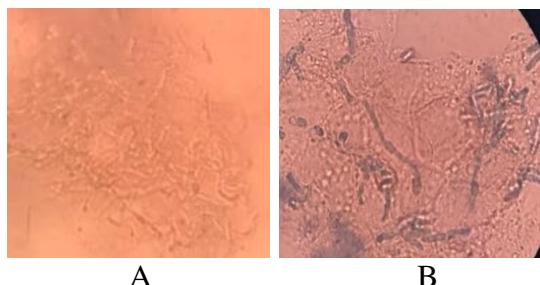


Figure 2. The observation of pityriasis versi color using KOH (A) and CSB (B)

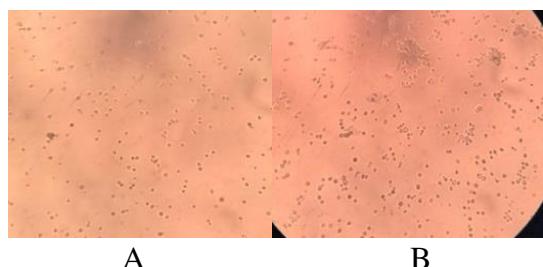


Fig 3. The observation of superficial candidiasis using KOH (A) and CSB (B)

The early diagnosis for superficial dermatomycosis could be performed using anamnesis, physical examination and supported by the direct observation of the samples. The most common method for routine observation is using potassium hydroxide 20% solution (KOH 20%). Beside KOH, the direct observation of the samples could be performed using another reagents, such as Chicago Blue Sky (CSB). CSB is the combination of KOH solution and diazo dye.⁶

In this research, 30 patients met the inclusion and exclusion criteria, thus became the respondents of this research. It could be seen from the gender-wise distribution of the respondents that the female respondents were in higher number than the male respondents. The gender-wise incidence were varied in several countries. This fact was proved by Kakande (2019) research. The research showed that the male subjects prevalence in suffering a dermatomycosis infection was higher than the female subjects.⁷ On the other hand, the research by Citrashanty et al. (2011) that

was conducted in “Divisi Mikologi URJ Penyakit Kulit dan Kelamin RSUD Dr. Soetomo Surabaya” showed that the female subject distribution was higher than the male subjects. It was proved that the gender-wise distribution for superficial dermatomycosis patients was varied.⁸

Table 1 showed the highest age-wise distribution in this research was 31-40 years range and followed by 41-50 years range. These following age range are categorized as adult and productive age. In these age range, there are several predisposition factors such as activities, that cause the excretion of sweat, wet or damp condition, and trauma. These following factors could enhance the risk of suffering from superficial dermatomycosis in a higher trend compared to the other age range. The superficial dermatomycosis type of case was mostly dermatophytosis, followed by pityriasis versi color and superficial candidiasis (Table 5).

In dermatophytosis, the fungal elements found were hyphae/arthroconidia in both KOH and CSB methods. Table 6 showed the positive result of KOH was 75%. This number was lower than CSB method that was calculated to be 100% and gave the blue color on the fungal elements. Fig 1A demonstrated the appearance of fungal elements in KOH to be more transparent, thus it was more difficult to differentiate the fungal elements, the background and the artefacts around the fungal elements. This could be the possible factor to get 2 negative results from KOH observation. Meanwhile, using CSB (Fig. 2B), the fungal elements were dyed in purple on the pink background, so the fungal elements could be seen clearly. On the other hand, the research by Noviandini et al (2017) showed that the fungal elements were dyed in blue while using CSB method. The different concentration of CSB and the different light intensity of the microscope could be the factors of the different results in fungal elements coloration.⁴

In pityriasis versicolor, the fungal elements found were short hyphae or “spaghetti and meatballs”, in both CSB and KOH method. The observation using KOH and CSB gave 100% positive result (Table 6). Yet, the CSB dye gave a clearer color contrast in blue (Fig. 2B), so the fungal elements were easily detected. Meanwhile, the KOH method only gave the transparent appearance of the fungal elements (Fig. 2A).

In superficial candidiasis (Table 6), the observation using KOH gave 100 % positive results, meanwhile the CSB observation only gave 67.67% positive result. The negative result in CSB method was due to the lack of clinical sample (scrape), so the fungal elements were dissolved in reagents. It could be also caused by several *Candida* species that were slow in absorbing CSB dye. Fig. 3 demonstrated the fungal elements that appear in transparent color, in both KOH and CSB methods. Lim and Lim (2008) stated that *Candida* fungi within 20 minutes of CSB dyeing was not good in absorbing the color in comparison with dermatophytes.⁹ Yet, after one day *Candida* species could absorb the color well. Another research that support the fact of CSB unsuitability to diagnose superficial candidiasis was also stated by Noviandini et al (2017). The research by Noviandini et al (2017) showed that all candidiasis samples appeared as transparent-colored fungal elements under the microscope. It might due to the slower CSB dye absorption for several species. Thus, the candidiasis case would take longer time for the CSB observation compared to dermatophytosis and pityriasis versicolor.⁴

The KOH observation result from 10-20 minutes test of 30 respondents, 15 superficial dermatomycosis patients and 15 non-superficial dermatomycosis patients, showed the sensitivity and specificity of 86.67% and 100%, respectively. Meanwhile, the CSB observation showed the sensitivity of 93.33% and specificity of 100%. The results showed that CSB had a

higher sensitivity than KOH, but both CSB and KOH had the same specificity of 100%. The research by Afshar (2018), demonstrated that the CSB dyeing method also had a higher sensitivity (97%) and specificity (100%) compared to KOH method sensitivity (66%) and specificity (98%). The other research by Tambosis (2012) also gave the similar result with the higher sensitivity of CSB method (78%) compared to KOH method (48%). Meanwhile, the specificity of both methods were equal (96%).

CONCLUSION

The sensitivity and specificity of CSB observation were 93.33% and 100%, respectively. The sensitivity and specificity of KOH observation were 86.67% and 100%, respectively. It could be concluded from this research that the Chicago Sky Blue has a high sensitivity and specificity for diagnosing superficial dermatomycosis. Thus, it is expected that Chicago Sky Blue (CSB) could be used as the alternative tool for superficial dermatomycosis diagnosis in the future.

REFERENCES

1. Kelly, J. B. and Makkar, H. S. (2012) ‘Ethics in pediatric dermatology’, *Clinics in Dermatology*, 30(5), pp. 471–475. doi: 10.1016/j.clindermatol.2011.06.014.
2. Rosida, Fatma dan Evy Ervianti. 2017. *Penelitian Retrospektif: Mikosis Superfisialis*. Berkala Ilmu Kesehatan Kulit dan Kelamin-Periodical of Dermatology and Venereology. Vol 29, No. 2, p. 117-122.
3. Verma S, Heffernan MP. *Superficial Fungal Infection: Dermatophytosis, Onychomycosis, tinea nigra, piedra*. Dalam: Wolff K, Goldsmith LA, Katz SI, etc. 2008. *Dermatology in general medicine*. New York; McGraw Hill

4. Noviandini, Anggraeni, *et al.* 2017. *Pemeriksaan Pewarnaan Kalium Hidroksida (KOH) 20% + Tinta ParkerTMBlue-Black, Chicago Sky Blue (CSB), dan Kultur Jamur pada Dermatomikosis Superfisialis*. Berkala Ilmu Kesehatan Kulit dan Kelamin - Periodical of Dermatology and Venereology, Vol. 29, No.1, p. 21-29.
5. Morton, R. and Hebel, J. R. (1984) *study guide to epidemiology and biostatistics*. 2nd Ed. New York: University Park Press.
6. Afsharl, Parvaneh., Lale Vahedi., Hamed Rouhanizadeh. 2018. *A comparison of conventional rapid methods in diagnosis of superficial and cutaneous mycoses based on KOH, Chicago sky blue 6B and calcofluor white stains*. Iran J Microbiol. Vol. 10, No. 6, p. 433-440
7. Kakande, T. *et al.* (2019) ‘Prevalence of Dermatophytosis and Antifungal Activity of Ethanolic Crude Leaf Extract of Tetradenia riparia against Dermatophytes Isolated from Patients Attending Kampala International University Teaching Hospital, Uganda’, *Dermatology Research and Practice*, 2019. doi: <https://doi.org/10.1155/2019/9328621>
8. Citrashanty, Irmadita dan Sunarso Suyoso. 2011. *Mikosis Superfisialis di Divisi Mikologi Unit Rawat Jalan Kulit dan Kelamin RSUD Dr. Soetomo Surabaya Periode Tahun 2008–2010 (Superficial Mycosis in Mycology Division – Out Patient Clinic of Dermatovenereology Dr. Soetomo General Hospital Surabaya in 2008–2010)*. Berkala Ilmu Kesehatan Kulit & Kelamin. Vol. 23, No. 3, p. 200-205.
9. Lim, Christopher Seng-Hong, *et al.* 2008. *New Contrast Stain for the Rapid Diagnosis of Dermatophytic and Candidal Dermatomycoses*. Arch Dermatol. Vol. 144, No. 9.