

## New Contrast Stain for the Rapid Diagnosis of Dermatophytosis and Pityriasis Versicolor

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DOI: 10.1309/LM1BSUJ6LM2QPAAE

**Abstract**

**Objective:** To compare the efficacy of Chicago sky blue (CSB) stain with the routine potassium hydroxide (KOH) wet mount in the diagnosis of dermatophytosis and pityriasis versicolor.

**Methods:** Duplicate skin scrapings from patients with a clinical diagnosis of dermatophyte infection and pityriasis versicolor were examined with the KOH wet mount and the CSB stain.

**Results:** Thirty-six patients had dermatophyte infections, and 13 patients had pityriasis versicolor. After 30 minutes, 13 (36%) of 36 dermatophyte slides and 2 (15%) of 13 pityriasis versicolor slides became positive with 20% KOH. The corresponding data for CSB stain were 23 (64%) of 36 dermatophyte slides and 10 (77%) of 13 pityriasis versicolor slides.

**Conclusion:** Chicago sky blue stain is superior to the KOH wet mount for the diagnosis of

dermatophyte infection and pityriasis versicolor and is equally inexpensive. We recommend reading negative dermatophyte slides again on Day 2, but a 30-minute reading is adequate for pityriasis versicolor.

**Keywords:** tinea, dermatophytes, pityriasis versicolor, microbiology, microscopy

**T**he diagnosis of superficial mycoses, such as dermatophytosis and pityriasis versicolor, is often done clinically with clinicians requesting laboratory confirmation in more difficult cases. Current techniques to diagnose superficial mycoses include direct microscopic examination, culture, and molecular diagnosis.<sup>1</sup> Direct microscopic examination of samples from the affected area using potassium hydroxide (KOH) wet mounts is the most widely-used laboratory method. Although rapid, the KOH wet mount lacks a color contrast and requires considerable skill to interpret. In busy clinics, it is useful to have a rapid, reliable, and easy to interpret diagnostic method. Chicago sky blue (CSB) stain is a new contrast stain that has shown promise as a rapid and reliable diagnostic method for dermatomycoses.<sup>2,3</sup> It contains 1% CSB 6B and is used together with KOH as the clearing agent.

**Materials and Methods**

The study population was composed of male and female patients who were visiting the dermatology outpatient department of the Teaching Hospital Kandy, Sri Lanka. Patients diagnosed clinically by the consultant dermatologist to have dermatophytosis or pityriasis versicolor were included in the study. Exclusion criteria included atypical cases and those previously treated. Skin scrapings and hair or nail clippings composed of the distal free edge of the nail plate, including attached subungual debris, were obtained from the patients. Areas of skin to be scraped were first cleaned with an alcohol swab to remove traces of cream and surface bacteria. Adequate amounts of scales were taken with the blunt side of gauge 15 surgical blades and placed on 2 clean microscope slides. One drop of 20% KOH was added to 1 slide, and 1 drop each of CSB stain (K B Lim Skin Clinic Pte, Singapore) and 20% KOH was added to the other and then covered with a cover slip. All slides were placed in a humidifying chamber

(covered plastic container lined with moist paper towels) for 30 minutes. Slides were examined at ×10 magnification to locate fungal elements and at ×40 magnification to identify fungal spores and hyphae. *Malassezia furfur* was confirmed by the presence of short, blue-staining hyphae and spherical spores. Dermatophytes were detected at ×10 magnification as blue-staining fungal hyphae against a purple to pink cellular background and confirmed at ×40 magnification on the visualization of septate filaments. All negative slides were returned to the humidifying chamber and re-examined the following day. Two investigators (Fonseka and Bandara) read the slides and recorded their findings separately. If their findings were different, both investigators corroborated to make the final diagnosis. Cohen’s Kappa test was used to assess the agreement between the KOH wet mount and CSB stain.

**Results**

We examined slides from 49 patients. We were able to detect 10 more cases of dermatophytosis and 8 more cases of pityriasis versicolor with CSB stain than with the KOH wet mount at the 30-minute examination (Table 1). Thirteen (36%) of the 36 dermatophyte slides and 2 (15%) of the 13 pityriasis versicolor slides were positive with 20% KOH. The corresponding data for CSB stain were 23 (64%) of 36 dermatophyte slides and 10 (77%) of 13 pityriasis versicolor slides. The Kappa score was 0.352, indicating a low level of agreement between the 2 tests (Table 2).

All negative slides were reexamined for fungal elements on the second day. Out of 34 negative KOH slides, 3 became positive on the second day; whereas out of 16 negative CSB slides, 7 became positive on Day 2. All the slides that became positive on Day 2 were dermatophytes. If the 30-minute and Day 2 readings were combined, the CSB stain was able to detect 14 more cases of dermatophytosis than the KOH wet mount. No additional cases of pityriasis versicolor were detected on Day 2 using either method. While examining dermatophyte slides, blue-staining fungal hyphae were seen clearly against a pink cellular background (Image 1) even at a low magnification, and it was easy to locate them using the CSB stain, which was not the case with the KOH wet mount. *Malassezia furfur* was particularly easy to identify with the CSB stain at 30 minutes (Image 2). Chicago sky blue stain confirmed the clinical diagnosis in 10 (76%) of 13 cases, whereas the KOH wet mount confirmed the clinical diagnosis in only 2 (15%) cases.

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**Abbreviations**

CSB, Chicago sky blue; KOH, potassium hydroxide; PAS, periodic acid-Schiff; PCR, polymerase chain reaction

**Discussion**

Immediate diagnosis of superficial mycoses is desirable since specific treatment can be initiated without delay. Fungal

culture takes too long and is impractical for this purpose. The KOH wet mount using brightfield microscopy is quick, inexpensive, and routinely used for the immediate diagnosis of fungal infections. However, it does not produce a color contrast and requires some skill to interpret. Parker's ink has been added to KOH to provide a contrast, but this stain works better for *M. furfur* and yeasts rather than for dermatophytes.<sup>4</sup> Skin surface biopsies followed by periodic acid schiff (PAS) or Gram's stain<sup>5</sup> are more complicated, frequently painful, and impractical for obtaining samples from interdigital webs. Chlorazol black E in KOH, while specific for chitin,<sup>6</sup> is a potential carcinogen.<sup>7</sup> Calcofluor white with KOH is specific and sensitive,<sup>8</sup> but it requires a fluorescent microscope.

Molecular techniques using DNA probes, *in situ* hybridization, and polymerase chain reaction (PCR) are still being evaluated in several laboratories.<sup>1,9</sup> These techniques have been shown to have high degrees of sensitivity and specificity. However, they are technically demanding, require up to 48 hours to complete, and will not be available in third-world laboratories for the foreseeable future. In this situation, the availability of a rapid, reliable, and cheap staining method is invaluable.

Chicago sky blue stain and the KOH wet mount were both able to detect all cases of pityriasis versicolor at the 30-minute examination with no additional cases detected on Day 2. This suggests a 30-minute examination is adequate for detecting *M. furfur*. However, CSB stain detected 8 more cases of pityriasis versicolor than the KOH wet mount.

**Table 1\_Results at 30 Minutes**

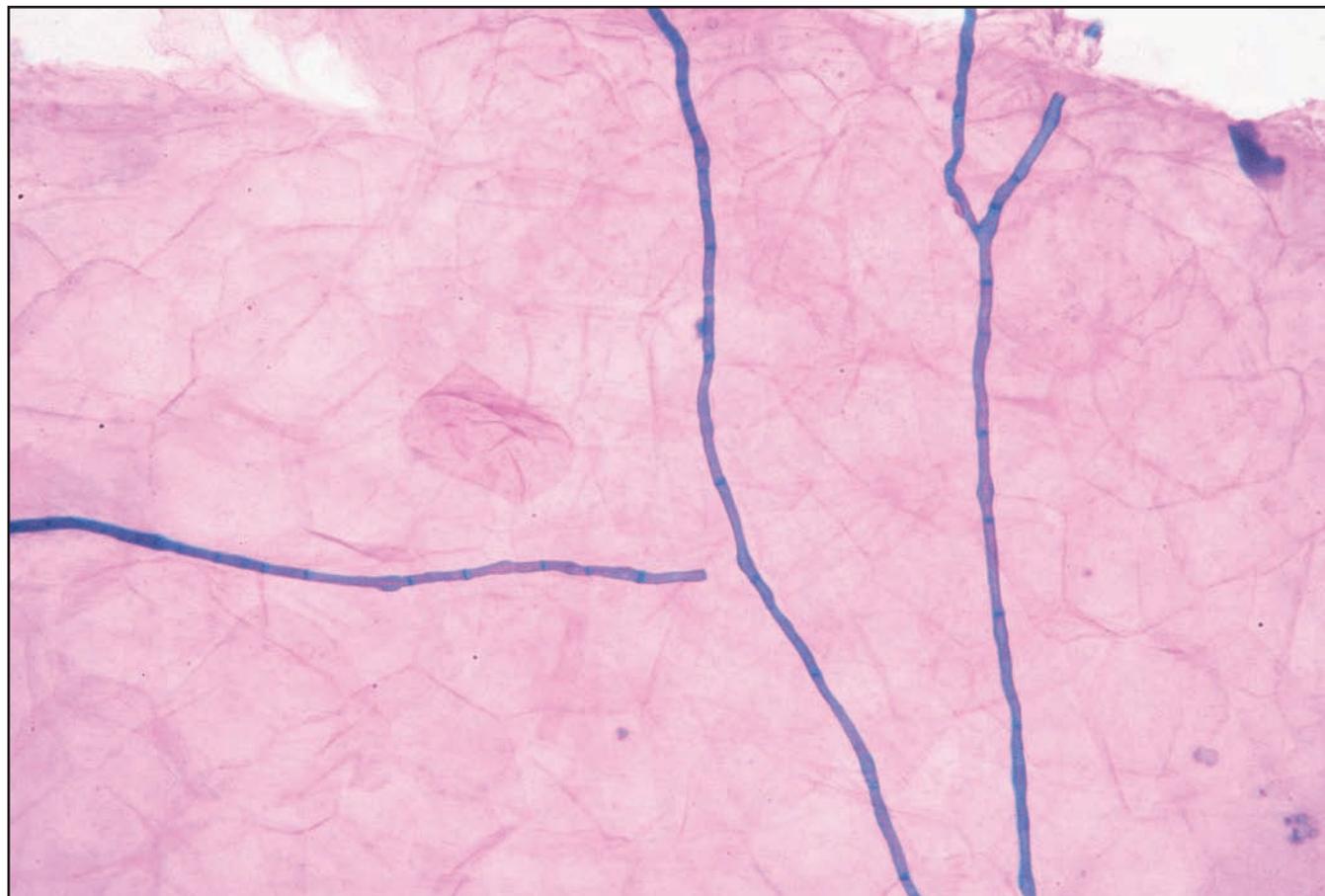
Diagnosis	KOH Wet Mount		CSB Stain	
	Positive	Negative	Positive	Negative
Dermatophyte	13	23	23	13
Pityriasis versicolor	2	11	10	3

**Table 2\_Agreement Between CSB Stain and KOH Wet Mount**

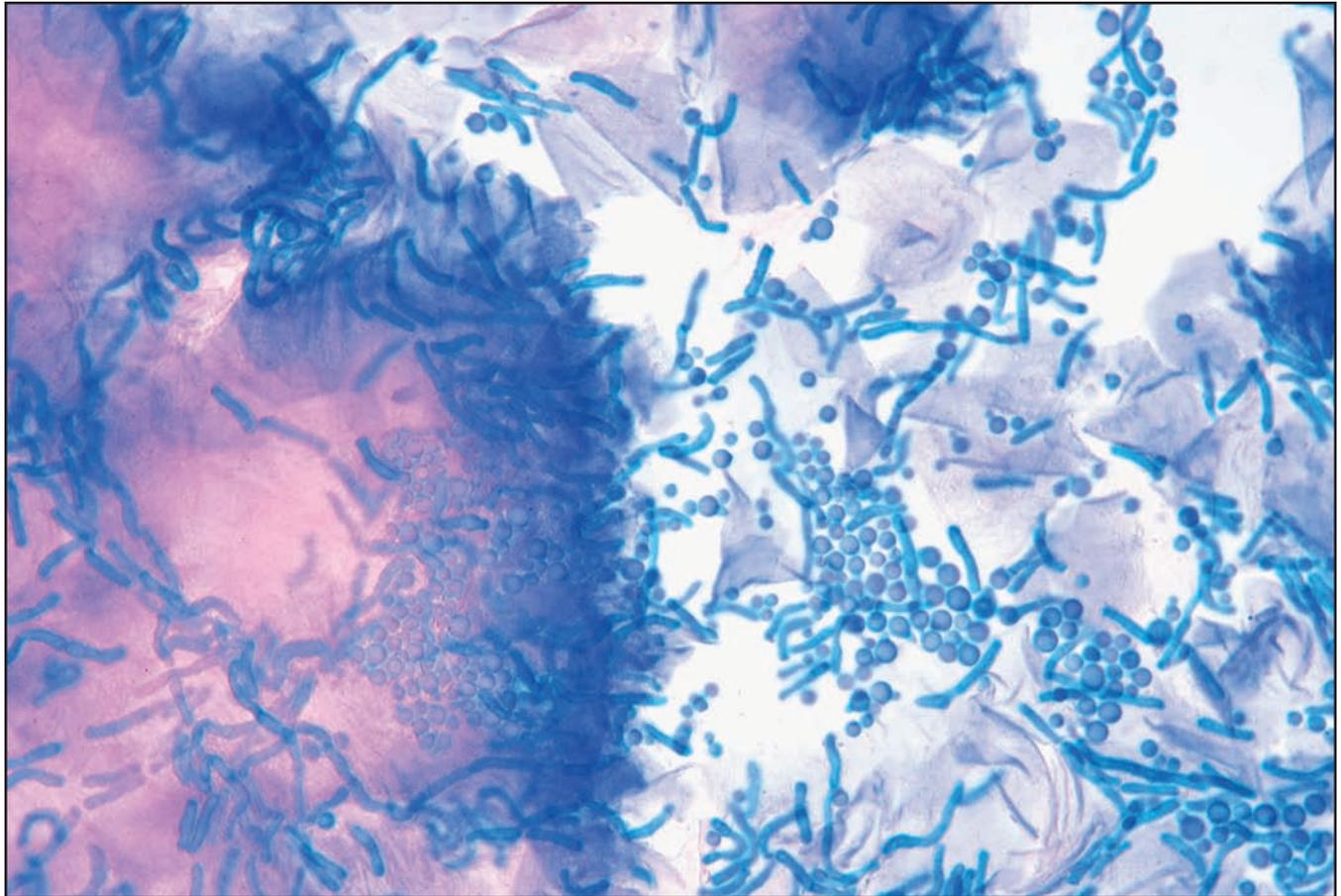
CSB Stain	KOH Wet Mount	Number of Pairs
+	+	15
+	-	18
-	+	0
-	-	16

*Kappa score=0.352.*

Ten more cases of dermatophytosis were detected with the CSB stain than the KOH wet mount at 30 minutes. If Day 2 and 30-minute readings were combined, CSB stain detected 14 more cases of dermatophytosis than the KOH wet mount. These results suggest CSB is superior to the KOH wet



**Image 1\_Dermatophytes as blue-staining fungal hyphae against a purple to pink cellular background (original magnification ×400).**



**Image 2** Short blue-staining hyphae and spherical spores of *Malassezia furfur* (original magnification  $\times 400$ ).

mount. We suspect some dermatophyte species may take up the CSB stain more slowly; hence, it is prudent to subject all negative dermatophyte slides to a second reading on Day 2, as was done in this study. Kah-Beng Lim, who formulated CSB stain, suggests lowering the condenser to look for refractile filaments in the same way as the KOH wet mount if investigators wished to avoid reading the slide again on Day 2.

In this study we compared CSB stain with the KOH wet mount, which are used routinely in our laboratory, and found a low level of agreement between the 2 (Kappa score of 0.352). This is not surprising since the KOH wet mount, although used routinely in many laboratories, is actually an imperfect standard. Chicago sky blue stain provides a color contrast, making interpretation easy and very affordable. A 12 cc bottle of CSB stain costs \$25 and is sufficient for 200 tests. We believe the new contrast CSB stain has the potential to replace the KOH wet mount as the routine method for the rapid diagnosis of superficial mycoses.  $\mu\text{M}$

**Acknowledgement:** We would like to thank Kah-Beng Lim, MRCP (U.K.), K B Lim Skin Clinic Pte, Singapore, for providing us with the CSB stain.

**Author contributions:** Sanjeevani Fonseka, MBBS, MD and Upendra N. Bandara, MRUN, MBBS, MD contributed to the acquisition, analysis, and interpretation of the data.

All 4 authors contributed equally to the study concept and design, drafting of manuscript, and critical revision of the manuscript for important intellectual content.

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