

## Challenge 1001KOH A and B

January 2010

Slide for direct microscopic examination: A) **POSITIVE** B) **POSITIVE**

### CMPT QA

These samples were verified by a reference laboratory. The laboratory used Calcofluor White method and both samples were positive.

### SURVEY RESULTS

All participants reported the slides as positive. Seven participants used Calcofluor White method; three laboratories reported using both Calcofluor White and KOH methods while one participant used only KOH method for the examination of the slides.

### METHODS

Issues of personal preference and the availability of fluorescent microscopy appear to prevail as to the choice of Potassium hydroxide (KOH) or Calcofluor White preparations.

#### KOH preparation

KOH solution (a strong base; NaOH may also be used) dissolves skin tissue cells and keratinized material allowing the fungal elements to be seen.

#### Methodology

Skin scrapings are placed in 1 or 2 drops of 10% KOH on a clean glass microscope slide, then a cover slip is placed on top.

To facilitate clearing of thick or viscous specimens it may be necessary to let the slides stand for up to 30 minutes or gently heat (but not boil) the mixture.

**Table-1:** Results – 1001 KOH challenge

| Report A / B       | No of labs | %          |
|--------------------|------------|------------|
| Positive /Positive | 11         | 100        |
| <b>Total</b>       | <b>11</b>  | <b>100</b> |

KOH preparations are not permanent, but the addition of 10% glycerol to the KOH, helps to preserve the preparation for several days<sup>1</sup>.

All samples should be examined under low power, and the findings confirmed under high power <sup>2</sup>.

Cottons swabs should not be used to prepare the slides because cotton strands may resemble hyphae<sup>1</sup>.

#### Calcofluor White

It is a fluorescent brightener that binds to  $\beta$ -1-3 and 1-4 polysaccharides, such as cellulose and chitin; when exposed to long-wave UV light these polysaccharides will fluoresce.

While the fluorescent stain allows for rapid detection, this does need to be counter-balanced against the cost, and intermittent difficulties of background fluorescence.

A drop of Calcofluor White (0.1% solution) may be added directly to the KOH drop on the slide <sup>2</sup>.

### REFERENCES

1. Larone DH. 2002. p. 296-298. *Medically important fungi A Guide to Identification*. 4<sup>th</sup> ed. ASM Press, Washington, D.C.
2. Summerbell RC. 2003. pp. 1798-1819. *Trichophyton, Microsporium, Epidermophyton, and agents of superficial mycoses*. In PR Murray PR et al. (eds.) *Manual of Clinical Microbiology*. 8<sup>th</sup> ed. Vol. 2. Ch. 119. ASM Press, Washington, DC.
3. Abdelrahman T, Letscher V Bru, J. Waller, G. Noacco and E. Candolfi. Dermatomycosis: comparison of the performance of calcofluor and potassium hydroxide 30% for the direct examination of skin scrapings and nails. 2006 J. Med. Mycol. Vol 16(2):87-91

### KOH vs Calcofluor White

According to a study by Abdelrahman et al (2006) that compared the performance of potassium hydroxide 30% (KOH) with slight heating and staining with 0.01% calcofluor for the diagnosis of dermatomycosis, the sensitivity of direct microscopy was significantly higher with calcofluor than with KOH (respectively, 88% and 72%,  $P = 0.0116$ ). Specificity was 93% with calcofluor and 88% with KOH ( $P = NS$ ). Calcofluor showed higher specificity on skin scraping than on nails samples (respectively, 100% and 89%,  $P = 0.0138$ ) while KOH specificity did not differ as a function of the type of specimen (respectively, 92% and 86%)<sup>3</sup>.