

Slide for direct microscopic examination of fungal elements

0709: positive

CMPT QA: 0709: KOH positive. The slides were validated by random lot sample.

Reference Laboratory: 0709A: Calcofluor White positive

Results received are shown in Table 1

Table 1. 0709 KOH Results received.	
Report	No.
KOH Positive	5
No report	1

KOH preparations ¹ A drop of 10% potassium hydroxide (KOH) is mixed with a small quantity of the specimen placed on a clean microscope slide, then covered with a coverslip. The proteinaceous components of the host cells become partially digested (cleared) by the KOH, leaving the fungal cell wall intact and easier to observe. The mixture on the slide should be gently heated (but not boiled). For thick or viscous specimens it may be necessary to let the slides stand for up to 30 minutes to facilitate clearing. A slide warmer set at 51-54°C for 1 hour may also be used to heat the slides. The clearing time depends on the thickness of the sample, the concentration of the hydroxide used, and the amount of heat (if any) applied.

The slide must be carefully examined microscopically. Precaution: Cotton strands or fibres may resemble hyphae.

KOH preparations are not permanent and the KOH will eventually destroy the fungi; however, a small drop of glycerol added to the preparation will preserve it for several days.

Calcofluor White (and related compounds such as Blankophor and Uvitex 2B ²) binds to β 1,3 and β 1,4-polysaccharides, such as cellulose and chitin (present in fungal cell walls), which fluoresces when exposed to long-wave UV light. Calcofluor White (which is considered a stain) may be used with or without KOH ^{1,3}.

Calcofluor White is useful for direct microscopic examination of samples and for exhibiting certain morphologic structures of fungi grown in culture. While the fluorescent stain allows for rapid detection, this does need to be counter balanced against the cost, and intermittent difficulties of background fluorescence. The required light source is a mercury vapor lamp. Quartz halogen bulbs are usually not suitable, due to low energy output¹.

Precautions ^{1,2}: Cotton fibres will fluoresce strongly and may be confused as fungal hyphae. Brain tumour biopsy specimens may fluoresce and resemble *Aspergillus* hyphae.

Studies comparing Calcofluor White and KOH with a variety of sample materials have not demonstrated a significant difference in the sensitivity or specificity with either technique. Issues of personal preference and the availability of fluorescent microscopy appear to prevail.

REFERENCES

1. Larone DH. 2002. p. 298, 317. *Medically important fungi A Guide to Identification*. 4th ed. ASM Press, Washington, D.C.
2. LaRocco MT. 2003 Reagents, stains, and media: Mycology. pp. 1686-1692. In PR Murray et al. (eds.) *Manual of Clinical Microbiology*. 8th ed. Vol. 2. Ch. 119. ASM Press, Washington, DC.
3. Haldane DJ, Robart E. 1990. A comparison of calcofluor white, potassium hydroxide, and culture for the laboratory diagnosis of superficial fungal infection. *Diagn Microbiol Infect Dis*. 13(4):337-9.