



### 0609 Skin scraping

Slide for direct microscopic examination: **positive** for fungal elements

**CMPT QA:** 0609 KOH examination: positive for fungal elements. The slides were validated by random lot sampling.

**Reference Laboratory:** 0609: Calcofluor White positive for fungal elements.

Table 1 lists the results received.

Positive for fungal elements	2
Indeterminate	1
Negative	2
No report received	1

**SPECIMEN COLLECTION** Disinfect the patient's skin with 70% alcohol prior to sample collection. If a characteristic dermatophyte "ring" is present on the skin, collect scrapings from the outer margin of the ring at its junction with the normal skin. Otherwise, collect samples from areas where the skin appears to be scaling. The skin scrapings may be placed in a sterile Petri dish or in clean paper envelopes; avoid using closed tubes as retained moisture may result in overgrowth of contaminants<sup>1,2</sup>. Transport kits that have black paper (so the scrapings may be easily visualized) are recommended. Ideally, samples should be delivered to the laboratory within 2 hours of collection as fungal viability may decrease with prolonged specimen storage. Samples may be stored at 4°C if bacterial contamination is likely. Commercially available transport kits are available for sending samples in the mail<sup>2</sup>.

**PROCESSING** The **direct microscopic examination** of samples submitted for fungal culture helps to provide the physician with early information regarding the presence of fungi and in providing early information that may be crucial for determining appropriate therapy for the patient.

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.

The high concentration of cellular chitin in the fungal elements is resistant to the denaturation effects of KOH. Chitin is a polysaccharide giant molecule consisting of smaller molecules of sugar strung together. Chitin means "*tunic*" in Greek and offers fungi structural and defensive functions.

**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. The clearing time depends on the thickness of the sample, the concentration of the hydroxide used, and the amount of heat (if any) applied. 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. A slide warmer set at 51-54°C for 1 hour may also be used to heat the slides. Dimethyl sulfoxide (DMSO), may be added to the KOH solution to accelerate the process in the absence of heat. 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>.

Observe the KOH preparation by brightfield microscopy. The illumination on a brightfield microscope should be carefully adjusted using the Kohler method. **Hyaline fungi will be difficult to see if the illumination is improperly adjusted.** Fungi have definite cell walls. 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** is useful for direct microscopic examination of samples and for exhibiting certain morphologic structures of fungi grown in culture. 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.