MYCETFLUO

FLUORESCENT REAGENT FOR MYCETES STAINING

INTENDED USE
MYCETFLUO is a fluorescent reagent able to stain fungal elements for direct microscopic examination of sample.

PRINCIPLE
Cutaneous mycoses are caused by yeasts or filamentous fungi (dermatophytes) which have an affinity for keratin. Lesions are confined to the superficial layer of the epidermis, hair, and nails.
Laboratory diagnosis of mycoses is based on direct examination and on sample culture of skin, hair or nails on Sabouraud medium.
Direct examination requires the use of reagents such as KOH, chlorolactophenol or detergent. The use of stains such as Chlorazole Black, Congo red or calcofluor is required as microscopic observation of fungal material is difficult in unstained preparations.
MYCETFLUO is dissociating agent and calcofluor based reagent. MYCETFLUO is able to dissociate components of samples of skin hair and nails. This reagent also stains mycelium filaments or spores so they can be detected with a fluorescent microscope.

KIT CONTENT
- 1 vial of dissociating solution
- 4 vials of lyophilized stain A
- 2 vial of diluter B
- 125 disposable sticks
- 4 blacks cards
- Package insert

MATERIAL REQUIRED BUT NOT PROVIDED
- Micropipette or other equipment delivering 25 µL and 50 µL
- Microscope Slides (76x26 mm)
- Coverslip
- Fluorescent Microscope (objective 10-40x)
- Container for contaminated waste

STORAGE CONDITIONS
Ready to use.
Store in the darkness at + 12°C to +37°C, reagents are stable until the expiry date indicated on the box.
Do not freeze. Do not expose the reagent to strong light. Keep the reagent below 37°C.
After reconstitution, stain A is stable for 8 weeks at room temperature and in the dark.

SAMPLES COLLECTION / HANDLING / STORAGE
For lesions on glabrous skin e.g. in the case of ringworm, take a sample from the peripheral lesion with a dermal curette. For nails, scrape or cut the affected part. In the case of tinea collect suspected hair.
Smalls samples or nail dust are best for direct examination, in particular pieces of nail. Air bubbles may appear with thick nail samples. Using thin samples will avoid air bubbles which would interfere with observation and coloration.
A curette is useful for scraping and collecting scale or thin strip nail

WARNINGS AND PRECAUTIONS
- For in vitro diagnostic use.
- Only for professional use.
- Do not interchange reagents from different kit lots.
- Follow the instruction for use.
- The sticks provided in the kit are single use
- In case of accidental spill of reagent clean the surface with absorbent paper, bleach and rinse with water. In case of environmental contamination with culture samples clean with bleach and absorbent paper.
- Avoid any contact of reagent with skin, eyes, and mucous membranes. Do not ingest.
- The samples, reagents as well as the contaminated materials and products must be eliminated in a container for contaminated waste, according to the prevailing recommendations and regulations.
- The diluter B and the dissociating solution contain < 0.09% sodium azide which may react with lead and copper plumbing to form explosive metal azides. Azide built-up can be avoided by flushing with large volumes of water following the disposal of reagents.

TEST PROCEDURE
a. Reconstitute the stain A by adding 650 µL of diluter B in the lyophilized stain A bottle. Incubate for 10 to 15 minutes at room temperature and shake gently. A limpid solution is obtained and store in the darkness at + 12°C to +37°C.
b. Put a microscope slide on a black card.
c. Place hair, scale or nail sample on the slide
d. Add 25 µL of dissociating solution. Tap on sample with a stick, to ensure that the sample is completely immerse in the dissociating solution.
e. After 15 or 30 minutes, big sample may need to be dissociating again with stick. Partial or complete dehydration of sample preparation may be observed, it's not disturbing the coloration.
f. Add 40 µL of stain A, homogenize all (sample + dissociating solution + stain A) and cover with a coverslip.
g. Incubate for 15 minutes at room temperature and observe the slide under a fluorescent microscope (objective 10 to 40).
h. Calcofluor-stained sections were observed with a microscope, equipped with a filter (excitation 330–380nm, emission >420 nm). Microscopic observation of fungal element appears blue. However green fluorescence may be observed from microscope with fluorescein filter system. To check that the microscope is compatible with calcofluor observation, it is advisable to test the reagent with yeasts or filamentous fungi (dermatophytes) sample from culture.

NOTES
a. Observation of the preparation may be put off for up to 24 hours.
b. In case of dry mounting or detachment of the coverslip, add progressively up to 50 µL of diluter B. To avoid air bubbles, add diluter B at the edge of the coverslip.

INTERPRETATION OF THE RESULTS
Samples are positive when fungal elements (mycelium filament, spores…) appear fluorescent (green or blue fluorescence according to the filter used)
Some fibres of vegetable origin which may be present in samples might also be colored by MYCETFLUO. These fibres can be distinguished from fungal elements by their being irregular in shape, lacking hyphal septa and being larger than fungal filaments.

PERFORMANCES
The results of a comparative study carried out of 50 samples treated with MYCETFLUO or Chlorolactophénol have shown that MYCETFLUO offers a sensitivity of 100% and a specificity of 100 %.

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