FUNGUS (MYCOLOGY) STAINS AND REAGENTS

PRODUCT:

Bottle:

Lactophenol Aniline Blue	R5955
Potassium Hydroxide, 10%	R6673

PURPOSE:

Lactophenol Aniline Blue and Potassium Hydroxide (KOH) are used in microscopic preparations of direct specimens or of isolated molds to distinguish fungal elements for the purpose of identification.

PRINCIPLE:

Lactophenol Aniline Blue is a reagent which stains the outer wall of molds, aiding in the microscopic examination of the fungal mycelium and fruiting structures. The stain has also been used as a counterstain for unfixed tissues, bacteria, and protozoa, in combination with other dyes. When used for the examination of fungus, the reagent contains phenol which is fungicidal, glycerol which makes a semipermanent preparation when examining molds, and lactic acid which acts as a clearing agent.

KOH is a reagent used in a rapid method for distinguishing fungal elements in microscopic preparations. It is particularly useful when examining thick mucoid material or specimens containing keratinous material such as skin scales, nails, or hair. The KOH digests the cellular material and background keratin, revealing the fungal elements and making them more visible.

FORMULAS:

Approximate ingredients per liter.

(1) Lactophenol Aniline Blue:

Water, Deionized	250.0 ml
Lactic Acid	250.0
Glycerol	500.0
Phenol	250.0 g
Aniline Blue	0.6

(2) KOH, 10%:

Water, Deionized	1000.0 ml
Potassium Hydroxide	100.0 g

PRECAUTIONS:*

For in vitro diagnostic use. Observe all safety precautions consistent with the hazard(s) stated on the product label and/or Material Safety Data Sheet. The carcinogenic potential of aniline blue is not known. However, the reagent should be handled with caution. Materials contained in the reagents are caustic. Avoid skin contact.

Storage: Upon receipt store at 10-30°C away from direct light. Stains or reagents should not be used if there are signs of deterioration or if the expiration date has passed.

Limitations: Prolonged exposure of a mold to KOH may result in distortion or destruction of the fungal elements.

The slide preparation should be stored in a moist chamber prior to reading in order to avoid drying.

Stain reagents may not result in a definitive identification. Further growth studies, biochemical, and/or serological studies may be needed to secure identification

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PROCEDURE:

Specimen Collection: Information on specimen collection and transport is found in standard reference material on the subject. In general, specimens should be protected from extremes of heat and cold and should be delivered to the laboratory without delay.

Method of Use, **Lactophenol Aniline Blue:** Place a drop of the staining reagent on a clean glass slide. Using a flamed needle or blade, cut a small amount of colony from the culture, preferably from the most granular area. A small amount of agar may be included. Place in the drop of Aniline Blue, and gently tease apart with a dissecting needle. Place a cover glass gently over the preparation. The specimen may be gently heated if agar has been included in the preparation; this will allow the fungus to be spread evenly and will remove air bubbles from the preparation. Examine under a microscope using a lowered condenser, first on low power and then on the high-dry objective.

Method of Use, **KOH Prep:** Place a portion of the specimen to be examined on a clean glass slide. Add one drop of 10% KOH. Place a cover slip on the preparation and allow to stand at room temperature for 5-10 minutes. Specimens held longer than 30 minutes must be placed in a moist chamber to prevent drying. Heating is not recommended since it may distort fungal elements in specimens. Examine for fungal elements under a low power objective using reduced light.

Interpretation:

For KOH:

Positive: Fungal elements are present.
Negative: No fungal elements are present.

For Lactophenol Aniline Blue: Consult appropriate references for the identification of fungal elements and fruiting bodies.

Materials Required but Not Provided: Standard microbiological supplies and equipment are not provided.

QUALITY CONTROL:*

Microorganisms Used (ATCC #):	**Expected Results:**
Microsporum canis	Typical structures,
Aspergillus niger (16404)	consults references.

User Quality Control: Check for signs of deterioration.

BIBLIOGRAPHY:

1. Campbell, M. C., and J. L. Stewart, *The Medical Mycology Handbook*, John Wiley & Sons, New York, 1980.
2. Koneman, E. W., et al., *Color Atlas and Textbook of Diagnostic Microbiology*, J. B. Lippincott, Philadelphia, 1979.
3. Lennette, E. H., et al., *Manual of Clinical Microbiology*, 4th ed., American Society for Microbiology, Washington, D. C., 1985.

*For more detailed information, consult appropriate references.

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