POTASSIUM HYDROXIDE (KOH) PREPARATION FOR DETECTION OF FUNGAL ELEMENTS

I. PRINCIPLE

The KOH Preparation of clinical material is used to facilitate the observation of fungal elements including yeast and ectoparasites. The microscopic examination of specimens for evidence of fungal elements may be used as a screening tool by the provider for fungal disease. 20% Potassium Hydroxide (KOH) is used to rapidly distinguish fungal elements in the microscopic preparations. The KOH preparation is made by emulsifying the specimen in a drop of 20% KOH on a microscope slide. The KOH digests the cellular material and background keratin, revealing the fungal elements and making them more visible. KOH is particularly useful when examining thick mucoid material or specimens containing keratinous materials such as skin scales, nails, or hair.

II. SPECIMEN COLLECTION AND PREPARATION

A. Specimens include non-vaginal sources such as skin, hair, and nails.
B. Lesions should be untreated with topical antifungal agents for at least one week prior to specimen collection.
C. Cleanse the skin to be sampled with an alcohol sponge (do not use cotton), to remove contaminating microorganisms.
D. The specimen to be tested will be at the discretion of the provider and will be collected using standard collection techniques.
E. If a characteristic dermatophyte ring is present on the skin, collect samples from the outer margin of the ring at its junction with the normal skin, or gently scrape up the entire periphery of the lesion with a blade, or collect samples from areas where the skin appears to be scaling.
F. Place sample onto a glass slide or other suitable container.
G. Transport covered specimen to microscope for examination.

III. REAGENTS AND EQUIPMENT

A. 20% Potassium Hydroxide (KOH). Store at room temperature. (15-30°C). Stable until the expiration date stated by the manufacturer.
B. Immersion oil
C. Microscope slides
D. Glass coverslips
E. Disposable plastic transfer pipette
F. Light Microscope with 10x and 40x objectives
G. Blades; dispose in sharp containers
H. Microscope

IV. QUALITY CONTROL

A. There is no liquid quality control available for the KOH prep. The KOH is visually and microscopically checked for observable contamination or deterioration each day of use. The reagent lot number and expiration date will be checked before use and recorded in LIS. Do not use past expiration date.
   1. **Satisfactory:** No sign of deterioration or precipitation, reagent is clear.
   2. **Unsatisfactory:** Contamination or deterioration is present. If contamination is suspected, discard KOH reagent. Repeat with a new reagent ampule. Do not continue with patient sample review until visual inspection is satisfactory.

V. SAFETY AND GENERAL PRECAUTIONS

Consider all specimens as potentially infectious.

A. Always wear appropriate PPE when performing a procedure or handling body fluids or tissues.
B. Wash hands with a disinfectant soap.
C. Use a biohazard red box to discard used glass slides, cover slips, blades, or other contaminated materials (Sharps Container).
D. All quality control and maintenance are performed and recorded by the personnel performing the test.

VI. PROCEDURE

It is the responsibility of the Provider collecting the sample to properly identify the patient and, unless collected and tested immediately the sample must be labeled with two full patient identifiers (full name, SSN, or DOB).

1. Place a portion of the specimen to be examined on a clean dry glass slide.
   a. Add one drop of immersion oil to aid in detection of ectoparasites.
2. Dispense 1-3 drops of 20% KOH onto the specimen on the slide.
3. Place a cover slip on the preparation.
4. Allow the smear to stand at room temperature for a period of 5 to 10 minutes. Specimens held longer than 30 minutes will potentially dry out.
5. Gentle warming may aid in the clearing of the smear.
6. Examine and scan the slide under low power objective (10x) to identify ectoparasites.
7. Use the high-power objective (40x) to confirm and identify suspected fungal elements.
8. **NOTE:** It is very important to avoid getting KOH on the microscope objective, as it will etch the lens. The diaphragm should be closed down and the condenser lowered as far as it will go.
9. Record the results in the patient medical record.

VII. RESULTS

A. The presence of hyphal elements, yeast cells, or ectoparasites indicates a positive test.
    1. Fungal elements present or absent
        a. Fungal elements should **not** be quantitated.
    2. Ectoparasites present or absent
    3. KOH Prep: Positive or Negative
B. Normal Reference Range
    1. Absent/No Fungal Elements Seen.
    2. Absent/No Ectoparasites seen.
C. Patient results are recorded in the patient's chart using Point of Care Result Entry in Powerchart.
    1. Lot number and expiration date of KOH is documented in LIS with the results
D. Patients tested and maintenance should be documented on a log for the laboratory review. (see Appendix A).

VIII. NOTES/LIMITATIONS

A. The reagent should not be used if KOH crystals are precipitated onto the wall of the ampule or are floating in the reagent.
B. The contrast between unstained fungal elements that may be present, and the background can be accentuated by narrowing the iris diaphragm to reduce the amount of incident light.
C. Cotton strands from cotton swabs may resemble hyphae.
D. KOH preparations are not permanent; the reagent will eventually destroy the fungi.
E. Overheating of the smear may cause crystallization of the KOH.
F. This test is performed only by providers such as Medical Doctors, Nurse Practitioners, or Physician Assistants specialized in the service.

IX. PROFICIENCY TESTING AND COMPETENCY ASSESSMENTS

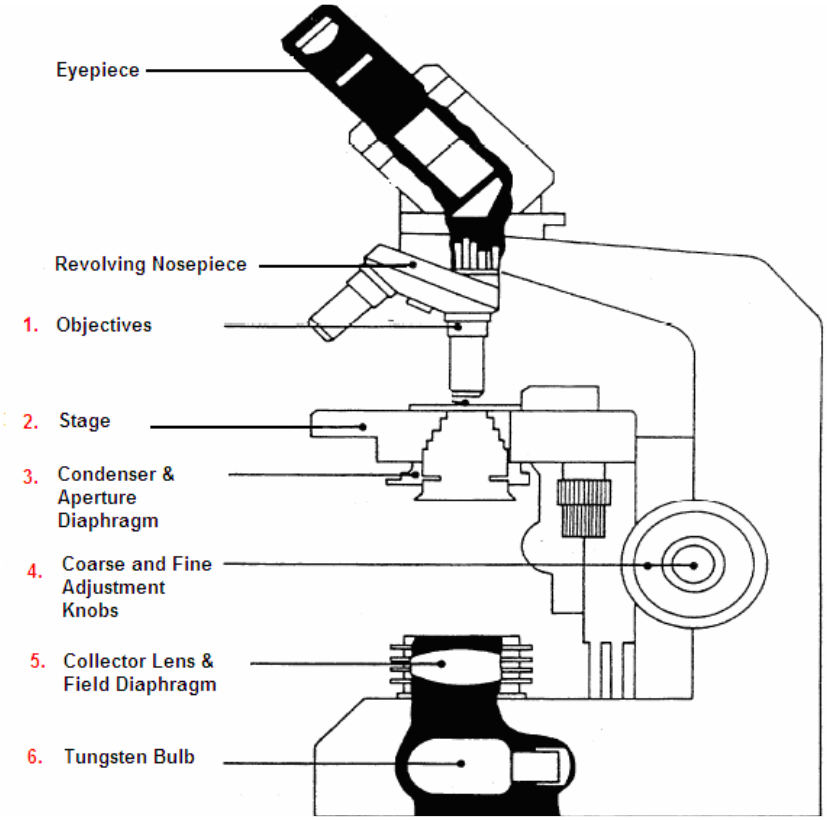
A. Proficiency Testing Procedure:
    1. Clinical proficiency is tested through the Pathology and Laboratory Medicine Service program of identification of unknown specimens. The PT samples will be rotated and distributed amongst the providers who perform KOH preps. There must be no inter-laboratory communication of Proficiency Testing data until after the deadline for submitting results to the proficiency testing provider. Proficiency testing specimens will not be referred to another laboratory. If a sample would normally be sent to a referral lab, our lab must submit a PT result of "Test Not Performed".
B. Proficiency Testing Review:
    1. Proficiency testing results will be reviewed by the Laboratory Director, Laboratory Manager, and the Ancillary Testing Coordinator (ATC). A copy of the proficiency testing results will be maintained by the ATC.

C. Competency Assessments:
1. Medtraining (MTS) competency tests are performed yearly by each provider that performs KOH Preps. This consists of five on-line slide micrographs. The provider must score 80% or better to pass. If the provider does not pass the tests corrective action will occur and is facilitated by the ATC.
2. Provider will be observed performing a procedure and evaluated using six methods.
3. The ATC maintains all competency records in the laboratory.

X. MAINTENANCE

A. Microscope adjustment
1. To darken the field, use the aperture adjustment and /or the rheostat to decrease the light source to its lowest setting.

B. Microscope Maintenance:
1. Clean microscope lenses and exterior surfaces as needed with lens cleaner, or at least monthly. Document on log sheet. (See Appendix A)
2. The microscope is cleaned and serviced annually by biomed.
3. Professional maintenance is performed at least every 2 years.
4. Any necessary repairs are performed by appropriate, designated personnel.
5. Center the light whenever the lamp is changed or as needed.
   a. Focus on an object using the coarse and fine drive.
   b. Close the field diaphragm. Loosen the stop screw and raise the condenser to its uppermost position with the height adjustment.
   c. Turn the condenser stop screw to lower the condenser until the edges of the field diaphragm appear sharp.
   d. Center the image of the field diaphragm with the two centering screws.
   e. Open the field diaphragm until it just disappears from the field of view.



XI. REFERENCES

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B. Modern Microscopy G.F.A. Culling, Butterworths, 1974."Optical Systems for the Microscope," Zeiss, 12/67.
C. Balows, A., et al., Manual of Clinical Microbiology, 5th ed., 1991, American Society of Microbiology, p 610.
D. Portland VAMC, KOH Prep Procedure manual.
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APPENDIX A

Provider Performed Microscopy Log

Location:

Patient Name	DOB	Date	QC*	RESULT*	CLEAN*	NOTES

*Tasks: Document for each day of use

QC- Observe Saline/KOH for acceptablitity. Document unaccepable results and resolution in NOTES column.

RESULT- Document results in patient chart

CLEAN- Wipe off microscope stage, clean lens, and cover microscope

Microscope Maintenance

DATE	REASON

Reviewed by:

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