KOH (Potassium Hydroxide) AND WET PREPARATION PROCEDURE

PURPOSE:
Potassium hydroxide digest or lyse epithelial cells, WBCs, RBCs, mucus, and various other proteinaceous debris, bleaches many pigments and dissolves the “cement” that holds keratinized cells together. This permits detection of fungal elements (yeast and pseudohyphae) that are present in vaginal secretions and keratinized tissue. Most fungi can be demonstrated in the KOH preparation.

Wet preps allow for microscopic observation of unfixed “wet mounts” of clinical specimens for the rapid detection of the presence of bacterial, fungal, and parasitic organisms. The presence of white blood cells and “clue cells” may also be identified.

SPECIMEN:

Patient Preparation:
Vaginal specimens- collect during an internal examination of female genital tract.
Nails- clean with 70% alcohol, scrape away the outer portion and obtain scrapings from the deeper infected areas
Hair- Select infected area. Remove at least 10 hairs. Entire hair shaft if necessary. Place hair between two clean glass slides. Label with patient’s identifier.
Skin- Clean skin with 70% alcohol, scrape away the outer portion and obtain scrapings from the deeper infected areas.

Type:
1. Skin
2. Hair
3. Nails
4. Vaginal mucosa and vaginal pool or scrapings

Handling:
1. Swabs are used to collect specimens for vaginal specimens.
   1. If the test is to be analyzed immediately, the scrapings are placed on to a microscope slide.
2. If sent to the laboratory, place 0.5-1ml of sterile saline in tube. Label tube with patients’ name and birth date/history number.
3. After obtaining specimen, clinician places swab(s) into saline avoid touching sides of the tube and resealed.
4. It is important the slide be examined within 15 minutes of collection.

To preserve the motility of Trichomonas specimens should not be refrigerated.

Examinations are to take place as soon as possible following collection.
EQUIPMENT AND MATERIALS:

Materials
1. 10% potassium hydroxide- solution in dropper bottle, NOTE: Caution Highly corrosive liquid use with gloves and eye/face protection
2. Microscope slides with frosted edge and Cover slips
3. Cotton-tipped swabs for vaginal secretions, Scraping implement for skin and nails
4. Tubes or vials with 0.9% sterile saline for vaginal samples
5. 70% Alcohol
6. Gloves

Equipment
Microscope with 10X and 40X objectives

Storage Requirements:
All materials are to be stored at room temperature.

QUALITY CONTROL:
Commercial controls not available for wet mount preps. Check KOH for expiration date prior to use.
Providers performing microscopic testing are assessed twice a year to assure competency is maintained.
Each provider should participate in the online competency program MTS managed through the Pathology Department in which 5 microscopic images are examined every six months. Minimum passing grade is 80%

PROCEDURES:

WET PREP
1. Mix the swabs in the tube of saline.
2. Place a large drop of mixture on slide.
3. Place a cover slip over the specimen- saline mixture on the slide. Avoid air bubbles as they will cause the specimen to dry out rapidly.
4. Examine under low power 10X and high power 40X.
5. Scan the specimen for bacteria, fungi, parasites and human cellular elements.

KOH PREP
1. Place the specimen on a clean glass slide
2. Add 1 drop of 10% KOH. Before adding coverslip, smell mixture for the “whiff” test for bacterial vaginosis. If fishy odor it is suggestive of bacterial vaginosis.
3. Place cover slip over the specimen KOH mixture on the slide.
4. Let the slide stand for 2-5 minutes for clearing and digestion to occur.
5. Examine the KOH mount using reduced lighting on the microscope.
6. Use the low power objective 10X, first to locate any fungal elements on the slide. Look for fungal forms (i.e. yeast, hyphae, or mycelium) on the slide.
7. Switch to the high power objective to better visualize and quantitate fungal elements (budding yeast cells and pseudo hyphae).
8. Document results in patient records
REPORTING RESULTS:
Results are to be documented in the patient record along with the initials of the person performing the test.

Wet Prep quantitation:

<table>
<thead>
<tr>
<th>Quantitation, Direct Exams</th>
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<tbody>
<tr>
<td>1+ occasional</td>
</tr>
<tr>
<td>1-5 organisms or cell/hpf</td>
</tr>
<tr>
<td>2+ scanty</td>
</tr>
<tr>
<td>1-5 organisms or cell/hpf</td>
</tr>
<tr>
<td>3+ Moderate</td>
</tr>
<tr>
<td>6-30 organisms or cell/hpf</td>
</tr>
<tr>
<td>4+ Heavy</td>
</tr>
<tr>
<td>&gt; 30 organisms or cell/hpf</td>
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Wet Prep- note organisms observed and quantity

KOH Prep –
Normal results – Negative/ No fungi (no dermatophytes or yeast).
Abnormal results – Positive/ Dermatophytes or yeast seen on KOH test indicates fungal infection.

LIMITATIONS OF THE PROCEDURE:
1. If oil droplets are present interpretation of direct wet mounts difficult. In such cases a gram stain can be helpful.
2. Microscopic lighting is critical in the performance of a KOH examination as too much light can wash out fungi, resulting in false negatives.
3. Many artifacts are generated by the action of KOH on cellular material so care must be taken to ensure true fungi are observed.
4. Examine the entire cover slipped area. Random examination of smears may result in a false negative result.

REFERENCES: