Keywords: KOH, saline, trichomonas, urethra, vaginal

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I. PURPOSE

This procedure provides instructions for collection of specimens for use in performing microscopic examination for the presence of bacterial, fungal and parasitic organisms in a saline and KOH suspension of vaginal secretions or skin scrapings.

II. PRINCIPLE

Microscopic observation of unfixed "wet mounts" is the most rapid, cost-effective diagnostic aid used to quickly identify the causative agent of well-recognized pathological conditions so that treatment may begin. Fungi are usually larger than bacteria, and in material from skin, hair or nails, they can be seen by direct microscopy, provided the material is first softened and cleared with a strong alkalai such as 10% potassium hydroxide (KOH). The purpose of the alkalai is to digest the keratin surrounding the fungi so that the hyphae and spores can be seen.

III. SAFETY PRECAUTIONS

A. Follow Standard Precautions and CDC hand washing guidelines when performing this test.
B. Sharps such as needles, blades, and glass slides are to be deposited in puncture-proof containers.
C. Visibly contaminated items or items saturated with blood are to be disposed of in appropriate biohazard containers.
D. Refer to ICPM IFC023 Infection Control and Prevention: Standard and Isolation Precautions

IV. MATERIALS

A. Sterile cotton tipped swabs
B. Speculum (for vaginal collection)
C. Rayon swabs on thin wire (for male urethral collection)
D. Disposable gloves
E. Glass slides
F. Cover slips
G. 70% ethanol
H. pH Nitrazine paper
I. Immersion oil

V. REAGENTS

A. 0.85% Sterile Physiological Saline
B. 10% potassium hydroxide (KOH)

NOTE: Concentrated KOH is a highly corrosive liquid that must be used with appropriate Personal Protective Equipment (PPE) which includes gloves and eye and face protection and an available eye wash. It is recommended that for use in the PPM environment 10% KOH be purchased commercially or obtained from pharmacy/laboratory sources. Instructions for the use of personal protective equipment and post exposure care may be found in the material safety data sheet (MSDS).

NOTE: The physiological saline and the KOH should be sterile and clear in appearance. Do not use either if cloudiness is evident because this could indicate contamination.

VI. STORAGE REQUIREMENTS

A. All reagents (KOH and saline) are to be stored at room temperature. KOH is to be discarded if precipitation occurs.
B. No reagents are to be used past their expiration date.

VII. SPECIMEN TYPE

Specimen types may include:

A. Swabs of the vaginal mucosa and vaginal pool
B. Skin scrapings
C. Hair clippings, scrapings, or plucking
D. Nail clippings or scrapings
VIII. SPECIMEN COLLECTION

Standard (universal) Precautions must be followed when collecting and handling potentially infectious specimens. Gloves should be worn when handling specimens.

A. Vaginal Wet Mount or KOH Collection
   1. Specimens collected for wet mounts are usually collected during an internal examination of the female genital tract.
      a. Place the female patient in the customary position for a gynecological exam.
      b. Insert a speculum and expose the cervix.
      c. Insert sterile cotton tipped swab into the vagina and collect a sampling by passing swab through and along the area of concern.

B. Nonvaginal Wet Mount or KOH Collection
   1. Skin Scraping
      a. Remove all powders, ointment, and creams using 70% ethanol.
      b. Allow the area to thoroughly dry.
      c. With a blade, scrape the skin area: Scales on the area bordering "normal" skin, Blisters should have their lids removed and the contents of the blisters as well as the scraping collected
      d. If more than one lesion is present, several should be scraped.
      e. Scrape into a sterile tube for transport , or onto a clean colored paper if the area's shape will not allow direct collection into a test tube. Scraping should occur until the dermis is exposed and bleeding begins.
      f. If paper was used in the collection, transfer the contents of the paper into a sterile tube.
      g. Once scraping is complete, securely cap the test tube
      h. Label the test tube with the patient's name and a unique identifier.

   2. Nail Clipping and Scraping
      a. Using clippers, remove the nail to the bed.
      b. Using a blade or clippers, clip or shave the nail bed at any separations or white patch.
      c. Clip or shave the nail and nail bed into a sterile tube
      d. Once collection is complete, securely cap the tube
      e. Label the tube with the patient's name and a unique identifier

   3. Hair Collection
      a. Using a clean hemostat, grasp 30 to 50 hairs in the region most affected, or if bald spots are occurring, the area most adjacent to the bald spot.
      b. If the hair bulb is required, using the hemostat, with a quick, sharp tug, pluck the hairs from the head.
      c. If the bulb is not required, with a clean blade scrape the skin and cut the hairs.
      d. Place the hemostat and hairs in a clean tube for transport.
      e. Securely cover the tube.
      f. Label the tube with the patient's name and a unique identifier.

   4. Skin Scraping for Ectoparasites (including lice, scabies, chiggers and dermatophytes)
      a. Remove all powders, ointment and creams using 70% ethanol.
      b. Allow the area to thoroughly dry.
      c. Drop 1 to 3 drops of immersion oil onto the lesion or affected site.
      d. With a blade, scrape the skin.
      e. With each scrape, transfer the blade contents to a clean slide.
      f. Continue scraping until the dermis is exposed and bleeding begins.
      g. Once scraping is complete, apply a coverslip on each slide.
      h. Label each slide with the patient's name and a unique identifier and its order of collection (ie, 1,2,3)
IX. SPECIMEN HANDLING

A. Vaginal Specimens
1. To preserve the motility of trichomonads, specimens should not be refrigerated and should be examined within two hours of collection.
2. Specimens from the vagina should be tested with pH paper before being placed in saline. A pH greater than 4.5 is associated with bacterial vaginosis or trichomoniasis. The pH should be documented on the report. NOTE: Pooled vaginal fluid potentially containing amniotic fluid or seminal fluid may present with a pH of 7.0 or greater.
3. Place 1-2 of normal saline on one 1/2 of slide labeled with the patient's name and a unique identifier and swish swab to dispel material into solution. It is important to dislodge particulates from the swab tip by twirling the swab vigorously in the solution. Failure to adequately dislodge particles may lead to erroneous results. Cover with a cover slip.
4. Add one drop of 10% KOH to other side of slide and swish swab to dispel material into solution.

B. Nonvaginal Specimens
1. Specimens from sources other than the vaginal tract should be placed immediately on a prepared slide or specimen container for examination. If the specimen must be transported to a testing area, place the specimen in a sterile, dry, covered container labeled with the patient's name and a unique identifier for examination.

X. LIMITATIONS OF THE PROCEDURE

A. Many intravaginal medications will leave oil droplets which can make interpretation of wet mounts difficult. It is often useful in such situations to perform a Gram stain for the detection of yeast or to test for the presence of bacterial vaginosis.
B. Many vaginal infections are uncomplicated and can be diagnosed using wet mount/KOH alone. If there are unusual circumstances, a Gram stain can be a very helpful aid for the detection of yeast or to test for the presence of bacterial vaginosis.

XI. REFERENCES

B. Clinical Diagnosis and Management by Laboratory Methods, 18th edition, John B. Henry, W.B. Saunders Company, Philadelphia, PA, 1991

XII. SIGNATURES

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Reviewed by Laboratory Director:

William Clarke PhD

On: 5/9/13
Specimen Collection Procedure for Saline and KOH Slides

Original signed document on file.