



Technical Bulletin

North Carolina State Laboratory of Public Health | Laboratory Improvement Unit

Testing at a Glance: Vaginal Wet Mount

The direct examination of a clinical specimen by wet mount is a rapid and cost effective diagnostic aid in the laboratory. The visual examination by the laboratory assists the clinician in quickly identifying the causative agent of infection so treatment may begin.

Wet mount samples may be collected from various clinical sites. This bulletin will discuss the vaginal wet mount which is a common test performed by the laboratory. Vaginal wet mounts aid in determining the causative agent of vaginitis, or inflammation of the vagina.

Training

Reliable wet mount examinations are largely dependent on the expertise of the reader. A well-trained, experienced microscopist is an asset to the laboratory. Personnel who perform this procedure must be adequately trained and demonstrate proper techniques for specimen preparation and use of the microscope. In addition, they should demonstrate thorough knowledge of organism morphology and interpretive skills.

Specimen and Handling

Proper specimen collection and handling is the first crucial step in accurate vaginal wet mount results. Specimens should only be collected by qualified personnel. The clinician will use a Dacron or sterile cotton swab to swab the vaginal vault and walls. The swab should be emulsified immediately into a small tube labeled with appropriate patient information containing 0.5-1.0 mL of warm (room temperature), sterile 0.9 percent normal saline.

Alternatively, the swab can be applied directly to a glass slide and diluted with one to two drops of 0.9 percent normal saline solution and covered with a coverslip.

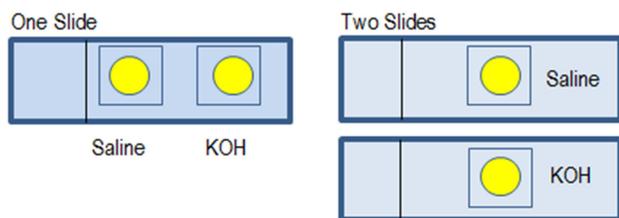
If vaginal pH is desired, the swab should be touched to pH paper before adding to the sterile saline. The pH is determined by matching the color change to the color chart supplied with the pH paper.

Test Method

The wet mount specimen should be maintained at room temperature and transported immediately to the laboratory for examination. Ideally, examination should begin within 15 to 20 minutes and no more than 60 minutes after collection. Slides should be prepared one patient at a time by vigorously mixing the swab or pipetting in and out of the saline with a plastic pipette.

A potassium hydroxide (KOH) preparation is often made in conjunction with the saline wet mount. KOH lyses the cell membranes of bacteria, trichomonads, white blood cells (WBCs), and red blood cells (RBCs). Yeast, if present, remains intact making the yeast more easily distinguished. When exposed to KOH for a prolonged period of time, the yeast will also be lysed by the KOH.

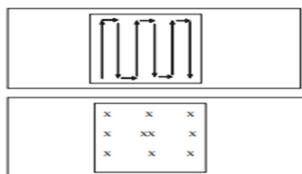
The saline and KOH wet preparations may be prepared on a single microscopic slide or on two separate slides depending on laboratory procedures.



If requested, the KOH preparation is also used to perform an amines (Whiff) test. The KOH preparation should be smelled by gently wafting the vapor layer above the surface of the slide and assessed for the presence of amines which have a “fishy” odor. A positive amine odor is characteristic of the presence of *Trichomonas vaginalis* and *Gardnerella vaginalis*.

Examination

Once the wet mount slide has been prepared, the slide is examined microscopically. Examination is made using both the low power (10X) objective to locate and focus on the specimen and high power (40X) objective to perform a systematic examination for the presence of cellular elements. A systematic pattern of examination is to examine three sites on each edge of the coverslip and two sites in the center. Both the saline and KOH-treated specimens should be examined in the same manner.



Interpretation

Examination of the wet mount may reveal the following observations: epithelial cells, white blood cells, red blood cells, clue cells, yeast, trichomonas, and bacteria. Based on these observations and the physical examination, a differential diagnosis of vaginitis and/or vaginosis can be made by the clinician.

Epithelial (epi) cells are the predominant cell found in vaginal mucosa. They are large, thin, and flagstone-shaped in appearance, with a small central nucleus about the size of a red blood cell. As epithelial cells degenerate, they can be misidentified as clue cells. Epithelial cells will be lysed in KOH-treated specimens.

White Blood Cells (WBCs) are not usually present in great numbers in healthy women. WBCs play a role in fighting infection and can be elevated in some infections including *Chlamydia*, *Trichomonas vaginitis*, yeast infections (Candidiasis), and *Neisseria gonorrhoeae*. WBCs will be lysed in KOH-treated specimens.

Red Blood Cells (RBCs) may be apparent to various degrees in vaginal discharge. In fresh preparations, they appear highly refractile as compared to the granular appearance of WBCs and are slightly smaller. In older preparations, the cell membrane may appear crenulated. These structures are sometimes confused with yeast and will be lysed in the KOH treated specimen, which is useful for distinguishing between RBCs and yeast.

Clue Cells present in vaginal secretions are presumptive for a diagnosis of bacterial vaginosis (*Gardnerella vaginalis*). Clue cells are squamous, vaginal epithelial cells covered with many bacteria, giving them a stippled or granular appearance. Borders are obscured or fuzzy because of the adherence of the bacteria. So many organisms may attach to a vaginal epithelial cell that its entire border is obscured. Clue cells will be lysed in KOH-treated specimens.

Trichomonads are generally ovoid, slightly larger than a white blood cell, and have four flagella at the anterior end with whip-like motility. They are easily identified by their undulating swimming motion.

The presence of trichomonads is a definitive diagnosis for *Trichomonas vaginalis*; however, motility will slow and eventually cease as the sample ages. Non-motile trichomonads can be often mistaken for white blood cells; therefore, prompt review of the sample is imperative. Trichomonads will be lysed in KOH-treated specimens.

Yeast and/or pseudohyphae is presumptive of Candidiasis in vaginal secretions. Yeast are round or oval and vary in size, but are typically smaller than RBCs. Pseudohyphae are chains of budding yeast cells marked by constrictions. Identification of yeast in vaginal secretions is presumptive for Candidiasis; however, results should only be reported as yeast and/or pseudohyphae. Definitive identification can only be made with more conclusive testing such as culture or PCR testing. Yeast can be misidentified as RBCs, and pseudohyphae must be distinguished from other elements such as mucus threads, artifacts, or fibers. The use of KOH is helpful in the identification as it destroys other elements, allowing better visualization of yeast.

Bacteria in some amount are normal in vaginal secretions. *Lactobacilli acidophilus* are large, non-motile rods and are the most predominant bacteria in the female vagina. Other bacteria that may be present include *Gardnerella vaginalis*, small, non-motile coccobacilli, and *Mobiluncus* species, thin, curved motile rods. A number of anaerobic bacteria may also be found. Bacteria, if reported, should only be identified as such. Definitive identification can only be made with more conclusive testing, such as culture or PCR testing.

Errors and Limitations

Care should be taken in all phases of the wet mount process as errors can affect results and subsequently alter patient treatment. The following are examples of errors and limitations that will decrease the sensitivity of the wet mount:

- Oil droplets from intravaginal medications and douches that may be mistaken for yeast
- Collection of the specimen from the endocervix
- The use of saline that is not at room temperature or has been refrigerated
- Delay in reading the smear
- Contamination of the saline preparation with KOH
- Preparation that is too thin or too thick
- Untrained or inexperienced staff

Conclusion

In the advent of more sophisticated and targeted testing for vaginitis, the vaginal wet mount continues to play an important role in laboratory testing. There are several factors that may hinder the effectiveness of the wet prep such as improper collection, delays in transport, and the expertise of the microscopist. However, when proper procedures are followed, the vaginal wet mount is a rapid, cost-effective aid that assists clinicians in the effective diagnosis and treatment of vaginitis.

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