Vaginal Microscopy: Refining the Nurse Practitioner's Technique

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ABSTRACT

The sensitivity rate of vaginal microscopy (including wet mount, wet preparation, hanging drop, and vaginal smear) is primarily determined by the expertise of the clinician carrying out the test. Although clinicians perform this diagnostic procedure daily, many have not been taught how to do so optimally. This article provides a carefully researched technique for collecting, preparing, and viewing vaginal smears for microscopic analysis. Operation and maintenance of the office microscope are also reviewed. Key aspects of viewing the saline and potassium hydroxide samples are discussed, and normal and abnormal smears are described.

Key words: wet mount, vaginal smear, hanging drop, wet preparation, vaginal microscopy, vulvovaginitis

In the United States, vaginal complaints account for an estimated 10% of visits by women to their primary care providers (Deutchman, Leaman, & Thomason, 1994). Vaginal microscopy (wet mount, wet preparation, hanging drop, vaginal smear) is a diagnostic test carried out by many nurse practitioners to evaluate patients with vulvovaginal complaints. When properly performed, this test may be 80% sensitive (Secor, 1992). Refining vaginal microscopy skills can lead to a remarkable improvement in the clinician's diagnostic capability, which can make the difference between offering a patient specific treatment versus therapy based on empirical guesswork.

Microscope Operation and Maintenance

The following supplies are needed to perform vaginal microscopy: wooden or plastic spatula, cardboard double-slide holder, two frosted-edge glass slides, coverslips (1- or 2-inch), saline solution, 10–20% potassium hydroxide solution, vaginal speculum, and gloves. A quality office microscope with low-power (10 ×) and high-power (40 ×) magnification is also needed (Kaufman & Faro, 1991). The detail and dimensional quality of microscopic observations can be improved significantly with the addition of a phase-contrast condenser (Horowitz, 1991a; Kaufman & Faro, 1994), which also enhances the differentiation of organisms from artifact. Retrofitting an office microscope with such a condenser is highly recommended.

When not in use, the microscope should be covered to prevent buildup of dust on the optical surfaces. Gloves should be worn when cleaning, adjusting, or operating the microscope to prevent contamination from the specimens. The eyepieces and other glass surfaces should be cleaned daily with lens cleaner and special lens paper. The stage, where the slide is placed, should be cleaned using a 10:1 mixture of bleach or equivalent solution. It is best to clean the surfaces of the objectives with a saline-moistened cotton-tipped applicator or lens paper to avoid the risk of scratching, as may happen if a paper towel is used.

Several times per year, the microscope should be professionally adjusted, lubricated, and cleaned. The specific interval depends on the amount of use, daily maintenance, and number of clinicians using the microscope. A repair company can recommend a periodic maintenance schedule based on these factors.

Lack of expertise in microscope operation is a factor contributing to reduced sensitivity of vaginal microscopy. The number of adjustments required to enhance the clarity of the sample being viewed is likely to increase if several clinicians use the same microscope.

Clinicians should develop the following consistent routine for adjusting and operating the microscope (Table 1). After turning the microscope operating

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Table 1
Steps in Microscope Operation

1. Turn operating switch on
2. Adjust interpupillary diameter
3. Adjust each eyepiece
4. Raise light condenser close to stage
5. Close aperture to small diameter
6. Center low-power objective
7. Place slide on stage
8. Adjust stage to center slide
9. Adjust gross focus to locate sample
10. Adjust light and aperture as needed
11. Adjust fine focus until image is sharp

switch on, the distance between the eyepieces, referred to as the interpupillary diameter, is adjusted; this adjustment allows for dimensional or binocular viewing. Next, the low-power objective is moved into position. The stage is also adjusted so that the glass slide, when placed on the stage, will be centered under the low-power objective. This is the ready position. After the smear has been placed on the stage and brought into focus by using the gross then the fine adjustment, each eyepiece should be individually focused, by closing one eye at a time. The light aperature may be adjusted as needed to further sharpen the image.

Sample Collection

A wooden or plastic spatula is used to collect the sample of vaginal mucus; cotton-tipped applicators are not recommended because they may contaminate the sample with fiber artifact, which may mimic yeast forms. Use of a spatula also allows the clinician to prepare the saline and potassium hydroxide samples in the proper concentrations (Kaufman & Faro, 1994).

With the vaginal speculum in place, the wooden spatula is positioned to the side of the cervix and drawn forward along the lateral aspect of either vaginal wall. Several attempts may be required to collect an adequate sample on the tip of the spatula. Care must be taken to avoid sampling cervical mucus, since this may result in a false elevation of the vaginal pH reading and alter the microscopy results.

Vaginal pH should be assessed whenever vaginal microscopy is performed. According to Kaufman and Faro (1994), the importance of vaginal pH determination cannot be overemphasized. It is easy to perform, is inexpensive, and has a high predictive value. Vaginal pH is tested with a 1-inch strip of phenaphthazine (Nitrazine [Squibb, Princeton, NJ], ColorpHast [Em Science, Gibbstown, NJ], or Hydrion [Micro Essential Laboratory, Brooklyn, NY]) pH paper (Fig. 1) dipped

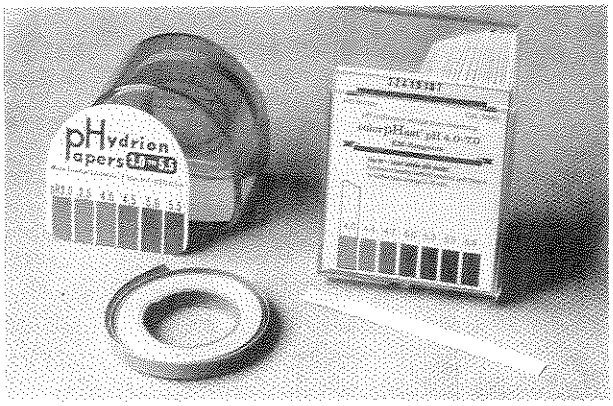

Fig. 1. Vaginal pH paper. Courtesy of Curatek Pharmaceutical Company.

into the discharge collected on the wooden spatula. The normal vaginal pH in women of reproductive age is 4.0, based on a 3.0–7.5 scale. In addition to cervical mucus, tap water (Kaufman & Faro, 1994), lubricating gels, and vaginal medications may alter the vaginal pH reading. Semen, with a pH of 9.0, may dramatically alter the vaginal pH (Thomason, et al., 1990). For these reasons, women should be advised to avoid intravaginal medications and sexual activity for a minimum of 2–3 days before an infection check or a routine examination. These instructions should be shared with patients during preventive visits, so that when a problem arises, the patient is more likely to avoid the use of intravaginal medications before an infection check. To aid insertion of the vaginal speculum, a woman's own vaginal secretions may be used as a lubricant. This may be accomplished by palpating the introital tissues prior to speculum insertion.

In patients with vulvovaginal candidiasis, the vaginal pH is 4.0–4.7, indicated by a medium to dark yellow color of the pH paper. A vaginal pH of 3.5–4.7 is noted in patients with no vaginal infection and those with cytolytic vaginosis. Patients with bacterial vaginosis will have a vaginal pH of 5.0–6.0, indicated by a color change ranging from light to dark olive green. In patients with bacterial vaginosis, a pH reading greater than 6.0 suggests contamination of the vaginal sample with cervical mucus or semen or concomitant infection with *Trichomonas* or *Chlamydia*. The majority of patients with trichomonal infections have a vaginal pH of 6.0 or higher, indicated by a bluish pH color (Kaufman & Faro, 1994).

Wet Mount Preparation

To prepare the wet mount, two frosted-edge slides are placed in a cardboard double-slide holder. One or two drops of saline are applied to the front slide and one

or two drops of 10–20% potassium hydroxide to the back slide. The wooden spatula containing the vaginal sample is dipped into the saline on the front slide, and several rotational stirs are made. The wooden spatula is then placed into the potassium hydroxide, and about 10 rotational stirs are made until the sample appears opaque white (Secor, 1992).

The whiff test (amine or sniff test) is performed next, by removing the wooden spatula from the potassium hydroxide mixture and noting the presence of a foul or fishy odor on the spatula (Bartleson, 1992). Such an odor is recorded as a positive result. This test is best performed immediately after mixing the vaginal sample with the potassium hydroxide since even a short delay may produce a false negative result.

Care should be taken to keep the slide holder level during transport to the microscope area; otherwise, the sample may spread across the slide and dry out prematurely. This causes crystal artifact to develop in the potassium hydroxide sample, and the saline sample may become hypertonic, immobilizing trichomonads. It is also more difficult to identify organisms in a nonmotile sample. Use of test tubes for collection of vaginal discharge, although popular, may lead to excessively dilute samples, thereby reducing the sensitivity of the test (Kaufman & Faro, 1994).

Viewing Principles

To promote motility and viability of the organisms, the coverslips should be applied just before viewing the smears (Secor, 1992). The slide coverslips are applied by placing one side of the coverslip on the slide and then gently dropping the coverslip onto the sample. This technique minimizes formation of bubbles under the coverslip. Use of 2-inch coverslips eliminates the multiple planes created by the overlapping edges when two or more 1-inch coverslips are used. Use of larger coverslips also decreases the risk of specimen leakage onto the microscope stage.

Liquid extending beyond the edges of the coverslips should be gently removed with a paper towel before the slide is placed on the microscope staging.

When moving the slide to view different fields of the sample, it is important to remain within the edges of the coverslips. This prevents contaminating or scratching the lens of the objective, which may be severe if the lens comes into direct contact with the potassium hydroxide solution.

Evaluating the saline and potassium hydroxide slides requires a systematic and consistent approach. Under low power, the quality of both smears is evaluated and recorded using such descriptors as proper concentration, too dilute, or too concentrated. Such observations help determine the sensitivity or reliability of the test results, since the sensitivity is highest when the saline and potassium hydroxide samples are prepared in recommended concentrations.

To achieve a maximum sensitivity rate of 80%, both the saline and potassium hydroxide samples are evaluated for a total of 3–5 minutes, during which time a minimum of 12 fields per sample are viewed (Cibley & Cibley, 1991; Secor, 1992).

Viewing the Saline Sample

The saline smear should be fairly dilute but contain at least several dozen epithelial cells per low-power field. The epithelial cells should be separated from each other and not excessively clumped together. This enables accurate evaluation of white blood cells (WBCs), lactobacilli, bacteria, and other organisms located in the intercellular spaces between the epithelial cells (Kaufman & Faro, 1994).

While the saline sample is being viewed under high power, the quantity and quality of lactobacilli are noted. Lactobacilli are identified by their straight, rod-shaped appearance and vary from very short to superlong. Superlong lactobacilli, previously termed *leptothrix*, are longer than the diameter of mature epithelial cells.

The quantity of lactobacilli is estimated using the Speigal scale of 0–4+ (Thomason, et al., 1990). An estimate of 1–2+ indicates scant lactobacilli (Fig. 2). An estimate of 3–4+ represents a moderate number of lactobacilli (Fig. 3), considered normal for women of reproductive age. Overgrowth of lactobacilli, an estimate of 5+ (modified Speigal scale), is associated with false clue cells, which are pathognomonic for cytologic vaginosis (Cibley & Cibley, 1991; Secor, 1992) (Fig. 4).

Next, the saline smear is examined for the quality and quantity of background bacteria present. These

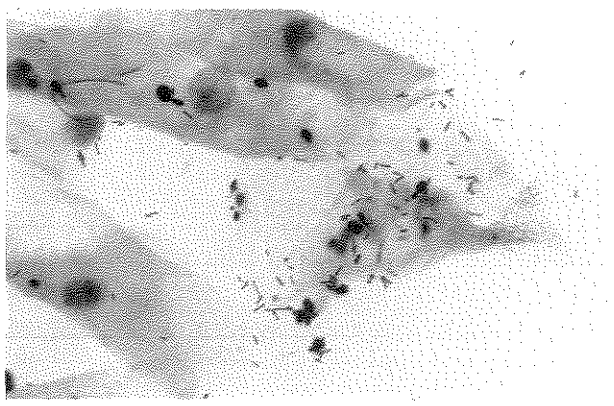

Fig. 2. Scant lactobacilli (1–2+), high power. Courtesy of Curatek Pharmaceutical Company.

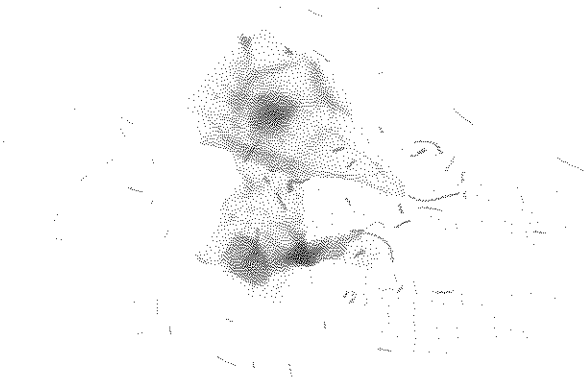

Fig. 3. Moderate lactobacilli (3–4+), high power. Courtesy of Curatek Pharmaceutical Company.

anerobes and facultative organisms appear as tiny dots, commas, or both, and are located between and overlying the epithelial cells. Again, using the modified Speigal scale, an estimate of 1-2+ indicates scant bacteria (Fig. 5). An estimate of 3-4+ indicates a moderate number of bacteria (Fig. 6) and is termed *intermediate flora*. An estimate of 5+ bacteria is associated with classic clue cells pathognomonic for bacterial vaginosis (Fig. 7). Normal flora are characterized by 3-4+ lactobacilli and fewer than 1-2+ background bacteria (Fig. 3).

While continuing to view the saline smear under high power, WBCs are identified next. Approximately equal in size to the nuclei of mature epithelial cells, WBCs appear dark and granular (Fig. 8). Generally round, WBCs may appear oval or teardrop-shaped in association with inflammatory conditions such as vaginitis or cervicitis. If trichomonal organisms are not motile (Fig. 8, center organism), it may be difficult to distinguish them from WBCs despite their slightly larger size.

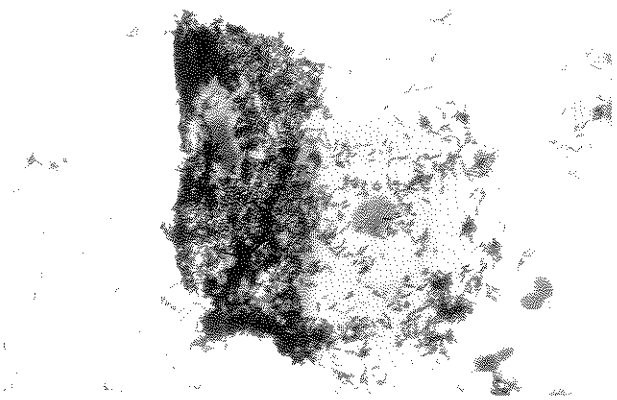

Fig. 4. False clue cells indicating cytologic vaginosis (4+), high power. Courtesy of Curatek Pharmaceutical Company.

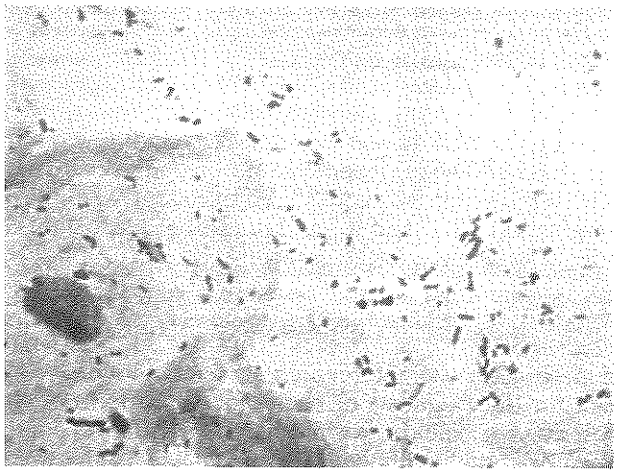

Fig. 5. Scant background bacteria (1–2+), high power. Courtesy of Curatek Pharmaceutical Company.

White blood cells are normal in small quantities, with a ratio of one WBC for every epithelial cell considered within normal limits (Sobel, 1994). Most sources state that more than 10 WBCs per high-power field is abnormal (Kaufman & Faro, 1994); however, this estimate does not take into consideration the concentration of a particular sample. For this reason, it is recommended that estimates of abnormal counts be based on a ratio of WBCs to epithelial cells, the method used to estimate the normal count of WBCs (ie, in blood) (Sobel, 1994). Expanding on this principle, I propose that a ratio of five WBCs to every epithelial cell (5:1) indicates possible mild inflamma-

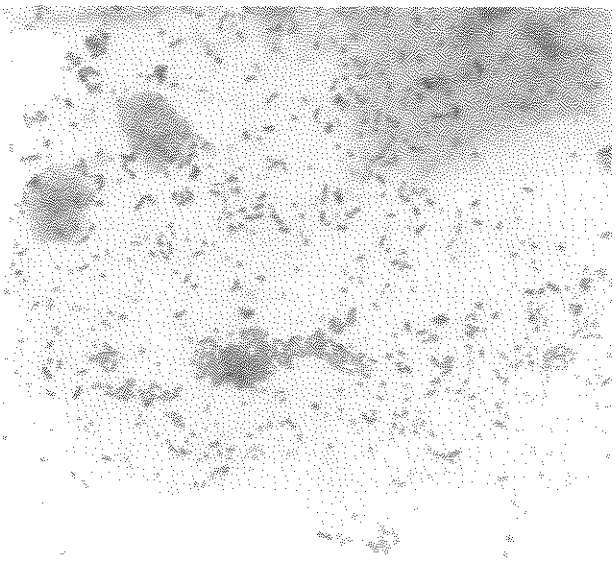

Fig. 6. Moderate background bacteria (3–4+), high power. Courtesy of Curatek Pharmaceutical Company.

tion. A ratio greater than 10:1 indicates possible moderate to severe inflammation, as seen in association with certain vaginal and cervical conditions.

While continuing to view the saline smear under high power, the epithelial cells are examined for evidence of classic clue cells associated with bacterial vaginosis and false clue cells associated with cytologic vaginosis. As a frame of reference, normal mature epithelial cells are characterized by cytoplasm that appears slightly grainy, with well-defined borders and nuclei (Fig. 3).

Sometimes described as peppered fried eggs, classic clue cells are epithelial cells coated with mixed coccobacilli (appearing as tiny dots), which cause the cell borders to lose their definition (Secor, 1994). When many bacteria are present, clue cells may be identified by locating multiple nuclei (Fig. 7). *Mobiluncus*, a type of anaerobic bacteria pathognomonic for bacterial vaginosis, are stubby, curved, comma-shaped bacilli, which characteristically twitch or vibrate in a small area (Fig. 9, central area) (Kaufman & Faro, 1994). Also of note are the reduced number of lactobacilli (straight, rod-shaped organisms) in the vaginal smears of women with bacterial vaginosis (Kaufman & Faro, 1994).

False clue cells are epithelial cells coated with and cytolyzed by normal, rod-shaped lactobacilli (Fig. 4). The borders of the epithelial cells are poorly defined, and the cell structures appear fragmented, blurry, and faint. These latter findings are a result of cytolysis or destruction of the epithelial cells by the acids produced from overgrowth of lactobacilli (Cibley & Cibley, 1991; Secor, 1992).

Excessive numbers of lactobacilli may also be present with vulvovaginal candidiasis (Kaufman & Faro, 1994). Therefore, the clinician may need to perform a yeast culture when the differential diagnosis includes cytologic vaginosis and vulvovaginal candidiasis, especially if saline and potassium hydroxide smears are negative for yeast.

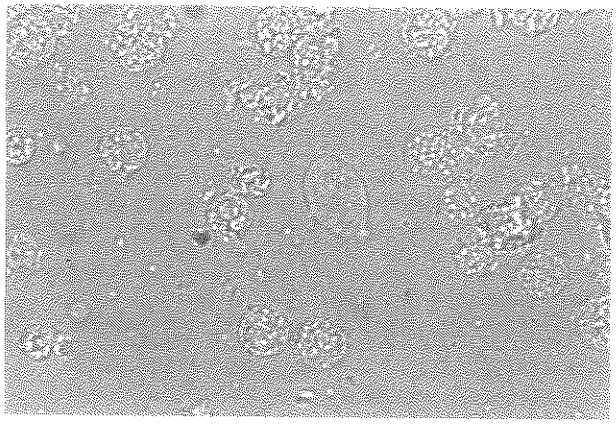

Fig. 8. White blood cells with *Trichomonas*, high power. Courtesy of Curatek Pharmaceutical Company.

Viewing the Potassium Hydroxide

Viewed under low power, the potassium hydroxide smear should be fairly concentrated, with the epithelial cells abutting one another like a cluster of balloons. Because potassium hydroxide destroys the cellular material, it distorts the shape of the epithelial cells, making them appear enlarged, rounded, and faint. These cells (ghost cells) become increasingly transparent with continuing exposure to the potassium hydroxide solution (Kaufman & Faro, 1994). Hyphal yeast forms (mycelia, pseudohyphae) become prominent and easier to identify, in contrast to the fading epithelial cells. Thus, the sensitivity rate for yeast identification is higher in the potassium hydroxide smear than in the saline smear.

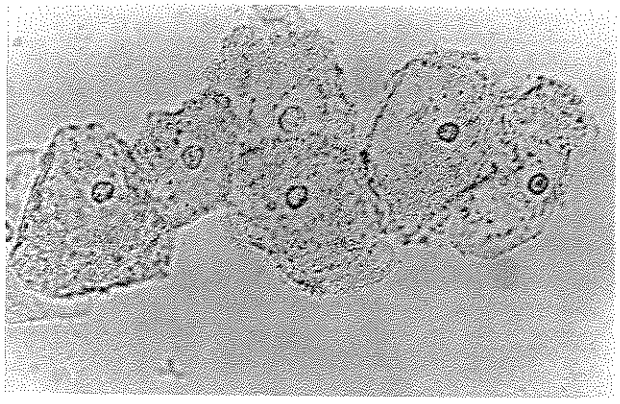

Fig. 7. Clue cells indicating bacterial vaginosis (4+), high power. Courtesy of Curatek Pharmaceutical Company.

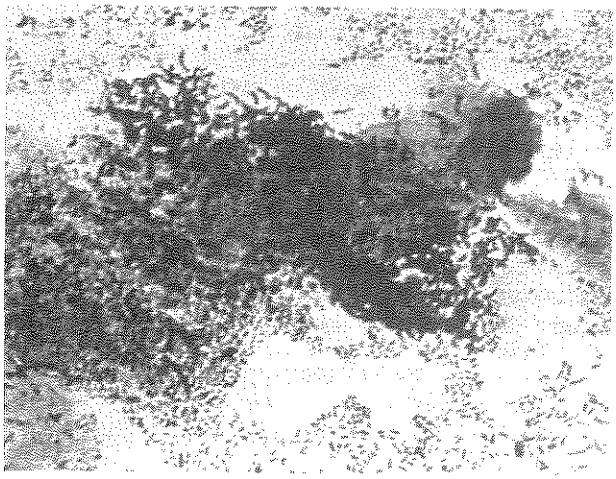

Fig. 9. Clue cells with *Mobiluncus*, high power. Courtesy of Curatek Pharmaceutical Company.

Yeast identification is performed by using both low- and high-power magnification. Under low power, hyphae and pseudohyphae appear as cobwebs or piles of twigs. A preliminary diagnosis of yeast may be made under low power, but morphologic characteristics are always confirmed under high-power magnification. Differentiating true yeast forms from various mimics (eg, fiber, long lactobacilli, hairs) is also easier under high-power magnification (Kaufman & Faro, 1994).

Viewed under high power, yeast appear in the branching hyphal form (mycelia, pseudohyphae, filaments) or in the round or oval bud form (blastospores, spores, conidia) (Fig. 10). *Candida albicans* and *Candida tropicalis* are dimorphic: they produce both hyphae and buds. *Candida glabrata* and other nonalbican species, however, are monomorphic, existing exclusively in bud form. Hyphal forms, under high power, appear tubular, thin, pale, and translucent. They are segmented, tapering like sausage links or tubular circus balloons at varying points along their lengths. Hyphae are characterized by short segments. In contrast, pseudohyphae are so named because of their longer, infrequent segments (Fig. 10) (Horowitz, 1991a). Buds are often subtle and difficult to identify, even under high-power magnification. Generally smaller than red blood cells, yeast buds appear spherical, smooth, and translucent. Resembling glass beads, they are often located around the borders of the epithelial cells and at junctions of hyphal segmentation (Fig. 10).

Differentiating yeast forms from artifacts and other mimics requires identifying the morphologic features of suspected yeast forms. These characteristics are more easily appreciated by using phase contrast microscopy (Horowitz, 1991a,b). Fibers, lint, hair, folded epithelial cells, and mucus strands are examples of artifacts and organisms that may mimic hyphal forms. Examples of yeast bud mimics include red blood cells, air bubbles, lipid globules, and vaginal medications (Kaufman & Faro, 1994).

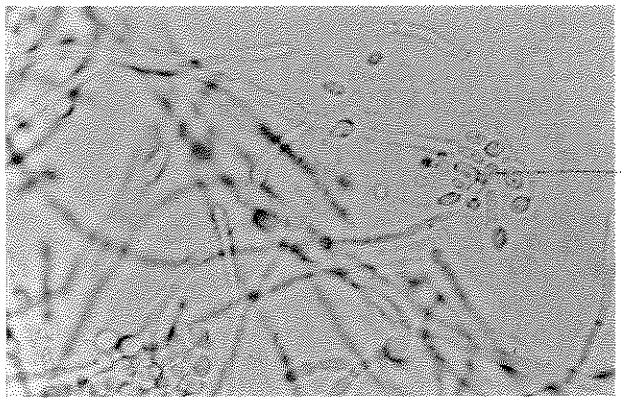

Fig. 10. Hyphae and buds, high power. Courtesy of Curatek Pharmaceutical Company.

Conclusion

Providing optimal care to women with vulvovaginal complaints requires that nurse practitioners refine their vaginal microscopy skills. With expert skills, an accurate diagnosis can be made and therapy initiated that is etiology-specific, not based on an empirical surmise. By using the recommendations described in this article for gathering, preparing, and viewing vaginal microscopic smears, a sensitivity rate of 80% may be achieved. This ensures that the majority of clients with vulvovaginal complaints will receive appropriate care and respond successfully to therapy.

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