Microscopy of the Bacterial Flora on Fresh Vaginal Smears

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Vaginal bacterial flora can be studied by microscopy of fresh vaginal fluid or of a fixed, stained smear. Classifying the flora by microscopy of fresh smears of vaginal fluid allows the pathology to be approached in a way other than by application of Nugent et al.'s criteria for Gram-stained specimens. Therefore, it may enable a more diverse and accurate assessment of the risk of preterm birth and other complications.

The classification is based on the relative quantities of lactobacillary morphotypes present. If lactobacilli of variable size predominate, the flora is considered normal (lactobacillary grade I Fig. 1). Sometimes, this grade-I flora leads to production of excessive amounts of lactate and acidity due to hydrogen peroxidase activity, even causing lethal damage to the epithelial cells (epitheliolysis). The bare nuclei of these torn cells can be seen, along with the debris of their cellular cytoplasm and with numerous lactobacilli (Fig. 2). Care must be taken to differentiate the cellular debris from coccoid bacteria and not to misinterpret the bare epithelial nuclei as leukocytes.

Lactobacillary grade II is an intermediate flora, with a mixture of lactobacilli and other morphotypes, usually coccoids. If the lactobacillary morphotypes still outnumber the other bacteria, this is called lactobacillary grade IIa (Fig. 3); if the lactobacillary morphotypes are less abundant than the other morphotypes, the term used is grade IIb (Fig. 4). Although grade II will partly overlap with the intermediate group of the bacterial vaginosis score according to Nugent et al. (score 4–6), it is certainly not identical to that group.

Lactobacillary grade III indicates complete disruption of the normal vaginal flora: other bacteria have completely overgrown the lactobacilli, which are no longer visible. The most obvious condition that would cause these lactobacilli to disappear is bacterial vaginosis, with its pathognomonic granular background appearance due to large numbers of anaerobic bacteria (Fig. 5) and the clue cells. It is not wise, however, to call every condition devoid of lactobacilli bacterial vaginosis. A search for trichomoniasis and other sexually transmitted diseases is also warranted. If the grade III flora consists of coarse, coccoid bacteria or is accompanied by numerous parabasal epithelial cells and increased vaginal leukocytosis (Fig. 6), the condition certainly does not correspond to the anaerobic condition Gardner and Dukes and Amsel et al. had in mind when discussing the clinical diagnosis of bacterial vaginosis. Again, it is unclear in which Nugent score this group belongs; most probably you would find it dispersed among the intermediate (score 4–6) or bacterial vaginosis (score 7–10) groups. Cultures often grow streptococci and gram-negative bacteria of enteric origin. We propose to call this condition “aerobic vaginitis,” a clinical entity clearly distinct from bacterial vaginosis and rather to be seen as a lesser form of “desquamative vaginitis.”

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**Fig. 1.** Lactobacillary grade I with abundant, normal appearing lactobacillary morphotypes of varying size, without other bacteria.

**Fig. 2.** Lactobacillary grade I with abundant, normal appearing lactobacillary morphotypes of varying size, in the presence of numerous cytoplasm organelles and nuclei derived from lysed epitheliocytes. The former may not be confused with coccoid bacteria, the latter not with leukocytes, both of which are infrequent in this slide.

**Fig. 3.** Lactobacillary grade IIa. Although the lactobacillary morphotypes are the predominant microbial flora, other bacteria are also frequently seen.

**Fig. 4.** Lactobacillary grade IIb. Although the lactobacillary morphotypes are evident, they are outnumbered by other coccoid bacteria.

**Fig. 5.** Lactobacillary grade III: bacterial vaginosis. Few, if any, lactobacillary morphotypes are seen, all of them being replaced by numerous staphococcoid bacteria (*Gardnerella* morphotypes). They form a granular carpet, as if “a sac of grain has been teared and spread on the floor.” Clue cells, not shown in this picture, can readily be found. Note the typical absence of leukocytes in uncomplicated cases.

**Fig. 6.** Lactobacillary grade III: aerobic vaginitis. Few, if any, lactobacillary morphotypes are seen, all of them being replaced by coccoid bacteria, often arranged in little chains or trains of 2 to 6 bacteria, such as in the case of group B streptococci. In more severe cases, as shown here, the numerosity of the leukocytes is striking, some of them filled with vividly vibrating granules, corresponding to lysosomes and phagosomes in these “toxic leukocytes.” In the most severe cases, 10 to 50 percent of the epitheliocytes are in an immature, parabasal state, such as epitheliocyte shown in lower right corner. This extreme end of aerobic vaginitis is also named “desquamative inflammatory vaginitis.”
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