

Predictive Value of the "Clue Cells" Investigation and the Amine Volatilization Test in Vaginal Infections Caused by *Gardnerella vaginalis*

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Although still controversial, an etiologic role of *Gardnerella vaginalis* is imputed in vaginitis. Besides isolation of the organism by culture, two alternative diagnostic procedures have been claimed to be useful: the investigation of "clue cells" in clinical specimens and the amine volatilization test or fishy odor perception in genital secretions. Herein we report on the findings of the simultaneous use of *G. vaginalis* isolation, the clue cell test and amine volatilization perception in specimens from 1,263 consecutive female patients referred to our clinic. Our results show that the simultaneous use of both alternative tests is very useful as a screening procedure. A negative result of both tests predicts a negative culture result in 99% of the cases. However, a positive result of either or both should be considered as an indication to proceed to culture and not as diagnostic of infection.

More than 3 decades ago, bacterial vaginitis could not be attributed to an etiologic agent. Since several different organisms (*Streptococcus* sp., *Staphylococcus* sp., diphtheroids, enteric pathogens) were found in genital specimens from these patients, the condition became termed nonspecific vaginitis.

In 1955, Gardner and Dukes (4) identified a small gram-negative bacillus in over 90% of women suffering from vaginitis, which was termed *Haemophilus vaginalis* and was thought to be the etiologic agent of this disease. Zinnemann and Turner (13) reported that this bacterium is gram-positive and therefore should be transferred to genus *Corynebacterium* as *C. vaginale*. However, Greenwood and Pickett (6) reported that it is both gram-intermediate and also unrelated to previously described genera and hence elected to place it in a new genus, *Gardnerella*, as *G. vaginalis*.

G. vaginalis appears to be a prevalent etiologic agent of nonspecific vaginitis. Its isolation from vaginal secretions is considered to be the cornerstone of diagnosis. However, two alternative procedures have been claimed to bear diagnostic value. One is the investigation of granular epithelial cells (clue cells) in freshly prepared smears of vaginal secretions (12), and the other is the perception of a fishy odor in genital secretions after the addition of potassium hydroxide, which results from the volatilization of certain amines thought to be specifically produced by *G. vaginalis* (1).

The purpose of this study was to evaluate the diagnostic value of the clue cell and amine volatilization tests in women with culture-proven *G. vaginalis* infection. Our results showed that the simultaneous use of both tests is very useful as a screening procedure.

Patients. We studied 1,263 consecutive female patients attending our clinic. Their ages ranged from 8 to 80 years with a median of 27. Most of the patients included in the study were referred to us because of some vaginal complaint; however, healthy women were also studied. The first 903 patients were studied by culture and the clue cells test; in the remaining 360 cases, the amine volatilization test was

added. In the latter group, more symptomatic women were included.

Swab smears were prepared from genital specimens, fixed with ethanol, and Gram stained. Epithelial cells with bacilli or coccobacilli attached to their surfaces were considered clue cells (12). We performed the amine volatilization (fishy odor) test by mixing equal volumes of genital fluid and a 10% KOH solution. The clear perception of a fishy odor was considered a positive result (1).

Culture isolation of *G. vaginalis*. Genital secretions were inoculated in IsoVitaleX-supplemented human and sheep blood agars. Suspicious colonies were reinoculated in human blood and chocolate agars. Gram stain, oxidase, and peroxidase activities were used as presumptive tests. If the isolated bacteria were pleomorphic, mainly bacillar, and negative for both enzymes, confirmatory tests were performed. These consisted of the inoculation of cystine tryptose agar medium with 1% potato starch, 1% raffinose, and a 1% aqueous sodium hippurate solution. The presence of hydrolysis in the first and third tests and its absence in the second was considered definitive of *G. vaginalis* isolation (2, 5, 7, 9, 10, 11).

Statistical analysis. Distribution of data was analyzed by the tetrachoric association coefficient and the chi-square test. When pertinent, the latter was Yates corrected. Predictive values were calculated by the method of Griner et al. (8).

G. vaginalis was isolated from 152 of 903 patients in the first phase of this study; 124 (81.6%) of them were clue cell positive and 28 were clue cell negative. Of 751 culture-negative patients, 114 were clue cell positive also. The association coefficient for isolation of *G. vaginalis* and the presence of clue cells was 0.9223—a statistically insignificant value. This was consonant with the great heterogeneity of the distribution of positive results ($\chi^2 = 287.14$; $P < 0.00025$). The positive predictive value of the clue cell test alone was 52.1%, and its negative predictive value was 95.8%.

In the second population of 360 female patients, *G. vaginalis* was isolated from 86 specimens, and 37 (43%) of them were positive in the amine volatilization test. Of the

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TABLE 1. Association between results of the isolation of *G. vaginalis* with the presence of clue cells and results of the amine volatilization test in genital specimens from 360 female patients

Results of isolation by culture/no. of patients	No. of patients with positive results by:			No. of patients with negative results by clue cell and amine volatilization tests
	Clue cell and amine volatilization tests	Clue cell test	Amine volatilization test	
Positive (86)	32	76	37	1
Negative (274)	38	35	10	131

remaining 274 culture-negative patients, 10 had positive amine volatilization tests. Again, the association coefficient was high ($r = 0.9000$) but statistically insignificant, for the positive results were also very heterogeneous in distribution ($\chi^2 = 89.39$; $P < 0.00025$). The positive predictive value of the amine volatilization test used alone was 78.7%, and its negative predictive value was 84.3%.

The results of the simultaneous use of the clue cell investigation, amine volatilization test, and culture isolation of *G. vaginalis* in 360 specimens are shown in Table 1. The positive predictive value of a positive result of both alternative tests was only 45%; however, the negative predictive value of a negative result of both alternative tests was 99.24%. The heterogeneous distribution of these data ($\chi^2 = 151.769$; $P < 0.00025$) reflects the large number of false-positive results obtained by both alternative tests.

Our findings indicate that neither the clue cell investigation nor the amine volatilization test has sufficient sensitivity and specificity to substitute for culture isolation for the diagnosis of infection by *G. vaginalis*. The main reason why the clue cell investigation yields false-positive results (18.5%) may be that bacteria other than *G. vaginalis* can become attached to epithelial cells. On the other hand, the number of cases (10%) in which they are absent despite positive isolation by culture may result from the inhibition of the attachment of bacteria to epithelial cells known to be caused by immunoglobulin A (3). The high proportion of cases (57%) in which the amine volatilization test was positive in the absence of the organism supports the idea (1) that the amines involved in fishy odor perception are produced by mixtures of aerobic and anaerobic bacteria and not by *G. vaginalis* exclusively.

If both tests are used simultaneously, they can be useful as a screening procedure. A negative result of both predicts negative isolation by culture in over 99% of the cases; hence, isolation could be unnecessary. However, a positive result of either or both tests should be considered as an indication to attempt isolation. Positive results should in no instance be imputed diagnostic value.

Since both alternative tests are easy to develop and rapid to perform, they might prove to be particularly suitable in first-contact clinics. Their use could rule out the need for isolation by culture in a large number of patients and select a population of women that should be referred to large medical centers for bacteriological studies.

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