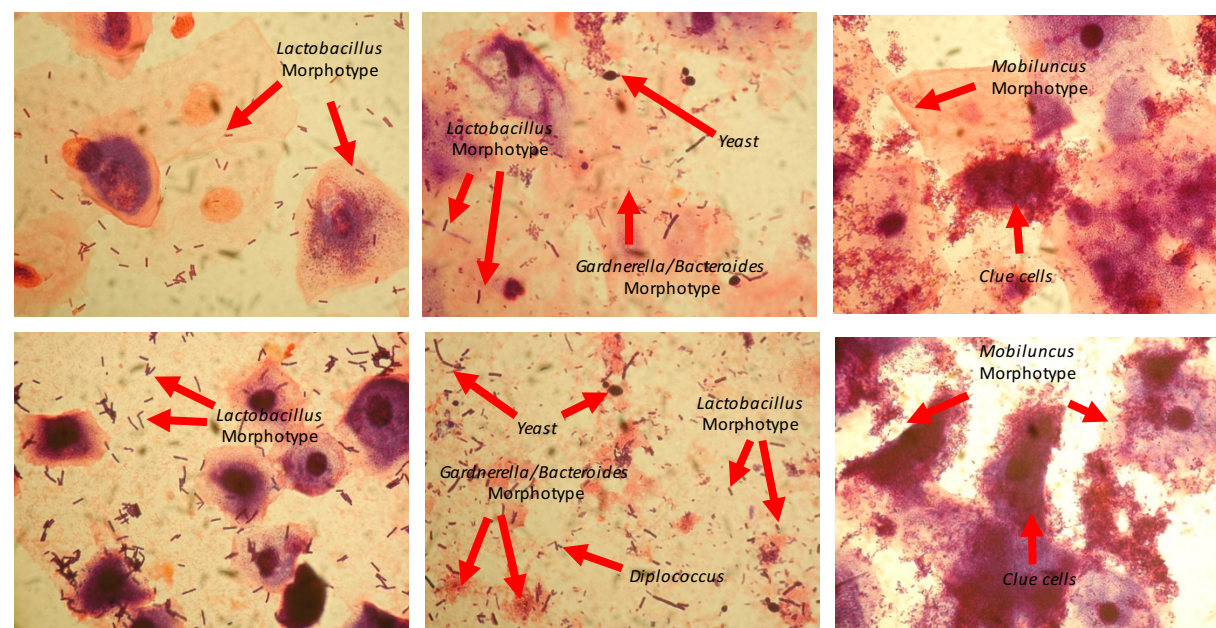


## BACKGROUND

Bacterial Vaginosis (BV) is the most common cause of abnormal vaginal discharge among women in reproductive age. BV is characterized by a change in the vaginal normal flora; a mixed microbial flora replaces the dominant *Lactobacillus* flora of the vagina. Nugent's scoring system is considered as the gold standard for the diagnosis of BV.

10 µl

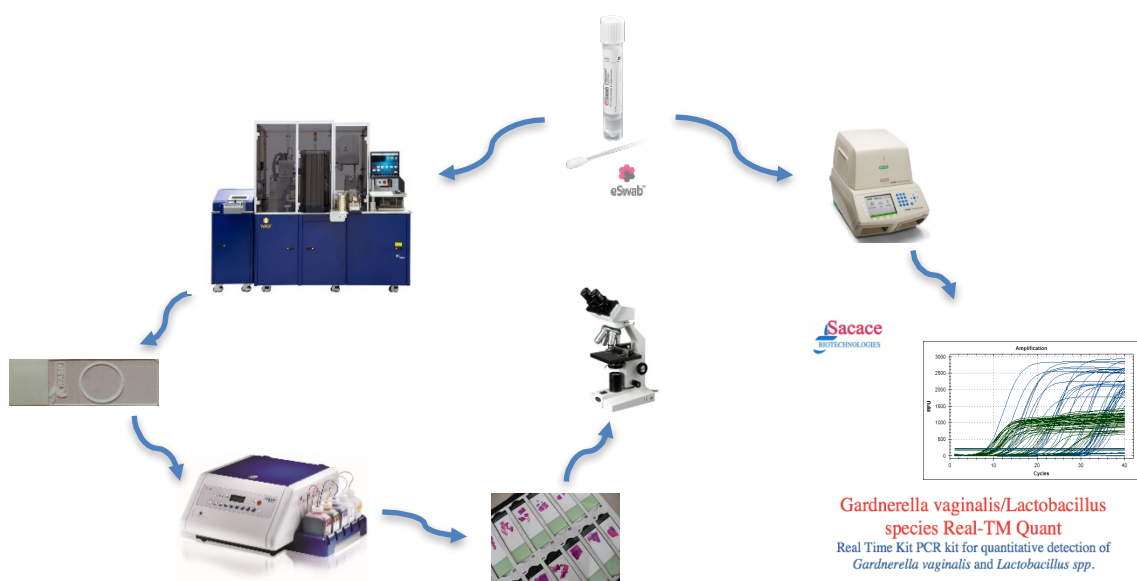
Grade I/Normal    Grade II/Intermediate    Grade III/BV



Grade I/Normal    Grade II/Intermediate    Grade III/BV

30 µl

The WASP automation and Liquid based Microbiology (LBM) concept have been introduced in the microbiology laboratories by the advent of the ESwab system. In our laboratory we have adopted the LBM system since 2008 and currently are used for a variety of tests, including culture, Gram and molecular.



The aim of this study was to compare Nugent's grading score to the Hay/Ison criterion in WASP™ automation prepared smears using 10µl and 30µl of vaginal samples collected in ESwab™.

## METHODS



100 vaginal samples were collected with ESwab™ (COPAN)

The WASP™ was used to prepare smears on slides with the 10 µl and 30 µl loop from each ESwab™ sample

All slides were alcohol fixed and Gram-stained

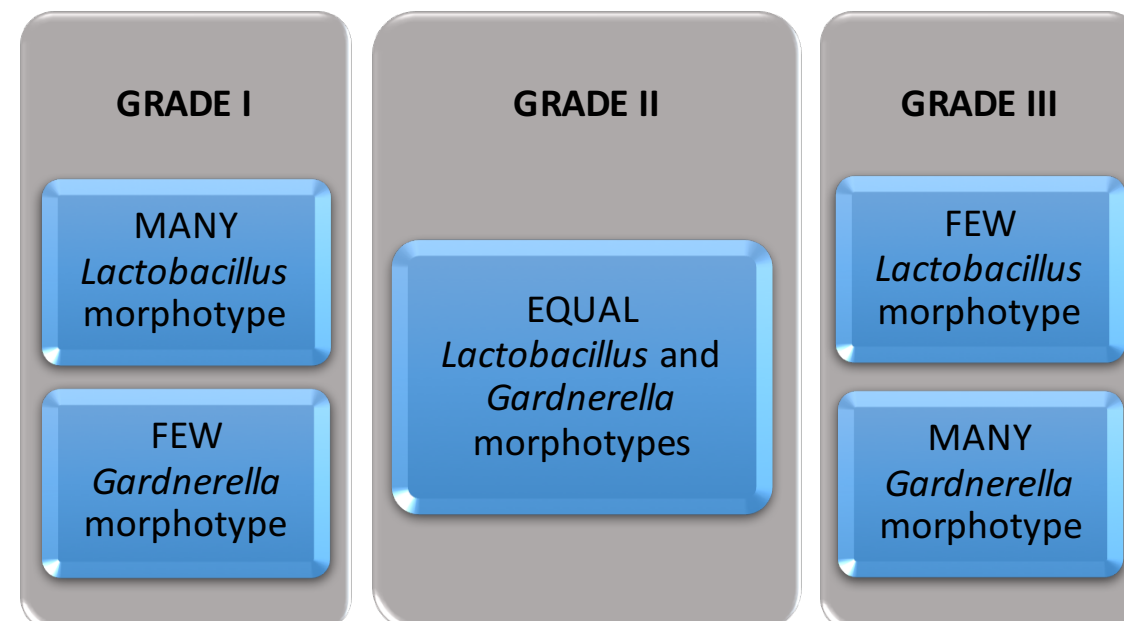
**MICROSCOPIC EXAMINATION.** All slides were examined at 1000x magnification and graded using both Nugent and Hay/Ison criteria. The slides were viewed by two different readers and reviewed by a supervisor. Nugent's score was categorized into 3 stages: 0-3 normal, 4-6 intermediate with or without Clue cells (BV or not-BV, respectively), and 7-10 BV (Table 1). The Hay/Ison was categorized as: Grade I (normal flora), composed only by *Lactobacillus*; Grade II (intermediate flora), mixed bacterial morphotypes and reduced *Lactobacillus*; Grade III (BV), mixed bacterial morphotypes with few or absent *Lactobacillus* morphotype (Table 2). In addition, the presence of clue cells was recorded.

LACTOBACILLUS MORPHOTYPE	SCORE	GARDNERELLA/BACTEROIDES MORPHOTYPES	SCORE	MOBILUNCUS MORPHOTYPE	SCORE
>30	0	0	0	0	0
5-30	1	<1	1	<1	1
1-4	2	1-4	2	1-4	1
<1	3	5-30	3	5-30	2
0	4	>30	4	>30	2

Σ SCORE	CLUE CELLS	REPORT
0-3		NO BV
4-6	Not Present	NO BV
4-6	Present	BV
7-10		BV

**Table 1: Nugent's score.** Every morphotype is classified in 5 scores, which represent different microorganism's amount (A): *Lactobacillus* and *Gardnerella/Bacteroides* have a score range of 0 to 4; instead, the *Mobiluncus* spp. is classified from 0 to 2. The final report is obtained by considering the addition of the three different morphotype scores and the presence of Clue Cells (B).



**Table 2: Hay/Ison criterion.** The vaginal flora is assessed in three different grades:

- Grade I (normal flora), describes the presence of *Lactobacillus* spp. morphotype only;
- Grade II (intermediate flora), reduced number of *Lactobacillus* spp. morphotype and similar amount of mixed bacterial morphotypes;
- Grade III (bacterial vaginosis), few or absent *Lactobacillus* morphotype and abundant presence of mixed bacterial morphotypes.

## RESULTS

Considering 10 µl ESwab™ smears (Figure 1A), the Nugent's scoring results were: 90 patients (90%) with normal vaginal flora, 5 (5%) intermediate flora and 5 (5%) BV. Instead, the Hay/Ison criterion's results were: 83 patients (83%) had Grade I, 12 (12%) Grade II and 5 (5%) Grade III (BV). About the 30 µl ESwab™ smears (Figure 1B), the Nugent's scoring results were: 92 patients (92%) with normal vaginal flora, 3 (3%) intermediate flora and 5 (5%) BV. Differently, the results obtained by using the Hay/Ison criterion were: 84 patients (84%) had Grade I, 12 (12%) had grade II and 4 (4%) had grade III (BV). Clue cells were detected in all the BV cases. Only one BV was diagnosed by Nugent's and missed by Hay/Ison criterion.

Figure 1A

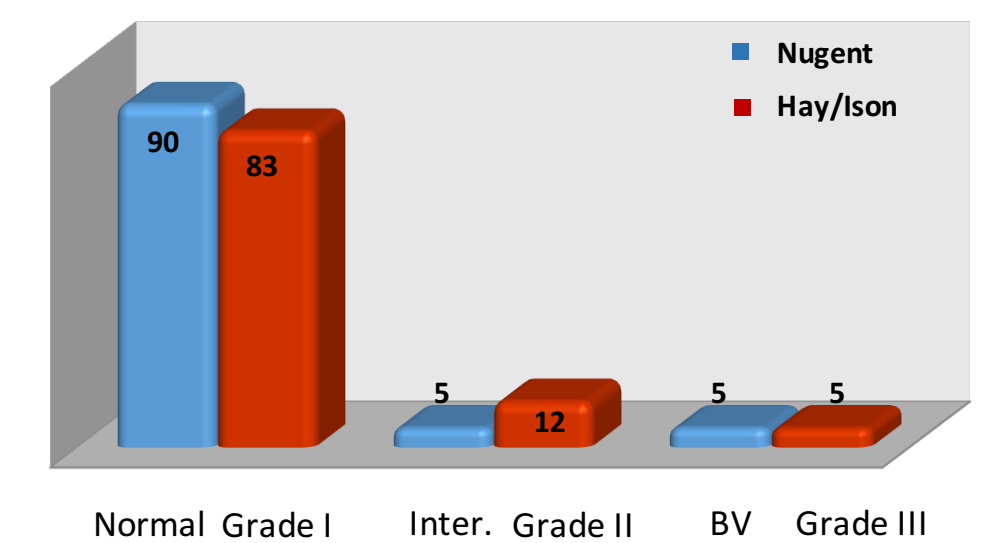
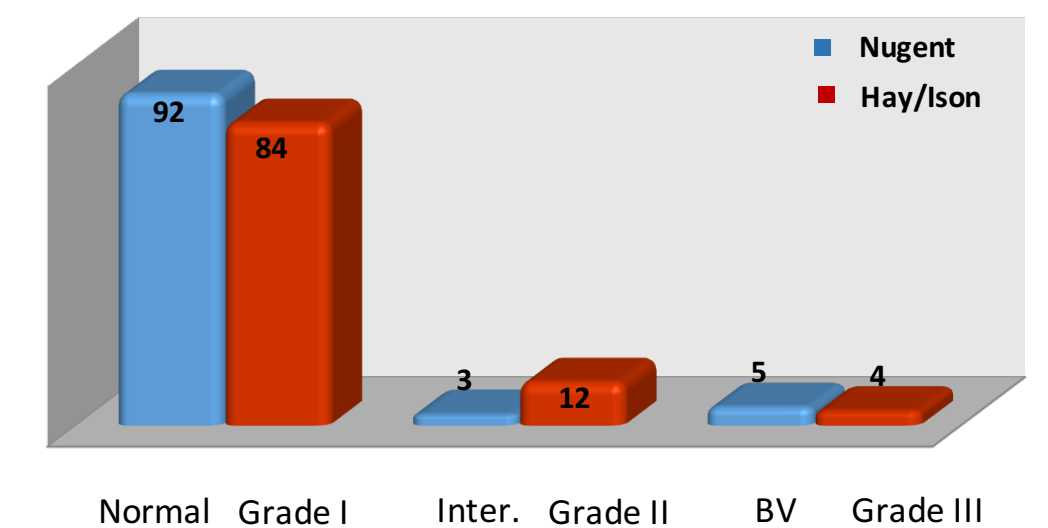


Figure 1B



## CONCLUSIONS

In this study, all cases of Bacterial Vaginosis, except one, were diagnosed in both smears prepared with 10 µl and 30 µl ESwab™ samples by both Nugent's and Hay/Ison criteria.

The only discrepant result was scored as BV according to the Nugent's in the smear prepared with 30 µl of ESwab™ sample, while was scored as Grade II according to the Hay/Ison criteria. This might be related to the simplified assessment of the Hay/Ison criterion, since the discrepant result was diagnosed as BV because of the presence of Clue cells in intermediate flora according to the Nugent's score.

The data obtained demonstrated that smears prepared by WASP™ automated system with 10 µl and 30 µl of ESwab™ vaginal samples are suitable to assess Nugent's and Hay/Ison criteria for the diagnosis of BV diagnosis.

Smears prepared with 10 µl or 30 µl of ESwab™ vaginal samples are suitable for detecting bacteria vaginosis with both Nugent's and Hay/Ison criteria