Gram-stain by a new specimen collection system

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Abstract

A comparison of results of Gram stain smears prepared from clinical specimens collected with flocked swabs and transported in an ESwab system with those collected with traditional rayon swab in Amies Gel Transystem (Copan Italia) was made on 240 slides prepared from specimens collected from 80 patients and showed that the quality of smear preparation from the ESwab system, allowed for easier identification of human cells and identification of greater number of microorganisms. Organisms more readily seen in ESwab preparations were: yeasts, Gram-negative bacilli, and Gram-positive and Gram-negative diplococci.

Background

In recent years, there has been increased emphasis placed upon the relevance of clinical microbiology. This has included preliminary evaluation of specimens to determine their value and quality for culturing pathogenic microorganisms. The Gram stain is used to classify bacteria on the basis of their forms, sizes, cellular morphologies, and Gram reactions; in a clinical microbiology laboratory, it's an additional critical test for the rapid presumptive diagnosis of infectious agents and serves to assess the quality of clinical specimens. Interpretation of Gram-stained smears involves consideration of staining characteristics and cell size, shape, and arrangement. These characteristics may be influenced by a number of variables, including culture age, media, incubation atmosphere, staining methods, and the presence of inhibitory substances. Similar considerations apply to the interpretation of smears from clinical specimens, and additional factors including: different host cell types and possible phagocytosis. Relevant is the appropriate specimen collection and transport, essential for accurate laboratory diagnosis of bacterial infections. This can be accomplished using the Copan ESwab collection and transport system. Van Horn *et al* have recently evaluated the ESwab system, on the basis of the CLSI acceptance criteria, concluding that it's an acceptable swab transport system for maintaining viability of both aerobes and anaerobes. To our knowledge no studies have been performed in order to evaluate the ESwab system compliance with the Gram-stain.

Objective

The objective was to compare smears of clinical specimens collected and transported in the ESwab system to traditional clinical specimens collected and transported in Amies Gel Transystem for detection and differentiation of bacteria with the Gram staining method

Table 1

Specimen	Microscopic examination of E Swab gram -slide s versus Amies Gel slides Results are expressed as: no. of slides presenting differences in human cells and /or microbial elements / no. of samples tested		
	ESwab Volumes for slides 100µl*	preparation 50µl*	Amies Gel slides
Vaginal Swab (32)	32/32	26/32	16/32
Cervical Swab (27)	27/27	25/27	15/27
Urethral Swab (11)	11/11	11/11	8/11
Wound Swab (10)	10/10	10/10	7/10
Total (80)	80/80 (100%)	72/80(90 %)	46/80 (57.5%)
P value		P=0.16	P=0.04

a = the results were the same even after 24 and 72h storage.

Table 3

Table 3 Human cells and /or microorganisms present in the microscopic examination of ESwab slides prepared using 100 µl of Amies medium, but not in the of ESwab slides prepared using 50 µl of Amies.

Specimens types (no.)	>10 Epithelial cells per field of view	5-6 Leucocyte s per field of view	Numerou s leucocyt es (20-30 per field of view)	Rare Red Blood Cells	Gram-positive Diplococci	Yeasts	Gram-negative Bacilli	Total
Vaginal Swab (32)	1	1			1	3		6
Cervical Swab (27)		1					1	2
Urethral Swab (11)								
Wound Swab (10)								
Total (80)								8/80 (10%)

Table 2

Microorganisms present in the microscopic examination of ESwab slides (using100µl of Amies medium) which were not in the Amies Gel slides.

Specimens types (no.)	Yeast cells	Gram-negative diplococci	Gram-positive diplococci streptococci	Trichomonas spp.	Clue cell due to Gram-negative coccobacilli	Mobilincus spp	Gram-negative bacilli	Total
Vaginal Swab (32)	6		6	1		1	3	18
Cervical Swab (27)	3		3		1		3	10
Urethral Swab (11)		1			1		1	3
Wound Swab (10)							1	1
Total (80)	9	1	9	1	2	1	8	29/80 (36.25%)

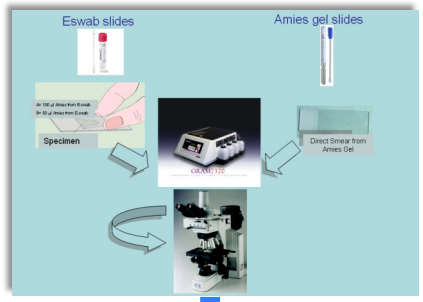
Materials & Methods

A total of 80 patients (32 vaginal swabs, 27 cervical swabs, 11 urethral swabs and 10 wound swabs) were collected and examined by Gram stain. Two swabs were collected from each patient, one using the conventional rayon swab of the Amies Gel Transystem (Copan Italia spa), the other using the nylon flocked swab of the ESwab system (Copan Italia spa). Once a swab sample was collected, it was placed immediately into the **ESwab transport tube** containing transport medium. Specimens were transported directly to the laboratory. The ESwab specimen tube was briefly vortexed then the swab was removed. An aliquot of the liquid Amies medium was used for routine culture procedures, and another was used to prepare two slides for microscopic examination. The first slide was prepared using 100 µl of liquid Amies medium from the ESwab tube, the second using 50 µl. The aliquot of liquid Amies medium was spread onto the surface of the slide with the help of a second slide (using a technique similar to that employed for making blood smears). The slides were air dried and fixed with the use of 70%-95% methanol for 1 minute (avoiding heat which alters cell morphology and makes organisms more susceptible to over-decolourization). The methanol was drained off, and the slides were air-dried. We named these slides: **ESwab-slide**.

The slides were prepared directly by rolling and smearing the swab. The slides were air dried and fixed with 70%-95% methanol for 1 minute. The methanol was then drained off, and the slides were air-dried. We named these slides: **Amies Gel-slides**.

All the slides were Gram-stained using the AEROSPRAY® MICROBIOLOGY SLIDE STAINER (Delcon Italy),

In order to evaluate the impact of a delayed microscopic examination of samples collected using the ESwab system, two sets of slides were prepared at 24 and 72h after the collection time (during this time the ESwab were stored at 5°C). The slides were prepared, stained and observed as reported above.



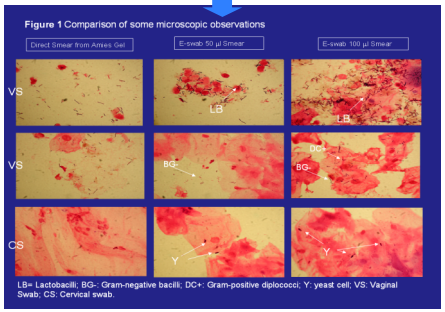


Figure 1 Comparison of some microscopic observations

LB= Lactobacilli; BG- Gram-negative bacilli; DC+: Gram-positive diplococci; Y: yeast cell; VS: Vaginal Swab; CS: Cervical swab.

Results

Microscopic examination of 240 slides from 80 specimens showed that the quality of smear preparation from the ESwab were superior to those obtained using the Amies Gel Transystem (**Table 1**). The ESwab slides prepared using 100 µl of Amies medium (22/80) were characterized by better details of the human cells such as: epithelial cells, leucocytes, red blood cells. They also contained bacteria or fungi not present in the Amies Gel slides (29/80) (**Table 2**). Differences among the ESwab slides and Amies Gel slides were statistically significant, being *p* value ≤0.04. The ESwab slides prepared using 100µl of liquid Amies medium were better then the one prepared with 50µl (**Table 3**). In addition, the slides prepared from the samples collected in the ESwab exhibit a very good preservation of cells. The micro-organisms which were more frequently observed in ESwab slides and not in Amies Gel slides were listed in table 2 and figure 1 illustrated some examples. Bacterial morphology (shape, colour, shine, etc.) was often more distinguishable in ESwab slides, especially in distinguishing diplococci (either gram-positive or gram-negative diplococci) (**Table 2** and **Figure 1**) Comparing the results of fresh ESwab slides with those after 24 and 72h storage, no significant differences were observed (**Table 1**).

Discussion

This study showed that the ESwab system identified more human cell and bacterial species than the traditional Amies gel system. These differences are mainly attributed to the flocked swab collection device. The flocked swab collected bacteria by capillary action whereas the traditional swab absorbed bacteria into the cotton fiber matrix. The flocked swab demonstrates a superior absorption and release (onto the slide surface) as evidenced ESwab system, allowed for easier identification of human cells and identification of greater number of microorganisms. Organisms more readily seen in ESwab preparations were yeasts, Gram-negative bacilli, and Gram-positive and Gram-negative diplococci. Slides prepared from ESwab at 24 or 72 hours after collection were equal to those prepared when received in the laboratory.

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