

Identification of cervicovaginal flora in liquid-based *Surepath*[™]. Results of rodeo study.

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ABSTRACT

Aim of the study: To evaluate the morphological pattern of cervicovaginal flora elements in liquid based SurePath[™] cytology.

Material and methods: During the period between May, 2010 and August, 2011, 10.166 gynecological samples preserved in liquid based SurePath were evaluated at Barretos Cancer Hospital, in order to identify cervicovaginal flora.

Results: Data showed that *Lactobacillus sp* were observed in 48.92% of the exams, Bacilli in 25.19%, Cocci in 11.77%, *Candida sp* in 5.14%, *Gardnerella sp* in 11.41%, *Trichomonas vaginalis* in 0.57% and in 0.02% of the exams the effects of *Herpes virus*. Any microorganism was observed in 2.45% of the exams.

Conclusion: This study demonstrates that the liquid based preparation does not interfere with the identification of cervicovaginal flora.

Key-words: Cervicovaginal flora; liquid based cytology

INTRODUCTION

The *Papanicolaou* test was developed by the greek physician Georgios Nicholas Papanicolaou (or George Papanicolaou), who developed a cytological investigation focused in understanding the phases of the menstrual cycle and the hormonal activity¹. The *Papanicolaou* test has been used for several years to prevent cervical cancer, and since then has enabled the identification of both cellular changes and infectious agents of the female reproductive system².

In several countries, the conventional method used to prepare samples was replaced by the liquid-based cytology (LBC). The LBC was created with the aim of diminishing the technical limitations of the preparation of the slides, establishing a staining pattern and facilitating the detection of abnormalities through an automated (computer-oriented) or manual reading. It was so well-received that it is, to this date, widely used by practitioners, even without the automated reading system³. The most frequently used LBC methods are *SurePath*TM and *Thinprep*TM.

Besides the advantages associated with the preparation of the sample, the LBC enables a more successful randomization of the cells which are effectively transferred to the slides, preventing undesirable sample losses. Using the *SurePath*TM method the collected sample is almost entirely processed, and the result is a cell preparation with 13 mm of diameter on the slide⁴.

On the conventional preparation, it is possible to identify the presence of resident vaginal flora, in accordance with what was shown by a research made in Finland. This research proved that the Pap stained smear has a sensitivity of 0.85 and a specificity of 0.92 to identify bacterial vaginosis – *Gardnerella vaginalis*, *Mobiluncus* and *Prevotella*⁵. Another study, which compared the conventional smear test with the liquid-based *SurePath*TM method, revealed that the capacity of detecting *Candida sp* microorganisms in liquid-based environments was almost two times higher than through the conventional method. However, in what concerns to the identification of *Trichomonas* and *Gardnerella*, the conventional preparations enabled a better

detection⁶.

The microbiological types whose presence is more frequent in the cervicovaginal samples are: *Lactobacillus*, a gram-positive non-pathogenic bacillus, blue-stained through the *Papanicolaou* staining technique; *Gardnerella vaginalis*, a small bacillus associated with bacterial vaginosis, generally widespread and with a film-like appearance, which adheres to the cytoplasm of the squamous cell forming the “clue cell”³; *Trichomonas vaginalis*, a sexually transmitted pear-shaped protozoan; and *Candida sp*, an eosinophilic fungus which manifests itself in the form of hyphae filaments or spores³. Still worthy of a reference is the presence of the *Herpes simplex* virus, identified by the modifications it causes in cells, like multinucleation, molding of the glass-shaped nucleus and/or eosinophilic nuclear inclusions³.

As a consequence of the elimination of intervenient factors (such as mucus and blood) during the preparation of the LBC, doubts arise frequently in what concerns to the potential elimination of microbiota from the cervicovaginal preparations, which could be regarded as a disadvantage of the LBC when compared to conventional cytology. It should be stressed that the primary goal of the *Papanicolaou* test is to identify malignant and premalignant alterations of the cervix, not to identify etiological agents. However, still acknowledging the importance of this variable in some contexts, the aim of this work was to evaluate the efficacy in identifying microorganisms in cervicovaginal liquid-based examinations, collected through *Surepath*TM and stained with *Papanicolaou* method.

MATERIALS AND METHODS

The collection of the cervicovaginal samples was performed among women referred to the ambulatory service of the ‘Barretos Cancer Hospital’ (BCH), as well as women who were examined in the prevention services of the BCH Mobile Clinics, and women who were consulted by a gynaecologist in regions which submitted the exams to the BCH Pathology Department, between May 2010 and

August 2011.

From a total of 33.998 cervicovaginal cytological exams, collected in liquid-based medium and interpreted through the 2001 *Bethesda* System, 10.166 exams were selected by simple random sampling, and then retrospectively analysed.

The samples were collected through the liquid-based *SurePath™* method (TriPath Imaging, Burlington, NC, USA) and the slides processed by *PrepMate™* and *PrepStain™* systems.

The slides were examined by a group of four cytologists and two cytotechnicians with 2-12 years of professional experience (an average of 6.8 years) under a light microscope, in a traditional way. The Nikon Eclipse E200 with panoramic objectives (4X) was the chosen microscope, with 10X and 40X magnifications.

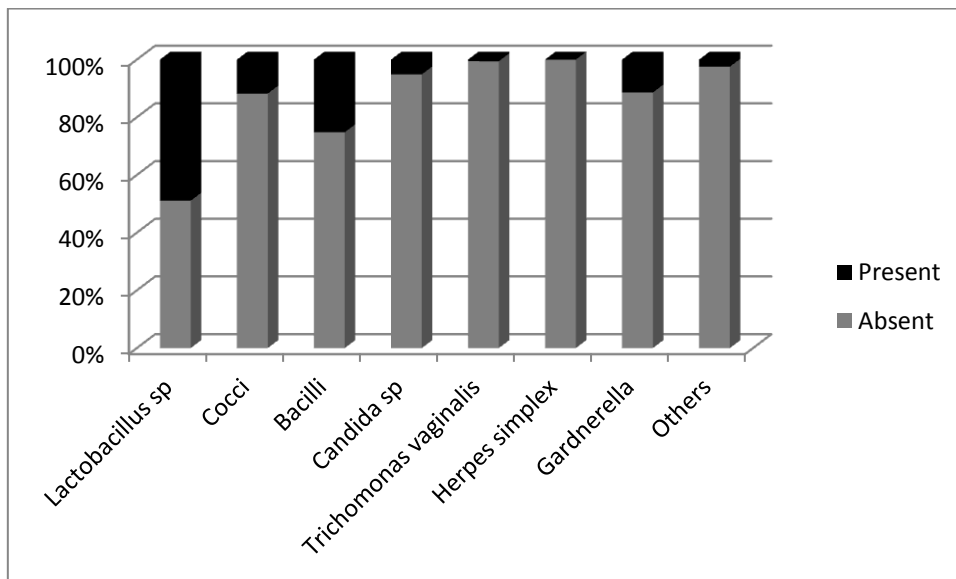
The data analysis was carried out by the SPSS software for Windows® v. 20.0 (Inc., Chicago, IL, USA). The *Mann-Whitney* test, the Chi-square and the T tests were applied to perform the statistical analysis.

RESULTS

After examining all the samples, it was possible to observe 4975 (48.92%) cases with *Lactobacillus sp*, 2560 (25.19%) with Bacilli, 1197 (11.77%) with Cocci, 523 (5.14%) with *Candida sp*, 1161 (11.41%) with *Gardnerella*, 58 (0.57%) with *Trichomonas vaginalis* and 2 (0.02%) with a cytopathic effect of the *Herpes* virus. In 249 (2.45%) cases no microorganisms were observed (Others) (**Graphic 1**).

The main characteristics of the patients who participated in this study are presented in **Table 1**. The average age of the females was 45 years old (sd = 13.9), varying from 13 to 96 years old. It should be emphasized that most of these women are aged between 31 and 60, have a low educational level and were born or living in the state of São Paulo (**Table 1**).

Table 2 indicates the average age of the participants and the most prevalent microorganisms associated with each one of them, and **Table 3** reports the frequency of the morphologically identified microorganisms in the cervical preparations preserved in liquid-based processed slides, according to the age group of the patients.



Graphic 1 – Percentage of the different microorganisms identified in the cervicovaginal samples.

Table 1- Characteristics of the patients

		n	%
Age group	11 a 20	367	3.6%
	21 a 30	1369	13.5%
	31 a 40	1917	18.9%
	41 a 50	2733	26.9%
	51 a 60	2271	22.4%
	61 a 70	1178	11.6%
	71 a 80	277	2.7%
	81 a 90	29	0.3%
	91 a 100	3	0.0%
Education	No information	1370	13.9%
	Illiterate	1015	10.3%
	Incomplete Elementary School	3644	36.9%
	Complete Elementary School	1355	13.7%
	Complete High School	1901	19.2%
	College/University degrees	595	6.0%
State of Origin	Goiás	124	1.3%
	Mato Grosso	325	3.4%
	Mato Grosso do Sul	506	5.3%
	Minas Gerais	502	5.2%
	Pará	13	0.1%
	Rondônia	550	5.7%
	São Paulo	7533	78.6%
	Others	34	0,3%

Table 2 – Evaluation of the presence of microorganisms according to the age of the patients.

	Presence of microorganisms	Average Age	Minimum	Maximum	P value
<i>Lactobacillus sp</i>	No	48	14	96	
	Yes	42	13	88	<0,01
Cocos	No	44	13	96	
	Yes	57	16	91	<0,01
Bacilli	No	45	13	91	
	Yes	47	14	96	<0,01
<i>Candida sp</i>	No	46	13	96	
	Yes	38	13	77	<0,01
<i>Gardnella sp</i>	No	46	13	96	
	Yes	41	15	86	<0,01
<i>Trichomonas vaginalis*</i>	No	45	13	96	
	Yes	48	20	69	0,71

Results of the Mann-Whitney test. (*For the *Trichomonas vaginalis* group, it was applied the T test)

Table 3 – Number of patients with infection by microorganisms according to the age group.

Age	<i>Lactobacillus</i>		<i>Cocos</i>		<i>Bacilli</i>		<i>Candida sp</i>		<i>Gardnerella sp</i>		<i>Trichomonas vaginalis</i>		Others	
	n	%*	n	%*	n	%*	n	%*	n	%*	n	%*	n	%*
11 a 20	212	2.1	9	0.1	81	0.8	48	0.5	64	0.6	1	0.0	0	0
21 a 30	823	8.1	32	0.3	302	3.0	111	1.1	206	2.0	2	0.0	6	0.0
31 a 40	1129	11.1	58	0.6	447	4.4	130	0.1	265	2.6	12	0.1	14	0.1
41 a 50	1524	15.0	163	1.6	649	6.4	143	1.4	368	3.6	20	0.2	23	0.2
51 a 60	880	8.7	474	4.7	636	6.3	72	0.7	197	1.94	16	0.2	81	0.8
61 a 70	338	3.3	370	3.6	333	3.3	13	0.1	50	0.5	7	0.0	82	0.8
71 a 80	55	0.5	81	0.8	98	0.9	6	0.1	7	0.1	0	0	32	0.3
81 a 90	5	0.1	8	0.1	10	0.1	0	0	2	0.0	0	0	3	0.0
91 a 100	0	0	1	0.0	2	0.0	0	0	0	0	0	0	0	0
Total	4966	48.9	1196	11.8	2558	25.2	523	5.1	1159	11.4	58	0.6	241	2.4

*Percentage regarding a total of 10,144 patients (no data on the age of 22 patients)

DISCUSSION

The present study was conducted in a reference hospital in the prevention and treatment of cervical cancer in Brazil, the BCH. Every year, an average of 150.000 cytology samples are performed at BCH, and subsequently observed and examined by a group of four cytologists and five cytotechnicians. The samples were observed during the daily routine at the BCH's Pathology Department. All the intervenient cytologists are highly qualified professionals, who received previous training in order to become familiar with the procedures for the analysis of the samples processed through liquid-based *SurePath*[™]. The aforementioned training consisted in theoretical and practical classes taught by BD (Becton Dickinson) qualified specialists.

The patients who participated in this study were aged between 13 and 96 years old. Of all these women (10.166) only 8.510 answered to the questions related to their educational level, and of all of those, the majority (43%) had not completed elementary school. The patient's most likely state of origin was São Paulo (78.6%), mainly because of the hospital's location.

Most of the patients presented *Lactobacillus sp* (48.9%). Lactobacilli are responsible for protecting the vaginal epithelium; they also convert sugars in lactic acid and maintain the acid pH, enabling innate immunity⁷. In

what concerns the tumour cells on the cervix, according to *Motivaseli et al*⁸, lactobacilli exert a cytotoxic effect independent of the pH and the lactate.

Concerning the presence of *Gardnerella sp*, *Candidas sp* and *Trichomonas vaginalis*, the obtained percentages were 11.41%, 5.14% and 0.57%, respectively. However, another study conducted in Fortaleza (Brazil) revealed higher rates of the presence of these microorganisms, namely 18.6% of *Gardnerella sp*, 5.7% of *Candida sp* and 3.0% of *Trichomonas vaginalis*⁹.

In the current study, the detection of *Trichomonas* (0.57%) was higher than the one registered in the study conducted with the *ThinPrep* - Cytic system (Marlborough, Massachusetts, U.S.A.), in which was noticed the presence of *Trichomonas vaginalis* in 0.2% of the collected samples between 2005 and 2008¹⁰.

According to the age group of the women involved in the study, the largest amount of *Lactobacillus* (15.0%), bacillus (6.4%), *Candida sp* (1.4%), *Gardnerella sp* (3.6%) and *Trichomonas vaginalis* (0.2%) was found in patients aged between 41 and 50, while the largest amount of cocci (4,7%) was registered in patients aged between 51 and 60. The fact that the highest concentration of microorganisms was found in patients aged between 41 and 50 can be explained, in a certain

extent, by the data which indicates that the majority of the women involved in the study belongs to this age group (26.9%).

Another study, conducted by the Federal University of Goiás (Brazil) in 2005, revealed a prevalence of 20%, 8% and 2% to *Gardnella vaginalis*, *Candida sp* and *Trichomonas vaginalis*, respectively. The patients from the most affected age group were aged between 21 and 40 years old¹¹.

CONCLUSION

The *Papanicolaou* test can be used not only to detect lesions which prompt the development of cervical cancer, but also to detect the presence of microorganisms. The results of this study clearly demonstrate that the process used to prepare the cervicovaginal samples through the liquid-based *SurePath*[™] method maintains the presence of the flora elements (resident or other) unaltered in most of the patients. The absence of any kind of microorganisms was evident in only 2.45% of the cases.

This study also demonstrated that, even with the purpose of avoiding interfering factors in the cytopathologic analysis, the liquid-based method *SurePath*[™] enables the observation of microorganisms in cervicovaginal samples.

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