

## Original Investigation

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### PAP SMEAR VS LIQUID BASED CYTOLOGY (CITOFEM®) PRAEVENTIO LABORATORY, SAN JOSÉ, COSTA RICA FROM 2003 TO 2009

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**Summary:** This document is about an observational descriptive study, which evaluates the cytological diagnosis analyzed at PRAEVENTIO laboratory, from 2003 to 2009.

In the present study the diagnoses of a total of 58 297 pap smears were compared, 31 601 were pap smears (conventional cytology) and 26 696 were Citofem® (liquid based cytology). The studies were evaluated by cytotechnologists and the pathologist. The results showed that Citofem® liquid based cytology, increased diagnostics for low-grade squamous intraepithelial lesions (LSIL) in 56% and 8.73% for high-grade squamous intraepithelial lesions (HSIL), reduces the inflammatory diagnoses in a 31.3% and increases the negative diagnostics for malignant cells in 34.6%

**Conclusions:** There is a frank inclination by the numbers favoring the liquid cytological diagnosis of the Citofem brand, where it is evident that lesions get detected, but it is as simple as a regular pap smear, having a similar or lower cost. The current study leaves the door open for more complementary studies.

**Keywords:** Vaginal cytological diagnosis, pap smear, liquid cytology, cervical cancer screening.

**Abstract:** This is a descriptive observational study which evaluated the cytological diagnoses analyzed in the PRAEVENTIO's Laboratory, from 2003 to 2009. In the present study was compared the diagnoses of a total of 58 297 Pap smears, of which of one were pap smears 31 601 (conventional cytology) and 26 696 were Citofem® (liquid based cytology). The studies were evaluated by cytotechnologists and the pathologist. The results showed that Citofem® liquid cytology, increased diagnostics for low-grade intraepithelial lesions (LSIL) in 56% and 8.73% for high-grade intraepithelial lesions (HSIL), reduces the inflammatory diagnoses in a 31.3% and increases the diagnostic of negative for malignant cells in 34.6%.

**Conclusions:** There is a clear inclination of the numbers for liquid cytology Citofem® where more lesions are detected but also a liquid cytology as simple and cheap as a pap smear. This study leaves the

door open for the completion of more studies.

**Keywords:** Pap smear, Pap test, liquid cytology, cervical cancer screening.

### Introduction

George Nicholas Papanicolaou introduced the diagnostic cytology from cervical smear of the cervix in 1940, the application of this screening test in massive form increased the detection of uterine cervical cancer, and the premalignant lesions in early stages, which made an important decrease in the incidence and mortality at different population groups (2-5). The incidence of the disease is estimated in a ratio of 40 cases out of 100.000 women in third world countries (6).

In Costa Rica, the pap smear has been under use by Social Security during many years, however, the liquid based cytology was introduced ten years ago, which has started to grow up at the private sector. In 2001, the Praeventio lab was the pioneer on its introduction in Costa Rica.

In a pap smear, the sample collection is made with two devices: the first consist of a wooden spatula for the collection of ectocervical cells, the second; consist of a brush (cytobrush) for the collection of endocervical cells. Once the sample has been taken, the cells are then transferred by smearing them in a glass slide. With this procedure, only a 10 to 20% of the cellular material of the patient is placed, and the rest gets disposed of. Immediately after, the material is to get fixed, generally using a known

solution such as carbowax, a mixture of alcohol and hair lacquer.

As an alternative to the conventional cytological diagnosis, the Liquid Based Cytology (LBC) was created 15 years ago and placed on the market. (1).

Today, there are several trademarks and one of them is Citofem®, which is known for being more simple and cheaper than other brands and has gotten into use in the majority of the pathology laboratories in Costa Rica. The other trademarks require expensive and sophisticated equipment, which Citofem does not require.

The Liquid Based Cytology sample is taken as the pap smear, but uses an anatomical collection device, with removable head, product of the combination of the plastic spatula and the endocervical brush, however, in contrast with the conventional cytological diagnosis where the sample is then smeared in the slide, in this method, the head of the brush is removed and introduced in a fixing dissolution where the cells are dispersed and the sample cleaning process is commenced. Once at the lab, the sample (cells), are collected and concentrated selectively through filters or centrifugation, so they can be then transferred to the slide for tincture and diagnosis. As the sample is immediately fixed after its collection and as in the process the materials which are able to obscure the evaluation of the epithelial cells, such as blood, mucus and inflammatory cells are eliminated, there are a few artifacts in the cellular morphology; furthermore, the cells are deposited in

a single cellular layer, which as in a mosaic, makes easier the cellular observation. (18, 19) One of the advantages gotten through this method was the decrease on inadequate acquired samples, according to a study it reduces the ratio of inadequate samples from 9 percent to 1-2 percent and decreases the time spent on interpretation as it makes easier the observation of the cells. (18)

The performed studies estimates that the specificity of the conventional cytology is of 0.98 (95% of confidence interval) and with a sensibility of 0.51 (95% of confidence interval). In relationship with the liquid based cytology, the few performed studies which used histological and colposcopy standards, reports sensibility and specificity within the reported ratios for the conventional cytologies, however, the studies compares the new technique with the conventional smear using only the cytology as a comparison standard. (18)

In the processing regard, the methods utilized by the different liquid based cytology trademarks can be classified in the following two: cellular separation through filtration and cellular separation through centrifugation. Between these two, there is an important difference on costs; the filtration system implies high tech equipment, thus, having a very high economical value. On the liquid based cytology trademarks, one of them promotes the usage of tilting or floating-like rotors, and a series of equipment of the type of vortex, sedimentation plate, analytical micropipettes, and glass slides

positively charged. However, Citofem does not require more than a conventional fixed angle centrifuge and a heating source to dry the slides, with a final price close to \$150.

### Materials and Methods

The present study is of observational descriptive nature of diagnosis performed at the Praeventio cytopathology lab in the period comprised between 2003 and 2009; for this purpose, **58297** cytological diagnosis, of which **31601** were pap smears (conventional cytology) and **26696** were Citofem® (liquid based cytology). The cytology diagnosis were taken at private gynecology consultancies, private clinics, enterprise medical consultancies and general medicine consultancies, located mostly at the San Jose central valley and others outside, such as Guanacaste, Puntarenas and Limón. The patient ages varied between 15 and 75 years old

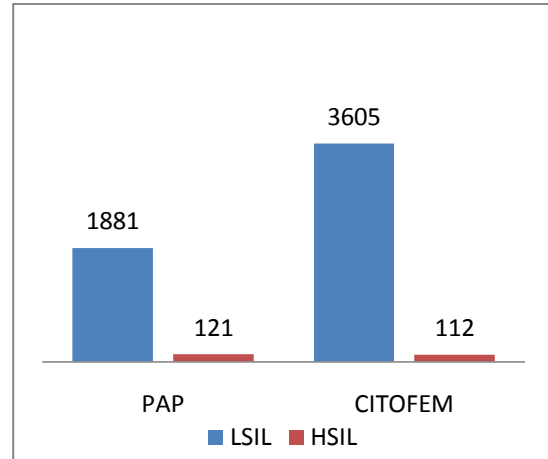
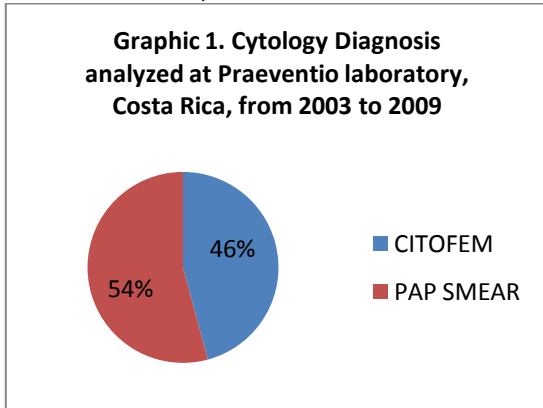
Two historical groups were selected (direct to vial): one screened with traditional cytology diagnosis (**31601**) and other with Citofem® (liquid based cytology) (**26696**). The diagnoses were tabulated following the Bethesda method by the pathologist and the cytotechnologists of the Laboratory. Furthermore, the findings were tabulated considering the derived data from the cytology diagnosis, which were integrated afterwards for relevant information procurement for the study.

### Results

The graphic shows that out of 58297 cytology diagnosis evaluated, 26696 (45,8%) corresponds to liquid based

cytology Citofem and 31601 (54,2%) to pap smears.

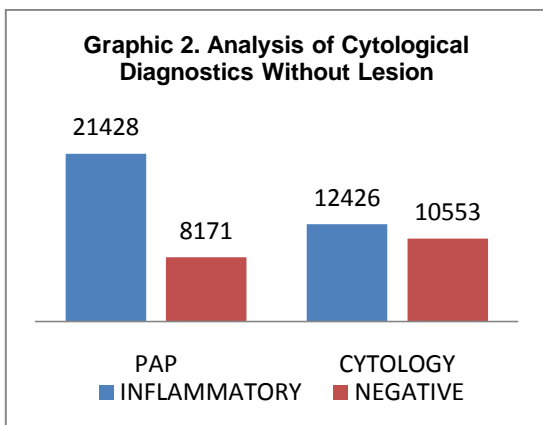
**GRAPHIC 1. CYTOLOGY DIAGNOSIS ANALYZED AT PRAEVENTIO LABORATORY, COSTA RICA, FROM 2003 TO 2009**



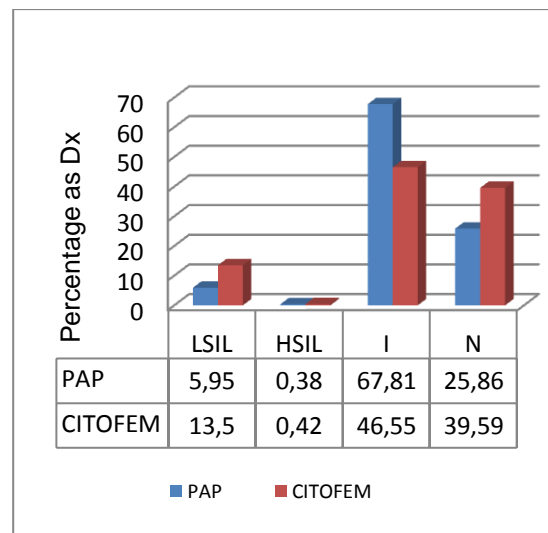
The graphic 3 shows that the liquid based cytology Citofem, doubles the diagnosis for the Low-grade Lesions (LSIL) and shows an adjusted percent for the High grade lesions (HSIL) between Citofem and pap smear or conventional cytology, which is necessary for the analysis of the percentage difference analyzed at graphic 5.

The Graphic no. 2 shows in the liquid based cytology Citofem, an increase on the negative diagnostics for malignant cells and a decrease in the inflammatory diagnostics.

**GRAPHIC 2. ANALYSIS OF CYTOLOGICAL DIAGNOSTICS WITHOUT LESION**



**GRAPHIC 4. PERCENTAGE ANALYSIS AS PER CYTOLOGICAL DIAGNOSTICS**

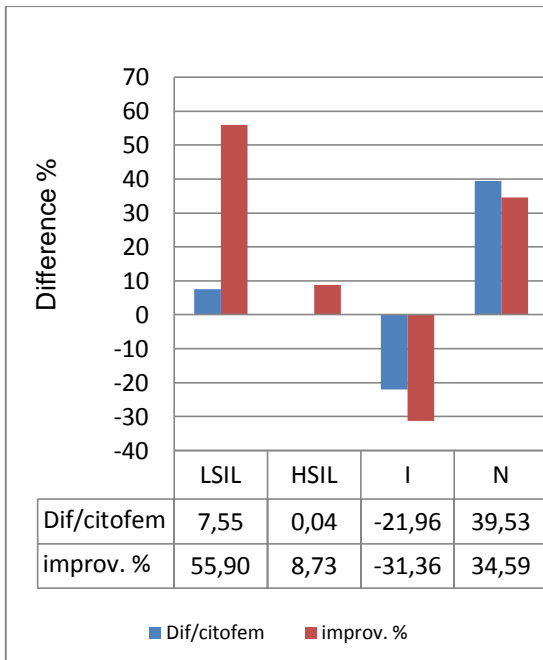


**GRAPHIC 3. INTRAEPITHELIAL LESIONS DIAGNOSIS ANALYSIS**

By analyzing graphic no. 4, we find a percentage difference favoring Citofem at diagnostic cytologies for

HSIL, which was not evident to the numerical analysis of graphic 3. Furthermore, an improvement in all diagnostics is evident.

**GRAPHIC 5. PERCENTAGE DIFFERENCE ANALYSIS FAVORING CITOFEM®**



Graphic 5, shows a factual percentage improvement as a result of the difference of the number of cytological analysis for high grade squamous intraepithelial lesions, close to 9%, and of 56% for low-grade lesions and a decrease of 31% on the inflammatory diagnosis

**Discussion**

This analysis shows us that the liquid based cytology of the Citofem trademark, valued by a team accustomed to the conventional cytology diagnosis, when reviewing the Citofem slides, increases the diagnostics for low and high grade precancerous lesions. The scientific publications mention us that the liquid

based cytology optimizes in general the quality of the smear at the processed slide, as cells arrange themselves next to each other, making a perfect mosaic, without obstacles decreasing the visibility of the precancerous cells, being the reason behind the increase of diagnosis, as these become more evident (see graphic 3, 4, and 5). These results are in accordance with the findings of other liquid based cytology diagnostics published around the world such as of those of Carpenter et al, and Papillo et al. (23-24)

The liquid based cytology eliminates considerably the amount of blood, mucus and inflammatory cells of the smear in comparison with the pap smear, therefore, it is logical the increase of the negative diagnostics for malignant cells and the decrease of inflammatory diagnostics (See graphic 2). (7-9, 18, 19)

Although being true that the liquid cytology has not reached the point of substituting the pap smear due to its lower cost (1), the Citofem can be seen as the modern substitute, as currently at the Praeventio Lab, is cheaper to receive, process, analyze and issue a cytology diagnostic for Citofem than for a pap smear. This experience can be analyzed by social security entities for their benefit. It is logical that the cost of the devices for taking the pap smear sample are very cheap, but when analyzing the total process up to the issuance of a report, we can realize that a pap smear is not as cheap as the Citofem.

One of the advantages of the processing of Citofem is that it is very

simple and eliminates the usage of toxic substances such as Xilol of the tincture recipients; furthermore, it increases the average lifespan of coloring solutions in such recipients. Another one of the advantages of Citofem is that the pap smears, generally comes fixed with substances from the medical consultancies, product of the mixture of 95% alcohol with hair lacquer, which generates residues contaminating the alcohol and helping to reduce the lifespan of the dyes.

Another considerable advantage of the Citofem system in regards to the cost is that it introduces as part of the process a resin (cubliq) which substitutes in total the usage of a mounting medium and the coverslip, making the functions of the two on half of the traditional price of the mounting medium, but with the great advantage of the savings made by not buying a glass coverslip. This biotechnology innovation contributes to the environment, as it makes possible the reutilization of the slides once the legal medical storage time and patient sample preservation time has passed.

At the social security level, the total cost of cytological analysis would be reduced considerably taking into account the following factors:

- The cytotechnologists making diagnosis, can only review and report 5 slides per hour, due to the smear being extended all through the slide and with a great deal of artifacts. With Citofem this amount can be increased in a negotiable and

pertinent way with the cytotechnologists.

- Those that process the pap smears at the Social Security can process a maximum of 75 cytology diagnosis per hour per person, a very low quantity taking into account that the step required is to apply the tincture. With Citofem at the Praeventio laboratory, 96 cytology diagnoses per person per hour are processed. This is done as Citofem shortens the steps of the current liquid based cytologies to two: centrifugation and mounting material in the slides. Making and administering the same work equipment used in such centers, the amount of slides processed per hour per person can be increased considerably.
- The total investment by Social Security implies:
  - a) Sampling supplies
  - b) Processing
  - C) diagnostic
  - d) This obviously carries an annual budget.

If we extrapolate this to the experience with the Citofem system we could show that:

- are
  - a) The devices for sampling a little more expensive than those of the pap smear (substantially cheaper and efficient than those of other liquid based cytology trademarks)
  - b) More cytology diagnosis processed per hour

- c) Savings on tincture and slide mounting supplies.
- d) More cytological diagnostics made per hour per cytotechnologists
- e) An annual budget, the price per diagnosed cytology would be reduced considerably.

diagnoses for low-grade and high-grade precancerous lesions. With the usage of liquid based cytology, with Citofem system, the amount of inflammatory diagnosis decreases and increases the amount of negative cases for malignant cells. The Citofem system reduces the cost of conventional liquid based cytologies, but with the processing simplicity and price of a pap smear.

### Conclusions:

The usage of Citofem as a substitute for pap smears increases the

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