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The Use of Liquid Based Cytology and Conventional Papanicolaou Smear in Cervical Screening: A Comparative Study

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ABSTRACT

The high rate of cervical cancer in women and the inadequacy in its results during diagnosis has necessitated the comparison of the two major techniques used in its diagnosis which are the liquid based technique and the conventional Pap smear method. The cytological techniques were compared in a group of 300 women who visited Ebonyi State University Teaching Hospital for cervical screening. The outcome of the two screening methods was compared with regard to the determination of the specificity and sensitivity of both methods using histopathology as gold standard. Out of the 300 cases screened 38 (12.67%) and 30 (10%) cases were diagnosed as low grade squamous intraepithelial lesion and high grade squamous intraepithelial lesion respectively by liquid based cytology and about 32 (10.67%) and 24 (10%)cases were diagnosed as low grade squamous intraepithelial lesion and high grade intraepithelial lesion respectively on conventional cytology. 250 cases were satisfactory for evaluation using the LBC and 140 cases were found satisfactory on conventional cytology. Sensitivity and specificity of LBC was 100% each and that of conventional cytology were 86% and 97% respectively. From the result above, the study has shown that the LBC gives more satisfactory samples. Though both methods showed high sensitivity and specificity, LBC still has a higher sensitivity and specificity when compared to conventional Pap smear.

Key Words: Cytology, Pap smear, Liquid based cytology, Specificity, Sensitivity, Cervix, Conventional test.

Cervical Cancer is cancer of the cells lining the cervix which is the passageway between the uterus and the vagina (Arbyn 2004). Cervical Cancer occurs when normal cells in the cervix change into cancer cells. It is usually caused by a chronic and persistent cancer causing type of human papilloma virus (HPV) infection, that leads to pre malignant lesions and progress to cancer. Screening is looking for cancer before a person has any symptom, cervical screening is a method of preventing cancer by detecting and treating early abnormalities which, if left untreated could lead to cancer in a woman's cervix (Davey et al 2006). Two screening tests can help prevent cervical cancer or find it early. They include; the pap test (or pap smear) which looks for precancers, cell changes on the cervix that might become cervical cancer if they are not treated and the HPV test which looks for the virus (human papilloma virus) that can cause these cell changes (Cotgan et al 2004).

Today there are two types of pap tests; the regular or routine pap test in which cells from a woman's cervix are smeared on a microscope slide and the liquid based pap smear, in which the cells are placed in a special liquid first and then into the slide. In both types, cells from the cervix are checked unto a microscope in order to find cervical cancer at a stage that is easy to cure. They can show early changes in the cells which can be treated to stop cancer from developing.

Cervical cytology was introduced by George Papanicolaou into clinical practice in 1940 (Papanicolaou 1940). In 1945, the Papanicolaou smear received the endorsement of the American Cancer Society as an effective method for the prevention of cervical cancer. Centre for cytology in Vancouver, British Columbia published data which confirmed that cytological screening leads to a reduction in the rate of invasive cancer of the uterine cervix.

Although organized screening programs based on the papanicolaou's (pap) smear have been very successful in reducing mortality a major problem emerged. Cervical cancer has not been eradicated and its incidence has remained virtually constant for several years (Nance 2006). In Switzerland, cervical cancer is still among the leading cause of cancer with 400 new cases and 1000 deaths annually, mostly occurring in women over 65years of age. The majority of cases had a pap

test or had false negative results from pap test, leading to death in routinely screened women. Results of one study showed that 14% of women with an invasive cervical cancer or HSIL had received a negative smear result within the two years prior to diagnosis (Hutchinson et al 1999). Approximately 2/3 of the false negative smears were related to sampling errors and the remaining were due to screening and interpretative errors mainly due to the small number of diagnostic cell present in suboptimal smear (Vassilako 1998). Several limitations of the conventional smear have been identified including inadequate transfer of cells to slide (Hutchinson et al; 1992), unhomogenous distribution of abnormal cells, presence of obscuring blood, inflammation or thick areas of overlapping epithelia cells (Bolick 1998) and low sensitivity and specificity (Nando 2000).

Liquid based cytology was developed as an alternative to address the limitations of Pap smear. It was developed to improve diagnostic reliability of papanicolaou's smear. For the liquid based Cytology (LBC) the cervical cells are collected with a sampling device and rinsed into a vial with preservation solution rather than being smeared on a slide. Liquid based cytology, rinses cervical cells in preservatives so that blood and other potentially obscuring material can be separated. Because only a representative portion of the sample is used, residual material in the vial may be used for ancillary testing such as HPV testing and other molecular test (parker et al 2001). The remarkable feature of LBC is that it reduces the number of inadequate tests and hence the number of women who have to be recalled for repeat testing. It will also reduce pressure on the cytoscreeners as they will have fewer inadequate smears to look at and cleaner samples to report (Luthra et al 2002).

Several studies comprising of more than 5,000 subjects have been carried out with a preponderance of data indicating a significant benefit of LBC in the detection of cervical cancer precursor lesions in the improvement of specimen adequacy (Richard and Bara 1990). The current investigation was carried out to evaluate the LBC technique and to compare LBC with the conventional Pap smear.

MATERIALS AND METHODS

Methods

The samples were collected from 300 female patients who reported for cervical cancer screening between June, 2009 and March, 2011 at Ebonyi State University Teaching Hospital, Abakaliki; Pap smears were collected from the cervix with Ayres spatula for conventional method and endocervical cytobrush for LBC. Smears were collected, processed and prepared as follows:

Conventional Method:

Patient was asked to lie down and her feet placed in stirrups to hold the feet in place during examination. Speculum was inserted into the patient's vagina using an Ayres spatula, sample was taken from the cervix by gently rotating the spatula through 360 degrees. Sample collected was used to make thin smears on grease free glass slides (4 smears for each patient). Smears were then fixed in 95% ethanol and were allowed to fix for 30 minutes and stained with the Papaniocolauo staining technique.

Liquid Based Method

The method for collection of sample is almost the same with that of the conventional method, the difference is in the instrument used for the sample collection and the method of preservation.

A brush like device known as the endocervical cytobrush was used to scrape the cervix, it was inserted into the cervix and rotated five times at 360 degrees in clock wise direction.

The head of the brush was thoroughly rinsed into the vial containing the fixative (Consisting of 95% ethanol, glacial acetic acid and conc. Hydrochloric acid). The sample was mixed, and then centrifuged at 1500 rpm for 15minutes. The sediment collected was re suspended and re spun 5 times. At the end of the centrifugation process, the supernatant was decanted and a drop of the suspension was used to make a thin film on a grease free glass slide (4 slides for each patient). Smears were then fixed in Pap fixative and stained by the Papanicolauo staining technique.

Staining

The smeared slides were stained using the Papanicolauo staining technique.

Procedure

Smears were hydrated in descending grades of alcohol (Absolute, 90% and 70%) for a minute each and rinsed in distilled water.

Smears were stained in Harris haematoxylin for 4 minutes and were rinsed in distilled water. Smears were differentiated in 1% acid alcohol for 15 seconds and rinsed in distilled water. Smears were blued in Scott's tap water for 5 minutes and were placed in 70% alcohol and 95% alcohol for 5 seconds each and stained in OG 6 for 2 minutes. Smears were placed in 95% alcohol; 2 changes for 10 seconds each. Smears were stained in 95% alcohol, 10 seconds each. They were dehydrated in absolute alcohol baths 1 and II for 10 seconds each.

They were cleared in 3 changes of xylene, 3 minutes each and mounted in DPX mountant.

RESULTS

Ninety four (94) (31.3%) cases studied belonged to the 41-50 age group. The minimum age of patients screened was 12 years and the maximum was 78 years. Out of the 300 cases studied, cytopathology and histopathology diagnosis confirmed abnormalities in 70 (23%) cases (table 1). 250 (83.3%) cases were satisfactory for evaluation on LBC, whereas 160 (53.3%) cases were satisfactory on conventional Pap smear. 16 (5.3%) cases were unsatisfactory for evaluation on LBC and 20 (6.7%) cases on conventional Pap smear.

There were only 34 (11.3%) cases which were satisfactory for evaluation but limited by factors like air drying artifact, obscuring blood and inflammation, cytolysis or absence of endocervical component on LBC, whereas 120 (40.0%) cases were in the same category on conventional Pap smear (table 2).

The most common cause of unsatisfactory smears on LBC was scanty cellularity in 10 (3.3%) cases and on conventional Pap test, thick smear was the commonest cause in similar percentage of cases. Infectious agents were detected in 50 (16.6%) cases on LBC and in 24(8.0%) cases on conventional Pap smear. Candida was the commonest infectious agent in 38 (12.7%) cases, followed by Trichomonas Vaginalis in 10 (3.3%) cases.

A comparative study of LBC, conventional Pap smear and histopathological findings were performed. 38(54%), 20 (29%) and 12 (17%) cases were diagnosed as LSIL, HSIL and carcinoma respectively on LBC while on conventional Pap smear 32(46%), 24 (34%) and 14 (20%) were diagnosed as LSIL, HSIL and carcinoma respectively on LBC while on conventional Pap smear 32 (46%) 24(34%) and 14(20%) were diagnosed as LSIL, HSIL and carcinoma respectively and histopathology confirmed 40 (57%), 18(26%) and 12 (17%) as LSIL, HSIL and carcinoma respectively (table 3).

A total of 58 (19.3%) cases were diagnosed as benign and 12 (4%) cases as malignant by histopathology while a total of 50 (19.3%) cases were classified as benign and 12(4%) cases as malignant on LBC and 56 (18.7%) as benign and 14 (4.67%) as malignant by conventional Pap smear (table 4).

Statistical Analysis

Sensitivity and specificity of the two techniques were calculated thus, using histopathological results as gold standard.

Where

TN = True Negative
TP = True Positive
FP = False Positive'
FN = False Negative

$$\text{Sensitivity} = \frac{TP}{TP+FN} \times \frac{100}{1}$$

For LBC

From table 4, TP, = 12 TN = 38 FP = O, F N = O

$$\text{Sensitivity is } \frac{12}{12+0} \times \frac{100}{1} = 100\%$$

$$\text{Specificity is } = \frac{58}{58+0} \times \frac{100}{1} = 100\%$$

For conventional Papsmear

From table 4, TP = 12 TN = 58FP = 2, FN = 2

Sensitivity is = $\frac{12}{12+2}$ x $\frac{100}{1}$ = 86%

Specificity = $\frac{58}{58+2}$ x $\frac{100}{1}$

The current study has shown that both the sensitivity and specificity of LBC was 100% each and that of the conventional Pap smear was 86% and 97% respectively.

Cytological Patterns in both conventional and Liquid Based cytology:

In the current study, slide 1a shows uneven distribution of cellular material, dirty background and thick clusters of cells associated with the conventional Papanicolaou pattern. There's squamous metaplasia, preponderance of neutroplils and areas of hemorrhage. Features although unsatisfactory, are consistent with acute cervicitis, cervical erosion.

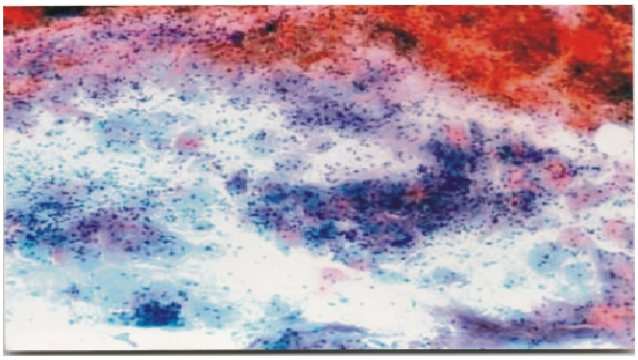

Fig. 1a: is a conventional Papanicolauo smear showing uneven `distribution of cellular material, dirty background and thick clusters of cells (unsatisfactory smear) associated with the conventional Papanicolauo patterns. Stained by Pap technique. X 10

Slide Ib represents the cytological pattern of the liquid based type showing even distribution of cells, clean background with no debris, nucleus or cell masking the abnormal cells.

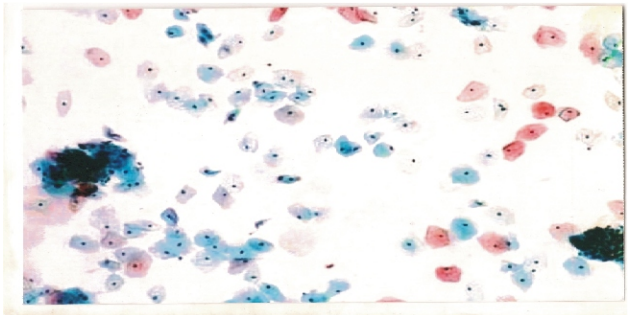

Fig. 1b:represents the photomicrograph of liquid based cytology smear from the same patients as the slide above showing even distribution of cells, clean background cells. Stained by papanicolauo technique. X10

Smear 2a was made form the conventional technique showing Candida albicans with hyphae, yeast cells, dirty background and thick cluster of squamous cells. Features are consistent with candidiasis.

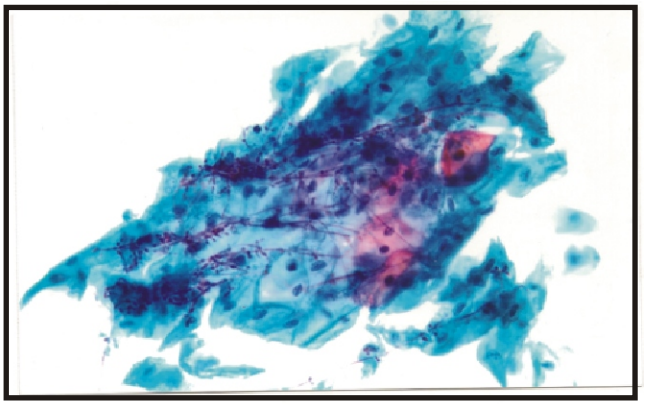

Fig. 2a: is a photomicrograph of the conventional papanicolauo smear showing candida albicans with hypae, yeast cells, dirty background and thick cluster of cells. Stained by Papanicolauo method. X 10

Smear 2b is another pattern shown by liquid based cytology displaying yeast cells, hyphae and clear background with no debris, mucus or blood cell masking any other cells. Cells are navicular. Features are compatible with candidiasis.

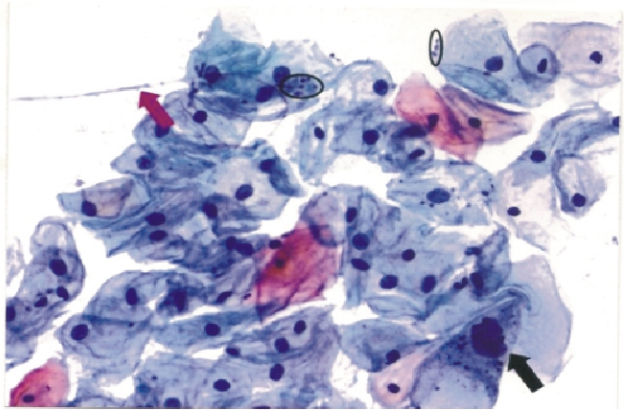

Fig. 2b: represents the photomicrograph of smear made from liquid based cytology technique displaying yeast cells, hyphae and clear background with no debris, mucus or blood cell masking the abnormal cells. Stained by Papanicolauo method. X200

Smear 3a was another one from the conventional technique showing reactive squamous cells associated with Trichomonas vaginalis. Cytomorphologic features include nuclear enlargement and cytoplasmic polychromasia.

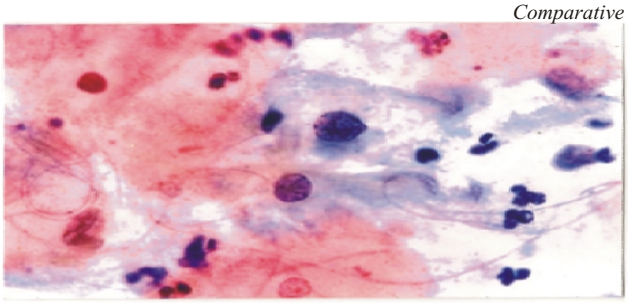

Fig. 3a: is a photomicrograph of smear made from the conventional technique displaying reactive squamous cells associated with Trichomonas Vaginalis. Cytomorphologic features include minimal nuclear enlargement and cytoplasmic polychromasia. Stained by papanicolauo technique. X200

Smear 3b shows liquid based one showing Trichomonas Vaginalis; a pear shaped oval to round cyanophilic organism that ranges in size from 15 30 µm. The nucleus is pale, vesicular and centrally located. Eosinophilic granules are often noted in the cytoplasm.

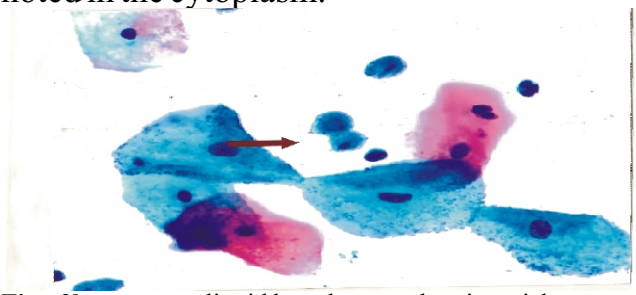

Fig. 3b: represent liquid based smear showing trichomonas vaginalis; a pear shaped oval to round cyanophilic organism that ranges in size from 15 3µm. The nucleus is pale, vesicular and centrally located. Eosinophilic granules are often visible in the cytoplasm. Stained by Papanicolauo technique. X 200

Smear 4a represents that of liquid based cytology showing basophilic and a few esinophilic squamous cells with perinuclear haloes surrounded by cytoplasmic thickening and with moderate nuclear enlargement and typical koilocytes.

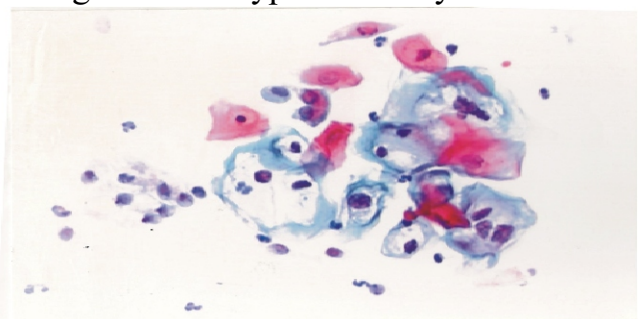

Fig. 4a: represents the photomicrograph of a liquid based cytology smear showing basogohilic and a few esinophilic squamous cells with perinuclear haloes surrounded by cytoplasmic thickening and with moderate nuclear enlargement mimicking typical koilocytes. Stained by Pap. Technique. X100.

4b is another smear from the liquid base cytology displaying esinohilic squamous cells with dense cytoplasm, parakeratosis and some typical koilocytes.

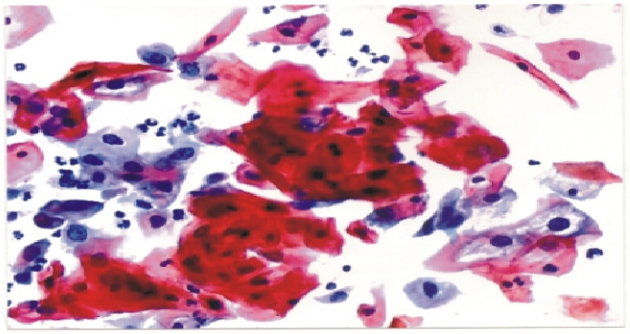

Fig. 4b: represents a liquid based cytology displaying esinophilic squamous cells with dense cytoplasm.

Smear 5a represents liquid based cytology type displaying parabased cells with nuclear enlargement, irregular nuclear outlines, with anisokaryosis and anisocytosis in a homogenous cell population.

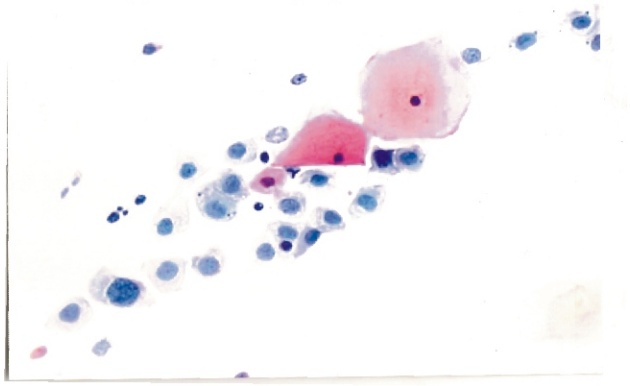

Fig. 5a: is the photomicrography of smear from the liquid based cytology showing parabasal cells with nuclear enlargement, irregular nuclear outlines, with anisokaryosis and anisocytosis in a homogenous cell population. Stained by Pap. Method. X100

Smear 5b is a liquid based cytology smear showing sheet of parabasal and basal cells with enlarged and hyperchromatic nuclei with irregular nuclear outlines.

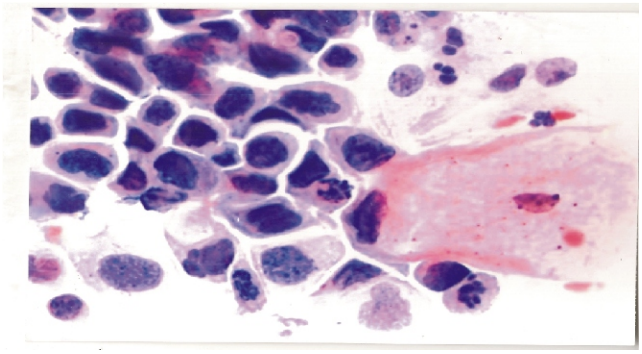

Fig. 5b: represents photomicrograph of a smear from liquid based cytology showing sheet of parabasal and basal cells with enlarged and hyperchromatic nuclei and irregular

Table 1: Age Distribution Pattern

Age	Table No of cases	Normal	Normal	%
11-20	6	4	2	2.00
21-30	32	26	6	10.66
31-40	70	50	20	23.40
41-50	94	80	14	31.30
51-60	32	22	10	10.67
61-70	46	36	10	15.30
71-80	30	12	8	6.67
Total	**300**	**230**	**70**	**100.00**

Table 2: Cytological Classification

Category	LBC		Conve	
	No	percentage	No	Percentage
Satisfactory	250	83.3	160	53.3
Unsatisfactory	16	5.3	20	6.7
Satisfactory but limited	34	11.3	120	40.0
Total	**300**	**100**	**300**	**100**

Table 3: Comparative Study Of Lbc. Conventional And Histpathology Results

Category	LBC	Conventional	Histopathology
LSIL	38	32	40
HSIL	20	24	18
Carcinoma	12	14	12
Total	**70**	**70**	**70**

Table 4: Benign Versus Malignant Lesions

Category	LBC	Conventional	Histopathology
Benign	58	56	58
Malignant	12	14	12
Total	**70**	**70**	**70**

DISCUSSION

The Papanicolauo has been utilized for cervical cancer screening for more than 50 years. Despite being credited with a 70% reduction in mortality for cervical cancer, the false negative rate is still a cause for concern. It is widely acknowledged that two thirds of the overall false negative rate can be attributed to sampling errors. Liquid based cytology has been developed to address the sampling problems of conventional Pap smear.

The current investigation was carried out to compare the LBC and conventional cytology. In this series, it was discovered that 80% of cells collected by conventional technique were not transferred on to the slide. Our finding is in conformity with those of Hutchinson et al (1992) which explains the high prevalence of true false negative rate. By rinsing the sample device into a liquid fixture in LBC technique helps the entire sample to be captured into the vial. In this study satisfactory smears on the conventional techniques was 53.3% as compared to 83.3% on the liquid based cytology method. This also agrees with the work of Weintraub and Morabia (2000), who reported more increased number of satisfactory cases (72.2% - 92%) on liquid based cytology than conventional smears. All drying artifacts and cytolysis were almost absent or minimal with liquid based cytology and specimen adequacy was greatly improved due to absence of limiting factors like blood, mucus and inflammatory cells.

Conventional smears had more unsatisfactory smears and this was due to thick smears, which was not a problem with liquid based cytology due to even distribution of cells. The microscopic details of infectious agents like Candida were enhanced on LBC which made it easy to be detected. In our series, sensitivity and specificity of LBC was 100% respectively and conventional Pap smear 86% and 97% respectively. This finding is in consonance with those of Beerman et al (2009) who reported sensitivity and specificity of LBC as 96.2% and 98.2% respectively, whereas on conventional Pap smear it was 92.0% and 97.8% respectively. Liquid based cytology was found to have higher diagnostic accuracy compared to conventional cytology in our present work. The study confirms previous reports of decreased members of unsatisfactory samples, increased satisfactory samples, and increased detection of LSIL, HSIL, carcinoma and true positive result with liquid based cytology.

Liquid based cytology is strongly advocated for the best interest of the public, it improves the quality of samples and reduces the likelihood of false negative result, thereby significantly improving early detection and treatment of cervical lesions.

Since there was a significant increase in the rate of detection of cervical lesions using the liquid based cytology technique, it is recommended that health organization changed to this method for better cervical screening.

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