Liquid based cytology

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Definition:

- Liquid-based cytology (LBC) is a thin-layer or monolayer slide preparation technology that has been introduced as a potential solution to overcome the shortcomings of conventional Papanicolaou (Pap) smears in cervical cancer screening.
- Liquid-based cytology from gynecologic samples uses a collection fluid that fixes, homogenizes, and rinses the cells.

Aim:

- To improve the transfer of cells from the collection device to a microscope slide
- To provide uniformity of the cell population in each sample.

Disadvantages of PAP smears:

Despite the demonstrated ability of cervical cytological screening to reduce cervical cancer, incidence and mortality, the conventional Pap test is less sensitive than is generally believed. The falsenegative rate of the conventional Pap test has been reported to be up to 50%.

Disadvantages of PAP smears:

• Approximately 67% of false-negative results are attributable to *improper sampling techniques or the poor quality of slides*. Sampling errors may result when a slide does not contain a representative sample of the cells from the cervix, or the *cells are obscured by mucous or blood*, or are *inadequately preserved*.

Disadvantages of PAP smears:

- Common findings on conventional Pap smears, includes:
 - -thicker and thinner smeared areas
 - -air-drying artifact
 - -variety of artifacts, such as "nuclear feathering"

I) Collecting devices:

• 1) Ayre spatula (wooden or plastic): Because wooden spatulas are porous, only about 20% of the exfoliated cells which are obtained are transferred to the Pap test slide. The plastic Ayre spatula has been shown to have a better transfer rate.

Ayre spatula is the <u>least effective device</u> for collecting cells from the endocervix.

I) Collecting devices (cont.):

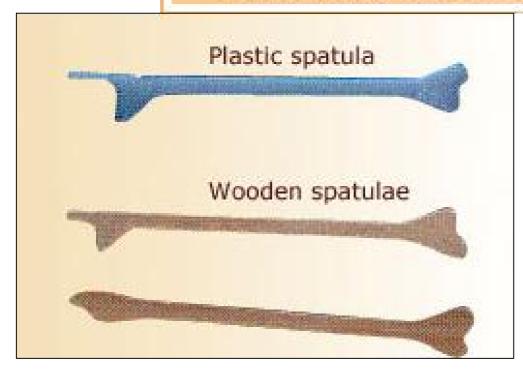
• 2) A saline-moistened, cotton-tipped applicator: has been used with <u>moderate success</u> to improve the adequacy of the sample.

• 3) Endocervical brush, or the cytobrush sampling of the endocervix <u>has improved the specimen</u> <u>adequacy</u>.

I) Collecting devices (cont.):

- 4)Another collecting device, the broom, was recently introduced. However, subsequent studies revealed <u>inadequacy of the endocervical</u> component when only the broom was used.
- 5)A combination of the broom and the cytobrush is the <u>most effective sampling technique</u> to collect exocervical and endocervical cells.

Different types of sampling device





Cervical brush



Cervical broom

II) Steps of LBC:

- 1) The sample is collected in the same manner as is used in the conventional Pap test, using either a broom-type device or a plastic spatula and an endocervical brush.
- 2) However, rather than smearing the cytological sample directly onto a microscope slide, the sample cells are *suspended in a methanol-based fixative solution*.

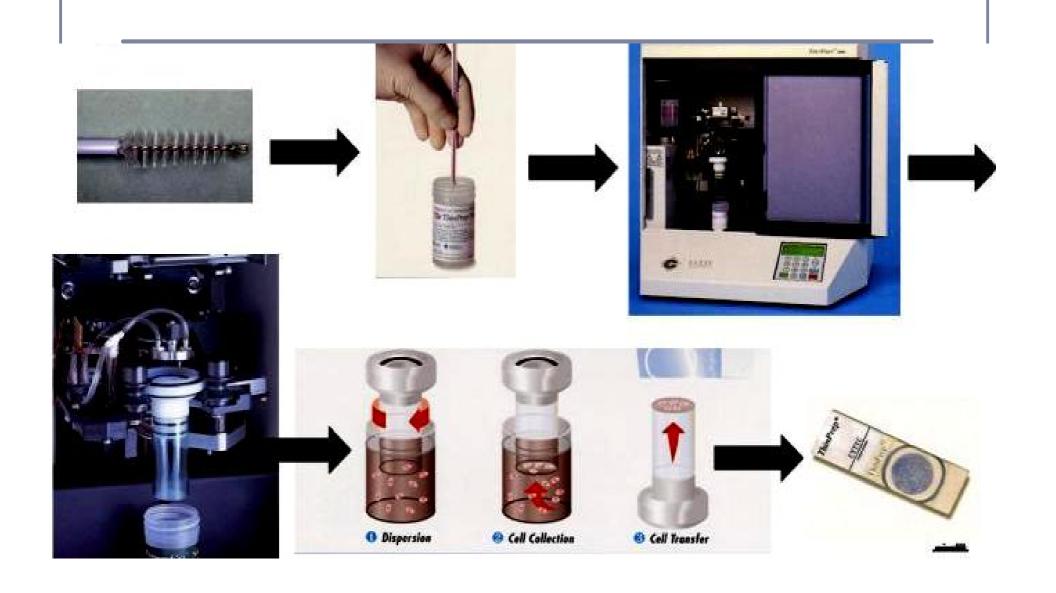
II) Steps of LBC . cont :

• 3)The *ThinPrep Processor* disperses the sample to separate mucous and debris from the cells.

• 4)Cells are collected onto a filter with a vacuum and transferred to a microscope slide for cytological interpretation.

Technique of LBC III) Automated, liquid-based sample preparation system:

After collection of the cervical sample, the tip of the brush is placed into a vial of ethanol-based preservative. The sample is then processed on the AutoCyte PREP System using a density gradient that removes a large portion of blood, mucus, and other potentially obscuring debris from the sample. The majority of cells collected are transferred by cytospin to a slide and stained.



Advantages of LBC

• 1) Provide *a more representative cervical sampling* than conventional smearing of the cervicovaginal specimen on a glass slide.

• 2)Because cytological samples are fixed immediately after collection, there are *fewer artifacts in cellular morphology* e.g.nuclear feathering artifact has not been reported when a liquid-based sampling technique is used.

Advantages of LBC (cont.)

- 3) The process reduces obscuring inflammatory cells and mucous, blood and debris
- 4) The cells are deposited on the slide in a monolayer.

 5) Clinical studies of the ThinPrep method have shown that test sensitivity is improved compared with conventional Pap tests.

Advantages of LBC (cont.)

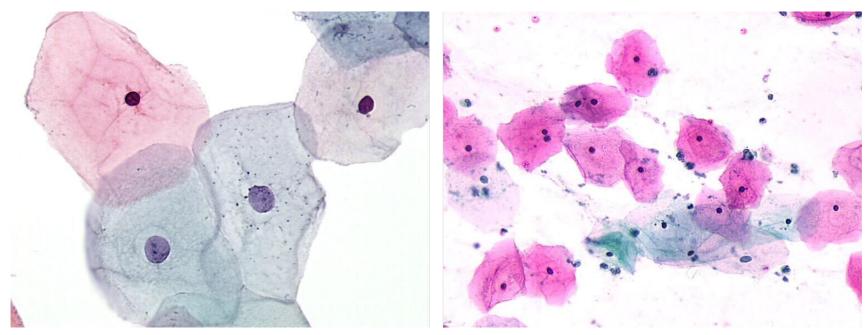
• 6) In addition, this method provides representative residual material in collection media that can be used for additional/adjunctive testing (e.g., HPV testing).

 7) The cells do not lie in thick layered deposits or in streaks of mucus. The mechanical distortions sometimes associated with smearing are missing.

Advantages of LBC (cont.)

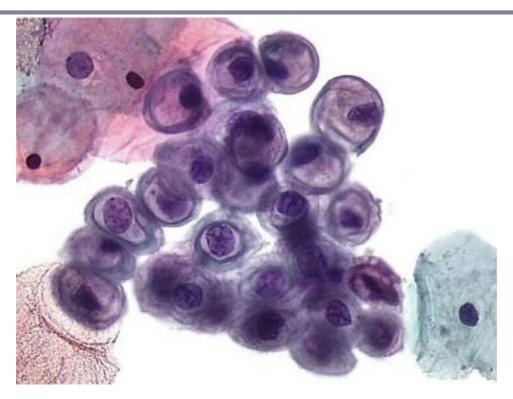
• 8) Histochemical analysis of a cyto-spin sample taken from the original vial, immunohistochemical analysis of liquid-based preparations or eventually, solution-based screening tests that would be suitable to exfoliated cells obtained by self-testing (cyclin E and an HPV antibody).

Liquid-based cytology microscopic features:



Liquid based preparation normal intermediate and superficial squamous cells (left); conventional Pap smear showing normal superficial eosinophilic cells. Note debris in the background (right).

Liquid-based cytology microscopic features:



Cervical specimen: Group of squamous metaplastic cells with perinuclear clearing; Pseudokoilocytes.

Disadvantages of LBC

 On LBC slides, cell nuclei often take on a more vesicular, delicate appearance. In addition, the LBC specimen has fewer landmarks than conventional Pap smears to guide the human eye during the screening process and can be more challenging and consuming per unit area to review. Liquid-based collection methods may cause some epithelial cells to round up and appear smaller.

Disadvantages of LBC (cont.)

• The rounding up of the cells will make the *nuclear-to-cytoplasmic ratio appear altered in favor of the nucleus*. Diagnostic groups and background clues (eg, tumor diathesis) are retained. Patterns such as *cytolysis and atrophy may look different than they do on conventional Pap smears*, but can be easily identified once recognized on the LBC.

Other uses of LBC

LBC ThinPrep system has found broad acceptance in **non- gynecologic cytopreparation** such as:

- Thyroid cyst fluid examination.
- Oral pathology: diagnostic for various types of oral lesions.
- Many laboratories have successfully applied this technique to body fluids (e.g. urine, pleural effusions), brushing samples (e.g. gastrointestinal tract, lung) and fine-needle aspiration.

