EVALUATION OF LIQUID-PREP ™ ENCAPSULATION METHOD FOR LIQUID-BASED CYTOLOGY: CELL LOSS ESTIMATES DURING PROCESSING

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ABSTRACT

Objectives:

Liquid-based cervical cytology procedures are characterized by the randomization of cervical samples collected in alcohol-based preservatives. Thin-layer cytology slides are prepared from aliquots of the dispersed cells and diagnostic material. In order to be representative, the diagnostic material must adhere quantitatively to the surface of the glass. Problems associated with currently available automated liquid-based cervical preparations relate to selective cell loss attributed to obstructed filters (mucus, red cell debris, leucocytes), mechanical transfer of cells to glass slides; suboptimal centrifugation of cells through starch gradients and 1 x gravity sedimentation of cells on poly-l-lysine coated slides. **Liqui-Prep™** resolves these potential biases by thoroughly randomizing the sample, optimizing the conditions of centrifugation and quantitatively encapsulating the cervical cells on the surface of the slide. The result is a diagnostically representative preparation. This study evaluates incidental cell loss during **Liqui-Prep™** processing.

Methods:

One hundred cervical specimens were collected using the **Rover's Brush™**. After preparation of the conventional smear, the head of the brush was removed and placed into a plastic vial containing preservative solution (split-sample). For preparation, the samples were mixed and centrifuged through a density cleaning solution at optimal centrifugal force and time (1000 x g for 10 minutes). The supernatants were decanted and the cellular pellets were uniformly mixed into an encapsulating (Cell Base) reagent. Fifty micro liter aliquots of the homogeneous suspensions were placed on clean microscope slides and spread into 17±4 mm circles. The slides were allowed to dry and stained. Cell loss was ascertained by comparing the number of cells in the original sample, to the number of residual cells in the emptied vial and decant.

Results:

There was a mean of 568,245 cervical cells (68,503 to 1,445,645) in the 100 cervical samples. A mean of 39,238 (10,812 to 76,760) cells were encapsulated for diagnosis on the slides. Analysis of residual material in the preservative vial showed a cell loss of 2.2 % (0.5 to 3.3 %) and 2.2 % (1.0 to 3.6%) in the decanted supernatant.

Conclusions:

This study shows that cell loss during routine Liqui-Prep processing of cervical samples collected in liquid-based preservative fluid is insignificant and in line with classic Saccomanno technique.

INTRODUCTION:

A significant factor contributing to the false-negative rate of conventional Pap smears is that the cervical specimen is not randomized on the collection device and a high percentage of the cells are discarded after the smear is made.[1-3] Some collection devices actually trap cervical cells.[4] Even if diagnostically important cells are transferred to the slide, they may be inadequately preserved and/or they maybe obscured by mucus, blood, inflammation and excessive cellularity.[5-7] Conventional Pap smears also suffer from cell adherence problems and an unpredictable number of nonrandomized cells are lost during staining.

Liquid-based cytology (LBC) procedures are characterized by the immediate preservation and randomization of the entire cervical specimen in an alcohol based preservative solution. Whereas cells smeared onto a glass slide may not represent the entire cell population collected, an aliquot of cells removed from the alcohol fixative is reproducibly representative of the entire cell population collected. [2] In order for these cells to form the basis of a useful diagnostic preparation, mucus, blood, inflammation, cellularity and cell adhesion have to be addressed. LBC manufactures have approached these technical issues in different ways. Table I summarizes the various approaches and compares them to those of **Liqui-PREP™**.

The purpose of this study is to investigate incidental cell loss during **Liqui-PREP™** processing. Further research into the mechanisms and optimization of LBC are underway.

Table I
Comparison of Liquid-Based Cytology Systems: Preparative Aspects

Technical Consideration	Liqui-PREP™	ThinPrep™	SurePath™
Mucus	Preservative and Cleaning Fluids	Preservative Solution	Preservative Solution and Density Reagent Separation
Red Blood Cells	Acetic Acid Treatment	Acidic Acid Treatment	Density Reagent Separation
Inflammation	Dispersal	Dispersal	Density Reagent Separation
Cellularity	Cell Pellet Dilution	Membrane Occlusion	Adjusted by Poly-l-lysine Concentration
Cell Adhesion	Encapsulated on Clean Glass Slides	Pressed onto Chemically Modified Glass Slides	Fixed on Poly-l-lysine Coated Glass Slides

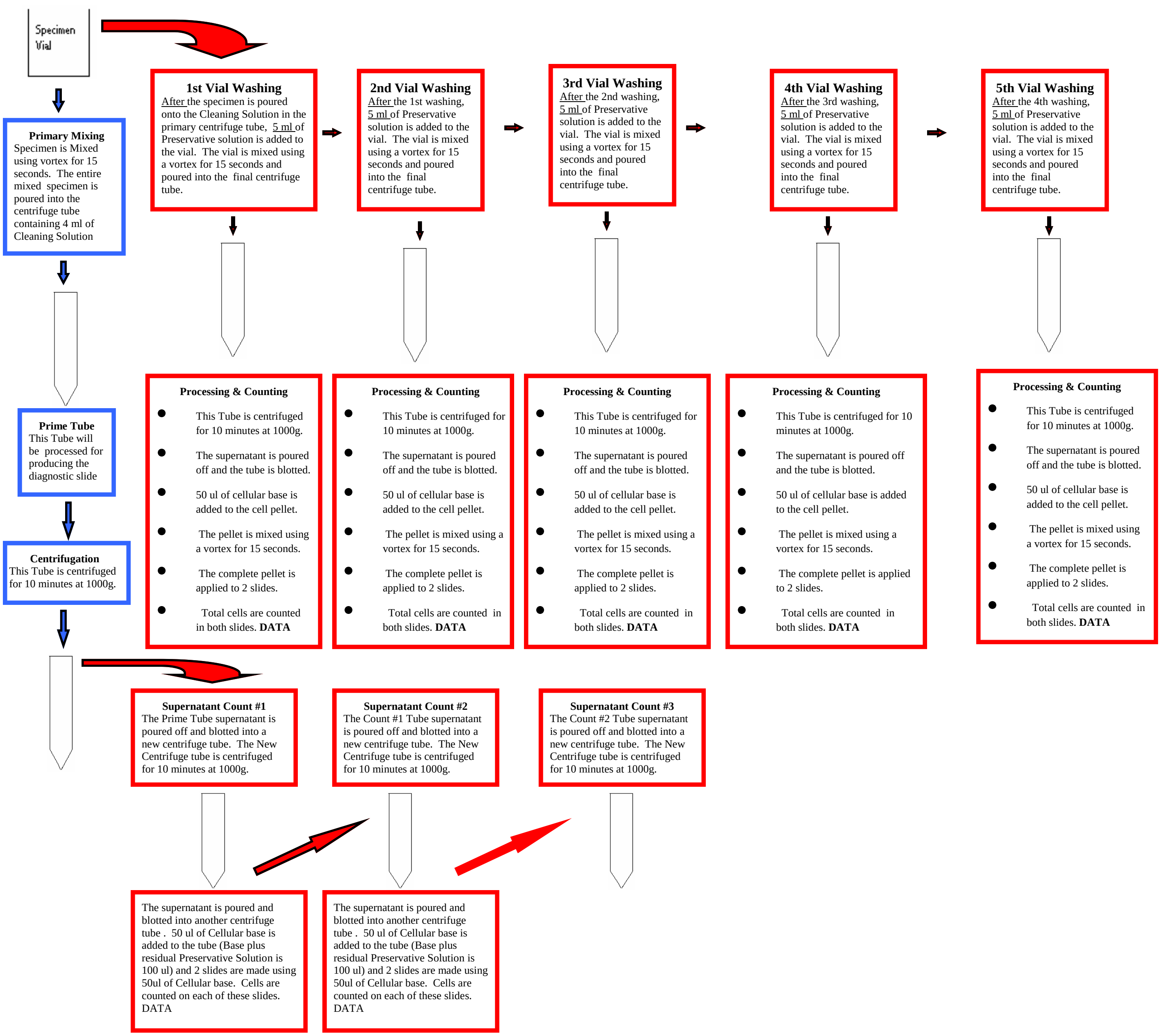
METHODS:

This study was carried out on 100 cervical samples from patients randomly accessed for routine conventional Pap screening and collected using the **Cervex Brush™** (Rover's, Oss, The Netherlands). After preparation of the conventional Pap smear, the head of the brush was detached and dropped into **Liqui-PREP™ Preservative Solution** (split-sample) and forwarded to the laboratory.

For preparation, the samples were mixed and centrifuged through the **Liqui-PREP™ Cleaning Solution** at optimal centrifugal force and time (1000 x g for 10 minutes). The supernatants were decanted and the cellular pellets were uniformly mixed into an encapsulating (Cell Base) reagent. Fifty micro liter aliquots of the homogeneous suspensions were placed on clean microscope slides and spread into 17±4 mm circles. The slides were allowed to dry and stained. Bloody samples were washed in preservative/acetic acid solution, prior to reprocessing.

Cell counts were performed on a Leica microscope (Germany) with a 40X objective. Ten random fields were counted on each **Liqui-PREP™** slide. This represented about 2% of the total area of the 17±4 mm diameter cell preparation. The total number of epithelial cells for each specimen was estimated by extrapolating the number of cells counted to the total area of the preparation.

Procedural Summary



RESULTS:

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Table II
Cervical -Vaginal Epithelial Cells Lost During Liqui-PREP™ Processing (n=100)

	Lowest	Mean	Highest
Number of epithelial cells observed on Liqui-PREP slides*	10,812	39,238	76,760
Total number of epithelial cells collected in the Split Samples	68,503	568,245	1,445,645
Percent of epithelial cells left in preservative vials	0.5%	2.2%	3.3%
Percent of epithelial cells left in the "pour-off" supernatants	1.0%	2.2%	3.6%
Percent of randomized epithelial cells lost during processing	2.2%	4.4%	5.9%

CONCLUSIONS:

1. **Liqui-PREP™** is a straight-forward cytologic procedure, relying on classic cell handling procedures.

2. Problems associated with other LBC systems, such as cell loss due to obstructed filters (mucus, red cell debris, leucocytes),[8] and suboptimal centrifugation of cells through starch gradients are avoided.[9]

3. Cellular material is encapsulated in a matrix material that assures quantitative, robust adherence to the slide.

4. The number of cells transferred to the slide is controlled by the cytologist and can easily exceed the 5,000 recommended for adequacy by Bethesda 2001.

5. Cervical samples collected and processed with LGM's Liqui-PREP system demonstrated compatibility with both molecular (HPV) and immunochemistry methods, (e.g. DakoCytomation's CINtec™ $p16^{INK4a}$)[10]

6. **Liqui-PREP™** is not expensive

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