Research Article

# A Comparison between Liquid-Based Cytology (LBC) and Cytospin Cytopreparatory Techniques in Urine Cytology

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## Abstract

*Introduction:* The simplest and non-invasive procedure for screening tumors of the bladder is assessing urine cytology as it shows high sensitivity for detecting higher grade urothelial lesions. Liquid based cytology (LBC), being a newer technique may be promising in assessing these lesions over conventional cytospin technique.

*Aim:* In the present study, we have assessed and compared the utility of LBC and cytospin preparations in urine cytology.

*Methods:* This study was carried out for a period of one year (June 2014-April 2015) in the Department of Pathology, JIPMER. We have analyzed 150 samples received in the cytology laboratory, which included hemorrhagic, turbid as well as, clear urine samples. Each sample, was processed for both conventional cytospin (CCS) and SurePath LBC techniques. The parameters analyzed were cellularity, cell distribution, cytomorphology, smear background and staining quality. Kappa statistics was used to study the parameters with a p value of <0.05 being taken as 'significant'.

*Results:* Amongst the parameters studied, cellularity and smear background showed good agreement between LBC and CCS techniques with a kappa value of 0.451 and 0.570 respectively, whereas cell distribution and staining quality showed poor agreement between both methods with a kappa value of 0.044 and 0.008 respectively. With regard to cytomorphology, cytospin method showed better cytomorphologic details even in smaller cells which were darkly stained by LBC method.

*Conclusion:* Liquid based cytology provided an excellent distribution of cells with a cleaner background, while the nuclear morphology is better appreciable with the conventional cytospin technique. There was no significant difference between the two techniques with respect to cellularity and smear background. Therefore, in our experience, the conventional cytospin technique is a better method for routine cytological examination of urine samples than the LBC techniques.

***Keywords:*** Bladder lesions, Urine cytology, LBC, Cytospin technique.

## Introduction

Urine cytology is a simple, non-invasive technique for screening of bladder cancer. Especially, it shows higher sensitivity for detecting carcinoma in situ and high-grade urothelial lesions. However, its sensitivity is low in identifying low-grade tumors and also it is limited by a large number of non-diagnostic samples. There are various cyto-techniques for the preparation and microscopic examination of urine sample. The techniques are cytocentrifugation, liquid-based cytology (LBC), Millipore filtration and direct smear.[1-3,6]

Cytospin is a conventional method that concentrates cells which contain low number of cells. However, it has certain limitations which include morphological distortion, obscuring inflammatory cells and blood.[1-5]

LBC is one of the newer and improved methods for gynecological and non-gynecological specimens. It has been introduced in non-gynecological cytology in the western countries and there are only a few studies comparing the conventional cytospin method with LBC.

Many studies have shown that LBC is better than conventional processing and it has higher sensitivity and specificity of more than 90%. The important advantage of LBC method is lesser screening area, cleaner background and improved cell recovery which enhances the quality of smear.[2-4]

In the present study, we have assessed and compared the utility of LBC and cytospin preparations in urine cytology.

## Objective

To compare the efficacy of LBC with cytospin method in cytologic examination of urine samples.

## Materials and Methods

This is a descriptive study which was conducted in the division of cytopathology of the department of pathology, JIPMER for a period of one year from June 2014 to April 2015. Urine samples (150) were collected from the patients in department of cytology constitutes the study material. Cellular samples were included. Gross nature of samples was noted and then each specimen was divided into two halves for further processing. One half of the samples were prepared by cytospin method and the other half were prepared by SurePath liquid-based cytology (LBC).

All smears prepared by both LBC and conventional cytospin methods were analyzed and five parameters like cellularity, cell distribution, cytomorphology, smear background, and staining quality were scored.

The kappa statistical test was used to analyze the parameters and p-value=<0.05 was considered significant.

## Results

The slides were prepared by conventional cytospin and LBC (SurePath) methods from 150 urine samples which were received in the division of cytology from June 2014 to April 2015 at JIPMER, Puducherry.

**Table 1.Age Distribution of 150 Samples**

| Gender | | | | | |
|---|---|---|---|---|---|
| | | Frequency | Percent | Valid Percent | Cumulative Percent |
| Valid | Males | 96 | 64.0 | 64.0 | 64.0 |
| | Females | 54 | 36.0 | 36.0 | 100.0 |
| | Total | 150 | 100.0 | 100.0 | |

The age of the patients included in the study ranged between 10 and 79 years with a mean age of 51 years. The male to female ratio was 16:9.

The visual appearance of all samples was noted and it was documented as follows: 65-clear; 67-turbid; 18-hemorrhagic.

## Details of Parameters Studied

### Cellularity

There was mild cell loss that occurred in conventional cytospin method as compared to the LBC method. The expression of cellularity in both methods showed a good agreement in the level of expression with kappa value of 0.451.

**Table 2.Agreement in the Level of Expression of 'Cellularity' between Conventional Cytospin (CS) and LBC (L)**

| Methods and Level Of Expression | | Liquid-Based Cytology (L) | | | Total |
|---|---|---|---|---|---|
| | | Low | Moderate | High | |
| Conventional cytospin (CS) | Low | 42 | 18 | 3 | 63 |
| | Moderate | 8 | 32 | 13 | 53 |
| | High | 1 | 11 | 22 | 34 |
| Total | | 51 | 61 | 38 | 150 |

## Cell Distribution

We observed that both methods showed a poor agreement in the level of expression with a kappa value 0.44. In comparison, LBC method showed more even cellular distribution than conventional cytospin method.

**Table 3.Agreement in the Level of Expression of 'Cell Distribution' between Conventional Cytospin (CS) and LBC (L)**

| Methods and Level of Expression | | Liquid-Based Cytology (L) | | Total |
|---|---|---|---|---|
| | | Uneven | Even | |
| Conventional cytospin (CS) | Uneven | 6 | 74 | 80 |
| | Even | 2 | 68 | 70 |
| Total | | 8 | 142 | 150 |

## Cytomorphology

In our study, we observed that both methods showed a poor agreement in the level of expression with a kappa value of 0.085. However, conventional cytospin method showed better nuclear details in comparison with LBC method.

**Table 4.Agreement in the Level of Expression of Cytomorphology between Conventional Cytospin and LBC**

| Methods and Level of Expression | | Liquid-Based Cytology (L) | | Total |
|---|---|---|---|---|
| | | Not Clear | Clear | |
| Conventional cytospin (CS) | Not clear | 2 | 18 | 20 |
| | Clear | 5 | 125 | 130 |
| Total | | 7 | 143 | 150 |

## Smear Background

With respect to smear background, both methods showed a good agreement in the level of expression with a kappa value of 0.570.

**Table 5.Agreement in the Level of Expression of Smear Background between Conventional Cytospin and LBC**

| Methods and Level of Expression | | Liquid-Based Cytology (L) | | | Total |
|---|---|---|---|---|---|
| | | Clean | Hemorrhage | Organism | |
| Conventional cytospin (CS) | Clean | 125 | 0 | 0 | 125 |
| | Hemorrhage | 14 | 10 | 0 | 24 |
| | Organism | 0 | 0 | 1 | 1 |
| Total | | 139 | 10 | 1 | 150 |

## Staining Quality

Statistically, we observed that both methods showed a poor agreement in the level of expression with a kappa value of 0.008.

**Table 6.Agreement in the Level of Expression of Staining Quality between Conventional Cytospin and LBC**

| Methods and Level of Expression | | Liquid-Based Cytology (L) | | | Total |
|---|---|---|---|---|---|
| | | Poor | Good | Excellent | |
| Conventional cytospin (CS) | Poor | 0 | 9 | 0 | 9 |
| | Good | 1 | 123 | 8 | 132 |
| | Excellent | 0 | 8 | 1 | 9 |
| Total | | 1 | 140 | 9 | 150 |

The diagnosis made for 150 samples were as follows:

3-urothelial carcinoma; 4-high grade urothelial lesion; 1-low grade urothelial lesion; 1-lymphoma; 23-suspicious of malignancy; 118-negative for malignancy.


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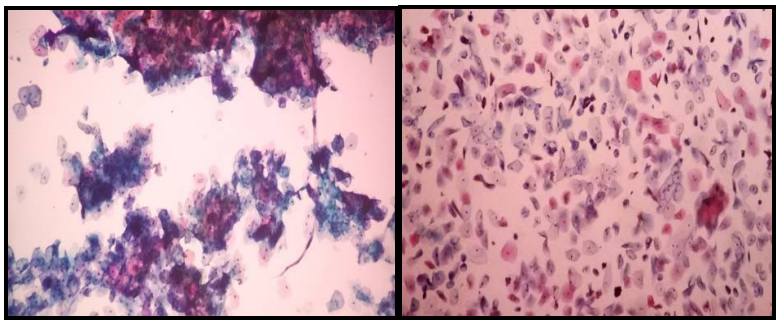

**Figure 1.Cellularity and Cell Distribution. (A) A Cytospin Smear Showing Numerous Unevenly Distributed Benign Squamous Cells (Pap Stain, x 10) and (B) LBC Smear from Same Sample as in Fig. 2A Showing High Cellularity with Well Distributed Cells in a Monolayered Fashion (Surepath Staining Kit, USA, x 10)**

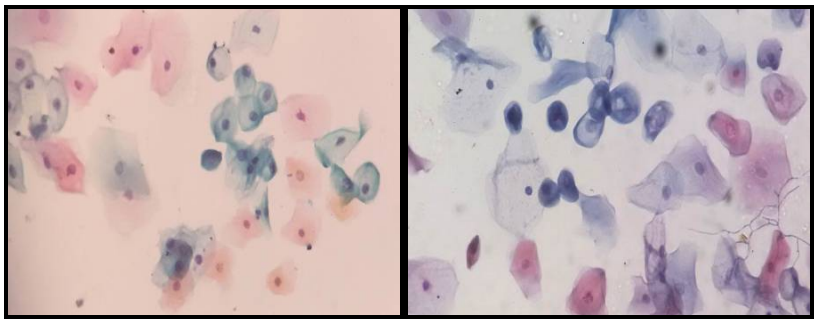

**Figure 2.Background. (A) A Cytospin Smear showing Benign Squamous Epithelial and Urothelial Cells in Clean Background (Pap Stain x 40) and (B) LBC Smear from Same Case as in Fig. 3A showing Benign Squamous and Urothelial Cells in Clean Background (Surepath Staining Kit, USA, x 40)**

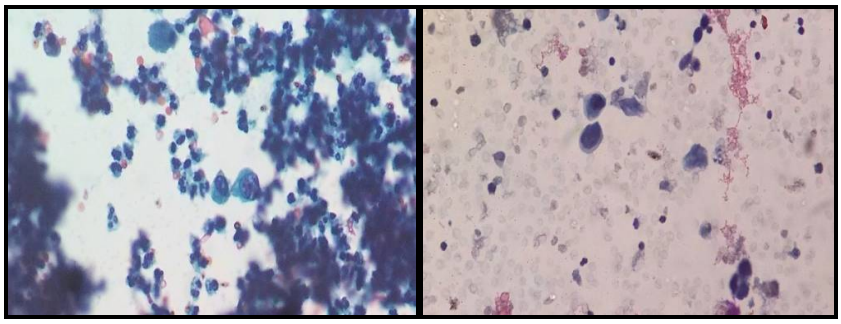

**Figure 3.Staining Quality (A) A Cytospin Smear showing Atypical Cells with Enlarged Nuclei and Irregular Coarse Chromatin, Highly Suspicious for Malignancy. (Pap Stain x 40) and (B) LBC Smear from Same Sample as in Fig. 4A Showing Nuclear Enlargement. Nuclear Details Are Not Well Appreciated because of Darkly Stained Nuclear Chromatin (Surepath Staining Kit, USA, x 40)**

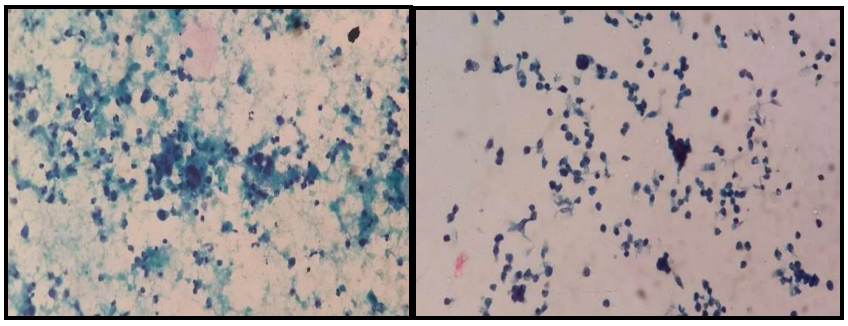

**Figure 4.(A) A Cytospin Smear showing Degenerated Cells with Mild Nuclear Atypia (Pap Stain x 40) and (B) LBC Smear from Same Sample showing Darkly Stained Nuclei with a Mild Nuclear Enlargement in a Cleaner Background (Surepath Staining Kit, USA, x 40)**

## Discussion

There are very few studies comparing cytospin and liquid-based cytology techniques for processing of urine samples.[1] Most widely used among them are ThinPrep and SurePath systems. In our study, we compared the SurePath LBC technique with the cytospin method for five different parameters like cellularity, cell distribution, cytomorphology, smear background and staining quality.

With respect to cellularity, our study showed equivalent results between the LBC and conventional cytospin techniques, although the number of samples with low cellularity was slightly higher in conventional cytospin method other than in LBC. The possible reason could be the vortexing of sample (collected in the liquid medium), which is performed twice as a part of the standard LBC procedure. The vortexing perhaps causes breakdown of cell fragments resulting in increase in the number of dissociated cells within the liquid medium. Moreover, in LBC method modified Poly-L-Lysine precoated slides were used in our study, which because of their increased adhesive property minimize the cell loss resulting in better cell recovery rate than the conventional cytospin method where egg albumin was used as adhesive agent to minimize cell loss.

LBC technique proved superior to conventional cytospin method. An even distribution of cells in LBC smears can be attributed to the effect of vortexing which results in homogenization of cellular elements and hence in their 'even' distribution on smears.

Gross nature of the sample is an important factor that influences cell distribution in cytospin technique.

In our study 'clear' urine sample, there was an absolute concordance between two techniques with regard to 'even' distribution of cells; while in turbid and hemorrhagic samples, cell distribution was not 'even' in the cytosine method. The use of saline in cytospin method for diluting the sample may help in achieving an 'even' cell distribution.

The cytospin method was proved to be effective in assessing the cytomorphological features and showed better nuclear features than the LBC method, in our study. Most of our urine samples contained predominantly benign squamous and urothelial cells and there was no difference in appreciating benign squamous and urothelial cells amongst both the methods. However, for appreciation of malignant cells and other smaller cells, conventional cytospin smears provided better nuclear morphology than in LBC. David et al. observed that SurePath technique resulted in three-dimensional cell clusters in smears in high-grade urothelial carcinomas, and cytospin preparations provided slightly better nuclear details.[1] Our study also showed similar concordance.

The smear background was superior with LBC method where 92% of the samples showed clear background. The principle of LBC (SurePath) technique is based on density-gradient cell-enrichment process. This reduces the RBC and inflammatory debris to a greater extent and provides cleaner background.

The reasons for 'poorer smear background' in conventional cytospin method could be attributed to the use of excessive albumin as adhesive and presence of hemorrhage. The solution to the previous can be avoiding the use of excessive amount of albumin while the latter can be tackled by the use of saline (to lyse red blood cells) before processing the sample by cytospin technique.

For the assessment of staining quality, both showed equivalent results. Care should be taken while staining LBC slides as pre-fixation may lead to darkly stained nucleus of smaller and malignant cells. Therefore, a pre-standardization of staining time needs to be set for LBC staining and LBC smears.

The overall staining quality is determined by buffer preparation in LBC method. It is always better to ensure the buffer pH range of 8–8.5, which yields better results with LBC method. It is also important to filter the staining solution before starting the staining procedure. Otherwise, staining deposits can obscure cell morphology.

## Screening Time

In the present study, 'assessment of screening time' was not included as a study parameter, as there is not much difference in the screening areas between the two techniques, logically (screening areas in LBC and CCS methods being 20 mm and 22 mm respectively). Wright et al. who included 'screening time' as also one of the parameters in their study did not find any difference between the two techniques.[7.]

## Conclusion

Liquid-based cytology provides an excellent distribution of cells with a cleaner background, while the nuclear morphology is better appreciable with the conventional cytospin technique. There is no significant difference between the two techniques with respect to cellularity

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and smear background. As for the interpretation of morphology, cytospin technique was found to be superior to LBC technique. Therefore, in our experience, the conventional cytospin technique is a better method for routine cytological examination of urine samples than the liquid based LBC technique.

## Conflict of Interest: None

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