# Use of Liquid-Based Preparations in Urine Cytology: An Evaluation of Liqui-PREP™ and BD SurePath™

Dear Dr. Bedrossian:

Conventional smears are tedious and time-consuming to screen due to nonuniform slide preparation and fixation. Features usually associated with conventional smears, such as, thick, overlapping cellular areas, obscuring inflammation, and blood and air-drying artifact result in poor cellular and nuclear preservation. Liquid-based preparations (LBP) are increasingly being used both for gynecologic[1] and nongynecologic cytology including urine[2] and fine-needle aspirations.[3] Urine cytology comprises a large proportion of nongynecological specimens processed in most routine cytology laboratories. A recent paper reported on a larger series comparing conventional urine preparations with LBP.[4–6] Liqui-PREP™ (LPR: LGM International, Fort Lauderdale, FL) and BD SurePath™ (BSP: BD Diagnostics, Franklin, NJ) are two commercially available LBP methods.

In this study, the differences between LPR and BSP were evaluated for a variety of parameters including cellularity, cytomorphology, background features, etc. The material consists of 51 fresh voided urine samples from 25 patients with known urothelial carcinoma, which were obtained from January 2008 to June 2009 at the Mihara Medical Associations Hospital and the Shigei Medical Research Hospital. All patients provided informed consent.

To equate cell density of LPR and BSP sample, urine samples were split equally. Each half of the urine samples was centrifuged at 3000 rpm for 5 minutes, and the supernatant fluid was discarded. Using a micropipette, 50 μL cell pellets were obtained and poured into a 15 mL centrifuge tube. Then, 1 mL of LPR preservation fluid and BSP CytoRich® Red preservative fluid were added into centrifuge tube, respectively. After 30 minutes fixation time, LPR and BSP sample tubes were centrifuged at 3000 rpm for 10 minutes, and the supernatant fluid was discarded. As for the LPR sample, 0.2 mL of LPR Cellular Base Reagent was added in LPR sample tubes, and the cell pellet was resuspended using a vortex for 10 second. Following this step, 100 μL of the suspension were pipetted onto the slide to form a 2.0 cm diameter circle. Two LPR specimens were prepared for each case and were then dried and stained by the Papanicolaou method (Fig. C-1). As for the BSP sample, 0.2 mL of distilled water was added in BSP sample tubes, and the cell pellet was resuspended using a vortex for 10 second. After resuspension, 100 μL of the suspension was transferred into small plastic chambers, mounted on microscope slides, and fixed with 95% ethanol. Two BSP specimens were prepared for each case and were immediately stained by the Papanicolaou method (Fig. C-1). Four of the authors (Y. N., N. K., Y. S., and T. K.) microscopically analyzed all the preparations.

1. Number of cancer cells: The cellularity of the samples was defined as "low" if <5 malignant cells were found; if the number of cancer cells was 5–30, it was defined as "moderate." If the number of cancer cell was >31, it was defined as "high." The number of cell clusters in the three types was calculated using the same criteria.
2. A cellular numerical difference between specimen of the first and the second: The number of atypical cells found in the first and the second specimen of each sample was recorded. Then, the number of cases in which a more than double cellularity between the first and the second sample were calculated.
3. Area of cell nuclei: At least 500 cells in randomly selected fields in each case were measured by using the image analysis software HANAKO (JustSystems Inc., Tokyo, Japan).

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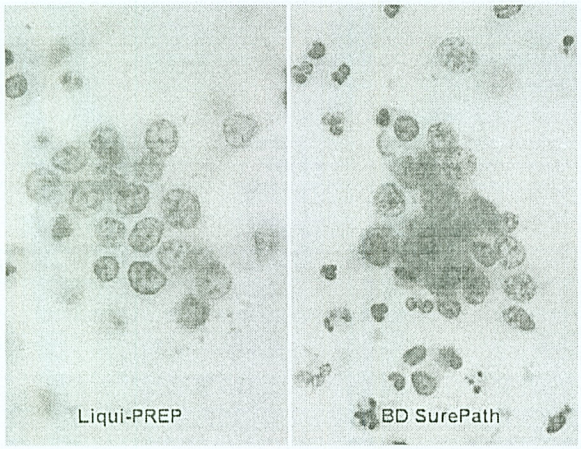


Fig. C-1. Urine, uroepithelial carcinoma. Right: BSP, Left: LPR (Papanicolaou, original magnification ×80).

4. The staining quality of nuclear chromatin: This was evaluated using image processing software ImageNos (http://www.vector.co.jp/soft/dl/win95/art/se065425.html). This was done by measuring brightness in at least 500 cells in randomly selected fields for each case.
5. The number of leukocytes: The leukocytes were counted in five high-power fields in each case selected at random.
6. Screening times: Average times for screening were calculated from all the preparations in a blinded fashion.
7. Diagnostic accuracy: Detection of abnormal cells was evaluated all the preparations in a blinded fashion.

Comparative data were analyzed using the chi-square test with Yates correction or Fishers exact test and the Mann-Whitney *U* test when necessary. *P* value $<0.05$ was considered significant. These analyses were made using the StatView system (Abacus, Berkeley, CA).

1. The comparison of the number of cancer cells: As for low cellularity, LPR (193.9 ± 347.2) had an higher absolute value in comparison with BSP (145.3 ± 254.5); however, the two findings were not statistically significant. As for moderate cellularity, LPR (32.9 ± 67.2) had an higher value in comparison with BSP (31.4 ± 54.0); however, two findings were not statistically significant. As for high cellularity, LPR (1.8 ± 3.9) had a higher absolute value in comparison with BSP (1.1 ± 2.2); however, the two findings were not statistically significant.
2. The comparison of cellular numerical difference between specimen of the first and the second: As for low cellularity, LPR (5.9%) had a lower value in comparison with BSP (11.8%); however, two findings were not statistically significant. As for moderate cellularity, LPR (13.7%) and BSP (13.7%) had the same values, and so the findings were not statistically significant. As for high cellularity, LPR (5.9%) had a lower value in comparison with BSP (17.6%); however, the two findings were not statistically significant.
3. The comparison of the nuclear area: LPR (92.8 ± 31.8 $\mu m^2$) had an higher absolute value in comparison with BSP (87.4 ± 24.9 $\mu m^2$); however, the two findings were not statistically significant.
4. The comparison of the staining quality of nuclear chromatin: LPR (125.4 ± 27.0, $P = 0.0118$) had a significantly lower value in comparison with BSP (141.0 ± 27.0).
5. The comparison of the number of leukocytes: LPR (33.1 ± 29.6, $P = 0.0066$) had a significantly lower value in comparison with BSP (58.9 ± 42.3).
6. Screening times: As for the average times for screening, LPR had 151.0 ± 12.3 second and BSP had 155.2 ± 17.9 second. The two findings were not statistically significant ($P = 0.4759$).
7. Diagnostic accuracy: As for the all preparations in both LPR and BSP, abnormal cells were detected.

Urinary tract cytology comprises a large proportion of nongynecological specimens processed in most routine cytology laboratories. However, both when ThinPrep and BSP were used, it was reported that laboratory costs were higher in comparison with the conventional cytospin.[6,7] The LPR preparation system is a new liquid-based method of cytology specimen preparation. Its viscosity is designed to allow nucleated cells to "settle" onto the bottom of the encapsulation matrix, and this creates a monolayer of nucleated cells for clear reading. The encapsulation matrix has a "adhesive nature," so as it dries it "sticks" to the glass. Because LPR does not need particular equipment and instrumentation, it seemed that to have better cost effectiveness than ThinPrep and BSP.

As for cancer cell cellularity, the LPR had a trend for higher absolute values in comparison with BSP; however, two findings were not statistically significant. In addition, as for the comparison of cellular numerical difference between specimen of the first and the second specimen, significant differences were not recognized between LPR and BSP. Therefore, as for the level of technical difficulty in the smear preparation, it seemed that LPR has equal ability in comparison with BSP. As for the urine sample, Wright and Halford[4] described that BSP was generally more cellular than ThinPrep and a detection rate for atypical urothelial cells of BSP comparable with ThinPrep was demonstrated. Papillo and Lapen[8] have reported the

number of diagnostic cells was increased on LBP slides, particularly for cytological specimens with low cellularity. As for the LPR, if urine sample had a low cellularity, it seemed that detection of atypical cell was possible. As for the cytologic morphology, the nuclear area was not statistically significant between LPR and BSP.

As for the staining quality of nuclear chromatin, LPR was significantly low value in comparison with BSP, and nucleus had a clearer tendency. Zardawi and Duncan[5] noted nuclear chromatin patterns were clearer and better preserved in the Cytospin method in comparison with LBP. If nuclear staining clarity is low, cellular detailed observation is disturbed. Therefore, it seems that nuclear staining clarity in the LPR may be helpful in detailed observation of a nuclear structure. Furthermore, as for number of leukocyte, LPR was significantly low value in comparison with BSP. Wright and Halford[4] described that erythrocytes and crystals were more markedly reduced among noncellular background elements in LBP as opposed to Cytospin specimen. In addition, Zardawi and Duncan[5] reported the LBP showed less blood and inflammatory cells and debris than in Cytospin specimen. If there are many leukocytes in the background of a specimen, cellular observation is disturbed. Therefore, it seems that specimen background clarity in the LPR may be helpful in cellular detailed observation.

Screening time was similar between LPR and BSP. Abnormal cells were detected as either atypical urothelial cells or urothelial carcinoma, and detection of abnormalities in this small study had no difference between LPR and BSP. Our experience demonstrated that cell preservation and quality of cell presentation in LPR was comparable with BSP. Therefore, an application of this method may be the useful tool in the detection of atypical urothelial cells.

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