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Comparison of Liqui-PREP™ and Conventional Preparations in Thyroid Fine Needle Aspiration

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Background : Liqui-PREP™ (LP) is a new liquid-based cytologic preparation that produces a thin layer of cells. **Methods :** Thyroid aspirates were obtained from 189 patients and divided to prepare pairs of conventional preparation (CP) and LP slides. The CP slides were routinely diagnosed by attending staffs and classified into the six categories. LP slides were independently evaluated by three cytopathologists and classified in an identical manner. Agreements between CP and LP diagnoses were investigated and interobserver variability of thyroid aspiration cytology results obtained using the LP method was determined using kappa values. **Results :** CP and LP slides from 155 patients (83%) were identically classified by all of three cytopathologists. Concurrences between CP and LP diagnoses for the three cytopathologists were 89% (κ=0.78), 92% (κ=0.87), and 85% (κ=0.70), respectively. Interobserver agreement among the three cytopathologists for LP slides ranged from substantial to almost perfect (κ= 0.84, 0.74 and 0.84). However, a lack of interobserver agreement was found for LP slides of the undetermined category as determined by original CP-based diagnoses. Moreover, cytomorphological alterations in the benign category appeared more worrisome for LP slides. **Conclusions :** An awareness of the novel cytomorphologic changes induced by the LP method is needed to avoid misinterpretations.

Key Words : Cytology; Thyroid; Fine-needle aspiration

Fine-needle aspiration (FNA) of the thyroid gland is currently considered to be the most cost-effective and accurate primary diagnostic procedure for thyroid nodules. In recent years, many approaches have been devised to improve the quality of thyroid FNA cytology, because the conventional preparation (CP) smear is subject to errors in sample collection and slide preparation. In particular, liquid-based, thin-layer techniques were developed to replace the CP method to overcome diagnostic difficulties in the gynecological cytology field.[1-4] The liquid-based cytologic preparations (LBP) provide optimal cellularity for evaluation purposes by adequately and rapidly fixing specimens, and by decreasing the number of red blood cells, inflammatory cells, and mucus. ThinPrep® (Cytyc Corporation, Marlborough, MA, USA) and SurePath® (TriPath Imaging, Burlington, NC, USA) are representative LBP. The Liqui-PREP™ Preparation system (LGM-International, Fort Lauderdale, FL, USA), a new LBP method, was recently introduced on the basis that it is more cost-effective than existing LBP methods.[5,6] The primary publications regarding LBP concern the gynecological cytology field, and as a few articles have been published on liquid-based techniques for thyroid FNA, most are concerned with the ThinPrep® (TP)[7-17] or SurePath® (SP) methods.[18-20] However, no study using Liqui-PREP™ (LP) has been performed on a thyroid aspiration cytology as of yet. Furthermore, little is known about the interobserver variability of LP or how it compares with the CP method in terms of thyroid FNA diagnoses. The aim of this study was to determine the degree of agreement between LP and CP regarding thyroid aspiration cytology. In addition, we also evaluated the interobserver variability of the LP method with regards to the diagnosis of thyroid FNA.

MATERIALS AND METHODS

This study involved 189 patients who were referred to the Samsung Medical Center for evaluation of a palpable thyroid lesion during the period from September 2007 to January 2008. Samples were obtained by ultrasound-guided FNA performed

by two radiologists. All specimens were prepared using the CP and LP methods. Initially, direct smears were prepared using the CP method, and then remaining aspirates in needles were mixed and centrifuged through the Liqui-PREP™ Cleansing Solution for 10 min. Supernatants were decanted and cellular pellet sizes were estimated. Pellets were then suspended in the encapsulating (Liqui-PREP™ Cellular Base) reagent. Cell density was controlled by estimating the ratio of cell pellet to Cell Base reagent added to each sample. Aliquots (50 μL) of each homogeneous suspension were transferred to clean microscope slides and spread into 17 ± 4 mm circles. After drying, slides were stained with either Papanicolaou or hematoxylin-eosin stains and coverslipped.

Original cytologic diagnoses were made using CP slides by attending staffs using the routine diagnostic procedure. LP slides were examined independently by three participants (two cytopathologists and a fellow) without clinical information or knowledge of CP diagnoses. The thyroid FNA samples prepared by LP were categorized into one of six groups according to the Bethesda reporting system[21], that is benign, follicular lesion of undetermined significance/atypia of undetermined significance (FLUS/AUS), follicular neoplasm/suspicious for follicular neoplasm (FN), suspicious for malignancy, malignant, and nondiagnostic. Original pathologic reports based on CP slide examinations were reviewed and similarly categorized. For statistical analysis, these six categories were assigned numbers from 1 to 6. Cohen's unweighted kappa statistic was calculated to adjust for the agreement between original CP and each LP diagnosis by three pathologists. Interobserver variability was assessed with the three observers for all LP slides and CP slides being in disagreement with LP diagnosis by any pathologist. The agreements were considered slight, fair, moderate, substantial and almost perfect for kappa values of <0.21, 0.21-0.40, 0.41-0.60, 0.61-0.80, and 0.81-1.00, respectively.

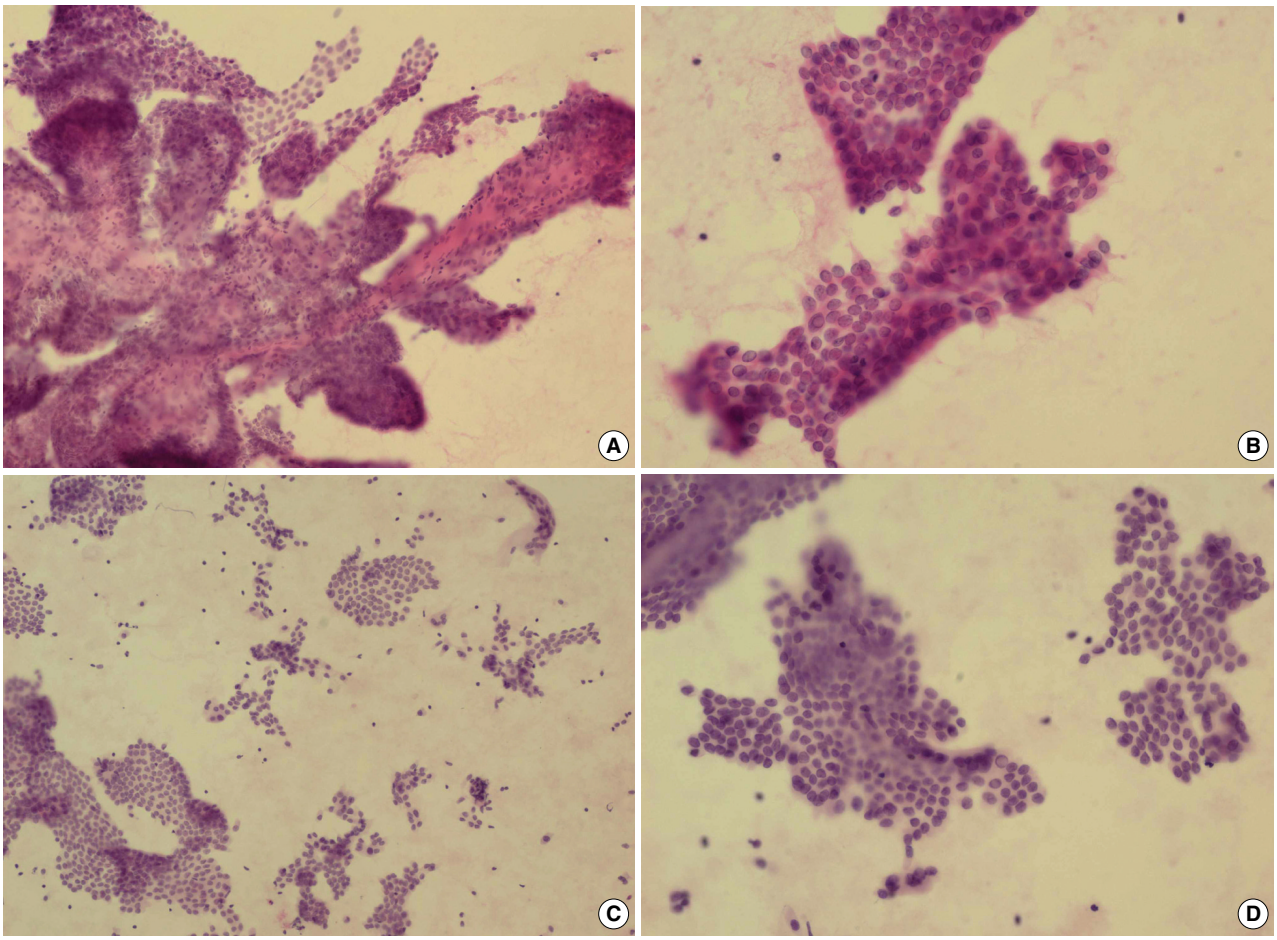

Fig. 1. Conventional smear (A & B) and Liqui-PREP™ preparation (C & D) of papillary carcinoma (H&E stain). The nuclear features of papillary carcinoma are also visualized by the Liqui-PREP™ preparation (C & D). Architectural features, such as papillary structures and flat-sheet arrangements, are also maintained by the Liqui-PREP™ preparation (C & D) as compared with conventional smear (A & B).

RESULTS

Amounts of extracellular materials, such as blood and colloid, were significantly diminished on LP slides, and follicular cells were more crowded into tighter clusters than on CP slides. In addition, nuclei appeared smaller and nuclear membrane irregularities were more obvious. Nuclear grooves and pseudoinclusions were less apparent in cases of papillary carcinoma on LP slides. However, papillae and branching fragments were relatively preserved (Fig. 1). Lymphocytes were usually dispersed and lymphocytic tangles were less frequent in chronic lymphocytic thyroiditis on LP slides. Occasionally, oxyphilic follicular cells had frayed cytoplasm and micronucleoli were more easily detected on LP slides (Fig. 2).

Table 1 summarized total numbers of diagnostic categories as originally determined using CP slides and by the three observers using LP slides. The CP based diagnoses of the 189 cases included; 117 benign, 13 FLUS/AUS, 2 FN, 3 suspicious for malignancy, 46 malignancies, and 8 nondiagnostic. Complete agreement

Table 1. Cytology results according to the Bethesda reporting system for conventional and Liqui-PREP™ slides

	Conventional preparation (n=189)	Liqui- PREP™		
		Cytopathologist A (n=189)	Cytopathologist B (n=189)	Cytopathologist C (n=189)
Benign	117	121	121	111
FLUS/AUS	13	6	9	4
FN	2	2	1	7
Suspicious for malignancy	3	5	4	13
Malignant	46	47	46	46
Non-diagnostic	8	8	8	8

FLUS/AUS, follicular lesion of undetermined significance/atypia of undetermined significance; FN, follicular neoplasm/suspicious for follicular neoplasm.

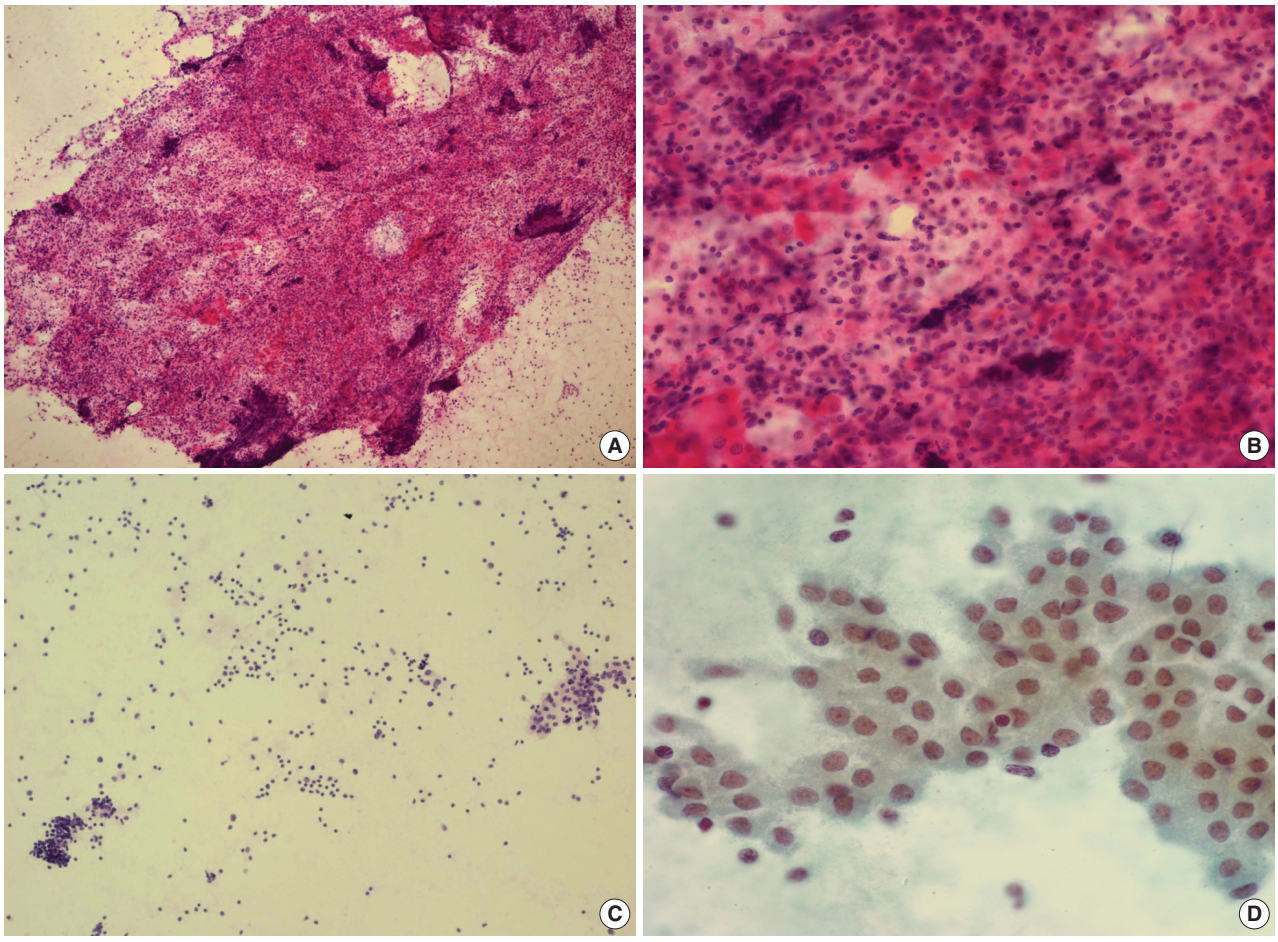

Fig. 2. Conventional smear (A & B, H&E stain) and Liqui-PREP™ preparation (C & D) of chronic lymphocytic thyroiditis. In contrast to conventional preparations, the lymphocytes are less conspicuous (C, H&E stain) in the Liqui-PREP™ preparation, and nuclear atypia of oxyphilic follicular cells are more evident (D, Papanicolaou stain).

Table 2. Comparison between conventional and Liqui-PREP™ cytological diagnoses

Original diagnoses	Discordant cases (n=34)	Disagreement in 1 of 3 observers	Disagreement in 2 of 3 observers	All disagreement
Benign (n=117)	17 (15%)	13	2	2
FLUS/AUS (n=13)	13 (100%)	1	6	6
FN (n=2)	1 (50%)	0	1	0
Suspicious for malignancy (n=3)	1 (33%)	0	1	0
Malignant (n=46)	2 (4%)	1	0	1

FLUS/AUS, follicular lesion of undetermined significance/atypia of undetermined significance; FN, follicular neoplasm/suspicious for follicular neoplasm.

with original CP diagnoses was achieved by all three observers in 155 (83%) of the 189 cases. The diagnoses made by the three observers using LP slides concurred with CP diagnoses in 169 (89%), 173 (92%), and 161 (85%) of the 189 cases, and their diagnostic concordances were high (Kappa values=0.78, 0.87, and 0.7).

Discordant cases were summarized in Table 2 according to CP-based diagnostic categories and in Table 3 after the three observers had reviewed paired CP slides. There were 17 discordant cases (15%) in the benign CP-based cytologic category, whereas FLUS/AUS by CP showed complete disagreement (100 %, 13 of 13 cases) with LP-based diagnoses. Thirteen of 17 discordant cases diagnosed as benign originally involved disagree-

Table 3. Observer diagnoses of 34 discordant cases

Case No.	OCD	Liqui-PREP™				Conventional smear			Repeated cytology/ resection
		A	B	C	No. who disagree	A	B	C	
1	1	1	1	4	One	1	1	1	–
2	1	2	2	4	Three	1	1	1	1
3	1	2	1	1	One	1	1	1	6
4	1	1	1	4	One	1	1	1	–
5	1	1	1	3	One	1	1	1	1
6	1	1	1	2	One	1	1	1	–
7	1	4	1	1	One	1	1	1	–
8	1	1	1	3	One	1	1	1	–
9	1	1	2	4	Two	1	2	1	–
10	1	1	1	3	One	1	1	1	NH
11	1	1	1	4	One	1	1	1	–
12	1	1	1	4	One	1	1	1	–
13	1	1	2	4	Two	1	1	1	–
14	1	4	1	1	One	1	1	1	–
15	1	1	1	3	One	1	1	1	–
16	1	5	2	5	Three	5	5	5	MC
17	1	2	1	1	One	1	2	1	–
18	2	1	1	1	Three	1	2	1	1
19	2	2	2	3	One	2	1	3	–
20	2	1	1	3	Three	1	2	1	2
21	2	1	1	1	Three	1	1	1	1
22	2	1	1	1	Three	1	2	1	1
23	2	1	1	1	Three	1	1	1	1
24	2	1	2	1	Two	2	1	1	6
25	2	4	2	4	Two	5	5	4	–
26	2	1	1	2	Two	1	2	3	FA
27	2	1	2	4	Two	2	2	1	–
28	2	1	2	1	Two	2	2	4	–
29	2	2	4	4	Two	2	2	2	NH
30	2	5	5	5	Three	5	5	5	PC
31	3	3	1	2	Two	3	2	3	2
32	4	1	1	4	Two	5	2	5	PC
33	5	5	5	1	One	5	5	5	PC
34	5	2	4	2	Three	5	5	5	PC

OCD, original cytologic diagnosis; 1, benign; 2, follicular lesion of undetermined significance/atypia of undetermined significance; 3, follicular neoplasm/suspicious for follicular neoplasm; 4, suspicious for malignancy; 5, malignant; NH, nodular hyperplasia; MC, medullary carcinoma; FA, follicular adenoma; PC, papillary carcinoma.

Table 4. Comparison of the interobserver variabilities of the pathologists: Paired kappa statistics in all 189 cases using LP, and 34 discordant cases using LP and CP

Pairs of observers	All 189 cases using LP (p-value)	34 discordant cases	
		Using LP (p-value)	Using CP (p-value)
A-B	0.84 (0.003)	0.28 (0.0985)	0.48 (0.0007)
A-C	0.74 (0.003)	0.03 (1.0)	0.67 (0.0001)
B-C	0.74 (0.003)	0.02 (1.0)	0.39 (0.006)

LP, Liqui-PREP™; CP, conventional preparation.

ment by only one of three observers. However, when CP slides were reviewed in these 13 cases, complete agreement with original CP diagnoses was achieved. The LP-based diagnoses of all three observers disagreed with original CP diagnoses in two cases (cases 2 & 16). One case was interpreted as a FLUS/AUS or suspicious for malignancy on LP slides, but was diagnosed as benign by all reviewers on CP slides. The other case was interpreted as medullary carcinoma or a FLUS/AUS on LP slides, but was diagnosed as medullary carcinoma by all reviewers on CP slides, which concurred with histological findings.

In terms of the diagnosis of suspicious for malignancy or malignancy by CP, disagreements between observers regarding cytological diagnosis only occurred in three patients. A case of suspicious for malignancy (case 32) was diagnosed as benign by LP by 2 observers. One (case 33) of two discordant cases for malignancy was interpreted as benign by LP slide by one observer. The other case (case 34) was classified as a FLUS/AUS (two observers) or suspicious for malignancy (one observer). However, all observers agreed with the original diagnosis after reviewing CP slides. These three discordant cases were histologically confirmed as papillary carcinoma.

Interobserver agreement between the cytopathologists ranged from substantial to almost perfect (Table 4). We also compared interobserver variabilities between reviewers for both CP and LP slides in the 34 discordant cases. The interobserver agreement for CP slide ranged from fair to substantial, but interobserver agreement was poor for LP slides (Table 4).

DISCUSSION

The liquid-based, thin-layer technique has become accepted as an alternative to conventional cytopreparatory methods in the gynecological cytology field. LBP has proven to be more efficient, effective, and easier to evaluate than CP because of slide clarity and smaller area required for microscopic examination. LBP has been attributed benefits such as increased cellularity and a lower rate of unsatisfactory or less than optimal specimens relative to CP in thyroid aspiration cytology.[7-9] However, its usefulness during aspiration cytology is controversial and most studies have used the TP. Although the LBP method can improve smear quality by reduction of the obscuring background, alterations of conventional morphological features induced by the different fixative solution and preparation method have led to misdiagnoses in FNA specimens.[10-13] In addition, the diagnostic accuracy of LBP has been reported to be slightly lower than CP.[10-12] Several studies have indicated that LBP reduces the sizes of cell clusters and causes large branching sheets to fragment.[10,12,13] Furthermore, it has been reported that diffuse colloid and lymphocytes are less obvious in LBP slides, and that intranuclear inclusions and grooves are less commonly observed.[10,11,13]

Few studies using SP method have been reported in thyroid aspiration.[18-20] Unlike TP method, the cells are separated as a result of the simple sedimentation of cells without any applied pressure in SP method. Jung *et al.*[18] reported that the atypical category showed a tendency to decrease in SP method and both Jung *et al.*[18] and Kim *et al.*[19] noted well visualized nuclear changes including nuclear pseudoinclusion and grooves in SP slides.

Unlike previous studies, we used the LP preparation system, a new liquid-based method of cytology specimen preparation. The LP System is based upon a series of chemical approaches. It utilizes a preservative for collection, transport, a cleaner to remove blood, mucus or cellular debris and an adhesive to apply cells to a standard laboratory slide. For the cleaning background, cells are separated by density gradient technology which traps blood, mucus or cellular debris on the surface of the cleaning solution and forms a compact pellet in the bottom of the centrifuge tube. Although the LP system is designed to maintain a classic cell morphology while discarding blood, inflammatory cells, and colloid, our observations show that the cytomorphology of LP methods are similar to TP method, based on the results of previous investigations.

Recent studies[7-9,14-17] have reported that the TP method alters cellular features, but that this does not influence diagnostic accuracy. Some have also demonstrated that TP-based cytologic diagnoses are better correlated with histologic diagnoses than CP-based diagnoses.[7-9,15-17] Biscotti *et al.*[14] analyzed paired FNAs from 41 thyroid FNA specimens to investigate the agreement between TP and CP diagnoses. They found that TP diagnoses agreed with CP diagnoses in 37 of the 41 (90%) cases. Similarly, we were able to observe that agreement between CP and LP

diagnoses for 189 FNA specimens of the thyroid lesion was 88% among our three observers.

In the present study, we determined that the discrepancy rate was low in the malignancy category because malignant cytomorphologic features are also easily detected in LP slides. The high discrepancy rate encountered in the undetermined category was expected due to the intermediate character of this category. However, some issues raised concern in the benign category. The discrepancy rate of LP was higher than expected. We observed this discrepancy was attributed to a cytopathologist who had less experience than others. Our results suggest that cytomorphologic alteration in LP slides may result in false positives and that LP-based determinations are likely to be more problematic in the benign category especially for inexperienced cytopathologists.

Three malignant cases by CP (suspicious for malignancy or malignancy) were underdiagnosed by the LP method and were histologically confirmed to be papillary carcinoma. We considered that decreased cellularity in LP slides might have caused this disagreement, because we prepared LP slides after CP processing. In addition, these misdiagnoses in LP slides were partly due to unfamiliarity with morphological artifacts. Nevertheless, one case of medullary carcinoma was detected by LP because of the clarity of the slide background.

We also examined interobserver agreements between three observers who assessed the LP slides of 189 FNAs. Although overall interobserver agreement was high, a lack of agreement between LP and CP based diagnoses was found for FLUS/AUS compared to the fair to substantial interobserver agreement on CP slides. After reviewing CP and histologic slides and follow-up FNA cytology findings, oxyphilic follicular cells, variable degrees of nuclear atypia, and nuclear enlargement in nodular hyperplasia or chronic lymphocytic thyroiditis were considered to be the main causes of disagreements on LP slides. These nuclear features appear to be more obvious in the relative clean background of LP slides and might result in interpretive difficulties especially for novice cytopathologists.

Summarizing, LP appears to be a valid means of processing thyroid FNA samples, mainly because it provides better nuclear detail and a clearer background. However, the LP method requires some interpretive experience in order to avoid diagnostic pitfalls. Some specific cellular features including the amount and distribution of colloid, nuclear, and cytoplasmic details differ for the LP and CP techniques. These morphologic differences must be recognized, and may necessitate diagnostic criteria modifications when LP is used to evaluate thyroid FNA specimens. We suggest that initially LP should be combined with CP in daily practice to facilitate the learning process and to avoid diagnostic pitfalls.

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