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Cervical Cytologic Smears in Pap Solution vs ThinPrep: Smear Characteristics and Diagnostic Agreement

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Background: The Pap smear has brought about a dramatic improvement in the prevention of cervical cancer in women worldwide. In an effort to decrease the occasional false negatives in the Pap smear and further increase the screened population, ThinPrep Pap Test (TP), a fluid-based cytology collection method, has been developed. With preservation of claimed advantages of TP, we have developed a Pap test solution for manual preparatory process and compared our manually processed fluid-based Pap smear with TP to identify cytologic similarities and differences between the two methods. **Methods:** Cervical swipes of 204 patients were prospectively collected in the 'Pap solution' and also in PreservCyt solution for TP. Diagnoses and smear characteristics were compared. **Results:** The diagnoses of the paired smears agreed in 190 of the 204 cases (93.1%). The smear characteristics regarding overall cellularity and background cellularities were similar in the two methods and the stainability of the cells was virtually the same. **Conclusions:** The 'Pap solution' has similar performance characteristics as TP in many aspects. With its advantages of cost-effectiveness and easier preparatory process, the 'Pap solution' can match previously implemented thin layer preparation.

Key Words: Pap smear; ThinPrep; Manual preparation

The Pap smear has been the most effective cancer screening test, playing a pivotal role in decreasing the cervical cancer rate in women worldwide.[1] The cervix cancer has become No. 8 cancer killer in women, a dramatic fall with regards to the fact that it was once No. 1 cancer killer of women before the introduction of the Pap smear.[1] Now, most cervical cancers affect unscreened or inadequately screened women.[2] Thus, it is obvious that the expansion of the screened population is the best public health policy regarding cervical cancer. However, there is one pitfall in the success of the Pap smear and that is the occasional occurrence of false negatives.[3] Although the possibility of a negative Pap smear being a false negative is only approximately 1 in 1,000 (0.1%), in a review of 1,545 negative Pap smears preceding a positive pap smear, 729 cases (42.7%) turned out to show atypical cells.[4] When the sampling errors as well as screening or interpretation errors are set aside, the only feasible way to decrease the false negative rates of Pap smears is improving the test itself.

Since its introduction in 1996, the ThinPrep Pap Test (TP; Cytyc Corp., Boxborough, MA, USA) method has attempted to improve the Pap test with fluid-based collection and processing, allegedly resulting in superior morphology, faster and easier screening, better sampling due to the cell dispersion step and the potential for multiple testing from a single sample.[5] There are still ongoing disputes on whether these said advantages are valid in the laboratory and also whether the method actually results in better outcome. However, clinical trials with split-samples for conventional Pap smear and TP in various centers have reported consistent results of increased detection rate of squamous intraepithelial lesions (SIL), especially low-grade SIL (LSIL), in the TP arm.[5-7] Specimen adequacy has also been reported to be improved with TP, with an 11% increase in satisfactory samples.[5] With preservation of such advantages of TP, but with shorter preparation time and simplified preparatory process, we have developed a Pap test solution for fluid-based collection of the Pap smear, which is processed manually instead of the automated process of TP. After years' experience with our own 'Pap solution,' we aimed to compare our manually processed fluid-based Pap smear with TP and further identify cytologic similarities and differences between the two methods of preparation.

MATERIALS AND METHODS

Cervical swipes of 204 patients who underwent gynecologic cancer screening test from January 3, 2011 to April 2, 2011 were prospectively collected in the 'Pap solution' and also in PreservCyt solution for TP. These samples were processed accordingly for manual processing of 'Pap solution' and for automated processing of TP. For the former, the cervical swipes are obtained with Pap scan brush and then put into the 'Pap solution.' After more than 20 minutes in room temperature, the cells on the brush are washed off by vortex. The brush is then removed and the remaining solution is centrifuged for 5 minutes at 1,500 rpm. After removing the supernatant, about 80-100 μL of the centrifuged material is pipetted, slowly smeared onto a slide, and fixed for more than 15 minutes in 95% ethylalcohol. Due to this manual smearing of the pipetted material, there is no preset and uniformly defined cellular area like TP (Fig. 1). The slide is then stained by the Papanicolaou method and mounted with cover glass. The entire process requires roughly 20 minutes for preparation of 24 slides. For TP, the cervical swipes are obtained with broom-style sampling device (Papete, Wallach Surgical Devices Inc., Trumbull, CT, USA). The brush is rinsed into PreservCyt solution, the preservative solution for TP, washing off the cells by ample rotation of the brush in the solution. After washing off the cells, the brush is discarded. After more than 15 minutes at room temperature, the bottle is put into the automated processor of TP using sequence 4, yielding one TP slide per case. The slide is fixed in 95% ethylalcohol for 15 minutes, and afterwards the slide is stained by the Papanicolaou method and mounted with cover glass.

The paired samples were separated and independently screened by one cytotechnologist and one pathologist, who had long-term training in both techniques, without knowledge of the findings on the paired sample. The screening was done with regards to the cell preservation status, quality of stain, cellularity, and diagnostic outcome. The cases were classified into Bethesda System categories for the diagnosis and cellularities of endocervical cells and white blood cell's including neutrophils and lymphocytes were estimated in a range of 0 to 3+ (0, none; 1+, 1-2 cellular clusters or no cluster but diffusely and evenly scattered; 2+, 3-5 cellular clusters; 3+, more than 6 clusters). The overall cellularities of exocervical cells were compared between the two methodologies by means of simple comparison in 5 fields of most cellular areas under ×100 magnification, i.e., greater than, less than, and equal to, and red blood cell (RBC)'s were determined to be either present or absent. Following the cytologic analysis, the results were tabulated and the cases interpreted as SIL by one method and negative by the other were reviewed again for consensus.

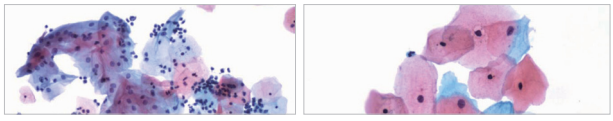

Fig. 1. Slide prepared by manual method of Pap solution. Due to manual preparation, there is no preset and uniformly defined cellular area.

RESULTS

Two hundred forty-four cases were obtained from 204 patients. The diagnostic results are summarized in Table 1. The diagnostic rates did not differ significantly between the two methods, although negative rate was slightly higher in TP (77.0% vs 79.4%) and the rates of LSIL and atypical squamous cells of undetermined significance (ASC-US) were slightly higher in 'Pap solution' (12.2% vs 10.8% and 7.8% vs 7.3%, respectively). Table 2 shows diagnostic agreement between the two groups and the diagnoses of the paired smears agreed in 190 of the 204 cases (93.1%). Though small in number, the two methods agreed exactly in high-grade squamous intraepithelial lesions (HSILs; 2 cases, 1.0%) and squamous cell carcinoma (1 case, 0.5%). One hundred fifty-seven cases (77.0%) were interpreted as negative or reactive cellular changes by 'Pap solution' and 162 cases (79.4%) were reported as negative or reactive cellular changes by TP. The 157 negative cases in 'Pap solution' were completely concordant with TP. However, of the

Table 1. Diagnosis by Pap solution and TP

Diagnosis	Pap solution	TP
NIL	157 (77.0)	162 (79.4)
ASC-US	25 (12.2)	22 (10.8)
LSIL	16 (7.8)	15 (7.3)
ASC-H	3 (1.5)	2 (1.0)
HSIL	2 (1.0)	2 (1.0)
Squamous cell carcinoma	1 (0.5)	1 (0.5)
AGUS/Adenocarcinoma	0 (0)	0 (0)
Total	204 (100)	204 (100)

Values are presented as number (%).
TP, ThinPrep Pap Test; NIL, negative for intra-epithelial lesion; ASC-US, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesions; ASC-H, atypical squamous cells-cannot exclue high-grade lesion; HSIL, high-grade squamous intraepithelial lesions; AGUS, atypical glandular cells of undetermined significance.

Table 2. Diagnostic agreement: Pap solution vs TP

Diagnosis by TP	Diagnosis by Pap solution						
	NIL	ASC-US	LSIL	ASC-H	HSIL	Carcinoma	Total
NIL	157	4	1	0	0	0	162
ASC-US	0	18	3	1	0	0	22
LSIL	0	3	12	0	0	0	15
ASC-H	0	0	0	1	1	0	2
HSIL	0	0	0	1	1	0	2
Carcinoma	0	0	0	0	0	1	1
Total	157	25	16	3	2	1	204

TP, ThinPrep Pap Test; NIL, negative for intra-epithelial lesion; ASC-US, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesions; ASC-H, atypical squamous cells-cannot exclue high-grade lesion; HSIL, high-grade squamous intraepithelial lesions.

Table 3. Overall cellularity: Pap solution vs TP

TP > Pap solution	TP ≒ Pap solution	TP < Pap solution	Total
31 (15.2)	160 (78.4)	13 (6.4)	204 (100)

Values are presented as number (%).
TP, ThinPrep Pap Test.

162 cases of negative's in TP, four cases were reported as ASC-US and one was reported as LSIL in 'Pap solution.' There was one case diagnosed as carcinoma by both methods, and there were two HSILs in the study group by both methods. Sixteen cases (7.8%) were reported as LSIL by 'Pap solution' and 15 cases (7.3%) were reported as LSIL by TP. Twenty-five cases were interpreted as ASC-US (12.2%) by 'Pap solution,' four of which were interpreted as negative or reactive and three of which were interpreted as LSIL by TP. Twenty-two cases (10.8%) were reported as ASC-US by TP, one of which was reported as atypical squamous cells-cannot exclue high-grade lesion (ASC-H) and three of which were reported as LSIL by 'Pap solution.' There were 3 cases of ASC-Hs (1.5%) in the 'Pap solution' group and 2 cases of ASC-Hs (1.0%) in the TP group. In the former, one was interpreted as ASC-US and another case was interpreted as HSIL by TP. In the latter, one was reported as HSIL by 'Pap solution.' There was no glandular abnormality in this study group. When the overall cellularities in five most cellular areas under magnification of ×100 were compared, 31 cases (15.2%) were more cellular in TP than in 'Pap solution' group, 13 cases (6.4%) were more cellular in 'Pap solution' group than in TP group, and 160 cases (78.4%) were almost the same in both groups (Table 3).

Table 4 shows the cellular composition of the smears by both methods in a range of 0 to 3+. There were no cases of 'adequate but limited by obscuring blood or inflammation,' 'air drying,' or 'scanty cellular material.' Endocervical cells tended to be present slightly more commonly in TP than in 'Pap solution' (67.7% vs 61.9%). The number of cases allocated to each score was similar between the two groups but for the fact that there were slightly more cases showing endocervical cells of 2+ in TP. The presence of inflammatory cells in the smear also scored similarly between the two groups. The presence and absence of RBC's were in exact concord between the two groups. There was no case showing RBC's in one method and none in the other.

Table 4. Cellularity and background: Pap solution vs TP

Cellular components	Pap solution	TP
Endocervical cells		
0	78 (38.2)	66 (32.3)
1+	51 (25.0)	49 (24.0)
2+	45 (22.1)	55 (27.0)
3+	30 (14.7)	34 (16.7)
Total	204 (100)	204 (100)
Inflammatory cells		
0	93 (45.6)	85 (41.7)
1+	42 (20.6)	48 (23.5)
2+	41 (20.1)	47 (23.0)
3+	28 (13.7)	24 (11.8)
Total	204 (100)	204 (100)
RBC's		
Absent	185 (90.7)	185 (90.7)
Present	19 (9.3)	19 (9.3)
Total	204 (100)	204 (100)

Values are presented as number (%).
TP, ThinPrep Pap Test; RBC, red blood cell.

DISCUSSION

TP is in its own terms a relatively revolutionary method in the field of gynecologic cancer screening. It is designed to prepare cytologic smears from a sample collected in a fluid suspension. Clinicians can obtain the patient specimen in a usual manner, the only difference being that the obtained cell sample must be rinsed into a provided preservative solution (PreservCyt).[8] The processor is a vacuum filtration device which deposits a single layer of cells with a uniform density over a specified 20 mm in diameter area of the slide surface. In so doing, not only are the cells well-preserved in the preservative solution, the preparation of the cell sample is also performed under uniform and controlled conditions in the laboratory, thereby insuring the preservation of morphologic uniformity from case to case.[9] In addition, common processing artifacts seen on conventionally-prepared cervical smears are well-eliminated, resulting in better diagnostic yield especially in SILs.[6,10-12]

Our 'Pap solution' is a specially developed preservative solution for liquid-based Pap smear with advantages similar to TP. Instead of automated process, the cells dispersed and preserved in 'Pap solution' are prepared manually by pipetting after centrifuge, hence no need for the automated processor and required disposables. Because the whole process is done manually by pipetting, the amount of centrifuged cells to be smeared on the slides can be regulated for the most optimal screening and the rate of unsatisfactory smears is decreased. In contrast to TP which employs filtration device to filter out nuclear debris, degenerated RBC's, and inflammatory cells and deposit onto the slide only those essential for screening and diagnosis,[13] cells in our 'Pap solution' are centrifuged and then smeared onto the slide so that all the cellular components contained in the original specimen are deposited onto the slide, only in cleaner background than conventional pap smear. In cases of malignancy, TP slides show a distinctive pattern of diathesis called 'clinging diathesis,' consisting of clumps of inflammatory and proteinaceous debris clinging to individual cells and groups of cells due to coagulation of the debris within the fluid suspension.[9] This coagulation of material is attributed for the cleaner overall background of TP. However, whether such clean smear results in better diagnostic yield is yet to be determined because the background cellular components other than the epithelial cells can sometimes be more informative than obscuring. Also, it can be a fairly subjective matter depending on the individual preference. In addition, TP has its cells in evenly distributed single layer with subtle morphologic change and a smaller number of diagnostic cells, and thus the methodology requires specially trained and well-experienced cytotechnologists and cytopathologists to screen and diagnose.[14] Unlike TP, the 'Pap solution' allows for the cells to naturally overlap as in conventional smears, resulting in slightly better detection rate of HSIL's at low power, though no difference is made in the final diagnostic yield. Moreover, since the cells do not have to pass through the filtration device, there is little morphologic change in regards to the individual cells smeared (Fig. 2). Additional intensive training and experience will not be needed, and those who are familiar with the conventional pap smears will find it easier to adapt.

Despite differences in the process of preparation and also in some of the smear characteristics, the stainability of both methodologies was virtually the same, in both nucleus and cytoplasm of smeared cells, and there was very good agreement in the diagnoses of paired TP and 'Pap solution' smears. The HSILs and carcinoma did not differ much between the two groups. We had

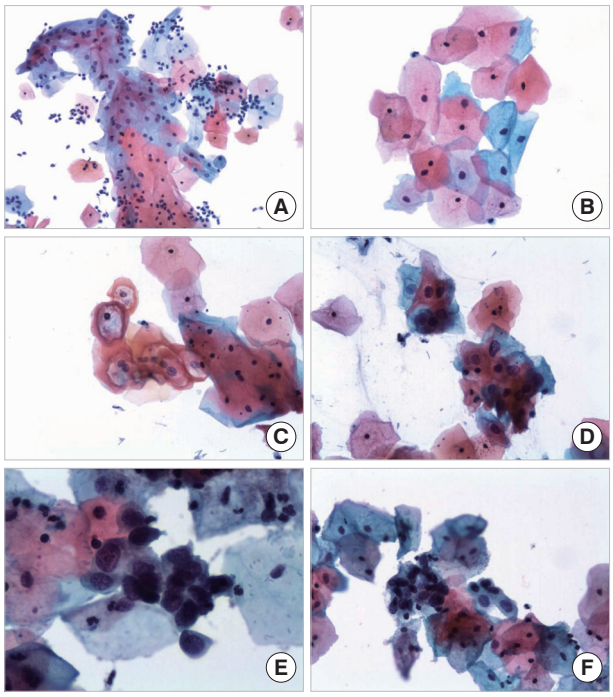

Fig. 2. Slides prepared by manual method of Pap solution. Little morphologic change is seen in each case. (A, B) Negative for intraepithelial lesion. (C, D) Low-grade squamous intraepithelial lesion. (E, F) High-grade squamous intraepithelial lesion.

one TP HSIL with a paired ASC-H in 'Pap solution' and vice versa. This may be easily overlooked because it is not a critical discrepancy. The major discrepancy, though small in number, was in ASC-US and LSIL. Four cases were seen as 'negative for intra-epithelial lesion (NIL)' and three cases were seen as 'LSIL' in TP whereas they were originally interpreted as 'ASC-US' in 'Pap solution.' Of all the cases interpreted as 'ASC-US' in TP, one was interpreted as 'ASC-H' and three cases were interpreted as 'LSIL' in 'Pap solution.' However, these are not critical discrepancies per se, and the true problem lies in the small number of false negatives in TP. Four cases of ASC-US and 1 case of LSIL in 'Pap solution' were interpreted as 'NIL' in TP. Upon review for consensus, there were no koilocytes or dysplastic cells suspicious of LSIL on TP slide. This might be attributed to the fact that pipetting of the sampled cells after centrifuge may contribute to the salvage of the entire cell sample onto the slide. The TP smears, on the contrary, have fewer cells in a single layer over a smaller area. This can contribute to the decreased screening times with TP, but it has the drawback of increased sampling error with potential loss of representative cells in the process of filtration. Moreover, we cannot be in haste to reach any conclusion because for one, LSILs and ASC-US lesions are especially prone to interobserver variation in cytologic interpreta-

tion and secondly, follow-up surgical biopsy was not available for confirmation. This lack of surgical biopsy is the limit to our study, even though biopsy follow-up studies may be limited by sampling problems and regression or progression of lesions prior to biopsy, precluding definitive assessment of the cytologic diagnosis and also precluding assessment of sensitivity and specificity of a given methodology. We had no unsatisfactory specimen in both methods. Regarding unsatisfactory rates, some studies report increased unsatisfactory rates with the TP and others report decreased unsatisfactory rates with the TP in comparison with the conventional smears.

Theoretically as compared to the conventional smear, both TP and 'Pap solution' methods decrease sampling error and thus reduce specimen inadequacy in the first place because clinicians no longer discard most of the cellular sample along with the sampling device and instead, they can simply put the sampling brush into the solution so that the cellular sample on the brush are all washed off and dispersed in the solution.

In conclusion, the 'Pap solution' has similar performance characteristics as TP in many aspects. With its advantages of cost-effectiveness, faster and handy preparatory process, the 'Pap solution' can stand comparison with any previously implemented thin-layer preparation.

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