Technical Aspect of ThinPrep

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ABSTRACT

Aim of Study: To analyze the common technical problems encountered in ThinPrep preparations.

Method: A prospective and retrospective study of eight hundred and fifty (n=850) conventional cervical smears with its corresponding paired ThinPrep specimens from July 1998 to December 1998.

Results: 139 ThinPreps were found to be technically suboptimal. Of these, 81 showed "patchy cells lost"; 18 showed "thick preparations"; 24 demonstrated "halo effect'" where the cellular material collected at the periphery of the cell circle, and 16 had "obscuring blood and amorphous debris", rendering the preparations "satisfactory for evaluation but limited" by the presence of the above artifacts.

Conclusion: Despite its many advantages in providing standardization of specimen preparation, superb cellular presentation, reduction in the number of unsatisfactory reports and increased lesion detection rate, ThinPrep has its own limitations in terms of technical problems, ease of operation and cost effectiveness.

Keywords: cervical smears, ThinPrep, technical problems, suboptimal

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INTRODUCTION

The Singapore General Hospital (SGH) laboratory, Cytology Section, reads an average 50,000 cervical smears annually over the past five years. Thirty-four percent (34%) of smears are from the Obstetrics & Gynaecology (O&G) Centre, SGH, while the remaining 66% are from the Primary Health Services.

Conventional cervical smear screening has always been regarded as tedious as "finding needles in a hay stack", especially when the critical parameters, such as cellular morphology, clarity and uniformity are obscured by excessive inflammatory exudate, blood and mucus. Studies show that sampling and preparation errors account for 53% - 90% of all 'false negative errors'[2]. The emerging "Thin-layer" technologies allow better control of the quality of smears, enabling 12% more lesions to be detected[8], and markedly reducing the number of unsatisfactory smears[7,8]. To date, many laboratories in Australia and the United States of America (USA) are offering ThinPrep Pap test as an adjunct to the conventional Pap smear. In the USA, the Food and Drug Administration has approved the ThinPrep test as a replacement for the conventional cervical smear. To assess the efficacy of this emerging technology, SGH, Department of Pathology, Cytology Section evaluated a "ThinPrep 2000" processor (Cytyc Corporation, Boxborough, Mass., USA) for a period of 6 months from July to December 1998. During the 6 months in-house trial, 850 split-samples of conventional cervical smears paired with its ThinPrep preparations were screened. By examining the conventional smears and the corresponding paired ThinPreps, cytoscreeners became familiar with the microscopic smear appearance and cellular morphology in ThinPreps. A total of 139 cases of ThinPreps showing a variety of technical problems were observed during the trial period. A cytological and histological correlation was not performed, as the number of cases studied was insufficient for statistical analysis.

MATERIAL AND METHODS

Specimen Collection Method

A total of 850 cervical smears were collected between July to December 1998 in the Obstetric and Gynaecologic Outpatients Clinic, Singapore General Hospital using the Cervex-Brush (Rovers B.V., The Netherlands). A conventional cervical smear was first made by spreading cellular material obtained with the brush onto the glass slide, followed by immediate spraying with Cytospray fixative (Kinetik, Australia). The remaining material on the brush was rinsed into a vial of PreservCyt Solution (Cytyc) which is an alcohol-based preservative. Both specimens were dispatched to the cytology laboratory together with one requisition form.

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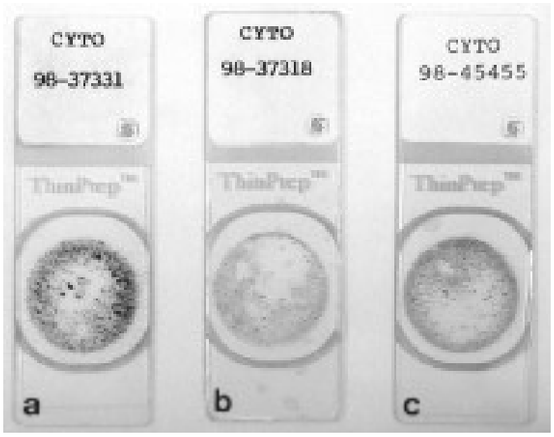

Fig. 1a-c ThinPrep illustrating patchy cell lost of (a-c) varying sizes and shapes.

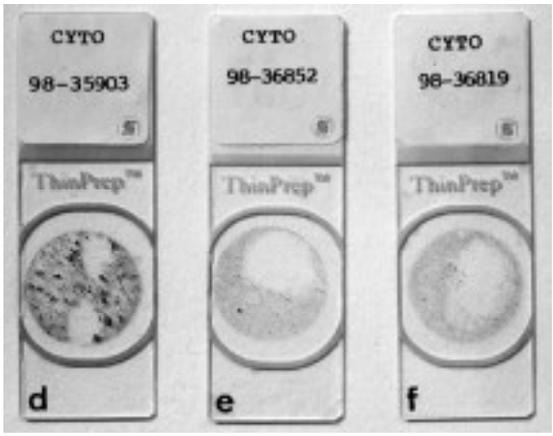

Fig. 1d-f (d) symmetrical shapes and (e-f) fairly similar shapes.

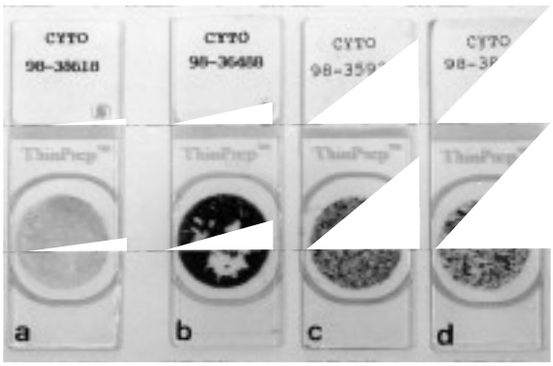

Fig. 2a-d (a) Homogeneous ThinPrep Vs (b-d) "thick preparations".

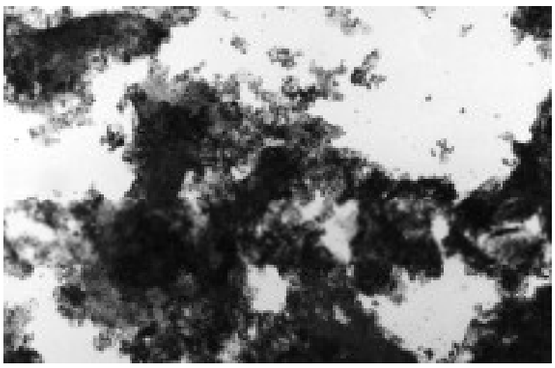

Fig. 2e Microscopic appearance of "thick preparation" (Papanicolaou stain, x40).

Processing of ThinPrep

The ThinPrep processor dispersed the cell suspension by rotating a TransCyt filter assembly in the solution[1]. A volume of the suspension was drawn onto the filter by negative pressure, and some components including debris, inflammatory cells and blood, were filtered and discarded. Approximately 50,000 cells were removed from the vial and the cells transferred onto a ThinPrep glass slide within a circular area measuring 22mm in diameter. A new TransCyt Filter and ThinPrep glass slide was used for each preparation. Both ThinPrep and Conventional slides were stained with the Papanicolaou stain, using the progressive and regressive methods respectively.

Trouble shooting during sample preparation

For sample containing excessive blood and amorphous debris, detected after the first ThinPrep slide had been screened, contents of the PreservCyt sample vial was transferred to a centrifuge tube and the specimen concentrated by centrifugation[1]. A 30ml solution containing 9 parts of Cytolyt solution with one part of glacial acetic acid was added to the centrifuged sample to lyse the red blood cells, and the specimen re-suspended by vortexing. The specimen was further centrifuged and the pellet added to a Preservcyt solution vial, and a new slide was prepared and stained as above.

Selection criteria for technically suboptimal smears

(1) All ThinPreps with cell lost of more than 50% were placed under the category of "patchy cell lost". (2) The criterion for "thick preparations" was extrapolated from the Bethesda system which considered the adequacy of a conventional cervical smear to be limited if thick overlapping cells preclude interpretation of 50% to 75% of epithelial cells[15]. Therefore ThinPrep that have 50% or more of its area covered by clumped, overlapping cellular material fulfilled the selection criteria of "thick preparations". (3) Smears that show "halo" effect have cellular material collected at the circumference of the cell circle, leaving a hollow impression on the smear. (4) Preparations that have 50% or more of its cellular material obscured by fragmented blood and amorphous material were grouped under the category "obscuring blood and amorphous debris".

RESULTS

A total of 139 (16%) ThinPreps were considered technically suboptimal and their distribution is listed in Table I. The most commonly observed technical

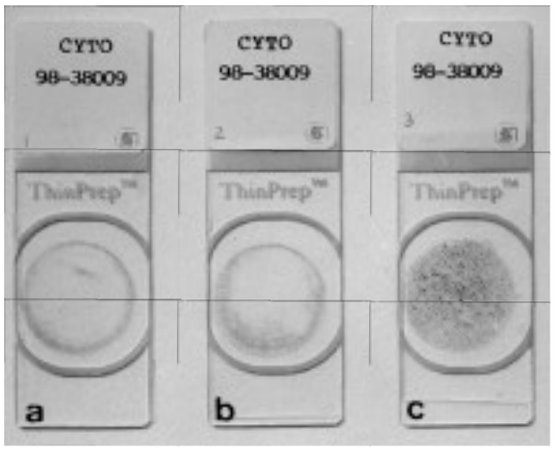

Fig. 3a-c (a) ThinPreps showing "Halo" artifact due to dense specimen; (b) First repeat smear of (a); (c) Second repeat smear illustrating homogeneous preparation (1:20 dilution of specimen with preservcyt).

problems were "patchy cell lost", comprising approximately 9% (n = 81) of the total preparations. These clear spaces were of varying sizes and shapes (Figs. 1a-c). Interestingly some of these clear spaces were symmetrical (Fig. 1d) or of fairly similar shapes (Figs. 1e-f). Two percent (n=18) of cases showed at least 50% of its smear area obscured by thick cellular material (Figs. 2b-e). Three percent (n=24) of cases showed the "halo" effect (Fig. 3a). ThinPreps with "obscuring blood and debris" comprised approximately 2% (n = 16) of total ThinPreps screened. These smears were suboptimal for screening while their corresponding conventional smears were satisfactory for evaluation.

Our results also showed that 16% (22 out of the 139) of ThinPrep preparations showed absence of endocervical cell component while their paired conventional smears showed presence of endocervical cells. Five cases had Candida spores identified on the conventional smears but not in the corresponding ThinPreps (see Table II). It was speculated that the lack of Candida spores might be related to the fact that 'left-over' material after PAP smearing was used for ThinPrep preparation.

All 139 of these technically suboptimal ThinPreps had corresponding negative conventional cervical smears, except 2 which had been categorized under 'thick preparation' and 'obscuring blood and amorphous debris'. In these two smears, the abnormal cells were few and singly dispersed. In one case, histologically proven to be an endometrial carcinoma, the diagnostic cells displayed hypochromatic nuclei that were obscured by fragmented and clumped proteinaceous debris. In another case, the diagnostic cells demonstrated lesser degree of nuclear abnormality when compared to its paired conventional cervical smear.

Table I. Distribution of suboptimal ThinPrep (n=850).

	Patchy cell lost	Thick Preparations	Halo effect	Obscuring blood/ amorphous debris	Total
Number	81	18	24	16	139
Percentage (%)	9%	2%	3%	2%	16%

Table II. Numbers of suboptimal TP with absent endocervical cells and infectious agents under each category.

	Patchy cell lost	Thick Preparations	Halo effect	Total
Abs of endocervical cells component	16	2	4	22
Absence of *Candida Species*	-	3	2	5

TP, ThinPrep; Abs, absence;

DISCUSSION

With the above observations, we sought to explain the likely causes for each category and the corrective measures performed to rectify each problem.

1. Patchy cell lost

The possibility of "falling off" of cellular material after hydrochloric acid bath was eliminated as the progressive method used in the staining of ThinPrep slides omitted the use of hydrochloric acid. Furthermore, immediately after the smears were prepared and before they were subjected to staining, close scrutiny of the preparations showed that these clear spaces already existed. Study of the corresponding conventional cervical smears showed that about 80% of these smears had moderate amount of mucin in the background. Mucinous material that was fixed in alcohol-based PreservCyt often showed less affinity to stick to glass slides. "Falling off" of the mucin fragments together with its attached cellular material could partially explain the empty spaces seen in some of these suboptimal ThinPreps. This phenomenon may be prevented from the outset by educating clinicians to wipe away the mucus plug at the cervical os; to avoid cell collection during menstruation and to limit the use of lubricants.

2. Thick preparations

"Thick preparations" ThinPreps are difficult to screen due to overlapping of cellular material obscuring cellular details. We noted that 'Thick preparations' usually occur in three consecutive specimens with 'sample is dilute' message appearing each time on the ThinPrep processor screen. Surveys done by Cytyc Corporation showed that "thick preparations" were common in countries with tropical climate especially those in the Asia-Pacific region. Users in Europe did

not encounter such problems. Cytyc Corporation has since incorporated an additional software device for use in hot climate that will monitor and better control the "thickness" of ThinPrep.

3. "Halo" effect

ThinPreps with "halo" effects were most unsatisfactory for evaluation due to paucity of cells.

Two possible explanations had been put forth for "Halo" effect. One of the reasons is that during transfer the pressure generated by the ThinPrep processor is insufficient to force the cellular material collected on the filter membrane to the glass slide. Another reason is that dense specimens due to excess epithelial cells, blood and/or inflammation may clump and hold the filter off the slide thereby inhibiting transfer in a particular area. The corrective action necessitates the dilution of the sample 20 fold (1ml of sample to a new solution vial of 20ml) and processing as usual. All repeat ThinPrep smears were satisfactory for evaluation (Fig. 3b-c).

4. Obscuring blood and amorphous debris

ThinPrep with "obscuring blood and amorphous debris" were also difficult to screen. To overcome this problem, the excessive blood and debris can be lysed and resolved with the use of Cytolyt solution which contains one part of Glacial Acetic Acid. This, however, leads to increased cost, time and labour in preparation.

CONCLUSION

We found ThinPrep processor that processes specimens singly requires manual handling at various stages and is unpractical for high volume work. While it is very simple to use, staff found it extremely tedious as only one sample can be processed at a time. The average time required for ThinPrep preparation is about 2 minutes, excluding time taken for the number of repeat procedures required for too dense samples. As the cell suspension is processed one at a time, the amount of technical time taken greatly exceeds the time saved in screening the monolayer smears.

The technical difficulties encountered during the processing of ThinPrep specimens lead to increased cost and time of preparation. It is anticipated that the number of repeat procedures and treatment steps would be increased with the direct placement of the entire brush content into the ThinPrep preservcyt (Direct-to-Vial method).

Future improvements in the technique, including the introduction of lytic agent into the specimen preservative, decreasing the need for consumables and use of fully automated, increased capacity sample processors, may improve the cost-effectiveness of this technology.

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