Malaysian J Pathol 2012; 34(2) : 115 – 121

ORIGINAL ARTICLE

Adequacy of cellular material in split-sampling of cervical scrapings for routine cancer screening: an analysis of 702 smears

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Abstract

Objective: The aim of this study was to examine cells (split-sample) that were retained on sampling devices used to collect conventional Pap smears (primary smears) in order to evaluate specimen adequacy and cytological diagnosis of scrapings that are routinely discarded. *Study design:* Cervical scrapings from women attending routine cervical cancer screening were obtained using a cervical brush. Following primary conventional smear preparation, the same sampling devices were rinsed in Preservcyt solution (Cytyc) for subsequent monolayered thin smear (split-sample/discarded sample). The smears (conventional and ThinPrep® monolayer) were examined independently by pathologists and classified using the Bethesda System. The diagnoses from discarded samples (split-sample smears) were then compared with the diagnoses made on primary conventional Pap smears. *Results:* 702 samples were studied. Cell abnormalities was found in 14/702 conventional smear and 12/702 split-sample thin smear. The adequacy of sampling in primary smears was 94.7% compared to 88.9% in split-sample smears. Six cases of Human Papillomavirus infection was found in split-sample smear, whereas only 5 cases found in primary smear. Cohen's Kappa was 0.61 showing substantial agreement between both sampling cytological results. *Conclusion:* The cervical brush discarded after conventional smear retain adequate number of cells for diagnostic purposes.

Keywords: Split-sampling, primary cytology, ThinPrep® cytology, discarded sample

INTRODUCTION

Cervical cytology is widely used for primary screening of cervical cancer. Early detection of precancerous lesions of the cervix have improved women's healthcare by enabling women to receive appropriate treatment before they become invasive cervical cancers. Hence, screening has led to a significant decrease in incidence and mortality rates from invasive cervical cancer in the screened population. It may reduce the incidence rate to as much as 70% to 80% and has been regarded as the most successful screening tool for cervical cancer.[1]

Cervical cytology can be prepared using different collection devices, techniques of collection, and methods of sample preparation. Cervical scraping can be collected either using cervix brush, cytobrush, plastic or wooden spatula.[1,2] The cellular material on collection devices can be directly smeared onto glass slides, known as conventional cytology or rinsed in liquid-based preservative medium to make liquid-based cytology (also known as ThinPrep® monolayer cytology). Collected cellular material that is directly rinsed into liquid medium for smear preparation is known as direct-to-vial sampling, whereas specimen that is split to prepare for conventional cytology and also liquid-based cytology is known as split-sampling. The liquid-based smear can be prepared using automatic processor which has been proven to reduce the probability of getting thick smears, poor fixation, blood obscuring and air drying or artifact.[3] The cytology result interpretation may vary depending on types of error introduced: sampling error, detection error and technical error in sampling or in fixation of the cervical cells.[4,5] The purpose of the present study was to determine if the throw-away sampling devices used for collecting primary conventional Pap smears would retain significant number of cells for diagnostic purposes. We then compared

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diagnoses made from cellular materials from the discarded devices with diagnoses made by conventional smears under routine conditions from an identical patient population without the need to take another specimen for repetition.

MATERIALS AND METHODS

The subjects were women who attended three main hospitals in Malaysia - Hospital Kota Bharu (HKB), Hospital Universiti Sains Malaysia (HUSM) and Hospital Kuala Terengganu (HKT), for routine cervical cancer screening and volunteered to be recruited into the study. The Pap smears were taken by doctors in the gynaecology clinics of these hospitals. Conventional Pap smear was collected and immediately smeared onto glass slides and alcohol-fixed. Immediately afterwards, the same cervical broom used was cut off and inserted into liquid solution for automated smear (ThinPrep® Pap smear) or termed Split Sample Smear. It was processed by the ThinPrep® 2000 slide processor (Cyctic). Both smears were stained with Papanicolaou stain using standard method. The cytologic diagnoses were classified according to the Bethesda System 2001 (TBS 2001) and reported independently by cytopathologists on rotation as per routine samples (the two smears; conventional and ThinPrep® monolayer smears need not necessarily be read by the same pathologist).

The smears were reviewed for the following features: (1) adequacy of sample, (2) presence or absence of endocervical component or endometrial cells, (3) epithelial cell abnormalities, and (4) miscellaneous findings (e.g., presence of micro-organisms). Sampling adequacy was either satisfactory or unsatisfactory for evaluation. Included in the category of "unsatisfactory smear for evaluation (USFE)" were either broken slides, scanty squamous cells (if cells are less than 10% of the smear), cells obscured by blood, thick areas, poor fixation, air-drying artifact or lack of endocervical cells/transformation zone component.

The Pap smear diagnosis was classified into "Within Normal Limits" (WNL) if it was interpreted as normal without inflammation or benign cellular changes. Benign cellular changes include reactive changes associated with inflammation, atrophy with inflammation, radiation, IUCD and chemotherapy or benign cellular changes due to infection by Trichomonas vaginalis, Candida spp., predominance of coccobacilli, Actinomyces spp., and cellular changes associated with herpes simplex virus (HSV). The epithelial cells abnormalities were either Atypical Squamous/Grandular Cells of Undetermined Significance (ASCUS/AGUS), Low /High-grade Squamous Intraepithelial Lesion (LSIL/HSIL), or Invasive Cervical Cancer (ICC) which is either Squamous Cell Carcinoma (SCC) or Adenocarcinoma (ADC).

This study was approved by the local institutional human ethics review board.

Statistical analysis

The discordance between the two methods (primary Conventional Pap smear and Split-Sample Smear) were compared by calculating percent of overall agreement, percent agreement on testing positive and kappa and tested for statistical differences ($p<0.05$) using an exact-symmetry or Mc-Nemar Chi-square (χ^2) test. Strength of agreement was judged according to Petignat and coworkers (2007)[6]: kappa <0: poor; 0 to 0.2: slight; 0.21 to 0.4: fair; 0.41 to 0.6: moderate; 0.61 to 0.8: substantial and 0.81 to 1.00: almost perfect.

RESULTS

Participant characteristics

Seven hundred and two (702) gynaecologic Pap smears were prospectively collected from women with ages ranging between 19 to 83 years during the study period. The mean±SD age of participants was 43±10.59 and 23.6% of the participants were younger than 34 years. Sixty-four percent were between 35-54 years and 11.8% were older than 55 years. The ethnic grouping of the women studied consisted predominantly of Malays who formed 85.3% of 702 women recruited. The other characteristics of the women studied are shown in Table 1.

Cytology diagnosis

The cytology diagnoses made on smears processed by both methods are shown in Table 2. The percentage of agreement between the two methods is 88.9%. The cytological diagnoses were further classified into 2 groups; (a) Negative (WNL and USFE), and (b) Positive (ASCUS/AGUS, LSIL, HSIL and ICC) (Table 3). There was a substantial agreement between primary conventional smear and split-sample smear (kappa = 0.61; $p\leq0.05$). The concordance (percent of agreement) between both smears increased to 98.6% when the results were classified into Negative and Positive groups.

TABLE 1: Demographics of women recruited into the study

Total number of samples (n)		702	
Age (Year)	<25	20	(2.8%)
	25-34	146	(20.8%)
	35-44	223	(31.8%)
	45-54	230	(32.8%)
	55-64	62	(8.8%)
	65 and above	21	(3.0%)
Ethnicity	Malay	599	(85.3%)
	Chinese	74	(10.5%)
	Indian	4	(0.6%)
	Other	25	(3.6%)
Education	Primary	82	(11.7%)
	Secondary	451	(64.2%)
	Tertiary	93	(13.2%)
	Did not respond to query	76	(10.8%)
Marital Status	Married	635	(90.5%)
	Widowed	32	(4.6%)
	Divorced	15	(2.1%)
	Did not respond to query	20	(2.8%)
Smoking	Yes	4	(0.6%)
	No	669	(95.3%)
	Did not respond to query	29	(4.2%)
Partner smoking status	Smokers	474	(67.5%)
	Non-smokers	119	(17.0%)
	Did not respond to query	109	(15.5%)
No. of sexual partners	1	621	(88.5%)
	2-3	50	(7.1%)
	>3	3	(0.4%)
	Did not respond to query	28	(4.0%)
Partner's use of condom	All the time	5	(0.7%)
	Sometimes	46	(6.6%)
	Never	547	(77.9%)
	Current use	14	(2.0%)
	In the past only	47	(6.7%)
	Did not respond to query	43	(6.1%)
Oral contraceptive use	Yes	76	(10.8%)
	No	594	(84.6%)
	Did not respond to query	32	(4.6%)
Pap smear done before	Yes	379	(54.0%)
	No	292	(41.6%)
	Did not respond to query	31	(4.4%)
Presence of vaginal discharge	Yes	108	(15.4%)
	No	560	(79.8%)
	Did not respond to query	34	(4.8%)
Household income	<RM1000	242	(34.5%)
	RM1000-RM3000	265	(37.7%)
	RM3000-RM5000	118	(16.8%)
	>RM5000	34	(4.8 %)
	Did not respond to query	43	(6.1%)

TABLE 2: Distribution of cytology diagnoses made by primary conventional smear and split-sample smear methods

	Primary Conventional smear n (%)	Split-sample smear n (%)
WNL	651 (92.7)	612 (87.2)
Abnormal		
ASCUS/AGUS	2 (0.3)	0
LGSIL	9 (1.3)	8 (1.1)
HGSIL	0	1 (0.1)
ICC	3 (0.4)	3 (0.4)
USFE	37 (5.3)	78 (11.1)

Percent agreement 88.9%. WNL, Within Normal Limit; ASC/AG-US, Atypical Squamous/Grandular Cell of Undetermined Significance; LGSIL, Low-grade Squamous Intraepithelial Lesion; HGSIL, High-grade Squamous Intraepithelial Lesion; ICC, Invasive Cervical Cancer; and USFE, Unsatisfactory Smear for Evaluation.

Presence of micro-organisms

The presence of *Candida spp* dominated in smears of both methods; 48.2% of primary conventional smear and 47.1% split-sample smears. The presence of human papillomavirus (HPV) was found in five primary conventional smears whereas the number increased in split-sample smear (6 smears). Equal incidence of *Trichomonas vaginalis* was seen in both smears (Table 4).

Sample adequacy

The significant difference between the two methods in terms of unsatisfactory smears was the absence of smears obscured by blood in split-sample smears compared to conventional primary smears. Other reasons for unsatisfactory smears were comparable (Table 5).

DISCUSSION

Liquid-based cytology (LBC) for cervical cancer screening is the method of choice in advanced countries because of it reduced inadequate smears by 80%, thereby requiring fewer women to re-attend clinics for re-sampling.[7] We conducted a split-sample study of cervical scrapings that were retained on Pap smear sampling devices after collection for primary conventional Pap smears. This was done to determine if discarded cells recovered from the sampling devices contained diagnostic material and whether the diagnoses made by these methods were comparable. The final diagnosis in the present study was not based on histology but on the diagnoses made by cytopathologists on conventional Pap smears (gold standard).

Our findings showed that split-sampling yielded adequate cellular material for diagnosis. The percentage of agreement between the two methods (Primary Conventional Pap smear and ThinPrep® Split-sample) was 88.9% (Table 2) when the cytological diagnoses were classified into 6 groups of lesions; Within Normal Limits (WNL), Atypical Squamous/Grandular Cells of Undetermined Significance (ASUS/AGUS), Low-grade Squamous Intraepithelial Lesion (LGSIL), High-grade Squamous Intraepithelial Lesion (HGSIL), Invasive Cervical cancer (ICC) and Unsatisfactory Smear For Evaluation (USFE). When the diagnoses were classified

TABLE 3: Concordance of negative and positive cytological diagnosis rates.

		Primary smear		
		Negative	Positive	
Split-sample smear	Negative	684	6	690
	Positive	4	8	12
		688	14	702

Percent agreement 98.6%, kappa = 0.61, p≤0.05 . [Negative is when the diagnoses made are Within Normal Limits or Unsatisfactory for evaluation; Positive is when diagnoses made are Low/High Grade Surface epithelial lesions [LSIL/HSIL] or invasive cancer [Squamous Cell Carcinoma or Adenocarcinoma]

TABLE 4: Presence of micro-organisms

Micro-organisms	Primary smear, N=56 n (%)	Split-sample smear, N=51 n (%)
Trichomonas vaginalis	2 (3.6)	2 (3.9)
Candida spp	27 (48.2)	24 (47.1)
Coccobacilli	19 (33.9)	17 (33.3)
Actinomyces	3 (5.3)	2 (3.9)
Human papillomavirus (HPV)	5 (8.9)	6 (11.7)

into two broad categories; Negative and Positive; Negative for Within Normal Limits or Unsatisfactory of Evaluation and Positive for ASCUS/AGUS, LSIL, HSIL and ICC there was a substantial agreement of 98.6% with kappa value of 0.61 (Table 3).

This study suggests that there is a higher probability of primary conventional Pap smear identifying abnormal cells in the ASCUS or AGCUS group compared to Split-sample smears. While four cases diagnosed as LSIL in primary conventional smears paired negative in split-sample smears, 3 LSIL in split-samples paired negative in primary conventional smear. Biscotti *et al* reported split smears had significantly more ASCUS results compared to primary conventional smears (9.5% vs. 6.3%, p=0.07).[8] They also stated that split smears had more LGSIL results compared to primary conventional smears (7.8% vs. 5.3%, p=0.03). Interestingly, they found that split-smear had no false negatives, while primary conventional smear had 3 false negatives among 17 confirmed cases of CIN 2 or above.[8] One sample that gave normal results by primary conventional smear was diagnosed HSIL in split-sample smear.[8] In our series, we had a case diagnosed as LSIL by conventional Pap smear but was diagnosed as HSIL in the ThinPrep® split-sample smear. Unlike the previously reported study, our study had nearly double the number of cases recruited.

In general we found micro-organisms are better detected by conventional Pap smears than by split-sample smears except for *Trichomonas* and HPV. Overall, among all samples included in the present study, 2.0% had abnormal results by primary smear and 1.7% by split-sample smear. Despite the small number of abnormal cases, we were able to demonstrate that diagnoses made from split-sample smears were comparable to the primary conventional smears. A study done by Rahimi *et al* also showed the same diagnosis between primary and ThinPrep® smear in their three patients with squamous cell carcinoma (SCC) (sensitivity 100%).[9]

Alves *et al* also compared ThinPrep® and conventional smears in a split-sample study. They observed an increased level of detection of SIL with split-samples compared to conventional Pap smears.[10] They detected 176 mild dyskaryosis by split-sample ThinPrep® smear compared to 125 such cases by conventional Pap smears, an incremental detection of 40.8%.[10] Regarding moderate dyskaryosis or greater, they found that ThinPrep® smears detected 70 cases whereas only 35 cases were identified by conventional Pap smears, corresponding to a 100% increased detection.[10] "High-grade" lesions (moderate and severe dyskaryosis) were detected in 66 cases by ThinPrep® smear, whereas only 32 cases were identified by conventional Pap smear.[10]

The main problem in cytology for cervical

TABLE 5: Unsatisfactory smears rates

Reasons for unsatisfactory smears	Primary smear, N=37 n (%)	Split-sample smear, N=78 n (%)
Scanty cells	2 (5.4)	10 (12.8)
Scanty squamous cells	26 (70.3)	60 (76.9)
Excess blood	5 (13.5)	0
Poor fixation, ThinPrep® sample too dilute, thick areas and air drying or artefact	4 (10.8)	8 (10.3)

cancer screening is sample adequacy. Patients need to re-attend clinics for second sampling if the first sampling is unsatisfactory for cytology evaluation. In our study, we used endocervical brush instead of Ayre spatula as the sample collection device. The endocervical brush is significantly more efficacious than the Ayre spatula in obtaining adequate cervical smears.[1] As proven previously, 97.5% smears taken with cytobrush contained endocervical cells compared with 91% of smears taken with an Ayre spatula.[1] The use of the cytobrush also ensures less repeat smears with a consequent reduction in workload for samplers and laboratories.[1] Our hospital, HUSM has implemented the use of endocervical brush in the past few years only for conventional Pap smear preparation.[11]

In the present study, the adequacy of sampling was 94.7% by primary smears and 88.9% by split-sample smears. The rates of unsatisfactory smears in split-samples are higher (11.1%) compared to primary smears (5.3%). The majority (>70%) of unsatisfactory smears in both smears was due to scant squamous cellularity. This percentage was in accordance with Othman and coworkers who reported that 42.8% to 70.4% of unsatisfactory smears did not contained neither endocervical cells nor presence of metaplastic cells.[12] The unsatisfactory smear due to the blood obscuring was not observed in split-sample smear compared with 13.5% in primary conventional smears. This shows split-sampling using ThinPrep® monolayer can overcome technical artifacts introduced in sampling.

The rate of unsatisfactory smears is variable; it has been reported to be increased[13,14] and decreased[15-19] in liquid-based methods. Some studies have reported no significant change[9] for the rate of unsatisfactory smears for liquid-based cytology. The interpretation of cytology is highly subjective. Therefore proper sampling technique and selection of the sampling instrument is of highly importance.[14] To avoid subjectivity a new method of cervical cancer screening has been introduced.[20]

CONCLUSION

In conclusion, split-sampling yielded adequate cellular material for diagnoses. The main advantage of using the split-sampling technique is that the ThinPrep® smears are less frequently compromised by blood components or cytolysis. Another advantage is for human papillomavirus and for other molecular tests.

ACKNOWLEDGEMENT

The authors would like to thank the women who participated in this study, the gynecologists of the 3 hospitals - Professor Nik Zaki Mahmood of HUSM, Dr Mohd Zulkifli Mohd Kasim of HKT and Dr Zainal Abidin Hanafiah of HKB - for providing cervical scraping samples, the cytologists and pathologists in Pathology Department HUSM Kubang Kerian who assisted in processing and interpreting pap smear samples. This study was funded by research grant (Grant No.: 06-02-05-1027 PR0024/09-01) of the Ministry of Science, Technology and Innovation, Malaysia.

Footnote: The first author is currently working at Institute for Medical Research (IMR), Kuala Lumpur, Malaysia

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