

Optimal Collection Technique and Devices for a Quality Pap Smear

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ABSTRACT

Background: Collection technique is critical in the performance of the Papanicolaou (Pap) smear as an important screening tool for cervical cancer. While superior devices have been established, less effective devices continue to be used for both conventional and liquid-based Pap smears. Our aim is to determine the performance of collection devices currently used in obtaining conventional Pap smears and whether sequence of collection is important for higher quality results.

Methods: This prospective, blinded, cohort study used 2 cytology labs to analyze Pap smears done within a 1-year period. Study participants were 128 clinicians who practice in Dane County, Wis, who send their Pap smears to either of the 2 study cytology labs. Participants included advance practice nurses, family physicians, and obstetrician/ gynecologists. Logistic regression was utilized for analysis.

Results: In conventional Pap smears, sequence of collection did not affect any quality indicators. The Cervex-brush™ (broom) was associated with absent endocervical cells (Odds Ratio=3.12, $P<.001$), limited or unsatisfactory results (OR=1.68, $P<.01$), and obscuring inflammation (OR=2.01, $P<.01$). Of those clinicians who had high levels of absent endocervical cells on their Pap smears (defined as $\geq 3/30$ Pap smears), 47% used the broom alone. The Cytobrush™ optimized quality indicators, and the combination of the Cytobrush™ for the endocervix and spatula for the ectocervix was superior. Presence of infectious agents also contributed to the absence of endocervical cells (OR=3.09, $P<.001$).

Conclusions: The combination of the Cytobrush™ (endocervix) and spatula (ectocervix) is superior for a quality Pap smear. The sequence of collection was not important in conventional Pap smears. The broom alone performs poorly. Presence of infection decreases quality.

INTRODUCTION

Numerous studies have investigated which collection devices best obtain the cells from the transformation zone (TZ) of the cervix for a quality Papanicolaou (Pap) smear.¹⁻⁹ Studies have shown that the Cytobrush™ for the endocervix and the extended tip spatula for the ectocervix lead to higher quality slides. The 2003 US Preventive Services Task Force, on the basis of the Martin-Hirsch meta-analysis on collection devices, recommend the Cytobrush™ (Figure 1) and extended tip spatula for optimum samples.^{2,10} Practitioners and researchers worldwide, however, continue to use the Cervex-brush™ (broom) (Figure 2) for conventional and liquid-based Pap smears.

One study demonstrated that sampling the endocervix first with the Cytobrush™ led to significantly increased blood on the slide.¹¹ Recommendations were made to the clinical community to obtain the ectocervical component first to minimize obscuring blood that can lead to unsatisfactory Pap smears and false negative results.

This study assesses the quality of conventional Pap smears in relation to collection devices and sequence of collection in a large prospective sample of Pap smears obtained by family physicians (FPs), obstetrician-gynecologists (OB/GYNs), and advance practice nurses (APNs).

METHODS

The study was conducted in 2 phases, both approved by the University of Wisconsin Institutional Review Board. The first phase, descriptive in nature, involved a survey of 564 OB/GYNs, FPs, and APNs who practice in Dane County, Wis, regarding their Pap smear col-

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Figure 1. Close up of a Cytobrush™.



Figure 2. Close up of a Cervex-brush™.

lection techniques, including the sequence of collection, instruments used, and management strategies. Phase 1 results were reported in a previous article.¹² Included with the survey was an invitation to participate in Phase 2, which asked participants for their consent to review up to 30 sequential conventional Pap smears they submitted to the cytology lab within a 1-year period.

Clinician inclusion criteria for Phase 2 were that practitioners do conventional Pap smears and process their Pap smears at Dane County Cytology Lab or the Wisconsin State Cytology Lab. Phase 2 data was collected for 1 year. After the investigators obtained consent, the practitioner's next 30 Pap smears were processed in the usual manner by the cytology lab and then examined prospectively. Each Pap smear was examined for quality indicators including degree of obscuring inflammation, degree of obscuring blood, presence of diagnostic cells, and presence of infectious agents.

To bring more experience to the interpretation, senior cytotechnologists were assigned to examine all study

Pap smears. If 50%-74% of the slide was obscured by blood or inflammation, it was judged limited for interpretation, and slides obscured >75% by blood or inflammation were judged unsatisfactory using the standards established by the Bethesda System (TBS) (prior to 2001).^{13,14} The 5 senior cytotechnologists involved in the study separately examined a set of 15 Pap smears, and a Chronbach's coefficient alpha of inter-rater reliability was calculated. One controversial slide was then re-evaluated by the group of 5 to reach consensus on the interpretation of the slide. The cyto-technologists were blinded to the results of the Phase 1 survey, with the clinicians assigned a numerical code for the purpose of entering data on the data sheet. This data sheet was stripped of all identifying patient information except Pap smear slide number (cytology reference number), patient age, patient menopausal or pregnancy status, and number of days since last menstrual period. Reports to practitioners were issued in the usual manner of the cytology lab.

Phase 2 results were analyzed using logistic regression analysis with the quality indicators of the Pap smear as dependent outcome variables. After adjusting for differences in patient age and menopausal and pregnancy status, the proportion of Pap smear slides limited or unsatisfactory from excessive inflammation, obscuring blood, improper fixation, or absence of diagnostic cells was regressed on the technique used to collect the sample, which included the instrument and order of cell collection. Dummy variables for instrument combination and collection order were assigned using centered-effects coding. Odds ratios and P-values reported represent odds of deviations from the global mean of all Pap smear results. Confidence intervals of 95% for the odds ratios are presented.

RESULTS

A total of 230 clinicians were eligible and completed the survey in Phase 1. Of these, 71% (N=163) agreed to participate in Phase 2, but only 128 clinicians met inclusion criteria. Of that 128 17% were APNs, 69% FPs, and 14% OB/GYNs. In total, 3657 Pap smears were examined (not all clinicians completed 30 conventional Pap smears in the 1-year study period). The majority of Pap smears were well woman or pregnancy-related, and few were secondary to prior abnormal Pap smears. The age range of the women who had Pap smears was 14-90, with an average age of 40 years. Four percent of the women were >65 years, and 2% of the woman were <18 years. There were no women included twice in the analysis.

Table 1. Adjusted Odds Ratio (95% Confidence Interval) of Limited Pap Smears by Collection Sequence

	N	Unsatisfactory or Limited	Excessive Inflammation	Blood Obscuring Slide	Endocervical Cells Absent
Collection Sequence					
1st ectocervix, then endocervix	2272	0.94 (0.84,1.05)	0.87 (0.76,1.00)	1.10 (0.89,1.35)	0.89 (0.69,1.16)
1st endocervix, then ectocervix	669	0.97 (0.83,1.12)	1.02 (0.85,1.22)	0.95 (0.72,1.24)	0.77 (0.53,1.11)
Both at same time	716	1.10 (0.96,1.27)	1.13 (0.95,1.33)	0.97 (0.74,1.26)	1.46*(1.09,1.97)

*P<0.05

Table 2. Adjusted Odds Ratio (95% Confidence Interval) of Limited Pap Smears by Collection Instrument

	N	Unsatisfactory or Limited	Excessive Inflammation	Blood Obscuring Slide	Endocervical Cells Absent
Instrument					
(Ectocervix/Endocervix) Broom/Broom	534	1.68*(1.30,2.17)	2.01*(1.44,2.81)	1.13 (0.71,1.81)	3.12*(1.56,6.23)
Wooden spatula (blunt end)/Cytobrush	240	0.97 (0.69,1.37)	1.17 (0.75,1.82)	1.11 (0.63,1.95)	0.48 (0.13,1.86)
Broom/Cytobrush	128	0.55†(0.32,0.92)	0.58 (0.28,1.19)	0.65 (0.28,1.51)	0.89 (0.23,3.48)
Plastic spatula/ Cytobrush	276	1.04 (0.74,1.44)	1.10 (0.71,1.72)	1.37 (0.81,2.32)	0.90 (0.33,2.48)
Wooden spatula (curved end)/Broom	60	1.51 (0.85,2.69)	2.68*(1.40,5.12)	1.07 (0.38,3.00)	0.21 (0.01,6.49)
Wooden spatula (curved end)/Cotton swab	55	1.35 (0.73,2.48)	0.69 (0.25,1.94)	0.40 (0.09,1.81)	9.71*(3.67,25.7)
Wooden spatula (curved end)/Cytobrush	2137	1.08 (0.88,1.33)	1.25 (0.94,1.67)	1.02 (0.72,1.46)	1.41 (0.73,2.72)
Wooden spatula (curved end)/Wooden spatula (curved end)	30	0.49 (0.19,1.30)	0.29 (0.06,1.31)	2.05 (0.60,6.98)	0.28 (0.01,8.57)
Cytobrush/Cytobrush	197	1.59† (1.15,2.20)	1.64† (1.12,2.41)	1.35 (0.77,2.38)	1.33 (0.60,2.94)

* P<0.01; † P<0.05

Inter-rater reliability among the 5 cyto-technologists evaluating the Pap smears was excellent, with a Chronbach's alpha of 0.94. Of all slides evaluated, 20% were limited or unsatisfactory for interpretation. For 12% of the slides, this was due to excessive inflammation; 3% had no endocervical component on the slide; and 5% had blood obscuring the slide. An additional 0.4% had drying artifact.

The collection sequence was not significantly associated with endocervical cells being absent, air drying artifact, or blood obscuring the slide (Table 1). Collecting both at once with the broom, however, was significantly associated with the absence of endocervical cells (Odds Ratio=1.46, $P<0.05$). Broom use was significantly related to unsatisfactory or limited pap smears (OR=1.68, $P<0.01$), excessive inflammation (OR=2.01, $P<0.01$), and absent endocervical cells (OR=3.12, $P<0.001$). It was not associated with blood obscuring the slide. For clinicians with high levels of absent endocervical cells on pap smears (defined as >3/30 Pap smears), 47% used the broom ($P=.001$). The average rate of Pap smears

with no endocervical component rate was 0.85/30 Pap smears. The Cytobrush™ was not associated with significant degrees of absent endocervical cells, inflammation, or obscuring blood. Absence of endocervical cells was negatively associated with postmenopausal status (OR=0.18, $P<0.001$), and positively associated with infectious agent present (OR=3.09, $P<0.001$). Blood obscuring the slide was also negatively associated with postmenopausal state (OR=0.57, $P<0.05$) and presence of infectious agent (OR=.17, $P<0.01$). Only 1 clinician used the spatula for endocervical cell collection, and 2 clinicians used the cotton swab for endocervical cell collection (Table 2).

DISCUSSION

In our study, obscuring blood on the slide, lack of endocervical component, and degree of inflammation were not affected by the sequence of collecting the Pap smear. In addition, the use of the Cytobrush™ for the endocervix was not related to an increase of blood on the slide. Eisenberg et al reported that if the

Cytobrush™ was used first in collecting the endocervical sample and the spatula second for the ectocervix, this sequence significantly increased blood obscuring the slide. They recommended from their results that the ectocervix be sampled first, prior to using the Cytobrush™ for the endocervix.¹¹ Our findings do not support this. Technique with the Cytobrush™, however, is critical. Blood produced with the Cytobrush™ may depend in part on how much it is rotated in the endocervical canal. Most studies report rotating the Cytobrush™ 360 degrees, but cytology guidelines recommend it be rotated only 90 degrees and then be completely rolled onto the slide for capture of the cells from the entire circumference of the endocervical canal.¹³ Eisenberg et al also found that the sequence of collection did not affect air-drying artifact,¹¹ which was supported by our data.

Presence of endocervical component on the Pap smear is often viewed as a quality indicator of a good Pap smear specimen.¹⁴ Curtis et al have shown that the presence of endocervical cells is correlated with a higher proportion of abnormal results.¹⁵ In our study, these cells were significantly absent when the broom was used as a collection device. In those clinicians who had the highest rates of absent endocervical cells, 47% used the broom. In Phase 1 of this study, 20% of clinicians used the broom for the endocervical sample and 21% for the ectocervical sample.¹² The broom was also associated with excessive inflammation, but not with blood obscuring the slide. This may be due to the tendency of the broom to collect more cervical mucus, and therefore, more inflammatory cells because of its design. The cost of repeat Pap smears and false negative results are significant issues. These findings have implications for the broom as a less than optimal collection device for conventional Pap smears, as well as for liquid-based Pap smears. Although the newer liquid-based Pap smears decrease obscuring inflammation and blood, they do not compensate for lack of diagnostic cells that are due to the collection device. Studies of conventional Pap smear collection methods have shown the superiority of sampling the ectocervix with a spatula and the endocervix with the Cytobrush™, rather than using either collection instrument alone or with another type of collection device.^{5,8,9} The cotton swab was shown to be inferior to the Cytobrush™ in obtaining endocervical cells and should not be used in Pap smear collection.⁵⁻⁸ Another study concluded that while the spatula was an effective tool, its sensitivity was improved by sampling the ectocervix first with the spatula and then the endocervix

with the Cytobrush™.¹¹ Effective collection technique and tools are key components to the success of any Pap smear. Our findings concur with these studies and the recommendations of the US Preventive Services Task Force that the extended tip spatula for the ectocervix and the Cytobrush™ for the endocervix are the superior collection tools for quality Pap smears.¹⁰ A recent Cochrane review on collection devices also concurs with these recommendations.¹⁶

Endocervical cells are also significantly absent when an infectious agent is present. Whenever possible, a Pap smear should be deferred until a patient has been treated for the infection, to optimize the accuracy of the Pap smear. In many circumstances and populations, however, this may not be possible.

Our findings have important implications for research on Pap smear technology. Studies that use the broom create an inherent limitation to the study findings. In worldwide cervical screening programs, the broom may at first appear to be an attractive collection device since it can sample both the endocervix and ectocervix at the same time and with just one instrument, but the number of unsatisfactory Pap smears and resulting false negative results negate its time- and cost-savings.

Limitations of this study include a well-defined geographic population, which may affect generalizability of the results. Of the clinicians in this study, 95% were associated with managed care, and they reported that their patient populations were generally low-risk ones.¹² Studying a low-risk population, however, may reduce the confounding variables when looking at how collection devices and sequence of collection affect the quality of Pap smears. Only conventional Pap smears were studied, but a poor collection device will affect both conventional and liquid-based Pap smear results. Although most practitioners used the conventional Pap smear in our study, 57% of practitioners used liquid-based technology in selected cases.¹² The study did not assess the technical skills of the practitioners directly, which could also lead to variability in the quality of Pap smears results.

In summary, the sequence of collection is not important to a quality Pap smear. Since only 2 studies have examined this issue, further research is warranted. The broom and cotton swab are inferior collection devices and their use should be discontinued. The superior tools for collection are the extended tip spatula for the ectocervix and the Cytobrush™ for the endocervix. These results have implications for both conventional and liquid-based Pap smear technology.

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