



# Better Pap Tests Using Liquid-Based Technology

Nancy Gardner, MT (ASCP)

Every year, approximately 12,800 American women are diagnosed with and 4,600 women die from invasive cervical cancer.<sup>1</sup> The good news is that incidence and mortality rates have decreased over the past several decades, mainly as a result of the widespread use of Pap smear screening.<sup>2</sup> The Pap test has been effective in detecting cervical pre-cancer and cancer lesions for over 50 years. Since its introduction, Pap test methodology has remained basically unchanged—until recently.

## Limitations of the Conventional Pap Test

Despite the success noted above, Pap smear testing has limitations. Generally, it is susceptible to two kinds of errors: (1) sampling and preparation errors, and (2) interpretation errors. Sampling errors can occur when cells are never actually collected or are discarded with the sampling device without being transferred to the slide. Studies have shown that, on average, more than 80% of the cell sample is discarded with the sampling device. This means that the cells that actually make it to the slide may be a nonrepresentative subsample of cervical cells.<sup>3</sup> Furthermore, making the smear itself produces results of variable quality. It is difficult to spread the sample evenly across the slide without creating some thick, dense areas, and other regions where cells begin to dry before they are fixed. Interpretation errors arise when blood, mucus, or other nondiagnostic debris obscures the slide. In addition, overlapping of cells and distortion due to air-drying complicate the evaluation of cell changes. The net result of these errors is that a large number of Pap smears give inconclusive or ambiguous results (reparative changes and atypical cells of undetermined significance).

The limitations associated with the conventional Pap smear increase the potential for false-negative readings (find-

ing “no evidence” of abnormal cells even though they are actually present). A study by Joseph et al<sup>4</sup> found that 90% of false negative Pap smear results could be attributed to sampling error. Gay et al<sup>5</sup> evaluated 339 patients diagnosed with cervical malignancy over a four-year period; 20% were found to have had false negative smears. On review and rescreening of patients, it was found that 62% of false negative smears were due to sampling error and 38% due to screening or interpretation error.

## New Pap Test Technology Offers Improvements

Liquid-based Pap tests have been developed to diminish both preparation and screening errors. In May 1996, the FDA approved the *ThinPrep* Pap test for use in gynecological Pap testing, noting that it was “significantly more effective than the conventional Pap smear for the detection of Low Grade Squamous Intraepithelial Lesions (LSIL) and more severe lesions in a variety of patient populations. Specimen quality with the *ThinPrep* System is significantly improved over that of conventional Pap smear preparation in a variety of populations.”<sup>6</sup>

In the *ThinPrep* process, the cell sample is collected in the usual manner using FDA-approved collecting devices (a combination of plastic spatula and endocervical brush or a cervical broom). The sample is rinsed from the collecting device directly into a vial of preservative solution. Virtually all of the cells are rinsed from the collecting device into the solution. The vial of preservative containing the cell sample is sent to the laboratory where it is placed into the *ThinPrep* processor. Gentle dispersion is used to break up blood, mucus, and nondiagnostic debris while thoroughly mixing the cell sample. The cells are collected under gentle vacuum on the surface of a specially designed filter and then transferred to a glass slide. After the slide is placed in a fixative solution, it is stained by the Papanicolaou staining method and examined under a microscope. As a result of this process,

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The author is Supervisor of the Cancer Cytology Laboratory at the NC State Laboratory of Public Health in Raleigh. She can be contacted at 919/365-7564; email: Nancy\_M\_Gardner@msn.net.

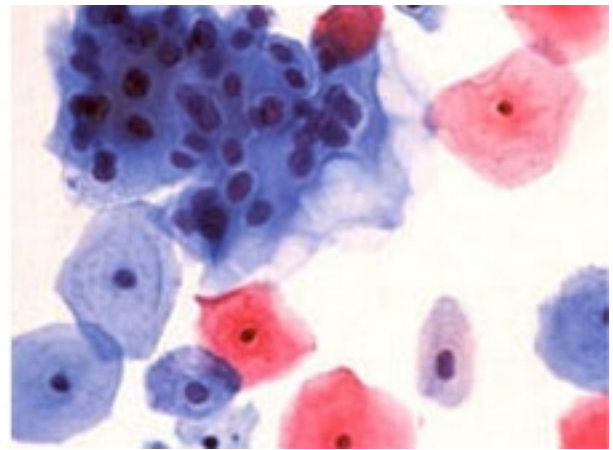
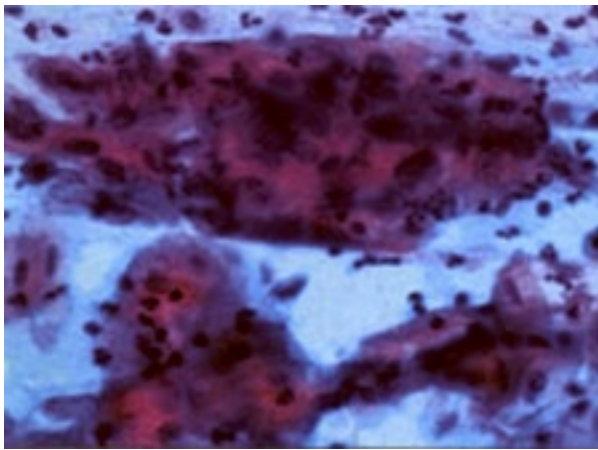


Figure. Cell samples gathered by using the conventional Pap smear (left) and by liquid-based *ThinPrep* method. *ThinPrep* gives more clearly defined cellular detail, with less nondiagnostic debris. Slides courtesy of Cytyk Corporation.

the *ThinPrep* slide contains a more representative cell sample; because it consists of a mono-layer of well-preserved cells, cellular detail is enhanced and blood, mucus, and nondiagnostic debris are gone (See Figure).

The Cytology Laboratory of the North Carolina State Laboratory of Public Health (SLPH) participated in very early clinical trials of the *ThinPrep* Pap test. In the study, 568 paired slides were examined.<sup>7</sup> Enhanced efficacy of the *ThinPrep* Pap test was evident in the improved quality of the slide; four abnormalities were detected on *ThinPrep* slides that were not found on reading of the matching conventional Pap smears.

Cytec Corporation, the maker of *ThinPrep*, conducted a second round of clinical trials. They collected specimens at six sites and included samples from both routine screening and high risk patients (97% of Pap tests done annually in the United States are for screening purposes). In these patients, detection of Low Grade Squamous Intraepithelial Lesions (LSIL) and more abnormal lesions increased by 65%.<sup>8,9</sup> In the high risk population, representing only 3% of annual Pap tests, the detection of LSIL and more severe lesions increased by 6%, and test quality was significantly improved.<sup>8,9</sup> With the *ThinPrep* Pap test, the number of slides classified as "Satisfactory, but limited by" (SBLB) decreased by 54%.<sup>8,9</sup> SBLB smears often must be repeated to obtain a more representative specimen, and this is costly (an SLPH survey of four local health departments found that costs of personnel time to locate the patient, patient education, supplies, etc. for repeat Pap smears ranged from \$90 to \$131).

Other studies of the *ThinPrep* Pap test have confirmed the increased detection of abnormalities and the reduction in number of tests that must be repeated.<sup>10,11</sup> A comparison of *ThinPrep* Pap test results with cervical biopsy results demonstrated excellent correlation, indicating that the increased number of squamous intraepithelial lesions detected with *ThinPrep* Pap testing were real abnormalities.<sup>12</sup>

A study of the ability of *ThinPrep* to detect glandular (rather than squamous) lesions indicated that there were no significant differences in the proportion of glandular lesions detected by *ThinPrep* and conventional Pap tests.<sup>13</sup> However, biopsy results indicated that the *ThinPrep* Pap test was more specific for the detection of glandular disease.

### SLPH Conversion to New Technology

The Cancer Cytology Laboratory of the North Carolina State Laboratory of Public Health was established in 1948. Every year it examines about 155,000 gynecological Pap tests sent from public health facilities, mostly local health department clinics. Most of the patients are at high risk for cervical cancer, meeting one or more of the National Cancer Institute risk factors for cervical cancer: beginning sexual activity at an early age, having multiple sexual partners, smoking tobacco, failing to have regular Pap test screening, infection with human immunodeficiency virus (HIV) or human papilloma virus (HPV), and poor diet. Inflammation is present in a large proportion (about 75%) of the smears (unpublished results, NC State Laboratory of Public Health Cytology Laboratory). As might be anticipated, a large proportion of the conventional Pap smears sent to the laboratory were of less than optimal quality and difficult to evaluate because of cellular drying, or obscuration from inflammatory cells, mucus, blood, etc.

Having already documented the improved Pap smear quality and increased detection of abnormalities, the SLPH Cytology Laboratory adopted the *ThinPrep* Pap test technology in August, 1999. By July 1, 2000, 100% of the health departments using SLPH for Pap screening services had converted to *ThinPrep*.

The SLPH *ThinPrep* Pap results were closely monitored and compared with conventional Pap test results obtained

**Table 1. NC State Laboratory of Public Health Pap test results calendar year 2000: conventional vs. ThinPrep**

Finding	Conventional (n = 51,872)	Thinprep (n = 101,498)
Reparative changes	4.0 (2,077)*	2.5 (2,555)
Reactive changes	8.1 (4,218)	2.4 (2,429)
Atypical squamous cells of undetermined significance	5.5 (2,828)	5.1 (5,131)
Human papilloma virus	2.7 (1,390)	4.2 (4,243)
Dysplasia	3.5 (1,839)	4.8 (4,853)
Atypical glandular cells of undetermined significance	<0.1 (19)	<0.1 (27)
Carcinoma in situ	<0.1 (37)	<0.1 (26)
Squamous cell carcinoma	<0.1 (2)	<0.1 (1)
Adenocarcinoma	<0.1 (2)	0 (0)
<i>Total abnormalities</i>	<i>6.3 (3,289)</i>	<i>9.0 (9,150)</i>
No endocervical component	3.7 (1,912)	2.9 (2,937)
Drying	7.6 (3,967)	<0.1 (20)
Thick smear	2.7 (1,395)	<0.1 (38)
Obscuring blood	1.4 (704)	<0.1 (30)
Total satisfactory, but limited by	19.1 (9,931)	7.4 (7,539)
Unsatisfactory tests	.4 (195)	1.9 (1,916)
<i>Total poor quality smears</i>	<i>19.5 (10,126)</i>	<i>9.3 (9,455)</i>

\*Values in parenthesis show actual number of smears with each finding. Smears may have multiple abnormal findings and poor quality factors. Listing does not include all poor quality factors.

previously (Table). Smear quality improved significantly. Samples classified as “Satisfactory, but limited by” were reduced by nearly two thirds, and benign cellular changes were substantially decreased. Overall, the number of results requiring a repeat Pap test was reduced by one half. Detection of abnormalities increased by about 50%. After gaining experience examining the *ThinPrep* slides, cytotechnologists found that it took less time to evaluate *ThinPrep* compared to conventional Pap smears. This is a benefit when there is a shortage of trained cytotechnologists.

A few problems emerged during the change to the new technology. Initially there was an increase in the number of Pap samples containing no endocervical cells. To improve endocervical sampling, the SLPH now recommends rotating the endocervical brush one-half to one full turn in one direction. Adequately rinsing the collection devices in the vial of preservative solution also lessens the problem. Once these recommendations were in place, we found fewer samples with no endocervical component using the *ThinPrep* Pap test than we found with the conventional Pap smear. We have noted an increase in tests that are unsatisfactory for examination (from 0.4% to 1.9%), mostly because the specimens on arrival in the laboratory contain too much blood (Table).

Improved Pap smear quality and increased detection of abnormalities using *ThinPrep* technology are available in private-sector health care in North Carolina. Thirty-seven cytology laboratories throughout North Carolina offer *ThinPrep* Pap testing. Some North Carolina cytology labo-

ratories offer TriPath, another liquid-based Pap test.

Collecting cells in liquid preservative offers the opportunity to use the preserved material for additional tests. Human papilloma virus testing of cells from the *ThinPrep* vial has been approved by the FDA. The presence of HPV can be suspected from cytological changes, but confirming its presence can be important in determining follow-up treatment. In the near future it should be possible to test for chlamydia, gonorrhea, and Herpes simplex virus (HSV).

## Conclusion

The *ThinPrep* Pap test technology improves Pap test quality and increases detection of abnormal cervical cells. These improvements can lead to more efficient treatment, less patient anxiety and a reduction in costly repeat tests. *ThinPrep* Pap test technology offers benefits to everyone involved in the testing process. The clinician collecting the sample is relieved of having to prepare a smear. Cytopathologists and cytotechnologists get a better quality smear that can be examined more quickly and with more confidence. There are fewer suboptimal smears and smears with benign cellular changes that require a repeat test. Fewer repeat smears save time and money. The patient is the ultimate beneficiary of fewer repeat smears, increased detection of abnormalities, and greater confidence that a “negative” result means no cancer is present.

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