Cervicovaginal Flora

Comparison of Conventional Pap Smears and a Liquid-Based Thin-Layer Preparation

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Key Words: Papanicolaou smears; Candida species; Bacterial flora shift; Trichomonas; Liquid-based thin-layer preparation

ABSTRACT

We compared the effectiveness of SurePath (TriPath Imaging, Burlington, NC) with that of conventional Papanicolaou smears (CP) to demonstrate microorganisms in cervicovaginal smears. Samples from 904 randomly selected cases were examined at the Medical Center of Louisiana clinics for 2 years—the year before and the year after the implementation of SurePath. One observer reviewed the CP and SurePath preparations for detection of microorganisms. Comparison of the 2 systems was made, taking into account patient age, ethnicity, and previous hysterectomy and seasonal variation in prevalence. A seasonal variation was observed in the prevalence of candidiasis. Trichomonas and a shift in bacterial flora were detected more often with CP than with SurePath (13.4% vs 8.3% and 38.7% vs 30.2%, respectively). In contrast, candidiasis was detected more frequently with SurePath than with CP (13.7% vs 7.7%). At the Medical Center of Louisiana clinics, CP was more effective for detecting trichomoniasis and bacterial vaginosis; SurePath was more effective for detecting candidiasis.

Liquid-based thin-layer preparations (LBPs) of cervical specimens are characterized by excellent fixation, homogeneous thin-layer dispersal of cellular material, crisp cellular detail, and a clean background. This technology is rapidly replacing the conventional Papanicolaou smear (CP) as the preferred method for the screening and detection of cervical carcinoma and its precursors. Two products, ThinPrep (Cytyc, Boxborough, MA) and SurePath (TriPath Imaging, Burlington, NC), have been approved by the US Food and Drug Administration and are available for clinical use in the United States for cervical cytology preparations. With SurePath, the samples are collected in an ethanol-based preservative fluid and homogenized by centrifugation. Next, they are concentrated by density gradient, a step intended to remove a large portion of blood, mucus, and other potentially obscuring debris from the sample. Finally, the cells are sedimented over a 13-mm-diameter controlled circle on a microscope slide.

Although it is not the main focus of cervical screening, reporting the presence of microorganisms is essential for a complete diagnostic evaluation of cervicovaginal specimens and may be clinically relevant in certain circumstances. In the 2001 Bethesda system under the category of “Organisms,” 5 microorganisms (Trichomonas vaginalis, Candida species, bacterial species [bacterial vaginosis], Actinomyces species, and herpes simplex virus) should be reported as part of the “nonneoplastic findings,” if present.1

Numerous comparative studies were conducted of LBP vs CP for the detection of cervical carcinoma and its precursors. A significant improvement in the detection of those lesions using LBP compared with using CP has been reported.2-11 However, to our knowledge, there was only 1 study comparing...
these 2 methods for the detection of microorganisms commonly seen in cervical cytology practice. The purpose of the present study was to compare the effectiveness of SurePath and CP in microorganism detection in cervicovaginal smears.

Materials and Methods

Data were obtained from 904 CP and SurePath specimens (3% of total cases) from randomly selected cases between June 2001 and May 2003 at the Medical Center of Louisiana clinics in New Orleans (MCLNO clinics). These clinics provide health care for an economically disadvantaged population, predominantly African American, with a high rate of abnormal cervical cytologic findings (17%-19%) and a high frequency of microorganism detection in cervicovaginal specimens.

At the MCLNO clinics, SurePath gradually replaced CP, beginning in May 2002. Accordingly, we compared 2 historic cohorts (CP and SurePath) of patients attending the MCLNO clinics—those who had CP obtained during the year before the implementation of the SurePath methods and those who had specimens processed with the new SurePath technology the following year. During the validation process, patients whose samples were tested in duplicate by CP and SurePath and unsatisfactory specimens with scant cellularity were excluded.

One observer (H.T.) with CP and SurePath experience examined all specimens for the presence of microorganisms. The presence of Trichomonas, Candida species (pseudohyphae and/or budding yeasts), shift in bacterial flora, Leptothrix, herpes, and Actinomyces was recorded for each specimen. For the purposes of this study, given that the bacterial background is virtually eliminated in the SurePath preparation, a shift in bacterial flora with SurePath specimens was defined by the sole presence of clue cells (squamous cells covered by a layer of cocccobacillary forms of bacteria that obscures the cell membrane). The examination of the selected specimens was conducted alternating small batches of CP and SurePath preparations. The impact of age, ethnicity, history of hysterectomy, and seasonal variation on the prevalence of microorganisms was first determined for the entire study population; then, the frequency of detection of microorganisms by the 2 systems was compared, accounting for these potential confounders.

Data analysis was done with SAS software (SAS Institute, Cary, NC). Continuous data were compared with the 2-tailed Student t test; noncontinuous data were compared using the χ² test. Statistical significance was defined as a P value of less than .05.

Results

A total of 904 specimens were selected for examination and analysis. There were 494 CP (54.6%) and 410 SurePath (45.4%) specimens. There were fewer SurePath than CP specimens because at MCLNO clinics, SurePath technology replaced CP gradually. The mean patient age was 35.6 years (SD, 13.7; SE, 0.62; age range, 13-79 years). Patients in the CP sampling group and those in the SurePath sampling group did not differ significantly with regard to age (P > .05).

The majority of patients (80.5%) were African American (white, 11.7%; Hispanic, 4.9%; and other ethnic groups, 2.9%). There was no significant difference in distribution of the ethnic frequency between patients sampled with CP and those sampled with SurePath (P > .05). This distribution also reflected the ethnicity of the general patient population attending the MCLNO clinics, as recorded in a previous study.

Among the 904 specimens, the prevalence of Trichomonas, Candida species, and a shift in bacterial flora was 11.1%, 10.4%, and 34.8%, respectively. No other microorganisms were found in significant numbers (Leptothrix, 2 cases; Actinomyces, 1 case; herpesvirus, 0). The prevalence of microorganisms was not significantly impacted by the history of previous hysterectomy, which was found in 13.8% of the patients, after accounting for age differences (P > .05).

As shown in Table I and Figure 1, a seasonal variation in prevalence was detected only for Candida species (P = .023; χ²), which was higher in winter (14.9%) and lower in spring (7.5%) and summer (7.9%). No significant seasonal variations were observed for Trichomonas or a shift in bacterial flora (P > .05).

Table I

<table>
<thead>
<tr>
<th>Trichomonas</th>
<th>Candida species</th>
<th>Shift in bacterial flora</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winter (n = 241)</td>
<td>Spring (n = 295)</td>
<td>Summer (n = 177)</td>
</tr>
<tr>
<td>22 (9.1)</td>
<td>34 (11.5)</td>
<td>19 (10.7)</td>
</tr>
<tr>
<td>36 (14.9)</td>
<td>22 (7.5)</td>
<td>14 (7.9)</td>
</tr>
<tr>
<td>75 (31.1)</td>
<td>107 (36.3)</td>
<td>67 (37.9)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Data are given as number (percentage). Winter: December, January, and February; Spring: March, April, and May; Summer: June, July, and August; Fall: September, October, and November.

<sup>b</sup> χ².
As shown in Table 2, there was a significant difference among races in the prevalence of a shift in bacterial flora ($P < .01; \chi^2$). African American and white women had a significantly higher prevalence of a shift in bacterial flora than women of other ethnicity. No significant difference was observed among ethnic groups in the prevalence of Trichomonas and Candida species.

As shown in Table 3 and Figure 2, younger women had a significantly higher prevalence of trichomoniasis, candidiasis, and a shift in bacterial flora than older women ($P < .05$). The shift in bacterial flora was unique because a significant difference was observed between the first 3 age groups (11-20, 21-30, and 31-40 years) and the last 3 age groups (41-50, 51-60, and >60 years).

As shown in Table 4, Trichomonas and a shift in bacterial flora were detected significantly more often with CP than with SurePath ($P < .05$). This significant difference persisted among African American women and women 50 years or younger after stratifying by ethnic and age groups.

In contrast, Candida species were detected more frequently ($P < .05$) with SurePath than with CP. This significant difference persisted among African American women and women 40 years or younger after stratifying by ethnic and age groups.

### Discussion

Most comparison studies of LBP and CP have documented that LBP offers an increased rate of detection of squamous intraepithelial lesions (SILs) and improvement of specimen adequacy, particularly in reducing obscuring factors such as background flora and debris.2-11 Most comparative studies analyzed data obtained with the direct-to-vial method or with the split-sample method. With a split-sample study design, a nonhomogeneous “subsample” was first removed for CP, and

**Table 2**

<table>
<thead>
<tr>
<th></th>
<th>African American (n = 728)</th>
<th>White (n = 106)</th>
<th>Other (n = 70)</th>
<th>Total (N = 904)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trichomonas</td>
<td>89 (12.2)</td>
<td>5 (4.7)</td>
<td>6 (8.6)</td>
<td>100</td>
</tr>
<tr>
<td>Candida species</td>
<td>84 (11.5)</td>
<td>6 (5.7)</td>
<td>4 (5.7)</td>
<td>94</td>
</tr>
<tr>
<td>Shift in bacterial flora</td>
<td>269 (37.0)</td>
<td>34 (32.1)</td>
<td>12 (17.1)</td>
<td>315</td>
</tr>
</tbody>
</table>

* Data are given as number (percentage).

**Table 3**

<table>
<thead>
<tr>
<th></th>
<th>11-20 (n = 117)</th>
<th>21-30 (n = 267)</th>
<th>31-40 (n = 200)</th>
<th>41-50 (n = 179)</th>
<th>51-60 (n = 96)</th>
<th>61 (n = 45)</th>
<th>Total (N = 904)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trichomonas</td>
<td>18 (15.4)</td>
<td>38 (14.2)</td>
<td>20 (10.0)</td>
<td>19 (10.6)</td>
<td>5 (5.2)</td>
<td>0 (0)</td>
<td>100</td>
</tr>
<tr>
<td>Candida species</td>
<td>30 (25.6)</td>
<td>30 (11.2)</td>
<td>14 (7.0)</td>
<td>9 (5.0)</td>
<td>4 (4.2)</td>
<td>7 (15.6)</td>
<td>94</td>
</tr>
<tr>
<td>Shift in bacterial flora</td>
<td>47 (40.2)</td>
<td>108 (40.4)</td>
<td>83 (41.5)</td>
<td>49 (27.4)</td>
<td>24 (25.0)</td>
<td>4 (8.9)</td>
<td>315</td>
</tr>
</tbody>
</table>

* Data are given as number (percentage).
then the residual material was submitted for LBP. Because clinical use is primarily in a direct-to-vial sampling mode, the direct-to-vial data are likely to be much more relevant than the split-sample data for judging the true potential of LBP to enhance detection. In the present study, our direct-to-vial data were collected during a 2-year period—the year before and the year after the implementation of the SurePath methods. This helped to eliminate the potential impact of seasonal variation of infectious agents on the data analysis.

At the MCLNO clinics, CP was more effective than SurePath for the detection of trichomoniasis and a shift in bacterial flora (bacterial vaginosis), whereas SurePath was more effective for the detection of candidiasis. No reports to date were collected during a 2-year period—the year before and the year after the implementation of the SurePath methods. This helped to eliminate the potential impact of seasonal variation of infectious agents on the data analysis.

At the MCLNO clinics, CP was more effective than SurePath for the detection of trichomoniasis and a shift in bacterial flora (bacterial vaginosis), whereas SurePath was more effective for the detection of candidiasis. No reports to date have compared LBP and CP methods for detection of microorganisms that are encountered commonly in cervical cytology practice, with the exception of 1 study by Howell et al in 1998. In this split-sample study, 2 methods (AutoCyte/CytoRich system [Burlington, NC] vs CP) were compared for the detection of candidiasis, trichomoniasis, and SIL. It was concluded that the new technology improved adequacy, increased detection of the 2 microorganisms, and was accurate in SIL diagnosis. Their conclusion, in part, is in disagreement with our data in the present study. This may be related to the difference in study design—use of a split sample vs direct-to-vial sample.

**Trichomonas** was identified more frequently in CP than in SurePath specimens. However, given the clean background with SurePath, the detection of *Trichomonas* was less laborious in SurePath. Because they are small (trichozoites, 4-32 µm), considerable numbers of *Trichomonas* organisms may be eliminated during the SurePath processing steps. SurePath claims to decrease the number of neutrophils, which are of comparable size to some of the trichomonal trophozoites. Consequently, we hypothesize that partial elimination of *Trichomonas* organisms during processing may explain why SurePath is less efficacious for detecting *Trichomonas* than CP.

Similarly, bacterial flora is clearly less abundant with SurePath than with CP preparations. As with *Trichomonas*, partial elimination of bacteria during processing may explain why SurePath is less effective than CP for detecting a shift in bacterial flora.

In contrast, *Candida* organisms, which are much larger than *Trichomonas* organisms and bacteria, may not be eliminated by the SurePath processing. In fact, the final step of sedimentation with SurePath processing may provide an increase in concentration of fungal organisms compared with CP. This may explain why SurePath is more effective than CP for the detection of candidal organisms.

We found a seasonal difference in the detection of *Candida* species but not in *Trichomonas* organisms or a shift in bacterial flora in patients at the MCLNO clinics. *Candida* species occurred most often during the winter (December-February) and least often during the spring (March-May) and the summer (June-August).

There have been a few studies published regarding seasonal variation in the detection of microorganisms in the cervicovaginal tract. The results vary from study to study. Willmott reported no seasonal variation in the detection of yeast forms of *Candida* species by a wet smear in patients attending a venereal disease clinic in London, England. Shradr et al reported no seasonal variation in the detection of *Trichomonas* based on 93,681 Papanicolaou smears examined at a medical school hospital laboratory. Rietveld et al studied a series of 504,093 cervical smears in the Netherlands, where the climate is temperate, and found that *Trichomonas* infections had a higher incidence during the winter (January-March) and a lower incidence during the summer (July-September). It is interesting that *Candida* species showed a higher incidence during fall (October-December) and a lower incidence during the spring (April-June). Sodhani et al studied the smears of 62,758 women in Delhi, India, and reported that *Trichomonas* infections exhibited a higher incidence during winter (November-February) and a lower incidence during summer (March-June) and that candidiasis showed a higher detection rate in the rainy season. Sociodemographic and epidemiologic factors should be studied to better understand the factors related to seasonal variation.

At the MCLNO clinics, CP was more effective than SurePath for the detection of *Trichomonas* and a shift in bacterial flora (bacterial vaginosis), whereas SurePath was more effective for the detection of candidiasis.
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References