Results of an Australian Trial Using SurePath Liquid-Based Cervical Cytology with FocalPoint Computer-Assisted Screening Technology


BD FocalPoint GS computer-assisted screening of BD SurePath liquid-based cervical cytology slides (SP + FP) was compared with screening an accompanying conventional cervical Papanicolaou (Pap) smear (CON) in a split sample trial of 2,198 routine specimens. The rate of unsatisfactory specimens in the SP + FP arm was 0.2% compared with 4.1% in the conventional Pap smear, a significant reduction. There was no statistically significant difference between SP + FP and CON for the detection of histologically confirmed high-grade (HG) lesions in the routine split sample specimens (n = 9). To further test the sensitivity of SP + FP for HG lesions, 38 SurePath slides from confirmed HG cases, without an accompanying CON, were interpolated among the routine smears. In every one of the 47 confirmed HG cases, either HG cells were present in the microscope fields selected by FocalPointGS for review by the screening cytologist (46 of 47), or full screening of the slide was indicated by the FocalPointGS (1 of 47), confirming the effectiveness of SP + FP technology for primary screening. In a small number of cases, the screening cytologist did not recognize the abnormality even though on review HG cells were present in fields selected by FocalPointGS. The overall detection rate was 93% for HG squamous lesions; 89% for known HG endocervical glandular lesions; and 91% for known endometrial carcinoma. In conclusion, the SP + FP detected 100% of HG abnormalities in the trial set; significantly reduced the rate of unsatisfactory specimens; and improved the overall screening rate of detection of HG abnormalities particularly of glandular lesions when compared with other screening technologies. Diagn. Cytopathol. 2011;00:000–000.

Key Words: liquid-based cervical cytology; unsatisfactory Pap smears; high-grade abnormalities of the cervix; SurePath

BD FocalPoint GS (FP) computer-assisted screening technology (formerly AutoPap) received United States FDA approval for primary screening of BD SurePath liquid-based cytology (LBC) slides (SP; formerly AutoCyte) in December 2008.1 The current SP and FP technology represents an advance on previous technologies, which have been shown in overseas studies to be effective for cervical screening, offering increased sensitivity for detection of an abnormality and a reduced rate of unsatisfactory specimens compared with the conventional Papanicolaou (Pap) smear.2,3

In Australia, the CON remains the basis of the publicly subsidized cervical screening program. LBC is not funded by Medicare.5 Thus, When LBC is used in Australia, the specimen is an optional adjunct to the conventional Pap smear. The CON smear is prepared first and the LBC sample is prepared from the residual cellular material remaining on the collection device.

For a SurePath specimen, the head of the collection device (eg the broom-type Cervex brush) is detached into
the vial of SurePath Preservative Fluid for transport to the laboratory. This ensures that the entire specimen adhering to the brush is available for the preparation of the LBC specimen. The preparation process of the SP slide in the PrepStain® System separates and reduces obscuring debris in the cell enrichment process. An aliquot of the washed and concentrated cell sample is settled onto a slide through a density gradient, which places a concentrated epithelial cell sample onto the slide as a 13-mm diameter circular specimen. The PrepStain® System stains the slides according to a modified Pap method.7

In FP-assisted screening of SP slides (SP + FP), slides are placed in the FocalPoint Slide Profiler® overnight in batches of up to 288 slides, for computerized screening. The Profiler selects 10 fields of view (FOVs) on each SP slide, the selected FOVs being fields at screening magnification (10× objective) deemed most likely to contain an abnormality.8 The FOVs are presented to the cytologist at the FP microscope Review Station by means of an automated microscope stage linked to the computer. If an abnormality is found in any of the 10 computer-selected FOVs, the cytologist fully screens the slide. Slides may be rejected as not assessable by the FP Profiler. For these cases, identified for “Process Review” (PR), no FOVs are displayed, and these slides must also be fully manually screened.

Information provided to the cytologist at the microscope workstation includes a quintile score. Quintile 1 indicates that the FP Profiler has placed the smear in the highest ranked 20% of smears for likelihood of an abnormality within that batch. Quintile 5 indicates the lowest 20% risk of an abnormality.

This study, at first in Australia, compares SP + FP screening to manual screening of an accompanying CON for detection of histologically confirmed high-grade (HG) abnormality and further examines the screening sensitivity of SP + FP for detection of confirmed HG abnormality using masked HG cases seeded among routine screening cases.

Materials and Methods

The trial patients were recruited from 23 clinics of general practitioners or specialists between August, 2009, and April, 2010. All patients who would be having a CON in the normal course of their care agreed to the residual material being used to prepare a split sample SP slide for examination using the FP computer-assisted screening system. Those who gave informed consent were included in the trial. Consecutive cases meeting inclusion criteria were accumulated. In all, 2,198 routine split sample cases consisting of a SP slide and an accompanying CON were included in the trial.

To enrich the presence of HG abnormalities within the population of screened specimens, 38 histologically confirmed HG SP slides were seeded into the SP + FP arm of the trial. The seeded cases were SP only with no accompanying CON. The seeded cases consisted of 18 HG cervical squamous abnormalities [17 cervical intraepithelial neoplasias grade 2 or 3 (CIN2 or CIN3), one squamous cell carcinoma (SCC), and 20 HG glandular lesions (six endocervical adenocarcinomas in situ (AIS), three endocervical adenocarcinomas, and 11 endometrial adenocarcinomas)]. Slides to be seeded were reviewed to confirm the presence of cells diagnostic or suggestive of a significant abnormality by JC or RB, who were not involved in screening. To disguise their origin, seeded cases were cleaned, relabeled, and accompanied by dummy request forms and worksheets. Cytologists were not informed that seeding was occurring. In order not to bias interpretations or unmask seeded cases, findings were blinded between SP + FP and CON arms. Different cytologists always screened the CON and the LBC slides, as is normal practice in our laboratory.

SP + FP screening was conducted according to protocols advised by BD.8 If any abnormality was detected in the 10 FP FOVs presented to the screening cytologist, or if the slide was rejected for PR, the cytologist fully screened the slide. In the CON arm, a cytologist fully screened the CON according to normal laboratory practice.

In each arm, if cases were considered negative (NEG) by the primary screener, they were not seen by a second cytologist. Smears deemed unsatisfactory by the primary screener in either arm were referred to a checker (a senior or supervising cytologist) or a pathologist for review and reporting. Smears deemed abnormal by the primary screener in either arm were referred, after full screening, to a checker for review, and then to a pathologist for reporting. Seeded cases were unmasked at pathologist level so that reports were not inadvertently issued for them.

Reports were issued according to the Australian Modified Bethesda System 2004 (AMBS).9 The following summarizes cytological categories used in the trial:

- NEG: Negative—no atypical or malignant cells present
- UNSAT—unsatisfactory for assessment
- LG—low-grade abnormality (comprising PLSIL, LSIL, AGUS)
  - PLSIL—possible LG squamous intraepithelial lesion
  - LSIL—LG squamous intraepithelial lesion— including HPV effect and CIN1
- AGUS—atypical glandular cells of undetermined significance
- HG—high-grade abnormality (Comprising PHSL, HSL, PHGL, AIS, MAL)
Follow-up information was obtained (a) by direct enquiry to referring doctors, (b) through our own histopathology service, and (c) from state Pap Test Register records. Trial cases for which histology revealed a HG lesion during a 10-month period following completion of the screening phase were analyzed.

The trial was approved by the Family Planning NSW Ethics Committee.

Results

The Slide Profiler rejected 68 (3.0%) of 2,236 SP slides and categorized them as PR for technical reasons such as insufficient reference cells, variations in slide or coverslip thickness, staining anomalies, or bubbles in the mountant. No FOVs were displayed for these slides, and according to protocol, all were fully screened manually.

There were four slides in the SP + FP arm that were considered unsatisfactory by the primary screener representing an unsatisfactory rate of 0.2% had the SP + FP specimen been the only specimen. Ninety-one (91) CON slides were considered unsatisfactory by the primary screener representing an unsatisfactory rate of 4.1% had the conventional smear been the only specimen. The difference between SP + FP and CON is highly significant ($P < 0.0001$).

Histologic follow-up of 23 routine split sample cases showed LG or HG changes. Nine of these, all reported HG, were histologically confirmed as HG lesions (all CIN2 or CIN3). No other cases from the trial were identified as having HG histologic follow-up. Histologic confirmation of a HG lesion was thus available for a total of 47 cases, nine routine cases, and 38 seeds.

Direct comparison between the detection of HG disease in the SP + FP arm versus CON was only possible for the routine cases, as the 38 seeded cases were SP only. All nine routine confirmed HG cases were detected on CON, and seven of the nine were detected by the SP + FP cytologist ($1^{	ext{st}}$ screener). The difference in detection rate between SP + FP and CON screening was not statistically significant ($P = 0.5$).

One of 47 (2%) confirmed HG cases was designated PR by the FP system (Table I). The protocol full screening prompted by its designation as PR led to detection of the abnormality by the SP + FP primary screening cytol-
Table II. Quintile scores of confirmed HG cases (N = 47)

<table>
<thead>
<tr>
<th>Quintile (Q)</th>
<th>Total HG cases</th>
<th>SP + FP, 1st screener, HG cases, detected</th>
<th>SP + FP, 1st screener, HG cases, missed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Highest risk of abnormality</td>
<td>41</td>
<td>39</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>2</td>
<td>1</td>
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<tr>
<td>3</td>
<td>0</td>
<td></td>
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<tr>
<td>4</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>5 Lowest risk of abnormality</td>
<td>1</td>
<td>1</td>
<td>1</td>
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<tr>
<td>Process review (PR)</td>
<td>1</td>
<td>1</td>
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gist, so this has been included as a detected case in the SP + FP arm of the trial. Overall, 43 of 47 confirmed HG cases were detected by the cytologist in the SP + FP arm, an overall screening sensitivity for HG abnormality of 91%.

Review of the FOVs presented to the screening cytologist in the four false-negative cases (two HSIL, one AIS, and one endometrial adenocarcinoma) in the SP + FP arm revealed the presence of HG abnormal cells in several FOVs in all four cases. In each case, the cells had not been recognized as abnormal by the primary screener. Seven of 47 confirmed HG cases were considered LG by the primary screener. In each of these seven cases, on referral to a checker and a pathologist, the HG abnormality was identified, and indeed HG abnormal cells were found to be present in at least one FOV in every case.

Squamous lesions, the principal targets of cervical screening, are by far the most common HG lesions encountered. Therefore, an indication of the practical sensitivity of SP + FP screening for HG cervical lesions is provided by considering only the detection of squamous disease (Fig. C-1). Of the 27 HG squamous cervical lesions (nine routine and 18 seeded), 25 were detected in the SP + FP arm, a screening sensitivity for cervical HG squamous lesions of 93% (Table I).

HG glandular abnormalities were not detected among the 2,198 routine cases in the trial, so no comparison of the SP + FP arm versus CON for detection of these lesions was possible. However, the seeded glandular cases allow an indication of the screening sensitivity of SP + FP for these lesions. Endocervical HG glandular abnormality (AIS/adenocarcinoma) was detected by the cytologists in the SP + FP arm in eight of nine cases—a screening sensitivity of 89%. This detection rate is promising in view of reported low sensitivity (70–85%) on the CON and ThinPrep for glandular lesions.10–12

Endometrial HG glandular abnormality (endometrial adenocarcinoma) was detected by the cytologists in the SP + FP arm in 10 of 11 cases—a screening sensitivity of 91%. For HG glandular lesions overall, screening sensitivity was 19 of 21 (90%). The detection rate is encouraging in view of reported low sensitivity for the cervical detection of endometrial adenocarcinoma (25–73%)13,14 (Table I).

Quintile scores provided to the screener at the time of SP + FP screening placed 41 of 47 (87%) of confirmed HG cases in Quintile 1, indicating that these cases were deemed by the FP Profiler to be in the highest 20% of slides for risk of being abnormal. Forty-five of 47 (96%) of HG cases were either in Quintiles 1 or 2 or were flagged for full screening for technical reasons (PR). One case, although placed in Quintile 5, was nevertheless detected by the SP + FP screener. The four missed seeded cases (2 × CIN3; 1 × AIS; and 1 × endometrial adenocarcinoma) were placed in Quintiles 1, 1, 2, and 4 (Table II).

Discussion
An unsatisfactory rate of 0.2% for SP + FP slides compared with 4.1% of CON smears is highly significant and represents a 95% reduction in unsatisfactory reports, providing substantial benefits in cost and convenience to patients, laboratories, and referring clinicians alike. This compares favorably to previous Australian experience of reported unsatisfactory rates for CON 3.5%, and manually screened ThinPrep(TP) technology 0.7%,15 and CON 3.1% versus computer-assisted TP screening 1.8%.16

It has been reported that the cell enrichment process in SP slide effectively clears blood and debris from the specimen and does so more effectively than the TP process.17 This may account for the superior performance of SP in reducing unsatisfactory specimens (Fig. C-2). Overseas studies also report comparable very low unsatisfactory rates, comparable with our findings, for SP slides in large series.2,17

FP technology performed as stated in its design goals of “detecting slides with evidence of squamous carcinoma, adenocarcinoma, and their usual precursors” and identifying “up to 10 FOVs that are most likely to contain abnormal cells.”8 Indeed, in every one of 47 cases of confirmed HG abnormality, the FocalPointGS system either selected FOVs containing HG cells for examination by the screening cytologist (46 of 47), or prompted a full review of the slide leading to detection of the abnormality (1 of 47). Thus, the screening cytologist was provided with the opportunity to make a correct diagnosis in every confirmed HG case. This finding confirms the effectiveness of the technology for primary screening of cervical specimens.

However, screening sensitivity did not reach the potential of 100% offered by SP + FP technology. In a small number of cases, the screening cytologist did not recognize an abnormality even though HG cells were present in FOVs selected by FocalPointGS system.

A detailed review of the cases undercalled by the primary screener in the SP + FP arm has been conducted. The review identified learning curve issues allowing us to
Figs. C-1–C-3. Fig. C-1. (a) High-grade squamous CIN3 in a SurePath (×40). (b) A negative SurePath sample (×10). Fig. C-2. (a) Conventional Papnicolaou smear reported as technically unsatisfactory due to excess blood (×10). (b) Accompanying ThinPrep reported as technically unsatisfactory due to insufficient cells despite repeat testing (×10). (c) The same ThinPrep sample re-processed using SurePath. Now a technically satisfactory slide. (×10). Fig. C-3. Statistical outcomes of Laverty Pathology SurePath trial.
develop refined criteria and screening strategies. These apply to SP LBC slides and to FP screening. This will allow us to incorporate these lessons into future cytologist training. Issues identified include the recognition in SP of small pale variants of HG abnormal cells. Although these have been well described for conventional smears, experience of such variants in SP slides is limited and more recent. We intend to publish the results of the slide reviews separately in the cytological literature.

Although it must be stressed that the number of HG glandular lesions in this trial was small, the sensitivities achieved are encouraging. For endocervical AIS/Adenocarcinoma, the SP + FP sensitivity of 89% exceeds that which we have previously reported for AIS with TP, either screened manually or by computer-assisted technology. It is interesting that in our TP Imager study, as in this study, the technology did place abnormal glandular cells in the selected FOVs in every case. However, even after a much longer experience with TP screening, cytologist errors reduced the potential sensitivity for AIS detection to 7 of 11 (64%), a greater rate of error than in the current BD study. The presence of HG abnormal cells in FOVs in each missed HG glandular case, and the lower error rate in this study, suggests that with more experience SP + FP technology may have particular potential for the detection of these difficult lesions (Fig. C-3).

In this trial, the Quintile scores were not actually used by the five of the six cytologists to influence their screening practice. The cytologists screened as if all slides showed a score of Quintile 1, that is, the highest probability of an abnormality. One cytologist however did have an increased alertness when given a score of Quintile 1 or 2. Quintile scores did however provide the potential for an additional prompt for suspicion in 87% of HG cases, including three of the four missed cases. Of the missed cases, in only one could the Quintile information have been of no help in raising suspicion. One seeded AIS was placed in the low risk Quintile 4. However, the FOVs selected did indeed contain HG abnormal cells, as in the other three missed cases.

The average screening time for cytologists to view the FPFOV was 1.61 min compared with an average of 7.40 min to screen PS in our laboratory. The FPFOV screening time included the time taken to fully screen those SurePath slides considered potentially abnormal. This 5-fold productivity gain in cytologists’ screening capacity is an attractive proposition in any laboratory.

These findings confirm the effectiveness of SurePath LBC together with FocalPointGS computer-assisted screening in reducing unsatisfactory cervical cytology reports and providing high technical sensitivity for HG disease. By addressing cytologist training issues identified in the trial, we will contribute toward achieving the high screening sensitivity offered by this technology.

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