Preliminary Study of a New, Fully Automated System for Liquid-Based Cytology: The NovaPrep® Processor System

Javier Esquivias López-Cuervo a, Estanislao Montalbán Beltran b, Jose Luis Cuadros Lopez b, Angeles Alonso Castillo c, Teresa Nieto Sanchez d

Departments of a Pathology and b Gynecology, Hospital Universitario San Cecilio, c Loja Metropolitan District Health Center, and d Alhama Metropolitan District Health Center, Granada, Spain

Key Words
Liquid-based cytology • Automation • Cervix • Screening

Abstract
Objective: To evaluate a fully automated system for liquid-based cytology (LBC): the NovaPrep® Processor System (NPS), which is based on the new concept of double decantation, versus conventional cytology (CC), the gold standard for cytology. Study Design: We performed a preliminary comparative study involving 1,129 female patients who underwent sampling for a Pap test; the sample was first smeared for CC and then, using the remaining specimen on the brush, for LBC with the NPS. The performances of CC and NPS were evaluated for accuracy and compared using the gold standard of a combination of one of the two methods of pathological cytology with screening for positive human papilloma virus, quantification of cells (normal and pathological), and improvement in the quality of samples and reading time. Results: The results showed improvement in sensitivity (3.81% for CC vs. 4.52% for NPS) with a specificity superior to 90% for both, a markedly decreased number of unsatisfactory specimens, notably samples containing too many inflamed cells (7.4% for CC vs. 0.5% for NPS), and a shortening of the reading time, which was three times less using NPS. Conclusion: This preliminary study showed a gain in sensitivity, a drop in the number of unsatisfactory specimens and a reduction in reading time with NPS. The results achieved using this fully automated LBC procedure are very promising and will hopefully reduce the overall cost of cervical cancer screening in the future.

Background
Screening for cervical and uterine cancer has been performed for more than 50 years according to the method described by Papanicolaou, known as conventional cytology (CC). In spite of the limited sensitivity of this screening smear [1], the incidence of cervical and uterine cancer has very distinctly dropped.

In order to try to improve the sensitivity of CC, new cytological techniques have been developed in the last 15 years, notably liquid-based cytology (LBC). LBC has two main advantages: it generates less unsatisfactory specimens [2] and offers the possibility of using the cellular specimen remaining in the vial for molecular biology techniques that allow detection of infectious agents such as human papilloma virus (HPV) [3].
Regarding an improvement in sensitivity, the results are disputed, and publications comparing it to CC in terms of quality of detection show mixed results [4]. Despite this controversy, these new LBC techniques have been developed in many countries and the economic impact of this technological change needs to be evaluated because of the potential extra technical costs entailed by its implementation [5]. Some studies do not recommend the use of LBC for screening [6]. Others advocate the introduction of LBC after analysis of cost-effectiveness parameters [7] either because of the overall savings related to the reduction in the number of callbacks as a consequence of the improvement in the quality of samples [8] or because of its greater sensitivity in the detection of pre-cancerous lesions [9]. The current trend is in favor of the use of new technologies such as LBC and screening for HPV with greater time intervals in the context of screening adapted to the patients’ age, thereby offering serious advantages over CC in terms of cost-effectiveness [10].

The NovaPrep® Processor System (NPS), provided by Novacyt® (Velizy Villacoublay, France), thus seems very interesting because of its full automation and the possibility of saving aliquots for HPV reflex testing on the same platform. This LBC preparation process is based on double decantation: (1) differential decantation in the vial to select cells of interest, and (2) decantation onto the slide to obtain a homogeneous smear. Automation relies on the use of a specific set of consumables and a pipetting/dispensing robot platform. There are currently two automated systems suited to the level of activity of laboratories: NPS 25 (20–25 samples/h) and NPS 50 (50–60 samples/h). The single-use consumables include a vial and a decantation chamber combined with an absorption system for extracting the supernatant. The vial illustrated in figure 1, allows easy detachment of the head of the sampling brush using an abutment to keep the brush in the vial throughout the whole process. Once sampling is performed by the cytopathologist, the vial is closed using a pierceable, self-sealing cap. Therefore, regardless of the handling performed in a laboratory, it will never again be opened. Furthermore, the vial has an integrated filter and a cone decantation system in order to perform selective filtration and differential decanting of the whole cell suspension. The automated process makes it possible to enrich the final sample with pertinent cells at the bottom of the decantation cone. The cell suspension selected in this manner is then placed in a decantation chamber whose periphery is surrounded by an absorption system that enables slow and gradual extraction of the supernatant. Once the supernatant has been extracted by absorption, the cell smear slide can be stained using the Papanicolaou method. Automation of the whole process is maintained by specific software that allows the use of various modes suited to the sample types (gynecological or nongynecological).

**Materials and Methods**

During the first semester of 2009, we performed a prospective comparative study between CC and NPS on 1,129 female patients asking to be screened for cervical cancer at the University Hospital of San Cecilio and in Health Care Centers for Primary Screening (Granada, Spain). A sample was taken using a detachable brush (CervexBrush®, Rovers, Oss, The Netherlands) allowing initial CC to be performed. Once CC was performed, the detachable head of the brush containing the remaining cell material was inserted into the NPS vial containing the fixative medium.

CC samples were fixed by spraying a fixative and stained using the Papanicolaou technique.

LBC samples were processed by an NPS50 instrument (fig. 2) in accordance with the manufacturer’s instructions. After the sampling, i.e. with the detachable head of the brush inside, the vials were labeled in the laboratory with a bar code sticker on the cap; the slides were labeled with a bar code sticker as well. Then, the vials and the slides were placed on specific deck trays (1–16 vials and slides) without removing the cap. The deck trays were placed on the worktable of the NPS50 instrument. As it is a ‘push-button-and-walk-away’ system, there is no need for a technician during the whole process that is performed by the specific software allowing 9 different modes from GYN to NONGYN. After automatic bar code reading and checking of the matching between vials and slides by the camera of the instrument (arm 2), the process to obtain the slides used 4 needles (arm 1), and included different steps described below:

1. First mixing to break clusters and to homogenize the cell suspension;
2. First decantation to differentiate small clusters and relevant cells from other cells;
3. Pipetting the relevant cells and small clusters from the bottom of the decantation cone;
4. Mixing them with the sticker (NovaStick) in the needle and dispensing them onto the slide, and
5. Second decantation for absorption of the supernatant by the blotter.

All the cervical samples were treated using a software program dedicated to cervical smears, i.e. ‘GYN’ mode, meaning that the first decantation took 15 min and the standardized volume of cells pipetted was 250 μl. If the number of cells on a slide was less than 5,000, an additional smear was done subsequently – entirely automatically – using the ‘Enrichment’ mode, which makes it possible to increase the total number of cells on the slide. At the end, the spot is round, 19 mm in diameter and ready for staining (fig. 3). The staining followed a classical Papanicolaou protocol, but hematoxylin had to be diluted by half in distilled water to reduce the darkness of the nuclei.

The cytological examination was performed by two cytologists from the Pathology Department, including the head cytopathologist. First, the two cytologists performed the readings inde-
pendently, without knowing the assessment made by the other cytologist. Then, the diagnoses were verified and if there was disagreement, the readings were done together to reach a consensus. Diagnoses were classified according to the 2001 Bethesda System. When one of the two cytological results (CC or NPS) was abnormal, the cytological specimen remaining in the LBC vial was screened for HPV with a polymerase chain reaction (PCR) HPV screening kit (Ref: MAD-003910M, Master Diagnostica®, Granada, Spain). A previous validation of the NovaPrep fixative medium for preservation of DNA for detection and genotyping HPV was published by Prébet [11].

In order to evaluate the rate of agreement of cytological diagnosis, the diagnoses made using CC and NPS were compared using the gold standard of combining one of the two methods of pathological cytology with screening for positive HPV. For cases diagnosed as positive by NPS and containing between 5,000 and 20,000 cells, a quantitative analysis of normal and pathological cells was performed in order to verify the ability of the NPS solution to improve the concentration of pertinent cells. The following were considered abnormal cells: koilocytes, significant nuclear hypertrophy (3× normal diameter), and/or hyperchromatism and/or irregular shape and/or contours of nuclei.

The satisfactory or unsatisfactory quality of the sample according to the 2001 Bethesda System received additional study involving the calculation of the total number of cells per slide, the presence or absence of endocervical cells, the difficulty of reading related to inflammation and/or hemorrhage. Calculation of the number of cells per slide was performed on 6 fields at 10× magnification representing an area of 18.84 mm². The area occupied by cells with the NPS solution was 283 mm², from which we deduced the total number of cells per sample.

Reading time was assessed after a minimum of training, i.e. on the last 197 smears. Total stopwatch time was divided by the number of smears in order to determine the mean reading time.

Results

The study was conducted on 1,129 female patients and lasted 6 months. Patients’ mean age was 42.5 years, with a minimum age of 15 and a maximum age of 80 years.

| Table 1. Pathologic findings of CC and NPS regarding HPV status |
| CC diagnosis | HPV+ | NPS diagnosis | HPV+ |
| ASC-US | 7 | 3 | 19 | 8 |
| LSIL | 35 | 35 | 41 | 38 |
| ASC-H | 1 | 0 | 3 | 2 |
| HSIL | 6 | 6 | 5 | 5 |
| AGUS | 1 | 1 | 2 | 1 |
| Total | 50 | 45 | 70 | 54 |

The diagnostic results of CC and NPS as well as the results of reflex testing for HPV are shown in Table 1. Out of the pathological samples detected by CC or NPS, it was impossible to perform HPV reflex testing on 4 smears. All of the pathological smears detected by CC were detected by NPS as well. The overall rate of concordance with CC, which is our reference method, is therefore 100%. The lesion classified as a ‘high-grade squamous intraepithelial lesion’ (HSIL) by CC corresponds to 1 of the lesions classified as ‘atypical squamous cell cannot exclude HSIL’ (ASC-H) by NPS and which was HPV positive. As to the 2 other lesions classified as ASC-H by NPS, only 1 was HPV positive and finally classified as HSIL. Out of the 6 smears classified as ‘low-grade squamous intraepithelial lesion’ (LSIL) by NPS and not detected by CC, 3 were HPV positive and were classified as LSIL. The 3 others were considered to be false positives, despite limited HPV genotyping and even though we did not have any histological results for these cases.

In all, the overall sensitivity – atypical squamous cell of undetermined significance (ASC-US), LSIL, ASC-H, HSIL, atypical glandular cells of undetermined signifi-
cance (AGUS) – of NPS is improved by 40.3% as compared to CC. Sensitivity besides ASC-US is improved by 18.6% for NPS (4.52% for NPS vs. 3.81% for CC), with a specificity of 90.2 versus 97.7% for CC.

The percentages of pathological cells among the 15 cases involving LSIL and HSIL (n = 5,000–20,000 cells) are compiled in table 2. The results show a total of 2.67% of abnormal cells.

The mean number of cells per slide was 15,699, with extremes ranging from 1,067 cells per slide to 50,517 cells per slide for the highest density. Among the 1,129 smears performed using the standard ‘GYN’ mode, 71 had less than 5,000 cells per slide, i.e. 6.2% of samples needed to be enriched. Of these 71 smears which were subsequently redone in ‘enriched’ mode, 8 again had less than 5,000 cells per slide, i.e. 0.7% unsatisfactory samples.

Furthermore, the difference between samplers for NPS was the same as that of samplers for CC. All the data collected by samplers were classified by cell density group as follows: 1 = <5,000, 2 = >5,000 in normal mode and <10,000, 3 = <20,000 and 4 = >20,000. These results, differentiating each sampler, are compiled in table 3. Of the 10 samplers, 2 produced markedly more cell-poor smears than the others, with at least 20% of samples needing to be enriched after the standard ‘GYN’ mode.

The ability of NPS to reduce the hemorrhagic and/or inflammatory component is compiled in table 4, where it appears that the number of very inflammatory samples was reduced by a factor of 14 (fig. 4), while the number of haemorrhagic samples was reduced by a factor of 2.5. The number of samples without endocervical cells collected by samplers were classified by cell density group as follows: 1 = <5,000, 2 = >5,000 in normal mode and <10,000, 3 = <20,000 and 4 = >20,000. These results, differentiating each sampler, are compiled in table 3. Of the 10 samplers, 2 produced markedly more cell-poor smears than the others, with at least 20% of samples needing to be enriched after the standard ‘GYN’ mode.

The ability of NPS to reduce the hemorrhagic and/or inflammatory component is compiled in table 4, where it appears that the number of very inflammatory samples was reduced by a factor of 14 (fig. 4), while the number of haemorrhagic samples was reduced by a factor of 2.5. The number of samples without endocervical cells

**Table 2. Percentage of pathologic cells for NPS smears**

<table>
<thead>
<tr>
<th>Studied fields</th>
<th>Ratio of pathological cells/normal cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.48</td>
</tr>
<tr>
<td>2</td>
<td>2.02</td>
</tr>
<tr>
<td>3</td>
<td>1.6</td>
</tr>
<tr>
<td>4</td>
<td>3.41</td>
</tr>
<tr>
<td>5</td>
<td>2.38</td>
</tr>
<tr>
<td>6</td>
<td>4.11</td>
</tr>
<tr>
<td>Total</td>
<td>2.67</td>
</tr>
</tbody>
</table>

**Fig. 4.** Ability of NPS to reduce the inflammatory component. HSIL (black arrowheads) represented by 3 clusters of pathologic cells subjected to NPS (a) that are easy to diagnose compared with isolated cells in inflammatory mucus for CC (b). Papanicolaou stain. a, b ×100.

**Fig. 5.** HSIL. Papanicolaou stain. ×400.
was greater in NPS, with a rate of 8.7% without endocervical cells versus 5% for CC, which is possibly due to the fact that it is a split sampling study with just the remaining material for NPS.

We tested reading time after a minimum of training, i.e. on the last 197 smears. The total time to read these 197 smears was 600 min, which corresponds to an average reading time for each cell smear of 3.05 min. Therefore, with sufficient training on NPS, the reader will be able to analyze approximately 20 slides per hour.

**Discussion**

The LBC techniques can be divided into three large groups: manual techniques, semi-automated or open automated techniques, and entirely automated techniques, which are closed and secured.

Manual techniques of slide centrifugation are derived from the technique marketed by Thermo-Fischer (PapSpin®). They present two major disadvantages: (1) for user safety with a high risk of viral contamination by aerosolization of HPV, and (2) for quality assurance, with a risk of a lack of tracking because of the absence of bar code identification.

Semi-automated or open automated techniques include the Surepath® preparation process distributed by Becton Dickinson (Franklin Lakes, N.J., USA), and the ThinPrep® using TP2000 and distributed by Cytyc-Hologic (Bedford, Va., USA). These techniques involve risks in terms of quality assurance with incomplete tracking of processed samples regarding, for example, the use of bar code identification.

Entirely automated processes have complete tracking thanks to integrated bar code identification, optimizing the chain of custody. The first instrument on the market was distributed by Cytyc-Hologic, with the TP3000 fol-lowed by the TP5000. The entirely automated NPS based on the principle of double decantation is an alternative to TP5000 and has a completely different design from that of ThinPrep, which uses a technique of filtration through a selective membrane.

Our preliminary study shows that the overall rate of concordance with the CC is 100%. This was the first essential point for validating NPS, i.e. avoiding false negatives versus the reference method. In addition, NPS has been shown to have better overall sensitivity; its sensitivity was also superior to CC (40.3%) for non-ASC-US diagnoses along with better sensibility (18.6%) for a specificity of 90.2 versus 97.7% for CC. The number of ASC-US cases, which was 2.5 times higher for NPS, shows the same proportion of HPV-positive smears as CC, i.e. 42%. The classification of a large number of lesions as ASC-US is probably related to our lack of experience in LBC. We should be able to improve the specificity in the future and more easily categorize these lesions as LSIL or HSIL. These results are all the more encouraging as the percentage of pathological cells was 2.67%, which avoids the problem of false negatives encountered with other technologies [12] due to rare pathological events within a set of normal cells that is too large. Larger studies will be necessary to judge the value of NPS in terms of quality of detection of NPS compared with other LBC technologies. Finally, we were able to demonstrate that with a mini-

### Table 3. Categorization of cell density regarding sampling for 10 practitioners

<table>
<thead>
<tr>
<th>Sampler</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>P4</th>
<th>P5</th>
<th>P6</th>
<th>P7</th>
<th>P8</th>
<th>P9</th>
<th>P10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>11</td>
<td>27</td>
<td>6</td>
<td>2</td>
<td>19</td>
<td>3</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>18</td>
<td>26</td>
<td>14</td>
<td>26</td>
<td>15</td>
<td>14</td>
<td>6</td>
<td>29</td>
<td>12</td>
<td>46</td>
</tr>
<tr>
<td>3</td>
<td>63</td>
<td>48</td>
<td>45</td>
<td>55</td>
<td>57</td>
<td>53</td>
<td>76</td>
<td>43</td>
<td>57</td>
<td>46</td>
</tr>
<tr>
<td>4</td>
<td>13</td>
<td>15</td>
<td>14</td>
<td>13</td>
<td>26</td>
<td>14</td>
<td>15</td>
<td>28</td>
<td>26</td>
<td>8</td>
</tr>
</tbody>
</table>

Values are percentages.

### Table 4. Comparison between CC and NPS for unsatisfactory smears

<table>
<thead>
<tr>
<th></th>
<th>CC</th>
<th>NPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Limitation by inflammation, n</td>
<td>84 (7.4%)</td>
<td>6 (0.5%)</td>
</tr>
<tr>
<td>Absence of endocervical cells, n</td>
<td>57 (5%)</td>
<td>99 (8.7%)</td>
</tr>
<tr>
<td>Limitation by hemorrhage, n</td>
<td>23 (2%)</td>
<td>9 (0.8%)</td>
</tr>
</tbody>
</table>
mum of experience with NPS smears, the reading time could be divided by three compared with CC. An experienced reader is thus able to read approximately 20 slides per hour.

The positive economic impact of these results in terms of diagnosis, optimization of the yield of cytological smears and reading capacity is increased by the possibility of performing HPV reflex testing directly on the remainder of the initial cytological sample. Moreover, pathological cases requiring cytological screening and typing of HPV added to cases diagnosed by CC requiring a second follow-up smear because the smears displayed too many inflamed or hemorrhagic cells represent an overall rate of second consultations of approximately 10% of the total number of initial consultations.

Besides diagnostic performance and economic considerations, it may be stressed that NPS offers a high level of user safety because the vial is never opened again once the sample has been assessed. This is a very important point. Indeed, several studies conducted in the past 20 years showed the possibility of aerosolization of viral or bacterial particles during centrifugation in open chambers/tubes or during agitation followed by opening of the vial [12]. In particular, contaminations by HPV of care-giving staff have been described [13] and several epidemiological studies are beginning to show a major role of HPV in cancers of the respiratory tract [14], notably the lungs [15].

In conclusion, this new concept in LBC opens up extremely interesting perspectives both by its standardization of cell suspension management and its potential of expanding automated pipetting and distribution to other technologies, particularly immunology/cytochemistry or molecular biology.

Acknowledgements

We are indebted to Carmen Prieto and Marga Merino for slide screening, to Mercedes Gonzalez and Gloria Gortz for technical assistance, and to Laura Fernandez from the Gynecology Department of the Hospital Universitario San Cecilio.

References