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Although cytological screening for cervical neoplasia has lowered mortality rates, current screening methods are plagued by sub-optimal sensitivity and/or specificity. The purpose of this study was to compare the performance of the new CellDetect staining technology as a potential screening tool. This initial, non-blinded study, utilized samples are taken at a community-based clinic. The diagnostic results using CellDetect were compared with the performance of Pap staining and human papilloma virus (HPV) testing on the same material, as well as the follow-up biopsies. These data were statistically analyzed in terms of sensitivity, specificity, predictive value (N.P.V and P.P.V), and inter-observer agreement. Bi-functional CellDetect staining revealed morphological details and tinctorial properties that permitted recognition of neoplasia even at low magnification. Performance-wise, CellDetect demonstrated non-inferiority for all statistical parameters to both Pap and HPV tests. Importantly, superior sensitivity compared with Pap staining was observed, as well as higher specificity than HPV testing with near equivalent sensitivity. We conclude that CellDetect is a promising approach to early detection of cervical cancer because of its bi-functional capabilities that afford high sensitivity and specificity. The data suggest that this new methodology warrants further and more extensive clinical evaluation. Diagn. Cytopathol. 2012;40:1054–1061.

One of the persistent problems in woman’s health is the incidence of cancer of the uterine cervix, and its consequent mortality. Fortunately, the incidence has been reduced over the last several decades because of widespread programs for cytological screening for intra-epithelial neoplasia, enabling detection of early, easily treatable lesions. These tests are effective because they evince positive predictive value (P.P.V.) for the outcome of definitive biopsy. The classical tool for this purpose is the universally used stain, introduced by Papanicolaou (Pap stain), which ushered in the current screening era.

Nonetheless, this test has major drawbacks as a diagnostic tool, and even as a screening approach. In many laboratories, the reported sensitivity is low, and the specificity is variable. Interpretation relies on expert cytoscreeners, which often are not available in under-developed countries.

To overcome these problems, several approaches have emerged. For instance, in wealthier countries, regular testing programs have been implemented. This is an ad hoc solution because it ultimately increases the burden on the medical system, without fundamentally resolving the critical issues of sensitivity and specificity. To address the latter goals, etiologically based directions have been pursued that enable use of more sensitive methodologies. Unfortunately, they often are balanced by decreased specificity, and the surrender of morphological assessment capabilities. A leading commercial product group exemplifying this direction—and its attendant limitations—is the DNA-based human papilloma virus (HPV) tests.

From the foregoing, it is clear that alternative methodological approaches to screening are still needed. For instance, a particularly useful advance would be a bi-functional stain that preserved morphology—comparable with Pap staining—while employing a sensitive and specific marker for early neoplasia. A staining technology

Key Words: cervical neoplasia; CellDetect stain; cervical screening

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Received 16 February 2011; Accepted 6 April 2011
DOI 10.1002/dc.21729
Published online 31 May 2011 in Wiley Online Library (wileyonlinelibrary.com).

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making this claim has recently been introduced, and commercialized as CellDetect®. In cell cultures and biopsies, this staining technology clearly differentiates neoplastic from non-neoplastic cells and tissues.9–11 However, its performance as a cytological screening modality for cervical neoplasia has yet to be reported.

Because of the potential importance of this issue, this new technology was subjected to an initial, non-blinded evaluation. The fundamental question addressed in this study was how the sensitivity, specificity, and P.P.V of CellDetect® compared with other standard methods, such as Pap staining and HPV-based assays.

This trial was based in a community clinic, where initial screening commonly occurs. Since patients with abnormal screening outcomes will be referred to tertiary centers for workup and possible treatment, the original samples will be examined by multiple observers. Consequently, we also were interested in a comparative assessment of intra-observer agreement for diagnoses based on CellDetect®. Ideally, the microscopic features appearing in a screening preparation should be comparable interpreted at all levels of the referral chain.

The results of all parts of this investigation support the potential usefulness of CellDetect® for cervical screening and justify a more extensive, further evaluation.

Methods

Patients

Cytological samples and corresponding biopsies were available from patients who underwent colposcopic screening for cervical cancer. The procedures were performed in a community-based Gynecology clinic affiliated with one of the major health funds operating in the Tel Aviv area. From these patients, 113 samples were examined distributed among ThinPrep® and conventional preparations, according to Table I. The diagnostic breakdown for cervical neoplasia found in the follow-up biopsy is also provided in Table I.

Staining Protocols

Papanicolaou staining protocol. Smears were dried and the following staining and washing steps were taken: Slides were washed with 70% ethanol for 2 minutes and then twice with tap water for 1 minute each. Slides were stained with Gill’s haematoxylin for 4 minutes and rinsed with Scott’s water for 2 minutes, washed with 70% ethanol for 1 minute, and then with 95% ethanol for 1 minute. Then slides were stained using Orange G for 2 minutes, and washed twice with 95% ethanol for 1 minute each, followed by 10 minutes in Eosin A (Eosin Azure, a counterstain), comprising three dyes: Eosin Y, Light Green SF yellowish, and Bismarck brown Y. Slides were washed twice with 95% ethanol, one minute each, immersed in xylene twice for 5 minutes each and mounted in Eukitt mounting media.

| Cytoplast Histochemical Color/Morphology Correlation Analysis |
| Neoplastic cells. In each CellDetect®-stained slide, all neoplastic cells were identified according to their morphological features, and were assigned a grade (ASCUS; LSIL; HSIL). For each such cell, the color of the cytoplasm was identified. Neoplastic cells that exhibited pink or red cytoplasm were considered to be positively correlated with the morphological status of such cells. The percent of positive correlation for each grade in the slide was calculated normal cells are abundantly present on these slides. To obtain an amount of ~100 cells per analyzed slide, we counted and analyzed normal single superficial, intermediate, and basal cells in 10 fields of view at ×40 magnification. Normal cells that had green-stained cytoplasm were considered to exhibit a positive correla-
The percent of positive correlation for each grade in the slide was calculated. For statistical analysis, we took into account every case that had at least one neoplastic cell (low or high-grade).

### Statistical Evaluation

The following tests were calculated with 95% confidence intervals (CI) for each test:

- **Test specificity** (conditional probability that the test will be normal if the condition is normal, calculated by the following formula: 
  \[
  \text{Specificity} = \frac{\text{True Negative}}{\text{True Negative} + \text{False Positive}} \times 100 
  \]

- **Test sensitivity** (conditional probability that the test will be positive if the condition is positive, calculated by the following formula: 
  \[
  \text{Sensitivity} = \frac{\text{True Positive}}{\text{True Positive} + \text{False Negative}} \times 100 
  \]

- **N.P.V.** (negative predicted value) probabilities for true negative, calculated by the following formula: 
  \[
  \text{N.P.V.} = \frac{\text{True Negative}}{\text{True Negative} + \text{False Negative}} \times 100 
  \]

- **P.P.V.** (Positive Predicted Value) probabilities for true positive, calculated by the following formula: 
  \[
  \text{P.P.V.} = \frac{\text{True Positive}}{\text{True Positive} + \text{False Positive}} \times 100 
  \]

In addition, we assessed how CellDetect® independently related to each of the other two procedures, by computing Spearman correlations. Because of the importance

Figs. C-1–C-2. **Fig. C-1.** Pictures A and B show non-neoplastic cervical cells. A—stained by Papanicolaou, and B—stained by CellDetect. In contrast to Pap stained cells, CellDetect stained cells exhibit monocolored green/blue cytoplasm. **Fig. C-2.** Example of low grade cervical neoplasia in cervical biopsy stained by CellDetect. Neoplastic epithelium exhibits red/pink color in contrast with non transformed epithelium stained blue.
of standardization in screening results, the extent of diagnostic agreement of two expert pathologists was examined by means of the weighted Kappa coefficient.

Results

General Properties of CellDetect® Staining

CellDetect® was found to have bi-functional capabilities. On one hand, stained preparations—both cytological and histological—readily enabled identifying and assessing cyto-morphological features of neoplasia microscopically. The images were not appreciably inferior to standard staining techniques, such as Pap and hematoxylin–eosin (H&E). On the other hand, this material also provided tinctorial information that related to neoplastic status. For normal cervical squamous epithelium and reactive processes, the cytoplasm stained green/blue. This phenomenon
was noted in both individual cells (Fig. C-1A) and in biopsy tissues (Fig. C-2). In contrast, morphologically recognizable neoplastic cells exhibited pink-magenta tinged cytoplasm (Figs. C-3A,B and C-4A,B). Thus, it was possible to tinctorially suspect neoplasia, even at low magnification, and then confirm that impression based on morphological criteria of the very same pink/red-stained cells. Because of excellent specificity of this method, potentially confusing conditions like reactive cell changes stained as normal cells, i.e., green (Figs. C-5A and B). Typical staining of different kind of cells is shown in Figure C-6.

Because of the potential relevance of such capabilities to clinical applications, the consistency of each of these features was explored quantitatively, and is reported in the following sections.

Quantitative Diagnostic Performance of CellDetect<sup>®</sup>: Morphology, Color

In this section, two basic issues were addressed. The first was the performance of the CellDetect<sup>®</sup> as a purely morphological stain. In that vein, we examined how consistently the following six diagnostic cytological features of HSIL could be identified in CellDetect<sup>®</sup> preparations:

- Nuclear/cytoplasmic (N/C) ratio
- Relative nuclear enlargement
- Nuclear membrane irregularities
- Abnormal chromatin pattern
- Prominent/multiple nucleoli
- Hyperchromatism

Ten cases of CIN 3 were screened with an average number of 94 diagnostic cells/slide. When a cell was encountered, the expression of each feature in that cell was graded 0–5, with five being equivalent to the appearance with the Pap stain; with six grade features, this gave a maximal “morphology score” of 30. We found that in CellDetect<sup>®</sup> preparations the mean score was 25.1, s.d.1.3. These results demonstrated that the stain consistently enabled the observer to discriminate the essential morphological features of CIN 3, similar to the capability afforded by the Pap stain.

The second basic issue was the performance of CellDetect<sup>®</sup> as a purely histochemical stain for neoplasia. Table II shows the tinctorial results as a function of neoplastic grade. The informative domain is the cytoplasm which in neoplastic cells generally stains pink, in distinction to normal and reactive populations where the cytoplasm is green-stained. These data demonstrate a strong trend showing that squamous cells exhibit consistent differential staining that reflects their biological status.

From all of these observations we can conclude two points. First, that CellDetect<sup>®</sup> is a bi-functional stain, providing consistent morphological and histochemical information. Second, that each of these functions can be used independently to assess cervical neoplasia.

Nonetheless, despite convincing trends, it is clear that the staining values show a minor degree of variability, i.e., neoplasia is not red-stained in 100% of samples. The clinical relevance of this variability was assessed by comparing the performance of CellDetect<sup>®</sup> with currently used screening methods in an actual clinical setting. Results of these investigations are described in the remaining sections of this report.

Quantitative Comparative Performance of CellDetect<sup>®</sup>, Pap, HPV

We examined the relative performance of each of these screening tests using two types of cytology preparation, viz conventional and ThinPrep<sup>®</sup>. For this analysis, sensitivity, specificity, and predictive value (negative and positive) relative to the follow-up biopsy were calculated with 95% CI. The data for both assessors were pooled in this phase, but were separately analyzed as well in a subsequent section. The definition of each of these statistical parameters is provided in the methods. In general, pooled data were used, unless a particular point required a sepa-
Table III.  Results of Statistical Analysis of the Comparison Between Screening Methods in the Two Types of Preparations, With the Calculated 95% Confidence Intervals. Data for Both Assessors Were Combined. The Abnormal Parameter Analyzed Was Total CIN

<table>
<thead>
<tr>
<th>Cytology preparation</th>
<th>Test</th>
<th>HPV vs. biopsy</th>
<th>CellDetect® vs. biopsy</th>
<th>Pap vs. biopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional</td>
<td>Specificity</td>
<td>42.9 (25.0, 62.6)</td>
<td>84.5 (72.1, 92.2)</td>
<td>84.5 (72.1, 92.2)</td>
</tr>
<tr>
<td></td>
<td>Sensitivity</td>
<td>88.9 (63.9, 98.0)</td>
<td>98.6 (92.6, 99.2)</td>
<td>84.5 (72.1, 92.2)</td>
</tr>
<tr>
<td></td>
<td>N.P.V.</td>
<td>85.7 (56.1, 97.5)</td>
<td>96.1 (85.8, 99.3)</td>
<td>84.5 (72.1, 92.2)</td>
</tr>
<tr>
<td></td>
<td>P.P.V.</td>
<td>50.0 (32.2, 67.7)</td>
<td>81.6 (67.5, 90.8)</td>
<td>78.6 (62.8, 89.1)</td>
</tr>
<tr>
<td>ThinPrep®</td>
<td>Specificity</td>
<td>36.8 (22.2, 54.0)</td>
<td>76.3 (64.9, 85.0)</td>
<td>80.3 (69.2, 88.2)</td>
</tr>
<tr>
<td></td>
<td>Sensitivity</td>
<td>88.0 (67.7, 96.8)</td>
<td>90.0 (77.4, 96.2)</td>
<td>80.0 (65.8, 89.5)</td>
</tr>
<tr>
<td></td>
<td>N.P.V.</td>
<td>82.3 (55.8, 95.3)</td>
<td>92.1 (81.7, 97.0)</td>
<td>85.9 (75.2, 92.7)</td>
</tr>
<tr>
<td></td>
<td>P.P.V.</td>
<td>47.8 (33.1, 62.9)</td>
<td>71.4 (58.4, 81.7)</td>
<td>72.7 (58.8, 83.4)</td>
</tr>
</tbody>
</table>

Table IV.  Results of Statistical Analysis of the Comparison Between Screening Methods in the Two Types of Preparations, With the Calculated 95% Confidence Intervals. Data for Both Assessors Were Combined. The Abnormal Parameter Analyzed Was CIN2/3

<table>
<thead>
<tr>
<th>Cytology</th>
<th>Test</th>
<th>HPV vs. biopsy</th>
<th>CellDetect® vs. biopsy</th>
<th>Pap vs. biopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional</td>
<td>Sensitivity</td>
<td>92.9 (64.1, 99.6)</td>
<td>93.3 (76.5, 98.8)</td>
<td>76.7 (57.3, 89.3)</td>
</tr>
<tr>
<td>Thin-prep</td>
<td>Sensitivity</td>
<td>100.0 (75.9, 100.0)</td>
<td>90.6 (73.8, 97.5)</td>
<td>84.4 (66.4, 94.1)</td>
</tr>
</tbody>
</table>

rated analysis. The results of this analysis are summarized in Tables III (total CIN) and IV (CIN 2/3).

These statistical tests revealed that there was no inferiority in using CellDetect® in comparison with the Pap and HPV tests. This was so across all the parameters and variables examined in this study. Moreover, clear trends of superiority for CellDetect® staining were also observed throughout the analysis. For instance, using conventional preparations, comparisons of “CellDetect® versus Biopsy” and “Pap versus Biopsy” showed a clear trend toward higher values of sensitivity and N.P.V. in CellDetect®-stained preparations. In addition, this trend was stable, regardless of whether the two assessors were evaluated separately or as pooled data. In the ThinPrep®, this trend persisted for sensitivity and N.P.V., but it was weaker than for the conventional preparations.

It is interesting to note a difference between Tables III and IV regarding HPV performance. In Table III, sensitivity for HPV testing is lower than expected, but at more typical levels in Table IV. The phenomenon expressed in Table III may partially reflect the relatively high portion of CIN 1 cases typical of community screening settings. In support of this interpretation are results of analyzing sensitivity for CIN1 separately for both preparations, (data not shown). In that case, an even greater difference in sensitivity for “CellDetect® versus Biopsy” was found in comparison with “HPV to biopsy” or “Pap to biopsy,” viz 93% versus 69% and 77%, respectively (n = 15).

In contrast, specificity and P.P.V values for CellDetect® (Table III) showed trends that were consistently higher then for HPV testing in all parts of this study. The P values obtained from the Fisher Exact Test confirmed that this trend was statistically significant: specificity, \( P < 0.01 \); P.P.V., \( P < 0.01 \). Importantly, in regards to Pap staining versus CellDetect®, the values for specificity and P.P.V. were essentially equivalent for both preparations.

As part of this analysis, we also assessed how CellDetect® independently related to each of the other two procedures, by computing Spearman correlations. These coefficients were found higher between “CellDetect® and Biopsy” as compared with the correlations between “Pap and Biopsy.” When data from both assessors was pooled, the calculated correlation was 0.79 for “CellDetect® versus Biopsy,” as compared with 0.66 for “Pap versus Biopsy.” In the ThinPrep®, the correlation coefficient was 0.73 for “CellDetect® versus Biopsy,” as compared with 0.66 between “Pap and Biopsy.” Similarly, “HPV versus Biopsy” was poorly correlated, with a coefficient of 0.33.

These statistical values indicate that the CellDetect® diagnoses are more highly correlated with the ultimate biopsy outcome than an analogous screening performed using the Pap stain or a HPV test.

Inter-Observer Variability Using CellDetect® Staining

This phase of the study addressed the question of whether diagnoses rendered using CellDetect® staining technology are reproducible and consistent across observers. Statistical results on the data for both assessors, separately analyzed, are found in Table V.

In regards to sensitivity and N.P.V, we found that a considerably higher level of intra-observer agreement was observed in CellDetect®-stained, conventional preparations than for Pap preparations (Table V). ThinPrep® demonstrated more inter-observer variability under all circumstances. The statistical analysis confirmed these impressions. The weighted Kappa coefficient (Cohen, 1960) was used, which has values from 0 to 1; 1 represents perfect agreement. Landis and Koch (1977)15 proposed that a Kappa value between 0.61 and 0.80 implied
Table V. Statistical Results on the Data for Both Assessors, Separately Analyzed. The Analysis Entailed Comparing Their Performance Between CellDetect®, Pap in the Two Types of Preparations, With the Calculated 95% Confidence Intervals. The Abnormal Parameter Analyzed Was Total CIN

<table>
<thead>
<tr>
<th>Cytology preparation</th>
<th>Test</th>
<th>Assessor 1</th>
<th>Assessor 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CellDetect® vs. biopsy</td>
<td>CellDetect® vs. biopsy</td>
</tr>
<tr>
<td>Conventional</td>
<td>Specificity</td>
<td>86.2 (67.4, 95.5)</td>
<td>82.8 (63.5, 93.4)</td>
</tr>
<tr>
<td></td>
<td>Sensitivity</td>
<td>95.2 (74.1, 99.7)</td>
<td>94.2 (76.1, 99.7)</td>
</tr>
<tr>
<td></td>
<td>N.P.V.</td>
<td>90.2 (78.4, 99.8)</td>
<td>86.2 (74.7, 95.5)</td>
</tr>
<tr>
<td></td>
<td>P.P.V.</td>
<td>83.3 (61.8, 94.5)</td>
<td>80.0 (58.7, 92.4)</td>
</tr>
<tr>
<td>ThinPrep®</td>
<td>Specificity</td>
<td>86.8 (71.1, 95.1)</td>
<td>65.8 (48.6, 79.8)</td>
</tr>
<tr>
<td></td>
<td>Sensitivity</td>
<td>88.0 (77.6, 96.8)</td>
<td>92.0 (72.5, 98.6)</td>
</tr>
<tr>
<td></td>
<td>N.P.V.</td>
<td>91.7 (76.4, 97.8)</td>
<td>92.6 (74.2, 98.7)</td>
</tr>
<tr>
<td></td>
<td>P.P.V.</td>
<td>81.5 (61.2, 92.9)</td>
<td>63.9 (46.2, 78.7)</td>
</tr>
</tbody>
</table>

“substantial agreement.” The weighted Kappa was 0.65 (CI = 0.51–0.79) for CellDetect® preparations, i.e., substantial agreement; for Pap staining, 0.58 (CI = 0.43–0.74) indicating moderate agreement. This was analysis utilized pooled data from both preparation types.

Discussion

This article described a community-based study that compared the predictive value of three technologies used to screen for neoplasia of the uterine cervix. The current gold standard remains the Pap-stained smear, which is frequently supplemented by etiologically based molecular detection of HPV high-risk strains. The first method is flawed by relatively low sensitivity, and the second by limited specificity. Consequently, a third, new staining technology was explored here as a possible adjunct to the current repertoire of screening tools. The new staining technology, referred to as CellDetect® (Zetiq, Israel), is claimed to be “bi-functional.” This term refers to the capability for identifying neoplasia histochemically (i.e., tinctorially), while concomitantly preserving the observer’s ability to assess cyto-morphology. As such, it could potentially afford unique advantages for screening.

To test these claims, the diagnostic results of CellDetect®-stained cytological preparations were statistically analyzed in relation to the performance of Pap staining and HPV testing on the same material in relation to the follow-up biopsy on each case. The statistical analysis of the data mainly derived from calculated values for sensitivity, specificity, and predictive value (N.P.V and P.P.V). We found that the CellDetect® never performed inferiorly compared with the other methods. On the positive side, CellDetect® exhibited clear trends of superiority to both Pap and HPV tests that were statistically significant in certain cases. These trends were observed in both conventional cytological smears as well as ThinPrep® preparations. Although the issue of differential performance of CellDetect® in LSIL versus HSIL is of ultimate concern, it was not the focus of this initial clinical trial. As evident from Table I, this study was specifically designed to assess the relative predictive performance of CellDetect® in relation to the outcome of the corresponding biopsy, on the one hand, and the comparable comparisons of HPV and Pap with biopsy, on the other. A larger follow-up study, is being planned that would include an examination of the question of SIL grade on CellDetect performance.

Another important issue, that was addressed here, concerned the potential for interpretative standardization of CellDetect® staining across observers, and even, between institutions. This is a relevant concern because, as in our study, initial screening is often performed within the community leading to referral to a larger center for treatment and follow-up. For that reason, a screening technology has to be robust enough to produce a high level of consistency across laboratories and observers. We found that the agreement between two experienced cytologists using CellDetect® was superior to Pap staining based on the weighted Kaplan coefficient.

We conclude that Zetiq’s newly introduced technology is likely to serve as a significant adjunct in cervical screening programs. We have demonstrated that CellDetect® possesses bi-functional morpho-histochemical capabilities, as well as superior sensitivity and intra-observer agreement compared with Pap staining. Similarly, in regards to HPV testing, CellDetect® demonstrated statistically significant superiority in specificity and positive predictive value.

In general terms, one could anticipate that this new approach could fill a void in the current battery of tools available for cytological screening. Based on the stain’s complementary capabilities, it offers the promise of becoming a key methodology to meet the challenge of early detection of a persistent and serious problem in women’s health care. The current results clearly justify a more extensive, blinded evaluation of this emerging technological route.

References


