Study on the Significance of Folate Receptor-Mediated Staining Solution (FRD) Staining in Screening High Grade Cervical Lesions

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Background:  
The aim of this study was to investigate the significance of folate receptor-mediated staining solution (FRD) in examination of cervical lesions during gynecological examination.

Material/Methods:  
A total of 404 patients participated in this study. FRD staining was applied to screen high grade cervical lesions. ThinPrep cytology test (TCT) and human papillomavirus (HPV) testing were also used for screening high grade cervical lesions. Coincidence rate and KAPPA value of different methods were compared by SPSS software.

Results:  
As for CIN2+ and CIN3+, sensitivities for HPV testing were (96.92% and 97.78%) > TCT classification 1 (90.77% and 91.11%) > FRD staining (80.00% and 86.67%) > TCT classification 2 (70.77% and 77.78%), respectively. While specificities for HPV testing were (7.08% and 6.44%) < TCT classification 1 (39.53% and 37.88%) < FRD staining (51.92% and 50.97%) < TCT classification 2 (70.80% and 69.36%), respectively. Coincidence rate and KAPPA value of FRD staining, TCT classification 1, TCT classification 2, and HPV testing for detecting CIN2+ results were 56.44% and 16.52%, 47.77% and 13.54%, 70.79% and 27.76%, and 21.53% and 1.36%, respectively. Coincidence rate and KAPPA value of FRD staining, TCT classification 1, TCT classification 2, and HPV testing versus CIN3+ results were 54.95% and 14.19%, 43.81% and 9.27%, 70.30% and 23.91%, and 17.08% and 1.12%, respectively.

Conclusions:  
FRD staining was capable of detecting cervical lesions rapidly and is a cost-effective method for routine cervical lesions screening.

MeSH Keywords:  
3T3 Cells • DNA Probes, HPV • I-kappa B Proteins • Silver Staining

Full-text PDF:  
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Background

Cervical cancer is a common malignancy in the female reproductive system because of human papillomavirus (HPV) infection. Most patients are diagnosed at a late stage and have thus lost the best chance for timely treatment. Therefore, the mortality rate of cervical cancer is high, and 275,000 deaths are reported to be due to cervical cancer each year [1]. Cervical intraepithelial neoplasia (CIN) is a premalignant lesion that is classified into CIN1, CIN2, or CIN3 by histo-pathological diagnosis. CIN2 or CIN3 (high-grade CIN) might progress to be cervical cancer without effective treatment. Lack of information and knowledge, as well as inadequate diagnoses and treatment, might be the reason of high mortality.

Persistent infection with one or more of the carcinogenic genotypes of HPV is a high-risk factor for cervical cancer [2]. It had been proposed that HPV testing could be a method for improve cervical cancer screening. HPV testing was recommended for follow-up of abnormal cytology in women over the age of 30 years and for the surveillance of patients after colposcopic treatment for CIN [3]. The other diagnostic method is Thin-Prep cytology test (TCT). But some studies have shown that TCT is considerably less sensitive than HPV testing to screen for CIN2+ and CIN3+ [4,5]. However, there are limitations for TCT and HPV testing, including low sensitivity of single smear TCT failed to detect high-grade precursor lesions and HPV testing has been shown to have low reproducibility that leads to increased false positives [6,7].

Folate receptor-mediated staining solution (FRD) targets the cervical cancer cell, and has been used for screening cervical lesions with a rapid, simple, effective diagnosis [8-10]. In this study, the cervical orifice and cervical canal were stained by FRD, and compared with TCT and HPV testing when a colposcopic biopsy used as the gold standard procedure. The significance of FRD staining in screening cervical lesions was evaluated in 404 patients.

Material and Methods

Patients

In this study, a total of 404 women had been seen from August 2015 to April 2016 at Third Xiangya Hospital, Central South University were included. Inclusion criteria were as follows: patients who needed a gynecological examination, who were between 20 and 69 years old, without pregnancy and not in menstrual period. Exclusion criteria was as follows: hysterectomy performed, received cervical surgery including conization, loop electrosurgical excision procedure (LEEP), infrared, microwave, or other physical therapy, pregnant, acute inflammation, or diagnosed with CIN2+. Colposcopy was performed for patients with positive HPV and/or TCT ≥ASCUS (atypical squamous cells ofundetermined significance). Before colposcopy, FRD staining was performed for each patient. Under colposcopy, all patients underwent biopsy. Patients with grade II–III of colposcopy and AGC (atypical glandular cells.) of TCT results received endo-cervical curettage (ECC). Finally, all patients had histo-pathological diagnosis. The flow chart of the methodology is shown in Figure 1. Each participant signed the written informed consent before undergoing any study procedures, which was approved by institutional review ethics boards of Xiangya Hospital.

TCT testing

TCT was performed using the ThinPrep system (Cytyc Corporation), with a dedicated cervical cell brush, head extended into about 1 cm of the cervical canal with 5 turns clockwise, and cells were removed into liquid cell preservation solution, according to standard procedure. Cytological diagnoses were performed as previously described [11]. Classifications included NILM (negative for intraepithelial lesion or malignancy), ASC-US (atypical squamous cells of undetermined significance), LSIL (low-grade squamous intraepithelial lesion), ASC-H (atypical squamous cells, cannot rule out high-grade squamous intraepithelial lesion), and HSIL (high-grade squamous intraepithelial lesions).

HPV testing

HPV testing was performed using the HPV GenoArray test kit (HybriBio Ltd.). A dedicated cervical exfoliative cell sampler was used to brush a certain number of cervical exfoliated cells, and cells were added into a 4°C specimen preservation solution. Digene Microplate Luminometer 2000 (DML2000;
Qiagen, Crawley, UK) was used for reading and calculating results of HPV testing.

**FRD staining**

FRD staining was performed in 2 areas of the cervix, the external cervical orifice and the cervical canal using folate receptor-mediated staining solution (Shaanxi Gaoyuan Medical Equipment Service Co., Ltd.). Colors were obtained: brown or green were negative, suggesting that there were no abnormal lesions (CIN\(^2+\)); blue, blue-black, or black were positive, suggesting that there was abnormal disease (CIN\(^3+\)) (according to the color standard ruler).

**Statistical analysis**

All data were collected in Microsoft Excel 2007, and SPSS 16.0 software was used for statistical analysis of data. The data were expressed as \(x\pm s\), and count data were \(x^2\) test. The Kappa test was used for the consistency test. \(P<0.05\) indicated that difference was significant.

**Results**

**Screening results of different diagnosis methods**

There were 404 patients included in the study, with age range from 20 to 69 years (mean 40.3 years). The histopathological diagnosis included inflammation (309 patients), CIN1 (30 patients), CIN2 (20 patients), CIN3 (36 patients), and cancer (9 patients). As for the 404 patients, results of FRD staining included negative (189 patients), positive for cervical canal (125 patients), positive of ecto-cervix (16 patients), positive of cervical canal and ecto-cervix (74 patients). TCT testing was performed for all patients, but only 5 types were identified: NILM (140 patients), ASCUS (119 patients), ASC-H (5 patients), LSIL (81 patients), and HSIL (59 patients). The number of negative and positive patients for HPV testing was 26 and 378, respectively (Figure 2).

**Diagnostic analysis of FRD staining, TCT testing, and HPV testing in different pathological conditions (CIN\(^2+\) and CIN\(^3+\))**

As for positive CIN\(^2+\): positive rates of FRD staining, TCT classification 1, TCT classification 2, and HPV testing were 80.00%, 90.77%, 70.77%, and 96.92%, respectively, and the HPV testing results were the best. However, for negative CIN2+, the negative rate of HPV testing (7.08%) was less than FRD staining (51.92%), TCT classification 1 (39.53%) and TCT classification 2 (70.80%), indicating that the negative rate of TCT classification 2 was the best. Similar results were found for CIN3+: the positive rates were HPV testing (97.78%) > TCT classification 1 (91.11%) > FRD staining (86.67%) > TCT classification 2 (77.78%), while the negative rates were HPV testing (6.44%) < TCT classification 1 (37.88%) < FRD staining (50.97%) < TCT classification 2 (69.36%) (Table 1).

**Consistency and diagnostic capability of FRD staining, TCT testing, and HPV testing results versus CIN\(^2+\) and CIN\(^3+\)**

KAPPA tests were performed for different diagnostic methods. In order to compare different methods, CIN\(^2+\) and CIN\(^3+\) were different pathological conditions which served as the gold standard. As for CIN\(^2+\) (Table 2), the coincidence rate and KAPPA value of TCT classification 2 (70.79% and 27.76%) was the highest among the 3 methods, and FRD staining (56.44% and 16.52%) was the second, while coincidence rate of HPV testing (21.53% and 1.36%) was the lowest. The diagnostic indicators including sensitivity, specificity, PPV, NPV, PLR, and NLR, indicated that FRD staining had comparable results with TCT classification 2. As for CIN\(^3+\) (Table 3), all results were similar in CIN\(^3+\).

**Stratified analysis results**

Stratified analysis was carried out to compare 2 different methods in different stratified patients. When FRD negative and positive results were stratified for analysis, the coincidence rate of TCT testing (47.62% and 47.91%) versus CIN\(^2+\) results were higher than that of HPV testing (14.81% and 27.44%) (Table 4). In ASCUS and LSIL patients (Supplementary Tables 1, 2), the coincidence rate of FRD staining (58.82% and 41.98%) versus CIN\(^2+\) results were higher than that of HPV testing (14.81% and 13.58%). But in HSIL patients (Supplementary Table 3), the coincidence rate of FRD staining (62.71%) versus CIN\(^2+\) results were lower than that of HPV testing (71.91%). In HPV positive and negative patients (Supplementary Table 4), the coincidence rate of FRD staining (65.38% and 55.82%) versus CIN\(^2+\) results were higher than that of TCT classification 1 (15.38% and 50.00%). The aforementioned results suggested that FRD staining and TCT testing methods were better than HPV testing.

**Consistency and diagnostic capability of results of combined methods versus CIN\(^2+\) results**

The combined consistency and diagnostic capability were also explored in this study. As shown in Supplementary Tables 5–7, the coincidence rate of FRD staining combined with TCT testing versus CIN\(^2+\) results were 69.06% when both were positive, FRD staining combined with HPV testing was 58.17%, and TCT testing combined with HPV testing was 52.72%. The results of combined consistency and diagnostic capability were better than only a single method, indicating that combined methods...
Figure 2. Diagnosis results of patients in this study. (A) Histopathological diagnosis; (B) FRD staining. Abnormality of both indicated abnormality of cervical canal and external cervical orifice. (C) TCT testing. (D) HPV testing. ASCUS – atypical squamous cells of undetermined significance; ASC-H – atypical squamous cells, cannot rule out high-grade squamous intraepithelial lesion; LSIL – low-grade squamous intraepithelial lesion; HSIL – high-grade squamous intraepithelial lesions; AGC – atypical glandular cells.
would be the best way to diagnose patients, especially the combination of FRD staining and TCT testing.

**Discussion**

In this study, FRD staining, TCT testing, and HPV testing methods were used for detecting high grade cervical lesions with histopathological diagnosis as the gold standard. As for CIN2+ and CIN3+, the positive rate of HPV testing was the best, in contrast, the negative rate of HPV testing was the worst. Coincidence rate and KAPPA value of FRD staining, TCT classification 1, and TCT classification 2 versus CIN2+ and CIN3+ results were better than HPV testing. Strengths of our study were the 3 methods that were used for comparing the same patients, so internal validity could be maximized. In addition, in order to avoid bias and increase the accuracy of results, pathology and the colposcopy were masked for screening results. Sensitivity and

Table 1. FRD staining, TCT testing and HPV testing results in different pathological conditions (CIN2+ and CIN3+).

| Methods          | CIN2+ Positive | CIN2+ Negative | CIN3+ Positive | CIN3+ Negative | Total Positive | Total Negative | Total |
|------------------|----------------|----------------|----------------|----------------|----------------|----------------|-------|
| FRD staining     |                |                |                |                |                |                |       |
| Negative         | 13 (20.00%)    | 176 (51.92%)   | 189 (46.78%)   | 6 (13.33%)     | 183 (50.97%)   | 189 (46.78%)   |       |
| Positive         | 52 (80.00%)    | 163 (48.08%)   | 215 (53.22%)   | 39 (86.67%)    | 176 (49.03%)   | 215 (53.22%)   |       |
| TCT classification 1* |                |                |                |                |                |                |       |
| Negative         | 6 (9.23%)      | 134 (39.53%)   | 140 (34.65%)   | 4 (8.89%)      | 136 (37.88%)   | 140 (34.65%)   |       |
| Positive         | 59 (90.77%)    | 205 (60.47%)   | 264 (65.35%)   | 41 (91.11%)    | 223 (62.12%)   | 264 (65.35%)   |       |
| TCT classification 2# |                |                |                |                |                |                |       |
| Negative         | 19 (29.23%)    | 240 (70.80%)   | 259 (64.11%)   | 10 (22.22%)    | 249 (69.36%)   | 259 (64.11%)   |       |
| Positive         | 46 (70.77%)    | 99 (29.20%)    | 145 (35.89%)   | 35 (77.78%)    | 110 (30.64%)   | 145 (35.89%)   |       |
| HPV testing      |                |                |                |                |                |                |       |
| Negative         | 2 (3.08%)      | 24 (7.08%)     | 26 (6.44%)     | 1 (2.22%)      | 25 (6.96%)     | 26 (6.44%)     |       |
| Positive         | 63 (96.92%)    | 315 (92.92%)   | 378 (93.56%)   | 44 (97.78%)    | 334 (93.04%)   | 378 (93.56%)   |       |

* TCT classification 1: Negative included normal patients, and positive included other patients; # TCT classification 1: Negative included normal and ASCUS patients, and positive included other patients.

Table 2. Consistency and diagnostic capability of FRD staining, TCT and HPV testing results versus CIN2+ results.

| Indicators          | FRD staining | TCT classification 1 | TCT classification 2 | HPV testing |
|---------------------|-------------|-----------------------|-----------------------|-------------|
|                     | Value       | 95% CI                | Value                 | 95% CI      | Value                          | 95% CI                        |
| Coincidence rate    | 56.44%      | 51.60–61.27%          | 47.77%                | 42.90–52.64%| 70.79%                        | 66.36–75.23%                 |
| KAPPA               | 16.52%      | 9.91–23.12%           | 13.54%                | 8.63–18.45% | 27.76%                        | 18.69–36.83%                 |
| Sensitivity         | 80.00%      | 70.28–89.72%          | 90.77%                | 83.73–97.81%| 70.77%                        | 59.71–81.83%                 |
| Specificity         | 51.92%      | 46.60–57.24%          | 39.53%                | 34.32–44.73%| 70.80%                        | 65.96–75.64%                 |
| PPV                 | 24.19%      | 18.46–29.91%          | 22.35%                | 17.32–27.37%| 31.72%                        | 24.15–39.30%                 |
| NPV                 | 93.12%      | 89.51–96.73%          | 95.71%                | 92.36–99.07%| 92.66%                        | 89.49–95.84%                 |
| PLR                 | 1.66        | 1.41–1.96             | 1.5                   | 1.34–1.69    | 2.42                          | 1.93–3.04                    |
| NLR                 | 0.39        | 0.23–0.63             | 0.23                  | 0.11–0.51    | 0.41                          | 0.28–0.61                    |

CI – confidence interval; PPV – positive predictive value; NPV – negative predictive value; PLR – positive likelihood ratio; NLR – negative likelihood ratio.
Specificity are statistical indicators of detection techniques relative to gold standards (pathological diagnosis), including misdiagnosis rates and missed diagnosis rates of response detection techniques. The KAPPA value is the consistency of the 2 methods (FRD and pathological diagnosis), that is, the probability that the 2 methods are consistent with the same sample. The closer the KAPPA value is to 1, the better the consistency of the 2 detection techniques.

TCT testing and HPV testing are used as common methods to detect high grade cervical lesions and cervical cancer [12–15]. FRD is a kind of living cell staining technique used in clinical human epithelial tissue cells in recent years [8,16]. It is mediated by folate receptor. The main components of the dye solution are folic acid derivatives, reduced methylene blue, acetic acid, and so on [17]. In this study, we compared FRD staining with TCT testing and HPV testing in screening high grade cervical lesions. When compared with HPV testing, coincidence rate, KAPPA value, specificity of TCT testing (TCT classification 1 and 2) were higher, but HPV testing had higher sensitivity than TCT testing. Previous studies showed similar comparison results of TCT and HPV testing [18,19]. When compared with histopathological diagnosis results (CIN2+ and CIN3+), coincidence rate of FRD staining and TCT testing were similar, but both were better than HPV testing, indicating that diagnosis results of FRD staining and TCT testing showed better consistency with the gold standard than HPV testing.

### Table 3. Consistency and diagnostic capability of FRD staining, TCT and HPV testing results versus CIN3+ results.

| Indicators     | FRD staining | TCT classification 1 | TCT classification 2 | HPV testing |
|----------------|--------------|----------------------|----------------------|-------------|
|                | Value | 95% CI | Value | 95% CI | Value | 95% CI | Value | 95% CI |
| Coincidence rate | 54.95% | 50.10–59.80% | 43.81% | 38.97–48.65% | 70.30% | 65.84–74.75% | 17.08% | 13.41–20.75% |
| KAPPA          | 14.19% | 8.60–19.78% | 9.27% | 5.25–13.29% | 23.91% | 15.50–32.31% | 1.12% | –0.0248 |
| Sensitivity    | 86.67% | 76.73–96.60% | 91.11% | 78.78–97.52% | 77.78% | 65.63–89.92% | 97.78% | 88.23–99.94% |
| Specificity    | 50.97% | 45.80–56.15% | 37.88% | 32.86–42.90% | 96.36% | 64.59–74.13% | 6.96% | 4.33–9.60% |
| PPV            | 18.14% | 12.99–23.29% | 15.53% | 11.16–19.90% | 24.14% | 17.17–31.10% | 11.64% | 8.41–14.87% |
| NLR            | 0.26 | 0.12–0.55 | 0.23 | 0.09–0.60 | 0.32 | 0.18–0.56 | 0.32 | 0.04–2.30 |

CI – confidence interval; PPV – positive predictive value; NPV – negative predictive value; PLR – positive likelihood ratio; NLR – negative likelihood ratio.

### Table 4. Consistency and diagnostic capability of TCT testing and HPV testing results versus CIN2+ results in FRD negative and positive patients.

| Indicators     | FRD negative patients | FRD positive patients |
|----------------|-----------------------|-----------------------|
|                | TCT testing | HPV testing | TCT testing | HPV testing |
|                | Value | 95% CI | Value | 95% CI | Value | 95% CI | Value | 95% CI |
| Coincidence rate | 47.62% | 40.50–54.74% | 14.81% | 9.75–19.88% | 47.91% | 41.23–54.58% | 27.44% | 21.48–33.41% |
| KAPPA          | 6.73% | 0.83–12.63% | 0.21% | –0.0454 | 15.50% | 8.38–22.62% | 1.49% | –0.0504 |
| Sensitivity    | 84.62% | 54.55–98.08% | 92.31% | 63.97–99.81% | 92.31% | 81.46–97.86% | 98.08% | 89.74–99.95% |
| Specificity    | 44.89% | 37.54–52.23% | 9.09% | 4.84–13.34% | 33.74% | 26.48–41.00% | 1.59–8.22% |
| PPV            | 10.19% | 4.48–15.89% | 6.98% | 3.17–10.78% | 30.77% | 23.53–38.01% | 18.86–30.65% |
| NLR            | 0.34 | 0.09–1.24 | 0.85 | 0.12–5.89 | 0.23 | 0.09–0.60 | 0.05–3.06 |

CI – confidence interval; PPV – positive predictive value; NPV – negative predictive value; PLR – positive likelihood ratio; NLR – negative likelihood ratio.
In stratified analysis of ASCUS and LSIL patients, FRD staining showed better consistency with the gold standard than HPV testing, and the opposite results were found in HSIL patients; the differences of both were not significant due to small sample size in HSIL patients. These results indicated that FRD staining was better than HPV testing in most TCT testing patients. As for HPV negative and positive patients, diagnosis results of FRD staining and TCT testing were similar, in addition to HPV negative patients, and the possible reason may be that only 26 patients were HPV negative. In FRD negative and positive patients, there was no doubt that TCT testing was better than HPV testing. The coincidence rate, KAPPA value, and specificity of TCT testing plus FRD staining, FRD staining plus HPV testing, TCT testing plus HPV testing were better than a single method, but all sensitivity was decreased in combined methods. All the aforementioned results indicated FRD staining was a comparable method to TCT testing, and showed more excellent diagnostic results than HPV testing. Moreover, combined methods would be a better choice for screening high grade cervical lesions. HPV detection for genotyping detection, a total of 21 HPV subtypes can be detected, including HPV high-risk types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68; HPV is low in types 6, 11, 42, 43 and 44; HPV virus types 53, 66, and CP8304 are common in Chinese populations. Statistical analysis was performed only for the presence or absence of HPV infection. HPV tests were positive and negative, and no statistical analysis was performed for each type. Many HPV virus infections are simultaneously infected with several subtypes, and the different types of HPV statistics have no effect on the sensitivity and specificity of FRD. Therefore, this study did not analyze the types of HPV high-risk, and statistically calculated the pathological diagnosis results.

**Conclusions**

This study investigated the clinical value of FRD staining in examination of cervical lesions compared with TCT testing and HPV testing, and the study results suggested that FRD staining had great diagnostic capability for screening high grade cervical lesions, had comparable results to TCT testing, and better diagnostic capability than HPV testing. In addition, FRD staining is cheap, rapid, easy, and effective diagnosis method, especially for China’s remote areas. Clinical laboratory personnel can be easily trained to operate FRD staining and it is worth it to further promote and apply this method in clinical practice. However, this study had a small sample size, especially for patients with cervical cancer. Therefore, further study is need in the future.

**Conflict of interest**

None.

**Supplementary Tables**

**Table 1.** Consistency and diagnostic capability of FRD staining and HPV testing results versus CIN2+ results in ASCUS patients.

| Indicators  | FRD staining       | 95% CI      | HPV testing   | 95% CI      |
|------------|--------------------|-------------|--------------|-------------|
| Coincidence rate | 58.82%             | 49.98–67.67%| 18.49%       | 11.51–25.46%|
| KAPPA       | 8.65%              | −4.07–21.36%| 0.41%        | −3.28–4.11% |
| Sensitivity | 61.54%             | 31.58–86.14%| 92.31%       | 63.97–99.81%|
| Specificity | 58.49%             | 49.11–67.87%| 9.43%        | 3.87–15.00% |
| PPV         | 15.38%             | 5.58–25.19% | 11.11%       | 5.18–17.04% |
| NPV         | 92.54%             | 83.44–97.53%| 90.91%       | 58.72–99.77%|
| PLR         | 1.48               | 0.91–2.41   | 1.02         | 0.86–1.21   |
| NLR         | 0.66               | 0.32–1.33   | 0.82         | 0.11–5.87   |

CI – confidence interval; PPV – positive predictive value; NPV – negative predictive value; PLR – positive likelihood ratio; NLR – negative likelihood ratio.
### Supplementary Table 2. Consistency and diagnostic capability of FRD staining and HPV testing results versus CIN2+ results in LSIL patients.

| Indicators         | FRD staining | HPV testing |
|--------------------|--------------|-------------|
|                    | Value        | 95% CI      | Value        | 95% CI       |
| Coincidence rate   | 41.98%       | 31.23–52.72%| 13.58%       | 6.12–21.04%  |
| KAPPA              | 2.41%        | -4.88–9.70% | -1.61%       | -6.62–3.39%  |
| Sensitivity        | 75.00%       | 19.41–99.37%| 75.00%       | 19.41–99.37% |
| Specificity        | 40.26%       | 29.31–51.21%| 10.39%       | 3.57–17.20%  |
| PPV                | 6.12%        | 1.28–16.87% | 4.17%        | 0.87–11.70%  |
| NPV                | 96.88%       | 83.78–99.92%| 88.89%       | 51.75–99.72% |
| PLR                | 1.26         | 0.69–2.28   | 0.84         | 0.47–1.48    |
| NLR                | 0.62         | 0.11–3.46   | 2.41         | 0.39–14.85   |

CI – confidence interval; PPV – positive predictive value; NPV – negative predictive value; PLR – positive likelihood ratio; NLR – negative likelihood ratio.

### Supplementary Table 3. Consistency and diagnostic capability of FRD staining and HPV testing results versus CIN2+ results in HSIL patients.

| Indicators         | FRD staining | HPV testing |
|--------------------|--------------|-------------|
|                    | Value        | 95% CI      | Value        | 95% CI       |
| Coincidence rate   | 62.71%       | 50.37–75.05%| 71.19%       | 59.63–82.74% |
| KAPPA              | 2.55%        | -19.50–24.61%| 18.92%       | -0.12–37.95% |
| Sensitivity        | 87.18%       | 72.57–95.70%| 100.00%      | 90.97–100.00%|
| Specificity        | 15.00%       | 3.21–37.89% | 15.00%       | 3.21–37.89%  |
| PPV                | 66.67%       | 53.73–79.66%| 69.64%       | 57.60–81.70% |
| NPV                | 37.50%       | 8.52–75.51% | 100.00%      | 29.24–100.00%|
| PLR                | 1.03         | 0.82–1.28   | 1.18         | 0.98–1.41    |
| NLR                | 0.85         | 0.23–3.22   | 0.00         | -.           |

CI – confidence interval; PPV – positive predictive value; NPV – negative predictive value; PLR – positive likelihood ratio; NLR – negative likelihood ratio.

### Supplementary Table 4. Consistency and diagnostic capability of FRD staining and TCT classification 1 results versus CIN2+ results in HPV negative and positive patients.

| Indicators         | HPV negative patients | HPV positive patients |
|--------------------|-----------------------|-----------------------|
|                    | FRD Staining | TCT classification 1 | FRD Staining | TCT classification 1 |
|                    | Value        | 95% CI      | Value        | 95% CI      | Value        | 95% CI      | Value        | 95% CI      |
| Coincidence rate   | 65.38%       | 47.10–83.67%| 15.38%       | 4.36–34.87%| 55.82%       | 50.81–60.83%| 50.00%       | 44.96–55.04%|
| KAPPA              | 6.40%        | -0.57       | 1.38%        | -0.0535     | 16.64%       | 9.89–23.39% | 15.25%       | 9.83–20.66% |
| Sensitivity        | 50.00%       | 1.26–98.74% | 100.00%      | 15.81–%     | 80.95%       | 71.26–90.65%| 90.48%       | 83.23–97.72%|
| Specificity        | 66.67%       | 47.81–85.53%| 8.33%        | 1.03–27.00%| 50.79%       | 45.27–56.31%| 41.90%       | 36.46–47.35%|
| PPV                | 11.11%       | 0.28–48.25% | 8.33%        | 1.03–27.00%| 24.76%       | 18.86–30.65%| 23.75%       | 18.37–29.13%|
| NPV                | 94.12%       | 71.31–99.85%| 100.00%      | 15.81–%     | 93.02%       | 89.22–96.83%| 95.65%       | 92.25–99.05%|
| PLR                | 1.5          | 0.34–6.70   | 1.09         | 0.97–1.23  | 1.65         | 1.40–1.94   | 1.56         | 1.38–1.76   |
| NLR                | 0.75         | 0.18–3.09   | 0.38         | 0.22–0.63  | 0.23         | 0.11–0.49   |

CI – confidence interval; PPV – positive predictive value; NPV – negative predictive value; PLR – positive likelihood ratio; NLR – negative likelihood ratio.
## Supplementary Table 5. Consistency and diagnostic capability of results of FRD staining combined with TCT testing versus CIN2+ results.

| Indicators  | One of positive results* | Both of positive results* |
|-------------|--------------------------|--------------------------|
|             | Value | 95% CI            | Value | 95% CI            |
| Coincidence rate | 35.15% | 30.49–39.80% | 69.06% | 64.55–73.57% |
| KAPPA       | 7.77%  | 4.77–10.76%       | 26.82% | 18.16–35.48%      |
| Sensitivity | 96.92% | 89.32–99.63%      | 73.85% | 63.16–84.53%      |
| Specificity | 23.30% | 18.80–27.80%      | 68.14% | 63.18–73.10%      |
| PPV         | 16.50% | 13.18–20.83%      | 24.77% | 21.03–28.50%      |
| NPV         | 97.53% | 91.36–99.70%      | 93.15% | 90.00–96.29%      |
| PLR         | 1.26   | 1.17–1.36         | 2.32   | 1.87–2.87         |
| NLR         | 0.13   | 0.03–0.52         | 0.38   | 0.25–0.58         |

CI – confidence interval; PPV – positive predictive value; NPV – negative predictive value; PLR – positive likelihood ratio; NLR – negative likelihood ratio. * FRD staining results or TCT testing are positive; # FRD staining results and TCT testing are positive.

## Supplementary Table 6. Consistency and diagnostic capability of results of FRD staining combined with HPV testing versus CIN2+ results.

| Indicators  | One of positive results* | Both of positive results* |
|-------------|--------------------------|--------------------------|
|             | Value | 95% CI            | Value | 95% CI            |
| Coincidence rate | 19.80% | 15.92–23.69% | 58.17% | 53.36–62.98% |
| KAPPA       | 1.06%  | –0.22–2.34%       | 17.45% | 10.53–24.36%      |
| Sensitivity | 98.46% | 91.72–99.96%      | 78.46% | 68.47–88.46%      |
| Specificity | 4.72%  | 2.46–6.98%        | 54.28% | 48.97–59.58%      |
| PPV         | 16.50% | 13.18–20.83%      | 24.77% | 21.03–28.50%      |
| NPV         | 94.12% | 71.31–99.85%      | 92.93% | 89.36–96.50%      |
| PLR         | 1.03   | 0.99–1.07         | 1.72   | 1.44–2.04         |
| NLR         | 0.33   | 0.04–2.42         | 0.40   | 0.25–0.64         |

CI – confidence interval; PPV – positive predictive value; NPV – negative predictive value; PLR – positive likelihood ratio; NLR – negative likelihood ratio. * FRD staining results or HPV testing are positive; # FRD staining results and HPV testing are positive.

## Supplementary Table 7. Consistency and diagnostic capability of results of TCT testing combined with HPV testing versus CIN2+ results.

| Indicators  | One of positive results* | Both of positive results* |
|-------------|--------------------------|--------------------------|
|             | Value | 95% CI            | Value | 95% CI            |
| Coincidence rate | 16.58% | 12.96–20.21% | 52.72% | 47.85–57.59% |
| KAPPA       | 0.19%  | –0.08–0.46%       | 16.14% | 10.44–21.85%      |
| Sensitivity | 100.00% | 94.48–100.00% | 87.69% | 79.71–95.68%      |
| Specificity | 0.59%  | 0.07–2.11%        | 46.02% | 40.71–51.32%      |
| PPV         | 16.17% | 12.57–19.77%      | 23.75% | 18.37–29.13%      |
| NPV         | 100.00% | 15.81–100.00% | 95.12% | 91.83–98.42%      |
| PLR         | 1.01   | 1.00–1.01         | 1.62   | 1.42–1.86         |
| NLR         | 0.00   | .              | 0.27   | 0.14–0.52         |

CI – confidence interval; PPV – positive predictive value; NPV – negative predictive value; PLR – positive likelihood ratio; NLR – negative likelihood ratio. * FRD staining results or TCT testing are positive; # FRD staining results and TCT testing are positive.
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