

# Effectiveness of novel folate receptor-mediated staining solution detection (FRD) for cervical cancer screening

Yuping Dai, MD<sup>a</sup>, Lirong Wang<sup>a</sup>, Donghong Li<sup>b,\*</sup>

## Abstract

Folate receptor-mediated staining solution detection (FRD) has been recently suggested as an effective tool in the cervical cancer screening. We aim to compare the accuracy of FRD to human papillomavirus (HPV) and thinprep cytology test (TCT) based on cytology-based biopsy.

During May 2016 and December 2016, we recruited women who presented for routine cervical lesion screening. The eligible cases were nonpregnant women and not in the menstrual period, aging between 25 and 65. All eligible women were screened with TCT, HPV, FRD testing, and colposcopy.

A total of 216 women include 137 (63.4%) cases of cervical inflammation, 27 (12.5%) cases of cervical intraepithelial neoplasia 1 (CIN 1), 51 (23.6%) cases of CIN 2, 34 (15.7%) cases of CIN 3, and 12 (5.6%) cases of cervical cancer. The sensitivity were 93.81%, 76.29%, 80.41% for HPV testing, TCT testing, and FRD testing, respectively. The specificities were 16.46%, 34.15%, and 68.29%, respectively. FRD has statistically significant higher specificity than HPV testing and TCT (both  $P < .05$ ). However, no differences were found in sensitivity (both  $P > .05$ ). The positive predictive value (PPV), negative predictive value (NPV), and Kappa consistency coefficient were 39.91%, 81.82%, and 0.08% for HPV testing, 40.66%, 70.89%, and 0.09% for TCT, and 60%, 85.5%, and 0.46% for FRD testing.

FRD had favorable accuracy and efficacy in detecting cervical cancer, and therefore could be used as an effective screening tool for cervical cancer screening.

**Abbreviations:** CR = coincidence rate, FRD = folate receptor-mediated staining solution detection, HC2 = hybrid capture 2, LEEP = loop electrosurgical excision procedure, NLR = negative likelihood ratio, NPV = negative predictive value, PLR = positive likelihood ratio, PPV = positive predictive value, TCT = thinprep cytology testing.

**Keywords:** cervical cancer, folate receptor-mediated staining solution detection, FRD, HPV, screening, TCT, thinprep cytologic test

## 1. Introduction

Cervical cancer is estimated to be the secondly leading causes of cancer among women worldwide, with approximately 530,000 new cases and 275,000 death each year occurring in women living in low- and middle-income countries.<sup>[1,2]</sup> Cytology-based screening programs have markedly reduced the incidence of

cervical cancer, especially in developed countries.<sup>[3]</sup> ThinPrep cytology test (TCT) is regarded as the most valuable cytology-based cervical screening to reduce the incidence of cervical cancer.<sup>[4]</sup> However, cytology-based cervical screening has been proven with some limitations, including low sensitivity in detecting high-grade of cervical cancer precursor lesions, and overall low specificity in detecting cervical intraepithelial neoplasia (CIN), which lead to high proportion of false-positive results.<sup>[5,6]</sup> More importantly, many low-resource settings are difficult to establish or maintain a high-quality, high-coverage cytology-based screening programs.<sup>[7,8]</sup> Human papillomavirus (HPV) test is another valuable test applied in the cervical cancer precursor lesion screening, however, the poor specificity has limited this methodology.

Recently, a novel approach named folate receptor-mediated staining solution detection (FRD) has been proposed as an effective testing to screen cervical intraepithelial neoplasia and cancer, proven with higher specificity than HPV and TCT testing.<sup>[9–11]</sup> The mechanism of this testing was based on the potential that folic acid receptor could be a target to capture cancer cells. Folate receptor conjugated with other agents and have been used to detect ovarian cancer.<sup>[10–12]</sup> Folate receptors are highly expressed in various cancers, especially in ovarian and lung cancers.<sup>[13]</sup> However, the efficacy of FRD has not yet been well established. The sensitivity and specificity of FRD observed in the previous study were 71.93% and 66.07%, respectively.<sup>[9]</sup>

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Informed consent was obtained from all individual participants included in the study.

The authors have no conflicts of interest to disclose.

<sup>a</sup> Department of Obstetrics and Gynecology, Affiliated Guangren Hospital,

<sup>b</sup> Department of Gynecology Oncology, The Affiliated Shaanxi Provincial Cancer Hospital, School of Medicine, Xi'an Jiaotong University, Xi'an, China.

\* Correspondence: Donghong Li, No. 309, Yantaxi Road, Yanta District, Xi'an 710061, Shaanxi, China (e-mail: lidonghong@yahoo.com)

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In the current study, we aimed to evaluate the efficacy of FRD via the cervical cancer screening, and compare with HPV and TCT testing.

## 2. Methods

### 2.1. Subjects

During May 2016 and December 2016, we recruited 216 women who were detected with positive HPV and/or abnormal TCT test result and need to undergo colposcopy in Guangren Hospital affiliated to School of Medicine, Xi'an Jiaotong University. All enrolled cases were nonpregnant women and not in the menstrual period, aging between 25 and 65. The exclusion criteria include: have been performed total hysterectomy, a condition of contact bleeding, had ever cervical surgery including conization, loop electrosurgical excision procedure (LEEP), chemotherapy using microwave or infrared ray, having acute inflammation for more than 7 days, and confirmed diagnosis of high-grade of CIN. All eligible women performed FRD test before they underwent colposcopy.

### 2.2. Cervical TCT

A specified cytobrush was placed into the cervical canal about 1 cm and rotated 5 cycles clockwise. And then it was taken out and put into the specified container. The liquid was sent to examination after the brush and washed for 10 times. ThinPrep automatic pelleter was used to make smears, automatic cell meter to disperse and the filtrate samples, and microscope was used to observe and diagnose by 2 independently experienced pathologists.

### 2.3. HPV testing

HPV testing was performed by Digene Hybrid Capture 2 (HC2, Qiagen, Crawley, UK) test, which was blind to TCT results. Digene Microplate Luminometer 2000 (DML 2000; Qiagen, Crawley, UK) was used for reading and calculating results of HPV testing.

### 2.4. Cervical cells FRD staining and detection

FRD staining (Shanxi Gaoyuan Medical Equipment Service Co, Ltd) was performed for recruited women before they underwent the colposcopy. The epithelium staining applicator was first immersed in the folate receptor-mediated staining solution for 30 seconds. Take the Epithelium Staining Applicator, and align the red-marked support bar on the proximal surface of the epithelium staining applicator to the virtual 12 o'clock position determined on the ecto-cervix. Insert the Epithelium Staining Applicator into the cervical canal until its proximal portion fully covers the ecto-cervix. Rotate the plunger 30° to the right, push the plunger into the cervical canal, and hold for 5 seconds. Hold the handle, press on the surface of cervix for 5 seconds, and push the plunger twice. Remove the applicator from the cervix and put it into the FRD colorimeter to detect the color changes of the applicator.

Subjects stained with the color including brown, and green were classified as low-grade CIN (CIN I). The staining color in blue, bluish black, or black was classified as high-grade CIN (CIN 2+).

### 2.5. Biopsy diagnosis

Biopsy samples were processed via paraffin-embedding, sectioning, and staining before the reading by pathologists.<sup>[14]</sup> CIN nomenclature was used to categorize the CIN as follows: CIN negative, CIN 1, CIN 2, CIN 3, and cervical cancer. In the current study, result of biopsy diagnosis was set as gold standard for the final diagnosis. CIN negative, CIN 1 were categorized as low-grade CIN, while CIN 2, CIN 3, and cervical cancer were set as high-grade CIN.

### 2.6. Statistical analysis

The sensitivity and specificity were analyzed and compared for FRD, TCT, and HPV test. The sensitivity was calculated by calculating positive results' ratios between HPV, FRD, or TCT testing and that of pathology diagnosis. The specificity was calculated by calculating the negative results' ratios between HPV, TCT, and FRD testing and that of pathology diagnosis. Positive predictive values (PPV), negative predictive values (NPV), Kappa, coincidence rate (CR), positive likelihood ratio (PLR) and negative likelihood ratio (NLR) were also calculated.  $\chi^2$  test was used to compare the differences in validity. The consistency between FRD, TCT, HPV result and golden standard histological result was calculated by Kappa test. Two-sided  $P < .05$  was regarded as having statistical significance. The SPSS (version 21.0, SPSS Inc, Chicago, IL) was used for all analyses. This study was approved by the Institutional Review Board of the Guangren Hospital affiliated to School of Medicine, Xi'an Jiaotong University.

## 3. Results

### 3.1. Baseline

A total of 216 women were enrolled, with mean age 41.11 (data not shown), including 137 (63.4%) cases of cervical inflammation, 27 (12.5%) cases of CIN 1, 51 (23.6%) cases of CIN 2, 34 (15.7%) cases of CIN 3, and 12 (5.6%) cases of cervical cancer (Table 1). CIN 2+, CIN 3+, and diagnosis of cervical cancer were classified as high-grade CIN ( $n = 91$ ), while inflammation, CIN 1 + were classified as low-grade CIN ( $n = 164$ ).

### 3.2. HPV screening

A total of 228 (87.35%) women were HPV positive, and 33 (12.64%) were HPV negative (Table 2). The sensitivity and specificity were 93.81% (95% CI 87.16%–97.13%) and 16.46% (95% CI 11.57–22.89) respectively (Table 3). The values of PPV and NPV were 39.91 and 81.82 respectively. The Kappa value

**Table 1**

**Pathological diagnoses of cervical intraepithelial neoplasia by folate receptor-mediated staining solution detection (FRD), thinprep cytology testing (TCT), and HPV testing.**

Diagnosis	HPV		TCT		FRD		Total
	Positive	Negative	Positive	Negative	Positive	Negative	
Inflammation	112	25	87	50	41	96	137
CIN1*	25	2	18	9	11	16	27
CIN2*	47	4	36	15	40	11	51
CIN3*	32	2	28	6	26	8	34
Cervical cancer	12	0	10	2	12	0	12

\* CIN = cervical intraepithelial neoplasia.

**Table 2**

**Diagnostic significance of folate receptor-mediated staining solution detection (FRD), thinprep cytology testing (TCT), and HPV testing in screening of cervical intraepithelial neoplasia and cancer.**

Test	Diagnosis of CIN 2+		Total
	Positive	Negative	
HPV test			
Positive	91	137	228
Negative	6	27	33
Total	97	164	261
TCT test			
Positive	74	105	179
Negative	23	59	82
Total	97	164	261
FRD test			
Positive	78	52	140
Negative	19	112	131
Total	97	164	261

CIN 2+ =cervical intraepithelial neoplasia and cancer.

measuring consistency between HPV test and pathologic diagnosis was 0.08 (95% CI 0.01–0.15) (Table 4).

**3.3. TCT screening**

A total of 179 (68.58%) women were TCT positive, and 82 (31.41%) were TCT negative (Table 2). The sensitivity and specificity were 76.29% (95% CI 66.93%–83.65%) and 34.15% (95% CI 27.33–41.69) respectively (Table 3). The values of PPV and NPV were 40.66 and 70.89 respectively. The Kappa value measuring consistency between TCT test and pathologic diagnosis was 0.09 (95% CI 0–0.19) (Table 4).

**3.4. FRD screening**

A total of 140 (53.64%) women were FRD positive, and 131 (46.36%) were FRD negative (Table 2). The sensitivity and specificity for FRD were 80.41% (95% CI 71.42%–87.09%) and 68.29% (95% CI 60.82–74.93) respectively (Table 3). The values of PPV and NPV were 60 and 85.5 respectively. The Kappa value measuring consistency between FRD test and pathologic diagnosis was 0.46 (95% CI 0.34–0.57) (Table 4).

No statistically significant difference in sensitivity was found between FRD and TCT. However, FRD had statistically higher specificity than both TCT (34.15, 95% CI 27.33–41.69) and HPV (16.46, 95% CI 11.57–22.89) tests (both *P* < .05).

**Table 3**

**Validity comparison of folate receptor-mediated staining solution detection (FRD), thinprep cytology testing (TCT), and HPV testing in screening cervical intraepithelial neoplasia and cancer.**

	HPV (%, 95% CI)	TCT (%, 95% CI)	FRD (%, 95% CI)
Sensitivity	93.81 (87.16–97.13)	76.29 (66.93–83.65)	80.41 (71.42–87.09)
Specificity	16.46 (11.57–22.89)	34.15 (27.33–41.69)	68.29 (60.82–74.93)
PPV	39.91 (33.77–46.39)	40.66 (33.79–47.92)	60.00 (51.41–68.02)
NPV	81.82 (65.61–91.39)	70.89 (60.09–79.75)	85.50 (78.46–90.51)
CR	45.21 (39.29–51.27)	49.81 (43.79–55.83)	72.80 (67.1–77.84)

CI=confidence interval.

**Table 4**

**Positive likelihood ratio (PLR), negative likelihood ratio (NLR), and Kappa values for folate receptor-mediated staining solution detection (FRD), thinprep cytology testing (TCT), and HPV testing.**

	HPV (95% CI)	TCT (95% CI)	FRD (95% CI)
PLR	1.12 (1.10–1.14)	1.16 (1.13–1.19)	2.54 (2.43–2.65)
NLR	0.37 (0.18–0.75)	0.69 (0.59–0.80)	0.29 (0.26–0.32)
Kappa	0.08 (0.01–0.15)	0.09 (0–0.19)	0.46 (0.34–0.57)

CI=confidence interval.

**4. Discussion**

Our study indicates that FRD test had higher specificity in detecting high-grade CIN and cervical cancer (CIN 2+) than that of conventional HPV and TCT testing, which indicates that FRD testing could be used as an effective screening tool for cervical cancer.

Although HPV and TCT seem to have higher sensitivity, higher proportion of false positive diagnosis was also observed in compared with FRD. As a single test, HPV testing had the highest sensitivity to detect high-grade CIN lesions and it detected 93.81% of CIN 3 lesions and 100% of cervical cancer in our study. HPV infection is well known as an important risk factor for cervical cancer. However, the false-positive rate of HPV testing was also extremely high. The specificity of HPV testing was only 14.46% in the current study. The positive results of HPV testing could be caused by a virus infection, but not necessarily the results of cervical cytological and pathological changes. The low specificity of HPV testing is a major limitation for its use as a sole screening test for cervical cancer. Therefore, it has been suggested to add a second test to reduce the high proportion of false-positive HPV tests, particularly when a “screen-and-treat” approach is used to treat positive lesions without colposcopy or biopsy triage.<sup>[15]</sup> The sensitivity and specificity of TCT observed in the current study were 76.29% and 34.15%, respectively. In comparison of previous Chinese study, the TCT testing in our study had higher sensitivity but lower specificity. The sensitivity and specificity were 53.13% and 79.01%, respectively in the previous study.<sup>[16]</sup> It has been concerned that TCT detection is affected by subjective factors, leading to different result reading from different cytological experts using the same smear. The potential reasons were mainly due to the lacking assessment of histological characteristics by TCT. The sensitivity of TCT was observed ranging from 55% to 80%.

The sensitivity of FRD testing reached as high as 80.64%, which was not statistically different to that of HPV and TCT testing. However, the FRD had the highest specificity of 68.29% among all the testing. The efficacy of FRD testing observed in our study is in line with that of previous study.<sup>[9]</sup> Folate conjugates with its receptors and multiple complexes, which are transferred to inner cells by endocytosis.<sup>[17]</sup> There are several potential advantages of FRD testing beside the high accuracy compared with other testing. Result reading for FRD testing is rapid and easily recognized without clinical pathological experiences. Furthermore, due to the cost-effectiveness of FRD, it could be widely used in the low-resource settings, where the colposcopy services and histopathologic laboratories are not available.

Although our study design maximized detection of true-positive disease in the study population by offering colposcopy to

all participants and directed biopsies in all cases of colposcopic abnormalities, the possibility of misclassification of disease outcomes due to subjective interpretations in colposcopy and histopathology cannot be entirely ruled out. Furthermore, all the women enrolled in the current study were nonpregnant, and therefore, the efficacy of FRD testing in detecting high-grade CIN is unknown for pregnant women. Another concern is the limited sample size in the current study. Further study with larger sample size shall be guarantee.

In conclusion, our study indicates that FRD has as good sensitivity, but higher specificity in screening high-grade CIN and cancer (CIN 2+), and could be considered a safe and effective screening test.

### Author contributions

**Conceptualization:** Donghong Li.

**Data curation:** Lirong Wang.

**Formal analysis:** Donghong Li.

**Investigation:** Yuping Dai, Lirong Wang.

**Methodology:** Yuping Dai.

**Project administration:** Donghong Li.

**Writing – original draft:** Yuping Dai, Donghong Li.

**Writing – review & editing:** Donghong Li.

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