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ORIGINAL RESEARCH

# Clinical Evaluation of Polymerase Chain Reaction Coupled with Quantum Dot Fluorescence Analysis for Diagnosis of *Candida* Infection in Vulvovaginal Candidiasis Practice

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**Purpose:** Time-consuming culture methods and wet-mount microscopy (WMM) with low sensitivity have difficulties in diagnosing Vulvovaginal candidiasis (VVC). Rapid and highly sensitive polymerase chain reaction coupled with quantum dot fluorescence analysis (PCR-QDFA) for the diagnosis of VVC has not been reported to date. This study was the first to evaluate the performance of PCR-QDFA for diagnosis of *Candida* strains in the leukorrhea samples from patients with suspected VVC.

**Patients and Methods:** Leukorrhea samples from all visited patients were taken from the vagina using vaginal swabs by clinicians. We evaluated patients admitted with suspected VVC who completed WMM for diagnosis and reported the diagnostic effectiveness of PCR-QDFA and *Candida* culture (gold standard) when testing leucorrhea samples.

**Results:** A total of 720 leukorrhea samples from 387 VVC-positive patients and 333 VVC-negative patients were included in this study. Of the 387 leukorrhea samples from the VVC-positive patients, 391 *Candida* strains were identified by culture. 99.23% (388/391) *Candida* strains were included in the PCR-QDFA list. The 388 *Candida* strains belonged to four different species of *Candida*, including *C. albicans* (n = 273, 70.36%), *C. glabrata* (n = 85, 21.91%), *C. tropicalis* (n = 16, 4.12%), and *C. krusei* (n = 14, 3.61%). PCR-QDFA diagnosed *Candida* strains in 340/384 (88.54%) of the leucorrhea samples with *Candida* strains infection. The sensitivity of PCR-QDFA for *C. albicans, C. glabrata, C. tropicalis*, and *C. krusei* was 89.01%, 85.88%, 81.25% and 92.86%, respectively. The specificity of PCR-QDFA for *C. albicans, C. glabrata, C. tropicalis* and *C. krusei* was 93.69%, 99.37%, 99.71%, and 99.57%, respectively.

**Conclusion:** The highly sensitive and specific PCR-QDFA technique can be exploited as a rapid (approximately 4 h) diagnostic tool for common *Candida* strains of leucorrhea samples from patients with suspected VVC.

**Keywords:** PCR-QDFA, VVC, NAC, diagnostic performance, *Candida* culture

## Introduction

VVC is a common mucosal infection of the lower female reproductive tract that afflicts many women.[1] Up to 75% women of reproductive age will experience VVC at least once in their lifetime, and up to 9% women experience more than three episodes annually.[2] Symptomatic VVC mainly results from the excessive growth of *Candida*, which is followed by epithelial invasion and the production of virulence effectors. The *Candida* strain can escape immune responses and resist many antifungal drugs by forming biofilms.[3–6] The majority of asymptomatic VVC patients are predominantly colonized by *Candida* strains.[7] When a patient's immunity is weak, colonized *Candida* strains can release virulence factors to trigger VVC.[8] Colonized *Candida* strains is an important prerequisite of symptomatic VVC.[9] The

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causes of Candida infection in VVC patients are well known and include overuse of glucocorticoids, immune disorders, diabetes, pregnancy, hormone replacement and behavioral risk factors.[10–12] Currently, the treatment of *Candida* infections usually involves azoles such as fluconazole, but *Candida* species may differ in their drug sensitivities.[13,14] Erroneous diagnosis and empirical drug treatment can lead to persistent growth and recurrent infection of resistant strains. Therefore, timely and exact identification of *Candida* species can facilitate accurate treatment of VVC.

Notably, accurate diagnosis and treatment of vaginitis is challenging due to similarities in clinical symptoms and vaginal discharge with other vaginal conditions. Usually, the diagnosis of VVC is based on the gold standard of *Candida* culture.[15] However, culture methods are reported to have low sensitivity and are time-consuming (approximately 40 h–57 h). Culture methods often require combined time-of-flight analyzer mass spectrometry (MALDI-TOF MS) technique to achieve high sensitivity.[16] However, expensive MALDI-TOF MS requires isolates of high purity to be tested. These are the elements that cause the patient's treatment to be delayed, which raises hospital expenses.[17] In addition, wet-mount microscopy (WMM) is often used as a quick, simple, and inexpensive method of VVC diagnosis. However, the observation background in WMM is complex and extremely demanding for the observer, which can easily lead to missed diagnoses.[7] WMM was also shown worse sensitivity and specificity.[18] Furthermore, *Candida* species cannot be differentiated by this method.

Several molecular diagnostic approaches for identifying *Candida* at the species or strain level have been established.[17,19] PCR-QDFA to identify *Candida* species is exactly new technique of a multiplex PCR assay using quantum dot luminescence to transduce a signal. The high sensitivity (86.10%) and specificity (100%) of PCR-QDFA applied to the diagnosis of bloodstream infections have been reported. PCR-QDFA exhibited high sensitivity (100%) and specificity (99.59%) for *Candida* strains in blood.[20] However, the use of PCR-QDFA for the diagnosis of VVC has not been reported. The purpose of the present study was to validate the performance of PCR-QDFA for the identification of *Candida* strains in leukorrhea samples from patients with suspected VVC.

# Materials and Methods

## Patients

Between April 2021 and October 2021, this study was carried out at the Zhejiang Provincial People's Hospital in Zhejiang, China. The leukorrhea samples of all patients enrolled in this study had been removed from the vagina using vaginal swabs by clinicians. All patients enrolled in this study had been diagnosed with WMM. WMM is to directly smear the leucorrhea sample on the glass slide, and then directly observe whether there are spores or hyphae under the microscope without staining agent. The entry criteria of the subjects included (1) being diagnosed with VVC by WMM. VVC was diagnosed when pseudohyphae or budding yeast were found with WMM.[21] (2) Being initially diagnosed with vaginitis by the gynecologist. (3) Exhibit clinical symptoms of VVC (eg, abnormal vaginal discharge, vaginal itching, vaginal redness, vaginal mucosal congestion, burning, dysuria).[8] Patients who met two of the above entry criteria were recruited for this study. Leukorrhea samples from all enrolled patients were performed for *Candida* cultures. The diagnosis of VVC was ultimately confirmed by *Candida* culture method.[22] Once one or more *Candida* strains were isolated from the leukorrhea samples by the *Candida* culture method, a positive VVC case was confirmed. Otherwise, one negative VVC case would be identified. Notably, leukorrhea samples with negative results of the first culture method and positive results of WMM were required to perform a second *Candida* culture. The diagnostic result of this leukorrhea samples was defined based on the result of a second *Candida* culture.

## Conventional Microbiological Methods

Vaginal split dilutions were evenly mixed and inoculated into 70 mm *Candida* chromogenic plates (Autobio Diagnostics CO., Ltd, China) with sterile cotton swabs. The *Candida* chromogenic plate can be replaced by sabouraud medium plate. The sabouraud medium mainly consists of peptone (10g/l), glucose (40g/l), agar (15g/l), chloramphenicol (125mg/l) and purified water. The 70 mm *Candida* chromogenic plates placed in an incubator for 48 hours at 37°C.[22,23] Three randomly selected *Candida* colonies with the same morphology were inoculated onto a new *Candida* chromogenic plate for further culture and finally identified by mass spectrometry (MALDI-TOF, bioMérieux, France).[23,24]

## Description of PCR-QDFA Setup

PCR-QDFA is a fully automated diagnostic technique that can directly diagnose the common *Candida* strains (*C. albicans, C. glabrata, C. tropicalis* and *C. krusei*) of leukorrhea samples from patients with suspected VVC. Biotin labeled PCR primers specifically amplify genes of different Candida strains. The nylon membrane has oligonucleotide capture probes that hybridize to amplified pathogenic targets. When the PCR amplified product was molecularly hybridized with the probe on the Nissl strip, the hybridization product was combined with that of QDs in the detection solution. Under fluorescence excitation at a specific wavelength, the quantum dots emit a fluorescence signal. The presence or absence of this pathogen in a specimen is identified by the finding that the probe hybridizes to this DNA fragment, as judged by the fluorescent signal at a specific position on the membrane strip.

Deoxyribonucleic acid (DNA) was extracted from 400μL vaginal split dilutions using nucleic acid extraction and purification reagents from Hangzhou Kilogene Biotech Co., Ltd, 8μL for following experiments. Ninety-six samples could be detected by each PCR-QDFA test. The 96 samples included 1 positive control (mainly including *C. albicans* DNA, HPV16 and the house-keeping genes of human), 1 negative control sample (human house-keeping genes) and 94 clinical leucorrhea samples to be tested. The positive control and negative control were from Hangzhou Kilogene Biotech Co., Ltd. The control sample and the extracted gene to be tested were subjected to the same amplification reaction and hybridization reaction as follows. 8μL of the extracted DNA was equally added to configuration tubes No. 1 and configuration tubes No. 2. Configuration tube No. 1 contained 20 μL reaction liquid I (Primer, dN (U) TP, UDG enzyme, buffer) and 1 μL reaction liquid III (Taq DNA polymerase, TAQ-Antibody) from Hangzhou Kilogene Biotech Co., Ltd. Configuration tube No. 2 contained 20 μL reaction liquid II (Primer, dN (U) TP, UDG enzyme, buffer) and 1μL reaction liquid III (Taq DNA polymerase, TAQ-Antibody) from Hangzhou Kilogene Biotech Co., Ltd. PCR-QDFA was mainly employed as a multiplex PCR combined with the hybridization reaction. PCR-QDFA was accomplished as a multiplex PCR reaction in a PCR machine (tc-96; Hangzhou Borge Science and Technology Co., Ltd.) (Figure S1).[16] PCR conditions included a uracil-DNA glycosylase reaction at 50°C for 2 min, pre denaturation at 95°C for 10 min, 40 amplification cycles and a further extension at 72°C for 5 min. Each amplification cycle consisted of a denaturation step at 95°C for 30 seconds and an annealing/amplification step at 55°C for 1 minute followed by an extension step at 72°C for 30 seconds. Automated operational hybridization detection was accomplished using an automatic hybridization detector (kj-96; Hangzhou Kilobase Biotechnology Co., Ltd.), as advised by the manufacturer.[16] Automated operational hybridization detection was performed using the following conditions: 1 hr hybridization at 48°C; 15 min wash at 48°C; 30 min incubation at room temperature; 5 min wash at room temperature; and final fluorescence detection on a fluorescence imager (js-680e; Shanghai Peiqing Technology Co., Ltd.).

## Statistical Analysis

The result of *Candida* incubation combined with MALDI-TOF MS was employed as a control to evaluate the efficacy of PCR-QDFA.[15] Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall percent agreement (OPA) were calculated for each target following comparison to the *Candida* culture results (which included adjudication of discordant results). The Kappa test was performed in the statistical package (Statistical Package for the Social Sciences) to calculate the statistical significance of sensitivity and specificity.[25]

# Results

## Characteristics of Patients Enrolled in This Study

A total of 720 suspected VVC patients were registered in this study. These patients were recruited from gynecological departments, obstetric departments, or reproductive departments. All registered patients were ≥17 years of age, with an average age of 26.5 years (17–69 years). The rate of patients with abnormal leucorrhea (34.11%) and vaginal Itching (47.92%) were relatively higher in the group of patients with VVC while the rate of patients with abnormal cervical secretion (61.56%), hyperemic in vaginal mucosa (9.01%), dysuria (4.20%) and dellyache (3.90%) was higher in the group of patients without VVC. Of the 720 patients with suspected VVC, 272 patients showed positive WMM results. Of the 387 positive VVC patients, 266 patients were diagnosed with WMM (Table 1).

**Table 1** Characteristics of Patients with Suspected VVC Enrolled in the Study

| Patients | Total (n = 720) | VVC (n = 387) | NO VVC (n = 333) |
|---|---|---|---|
| Age, median years (range) | 43 (17–69) | 43 (17–69) | 43.5 (18–69) |
| Abnormal leucorrhea (%) | 242 (33.61) | 131 (33.85) | 111 (33.33) |
| Vaginal Itching (%) | 323 (44.86) | 184 (47.55) | 139 (41.74) |
| Abnormal cervical secretion (%) | 328 (45.56) | 123 (31.78) | 205 (61.56) |
| The cervical secretions like beancurd dregs (%) | 40 (5.56) | 39 (10.08) | 1 (0.30) |
| Hyperemic in vaginal mucosa (%) | 52 (7.22) | 22 (5.68) | 30 (9.01) |
| Dysuria (%) | 16 (2.22) | 2 (0.52) | 14 (4.20) |
| Dellyache (%) | 29 (4.03) | 16 (4.13) | 13 (3.90) |
| WMM (%) | 272 (37.94) | 266 (69.27) | 6 (1.80) |

## Diagnostic Results of Traditional Laboratory Methods (*Conventional*)

Of the 720 patients recruited, 387 VVC-positive and 333 VVC-negative samples were confirmed by *Candida* culture. Of the 387 VVC-positive cases, one case of *Saccharomyces cerevisiae* infection and two cases of *C. parapsilosis* infection were excluded from the next performance evaluation of PCR-QDFA for *Candida* strains within PCR targets. Because *S. cerevisiae* and *C. parapsilosis* were not included within the PCR-QDFA diagnostic targets (only 4 species). Therefore, the remaining 717 samples containing 333 VVC-negative cases and 384 VVC-positive cases were included in this study. Of the 384 leukorrhea samples from the VVC-positive patients, 388 *Candida* strains were identified by culture. The 388 *Candida* strains belonged to four different species of *Candida*, including *C. albicans* (n = 273, 70.36%), *C. glabrata* (n = 85, 21.91%), *C. tropicalis* (n = 16, 4.12%), and *C. krusei* (n = 14, 3.61%) (Figure 1).

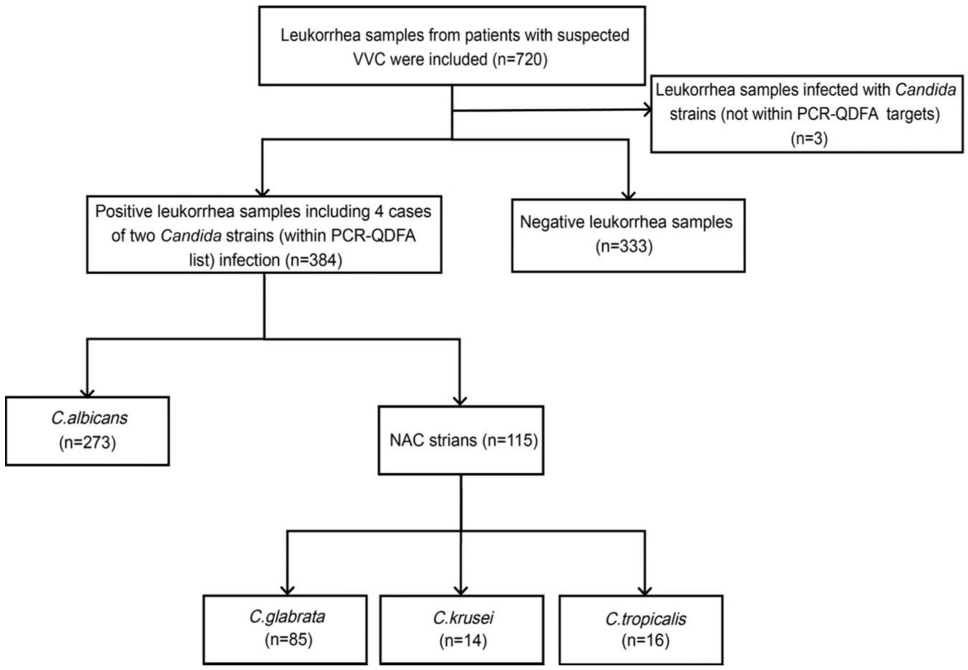


**Figure 1** *Candida* diversity analysis in leukorrhea samples from 720 suspected VVC patients.

## Overall Diagnostic Performance of PCR-QDFA for Candida Strains (Within the PCR Targets) in Leukorrhea Samples

Seven hundred and seventeen leucorrhea samples (384 positive VVC samples and 333 negative VVC samples) confirmed by culture method were used to evaluate the diagnostic performance of PCR-QDFA for *Candida* strains in leucorrhea samples. Three hundred and eighty-four leukorrhea samples of VVC positive cases included 380 leukorrhea samples infected with one *Candida* strain and 4 leukorrhea samples infected with two *Candida* strains. Among the 380 leukorrhea samples, which were diagnosed with one *Candida* strain by culture, 339 (89.21%) *Candida* strains were successfully identified by PCR-QDFA. Additionally, only 1 (0.25%) leukorrhea sample infected with two *Candida* strains was successfully detected by PCR-QDFA among 4 leukorrhea samples. Among the 384 positive VVC samples, 44 samples were false-negative samples diagnosed by PCR-QDFA. Of 44/384 (11.46%) leukorrhea samples, 8/44 (18.18%) leukorrhea samples were found with *Candida* strains by PCR-QDFA that were discordant with the culture method results. PCR-QDFA did not identify any *Candida* strains in the remaining 36/44 (81.82%) leukorrhea samples (Table S1).

Conversely, among the 333 leukorrhea samples from VVC-negative cases, *Candida* strains of 28 (8.4%) leukorrhea samples were identified by PCR-QDFA (Table S2). For 305/333 (91.59%) leukorrhea samples without *Candida* infection, the PCR-QDFA diagnostic results were consistent with the culture results. The sensitivity, specificity, and positive predictive value of PCR-QDFA assay for pathogenic *Candida* strains in leukorrhea samples from patients suspected VVC were 88.54%, 91.59%, and 89.96%, respectively. The kappa value of PCR-QDFA was 0.80 for pathogenic *Candida* strains in leukorrhea samples (Table 2).

## Diagnostic Performance of PCR-QDFA for *C. albicans* Strains in Leukorrhea Samples from Patients Suspected of VVC

Performance analysis of PCR-QDFA for different *Candida* strains was mainly divided into the diagnostic performance of *C. albicans* and NAC strains. Among the 273/717 (38.07%) leukorrhea samples where *C. albicans* strains were identified by culture method, the results of 243 samples diagnosed by PCR-QDFA assay were consistent with culture method, while the results of 2 samples were not. The remaining 28/273 (10.26%) *C. albicans* strains were unidentifiable by PCR-QDFA (Table S1).

Based on the culture method results, the 444/717 (61.92%) samples that were negative for *C. albicans* included 333 samples with no *Candida* infection and 111 samples with NAC strains infection. Furthermore, 25/333 (7.51%) samples were found with *C. albicans* infection by PCR-QDFA. Meanwhile, 3 (2.70%) leukorrhea samples were diagnosed with *C. albicans* infection among remaining 111 samples infected with NAC strains (Table S1). In summary, 28 (6.31%) samples with *C. albicans* infection were found by PCR-QDFA among 444 leukorrhea samples without *C. albicans* infection according to the culture method results. For 416/444 (93.69%) leukorrhea samples without *C. albicans* infection, the diagnostic results of PCR-QDFA were consistent with those of culture. The sensitivity, specificity, and kappa value of PCR-QDFA for *C. albicans* were 89.01%, 93.69%, and 0.83, respectively (Table 2).

**Table 2** Performance of PCR-QDFA for Diagnosis of *Candida* spp

| | N | TP | FP | TN | FN | Sensitivity (%) | Specificity (%) | PPA (%) | NPA (%) | Kappa value |
|---|---|---|---|---|---|---|---|---|---|---|
| *Candida albicans* | 273 | 243 | 28 | 420 | 30 | 89.01 | 93.69 | 89.67 | 93.27 | 0.83 |
| *Candida tropicalis* | 16 | 13 | 2 | 703 | 3 | 81.25 | 99.71 | 86.67 | 99.57 | 0.89 |
| *Candida glabrata* | 85 | 73 | 4 | 632 | 12 | 85.88 | 99.37 | 94.81 | 98.13 | 0.84 |
| *Candida krusei* | 14 | 13 | 3 | 704 | 1 | 92.86 | 99.57 | 81.25 | 99.86 | 0.86 |
| *NAC* strains | 115 | 99 | 7 | 595 | 16 | 86.09 | 98.84 | 93.40 | 97.38 | 0.88 |
| *Candida* group | 384 | 340 | 28 | 305 | 44 | 88.54 | 91.59 | 92.39 | 87.39 | 0.80 |

**Abbreviations**: *Candida* spp, different species of *Candida* strains within PCR targets; *Candida* group, all *Candida* strains within PCR targets; TP, true positive; FP, false positive; TN, true negative; FN, false negative; PPA, positive percent agreement; NPA, negative percent agreement.

## Diagnostic Performance of PCR-QDFA for NAC Strains (Within the PCR Targets) in Leukorrhea Samples from Patients Suspected of VVC

Among 115 leukorrhea samples with NAC strain infection, 99/115 (86.09%) NAC strains were identified by PCR-QDFA and consistent with the diagnostic results of the culture method. Among the remaining 16/115 (13.91%) samples where the NAC strains were not identified by PCR-QDFA, 6 samples were identified with *C. albicans* infection, while the other 10 samples were detected without candida strains. Among the 602 leukorrhea samples without NAC strain infection, 7 NAC strains were identified by PCR-QDFA, including 3 strains diagnosed as *C. albicans* by culture (Table S2). For the 595/602 (98.84%) leukorrhea samples without NAC strain infection, the PCR-QDFA diagnostic results were consistent with the culture results. The sensitivity, specificity, and kappa value of PCR-QDFA for NAC strains were 86.09%, 98.84% and 0.88, respectively (Table 2).

Of the 717 leukorrhea samples, 85 samples were diagnosed with *C. glabrata* strains by culture method. A total of 73/85 (85.88%) leukorrhea samples with *C. glabrata* infection were identified by PCR-QDFA. Of the 12 leukorrhea samples without *C. glabrata* infection, 5/12 (41.67%) samples were diagnosed with other *Candida* strains (Table S1). Among the 632 leukorrhea samples without *C. glabrata* infection, 2 leukorrhea samples with no *Candida* infection and 2 leukorrhea samples with *C. albicans* infection were found to have *C. glabrata* by PCR-QDFA. For the remaining 595/602 (98.84%) leukorrhea samples without NAC strain infection, the diagnostic results of PCR-QDFA were consistent with the culture results. The sensitivity, specificity, and kappa value of PCR-QDFA for *C. glabrata* were 85.88%, 99.37% and 0.89, respectively (Table 2).

A total of 16/717 (2.23%) leukorrhea samples with *C. tropicalis* infection were diagnosed by culture. *C. tropicalis* strains of 13/16 (81.25%) leukorrhea samples were identified by PCR-QDFA. Of the remaining 3 leukorrhea samples with *C. tropicalis* strains infection, only one *C. albicans* strain was diagnosed by PCR-QDFA. Among 368 leukorrhea samples with other *Candida* strains, 1 *C. glabrata* strain and 1 *C. albicans* strain were found to be *C. tropicalis* strains by PCR-QDFA (Table S2). For the remaining 699/701 (99.71%) leukorrhea samples without *C. tropicalis* strains infection, the diagnostic results of PCR-QDFA were consistent with the culture results. The sensitivity, specificity, and kappa values of PCR-QDFA for *C. tropicalis* strains were 81.25%, 99.71%, and 0.84, respectively (Table 2).

Among the 717 leukorrhea samples, 14 strains of *C. krusei* were identified by the *Candida* chromogenic culture method. Of these, PCR-QDFA diagnostic results were consistent with culture for 13/14 (92.86%) leukorrhea samples. In 703/717 (98.05%) leukorrhea samples without *C. krusei* infection, 3 strains of *C. krusei* were identified by PCR-QDFA (Table S2). For the remaining 700/703 (99.57%) of the leukorrhea samples without *C. krusei* infection, the diagnostic results of PCR-QDFA were consistent with the culture results. The sensitivity, specificity, and kappa values of PCR-QDFA for *C. krusei* strains were 92.86%, 99.57%, and 0.86, respectively (Table 2).

# Discussion

PCR-QDFA, a fully automated VVC diagnostic method with high sensitivity and specificity, takes only approximately 4 h for its diagnosis time. This study was the first to evaluate the diagnostic performance of PCR-QDFA for common *Candida strains* in the leukorrhea samples from suspected VVC patients.

This retrospective study was designed to evaluate the diagnostic performance of PCR-QDFA for several *Candida* strains in leukorrhea samples from patients with suspected VVC. Several studies suggest that the clinical diagnosis of VVC should be made mainly based on clinical symptoms combined with WMM results, and only uncertain cases should require further ascertainment by culture.[8] Of the 384 VVC positive patients in the study, only 69.27% were diagnosed by WMM. It has been reported that WMM with low sensitivity (48.50%) are prone to underdiagnosis of VVC.[26] WMM, a diagnostic method that cannot distinguish strain species is difficult to meet the needs of clinical precision medicine. Interestingly, the diagnostic performance of PCR-QDFA for all *Candida* strains within the PCR-QDFA targets was comprehensively evaluated. Among the 384 VVC-positive leukorrhea samples diagnosed by *Candida* culture, 88.54% of the *Candida* strains could be identified by PCR-QDFA. The sensitivity of other molecular diagnostic techniques was no more than 83.3% for *C. krusei* strains.[10,27] PCR-QDFA had higher sensitivity for *C. krusei* strains. The sensitivity of PCR-QDFA for *C. albicans, C. tropicalis, C. glabrata*, and *C. krusei* were 89.01%, 81.25%, 85.88%, and 92.86%,

respectively. The specificity of the PCR-QDFA was above 99.00% for the different *Candida* species among the NAC strains. The kappa values of PCR-QDFA for *C. albicans, C. tropicalis, C. glabrata*, and *C. krusei* were 0.83, 0.84, 0.89, and 0.86, respectively. The kappa values were within the interval of 0.81–0.99 for all strains, indicating that the results of the PCR-QDFA identifications were almost perfectly consistent with the results of *Candida* strains culture.[28] In addition, most of the operationally complex molecular diagnostic kits show lower sensitivity or specificity than PCR-QDFA.[10,29] BD MAX™ The vaginal panel (MAX VP) is currently the only reported fully automated molecular diagnostic technique (FAMDT) applied to VVC. The judgment of the diagnostic results of MAX VP requires expensive fluorometric systems. Only 24 samples each time (approximately 3 h) can be diagnosed by MAX VP with high sensitivity (96.2%) and specificity (96.1%).[10] PCR-QDFA is a new FAMDT for the identification of *Candida* species, which combines a multiplex PCR assay with quantum dot luminescence to transduce a signal. PCR-QDFA, a more efficient FAMDT than MAX VP, was able to diagnose 94 leukorrhea samples each time (approximately 4 h). Quantum dots (QDs), low toxicity and inexpensive nanomaterials, which often have strong and stable optical signals coupled with other components.[30] The judgment of PCR-QDFA diagnostic results only requires human interpretation of the stable quantum dot fluorescence signal in the UV imager. The QD luminescence signals on the nylon membrane strips in the PCR-QDFA procedure were manually interpreted. Human observations may lead to the occurrence of false-positive results. The high sensitivity of PCR-QDFA was considered as the cause of 8.40% false-positive results. When molecular diagnostic techniques find *Candida* strains that are not later recoverable, clinical uncertainties may occur.[27] Interestingly, all the patients with false-positive PCR-QDFA results were diagnosed with vaginitis by clinicians. 82.14% of these patients had symptoms related to VVC. And 46.43% of these patients showed vaginal itching. According to other literature, some clinical treatments are only able to reduce the expression of virulence factor genes (enzymes proteinase SAP5 and phospholipase PLB2) by 50–60%.[31] Therefore, symptoms of patients with false-positive PCR-QDFA results may be caused by toxic factors of inactivated *Candida* strains or other pathogenic microorganisms.

In addition, the present study also has some limitations. Because the actual number of leucorrhea samples infected by several Candida strains during this study was too small, only a small number of leucorrhea samples infected by a variety of Candida strains were included in this study. For PCR-QDFA, the purity and concentration of the *Candida* strains DNA in the samples to be tested were strictly required to be 1.4~2.2 and 0.2–100 (ng/μL), respectively. Leukorrhea samples with false-negative results by PCR-QDFA may have been mainly due to insufficient concentration or purity of pathogen-specific genes in the leukorrhea samples to be tested. This prompts clinicians to be advised to increase the volume of leukorrhea samples from different patients as much as possible. The required DNA concentration and purity standards for PCR-QDFA of leukorrhea samples may need to be more precisely determined. Therefore, the correlation between the luminescence intensity of QDs on the nylon membrane strip and positive results should be further investigated. Additionally, the PCR-QDFA targets were insufficient to cover all infectious strains. Therefore, the PCR-QDFA targets need further optimization.

## Conclusions

This study is the first to evaluate the diagnostic performance of PCR-QDFA for *Candida* strain identification in VVC. This study demonstrates that the diagnosis of suspected VVC patients with significant clinical symptoms needs to be aided by highly sensitive and specific PCR-QDFA techniques. PCR-QDFA, which requires only approximately 4 hours for the diagnosis of VVC, greatly complements the timeliness of the *Candida* culture diagnostic assay. Therefore, PCR-QDFA is capable of being used in VVC practice as a tool for *Candida* strain identification with excellent diagnostic performance.

## Ethical Approval

We followed the revised Declaration of Helsinki when performing the study. The Zhejiang Provincial People's Hospital Research Ethics Committee authorized the study. The ethical approval No. was QT2022297. Due to the fact that this was a retrospective study and patient data and medical records were collected anonymously, informed consent for inclusion was waived. Each participant was given a guarantee of privacy protection.

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## Author Contributions

Wen-jia Fan, Jie Li and Lingxia Chen performed the experiments and wrote the manuscript. Wenhao Wu made important contributions to data analysis and interpretation. Hong-ying Pan, Xi Li, and Wei-hong Zhong conceived the idea, designed the study, and revised the manuscript. All authors contributed to data analysis, drafting or revising the article, have agreed on the journal to which the article will be submitted, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

## Disclosure

The authors declare that they have no conflicts of interest.

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