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A performance evaluation of an immuno-latex chromatography card for the rapid detection of *Candida spp.*

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ABSTRACT

This report evaluates the performance and species specificity of an immuno-latex chromatography card (ICC) for rapid detection of *Candida* spp. Double-manipulator single-blind Gram staining smear examination (GSSE) and ICC were used to analyze 354 vaginal discharge specimens (VDS) (including 98 tested as positive by GSSE) from women with suspected candidal vaginitis, simulated specimens with a concentration gradient, and vaginitis causing organism suspensions (0.9% NaCl) of 22 species from nine genera. Limit of detection, semi-quantitative detection performance, total detection performance and species specificity were determined for ICC, and the results were compared with those of the GSSE method. The limits of detection of ICC for *Candida* spp. in organism suspensions with 0.9% NaCl and simulated specimens were 7×10^6 cells/L and 7×10^8 cells/L respectively. For species specificity, the results were positive for six *Candida spp.* (*C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. krusei* and *C. guilliermondii)* and negative for the remaining 16 species (*C. lusitaniae*, *Saccharomyces cerevisiae*; three Gram-positive coccus species, four Gram-negative bacillus species, three Gram-negative coccus species and four common microbes causing vaginal infection) from eight genera. The overall sensitivity, specificity, accuracy, positive predictive value and negative predictive value of ICC for VDS were 93.81%, 99.10%, 97.31, 98.14% and 96.90%, respectively. The above indicators in the 98 VDS evaluated as positive were 84.39%, 92.86%, 86.74%, 96.72% and 70.27%, respectively. In summary, ICC offered better specificity, sensitivity, positive predictive value and negative predictive value for the detection of *Candida* spp. in VDS.

1. Introduction

Candida spp. can cause infection of the skin mucosa of the genitals. Vaginal mycosis (VM), also known as candidal vaginitis (CV) (De Bernardis et al., 2018), usually presents as itchiness of the vulva and vagina with leukorrhea that is watery or resembles bean curd dregs. There are seven main pathogens involved in CV, including *C. albicans* which accounts for 80% of the cases, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. krusei*, *C. guilliermondii*, and *C. lusitaniae*. The latter six species account for about 19% of the cases (Engberts et al., 2008). CV shares some similarities with other sexually transmitted diseases, thus diagnosis should be made by a combined evaluation of the clinical manifestations and the laboratory tests. Refractory CV can be highly agonizing and may result from misdiagnosis, improper treatment (Engberts et al., 2008; Cernicka and Subik, 2006; Feng et al., 2010; Fan and Liu, 2011; Fan et al., 2008), drug resistance or repeated onset (Guzel et al., 2011; Mainini et al., 2011; Adib et al., 2011; Bahadoran et al., 2010).

Laboratory tests are of high clinical importance for confirming CV and the commonly used ones are the wet mount method (smear microscopy), smear culture (Kim et al., 2016), antigen detection such as latex agglutination (Evans et al., 1986) and nucleic acid-based methods such as PCR. The wet mount method is the most frequently used. Although smear culture is the most sensitive and specific for detecting *Candida spp.*, the detection procedures are difficult and time-consuming. Immunofluorescence and PCR techniques require expensive equipment and expertise, which also restrict extensive applications. Immuno-latex chromatography represents a new generation of immunoassays based on antigens. This method has the advantages of high specificity and accuracy without the demands for expertise and equipment (Matsui et al., 2009; Marot-Leblond et al., 2009).

A rapid immuno-latex chromatography card (ICC) for the detection

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of *Candida* spp. in vaginal discharge specimens (VDS, Nanjing Liming Biological Products Co., Ltd.) was assessed for its performance and species specificity.

2. Materials and methods

2.1. Specimens and specimen collection

From October 2013 to June 2014, 354 VDS were collected from women suspected of CV (aged 19–54 years) at the outpatient clinic of the First Affiliated Hospital of Fujian Medical University. Those treated with anti-candidal drugs 2 weeks before collection were excluded. The speculum was not lubricated during sample collection. VDS were collected from the vaginal wall using a sterilized swab and placed in 3 ml of sterilized normal saline for the Gram staining smear examination (GSSE) method and ICC.

Experiments were conducted by dividing the collected specimens into two groups. All collected specimens were evaluated for overall performance, and 98 specimens evaluated as weakly positive (+) or strongly positive (+ + +) by the GSSE method were employed for assessing the performance of ICC. The GSSE method and ICC were tested independently by two experienced researchers in double-blind trials.

2.2. Candida spp. suspensions for counting

Using 0.9% NaCl, the organism suspensions with the initial concentration of 7×10^{14} cells/L of *C. albicans* in simulated specimens were diluted into six more suspensions at concentrations of 7×10^{10} cells/L, 7×10^{9} cells/L, 7×10^{8} cells/L, 7×10^{6} cells/L, 7×10^{4} cells/L and 7×10^{2} cells/L.

2.3. Simulated specimens

Three negative VDS analyzed by GSSE were mixed evenly. This mixture was used to dilute the organism suspensions with the initial concentration of 7×10^{14} cells/L into three suspensions at concentrations of 7×10^{9} cells/L, 7×10^{8} cells/L and 7×10^{6} cells/L.

2.4. Equipment

An Olympus CH20 microscope (Japan) and an electric thermostatic incubator (Shanghai Yuejin Medical Instruments Co., Ltd.) were used. The ICC was manufactured by Nanjing Liming Biological Products Co., Ltd. (ICClm).

2.5. Smear microscopy with Gram staining

For smear microscopy with Gram staining, 20 low-power fields of view (10× eyepiece and 10× objective lens) were chosen under the microscope by two experienced researchers. For closer observation of suspected *Candida spp.*, high-power fields of view (10× eyepiece and 40× objective lens) were chosen. Specimens were considered positive if there were budding yeasts, pseudohyphae and/or hyphal forms seen under smear microscopy.

2.6. Examination of ICClm

The ICClm is based on the capture of Candida antigens from VDS using IgG mouse monoclonal antibodies (MAb 11D9) recognizing epitopes of antigens in the mannan of a wide range of *Candida spp.*, including *C. albicans* and *C. glabrata*, which are commonly implicated in CV. The MAb 11D9 are conjugated to gold particles in the mobile phase, whereas IgG mouse MAb 5F11 are used as capturing antibodies at the nitrocellulose paper in the card.

100 µl of the vaginal swab suspension was loaded onto the ICClm (Fig. 1), and the sample moved forward by capillary action through the pad containing the MAb-gold conjugate which specifically targeted the mannan in the sample. As the sample passed through the detection line, where immobilized MAb captured the mannan-MAb-gold complex to form a MAb-mannan-MAb complex, the test line turned pink or purple. In the absence of mannan in a negative sample, no MAb-mannan-MAb complex was formed and no color change of the test line was observed. Further detail of the procedure can be found in the literature (Marot-Leblond et al., 2009).

2.7. Genus specificity

Test strains were provided by Nanjing Dermatology Hospital of Chinese Academy of Medical Sciences. The 22 species belonging to nine genera were *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. krusei*, *C. guilliermondii*, *C. lusitaniae*, *Saccharomyces cerevisiae*; three Gram-positive coccus species, *Staphylococcus aureus*, *Enterococcus faecalis* and *Enterococcus faecium*; four Gram-negative bacillus species, *Pseudomonas aeruginosa*, *Escherichia coli, Proteus vulgaris* and *Salmonella typhi*; three Gram-negative coccus species, *Neisseria meningitidis*, *Neisseria gonorrheae* and *Moraxella catarrhalis*; and four common microbes causing vaginal infection, *Chlamydia trachomatis*, *Trichomonas vaginalis*, *Ureaplasma urealyticum* and *Mycoplasma hominis*. Using 0.9% normal saline, these microbes were diluted to six concentrations (7×10^{10} cells/L, 7×10^{9}cells/L, 7×10^{8} cells/L, 7×10^{6} cells/L, 7×10^{4} cells/L and 7×10^{2} cells/L).

2.8. Semi-quantitative microscopy method

All 354 specimens were assessed by smear microscopy and ICC. Criteria for semi-quantitative microscopy of *Candida* spp. were determined based on semi-quantitative analysis of erythrocytes in urinary sediments. Four levels were set up: negative (−) was defined as 0 in 20 low-power fields of view; weakly positive (+) as one organism in three high-power fields of view; moderately positive (+ +) as one to five organisms in each high-power field of view; strongly positive (+ + +) as five or more organisms in each high-power field of view. After testing the collected VDS in normal saline, the results were interpreted according to the user's instruction manual of ICC.

2.9. Comparison of ICC with semi-quantitative microscopy method

Ninety-eight VDS evaluated as + and + + + by the GSSE method were used for evaluating the performance of ICC. The intensity of the test line of ICC was graded from + + + to − (+ + +, purple, strongly positive; + +, red, moderately positive; +, pink, weakly positive; ±, light pink; faintly positive; −, negative or non interpretable).

2.10. Statistical analysis

Fourfold table analysis was applied to the data, and performed using SPSS v17.0 software (SPSS, Chicago, IL, USA). The indicators assessed were sensitivity, specificity, accuracy, positive predictive value and negative predictive value.

3. Results

3.1. Limit of detection for organism suspensions with 0.9% NaCl and simulated specimens using ICC

The limits of detection of GSSE and ICC were 7×10^{8} cells/L and 7×10^{6} cells/L, respectively, for diluted suspensions *with 0.9% NaCl* of *C. albicans*, and were 7×10^{9} cells/L and 7×10^{8} cells/L, respectively, for simulated specimens of *C. albicans*. Essentially, the limit of detection of ICC was 100 times and 10 times lower than that of GSSE for diluted organism suspensions and simulated specimens, respectively (Table 1 and Figs. 1, 2).

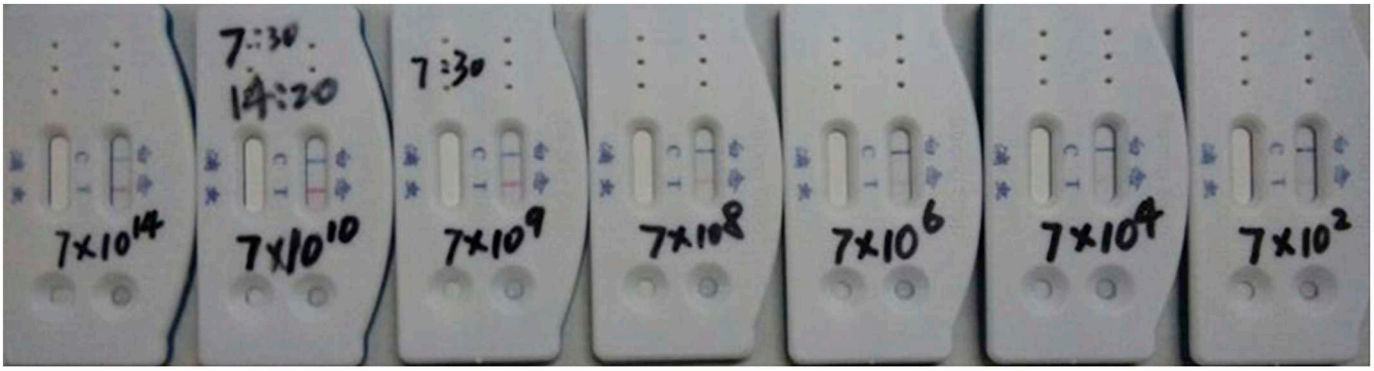

Fig. 1. Results of detection for *Candida* spp. in simulated specimens (bacterial suspension with 0.9% sodium chloride) by immunochromatography test in double-blind trials.

Table 1
Limit of detection of *C. albicans* in 0.9% NaCl and in simulated specimens of the two semi-quantitative methods.

	GSSE	ICC
Organism count in 0.9%NaCl (cells/L)		
7×10^2	−	±
7×10^4	−	±
7×10^6	−	+
7×10^8	+	+
7×10^9	+ +	+ +
7×10^{10}	+ + +	+ + +
7×10^{14}	+ +	+ +
Organism count in simulated specimens (cells/L)		
7×10^7	−	−
7×10^8	−	+
7×10^9	+ +	+ +
7×10^{10}	+ + +	+ + +

3.2. Genus specificity

Among the 22 species, six *Candida spp.* (*C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. krusei* and *C. guilliermondii)* were detected at the concentration of 7.0×10^6 cells/L or 7.0×10^8 cells/L, the remaining 16 species were not detected at the concentration of 7.0×10^{10} cells/L.

3.3. Comparison of detection performance between GSSE and ICC

Taking GSSE as the gold standard (Marot-Leblond et al., 2009), ICC demonstrated high specificity, sensitivity, accuracy, positive predictive value and negative predictive value (see Table 2). Although both methods demonstrated satisfactory performance overall, disagreement was found for a few specimens (those identified as + by GSSE). The two methods were further compared as follows.

3.4. Comparison of ICC with microscopy method

Ninety-eight VDS evaluated as + to + + + by the GSSE method were used for evaluating the performance of ICC. As indicated in Table 3, there were still differences between the semi-quantitative indicators, especially in sensitivity, accuracy and negative predictive value.

3.5. Comparison between the two semi-quantitative methods

Differences between the two semi-quantitative methods were mainly found in VDS determined as + (weakly positive) and + + + (strongly positive), and were less conspicuous in VDS specimens determined as − and + + (Table 4).

4. Discussion

Candida spp. is a yeast which inhabits the human skin, mucosa and gastrointestinal tract. Usually causing no symptoms in vagina, *Candida spp.* is found in 10% of non-pregnant women with leukorrhagia and 30% of pregnant women. However, the risk of CM increases under impaired immunity (deficiency of vitamin B complex, severe infectious diseases, diabetes and other consumptive diseases), long-term use of broad-spectrum antibiotics and adrenal cortical hormones (De Bernardis et al., 2018; Engberts et al., 2008; Cernicka and Subik, 2006; Feng et al., 2010; Fan and Liu, 2011; Fan et al., 2008; Guzel et al., 2011; Mainini et al., 2011; Adib et al., 2011; Bahadoran et al., 2010; Kim et al., 2016).

Very few rapid tests for *Candida* spp. have been developed so far (Evans et al., 1986; Matsui et al., 2009; Marot-Leblond et al., 2009), and a previously reported immuno-latex chromatography strip (Matsui et al., 2009) is not on the market. Marot-Leblond et al. (Marot-Leblond et al., 2009) were the first to evaluate the performance of the ICC and the CandiVagi assay (SR2B, Avrille, France) for *Candida* spp. with two monoclonal antibodies. The sensitivities of microscopic examination, culture and ICC for the diagnosis of CM were 61%, 100%, and 96.6%, respectively, while the specificities of the three methods were 100%, 82%, and 98.6%, respectively. ICC had a negative predictive value of 98.6%, a positive predictive value of 96.6%, and an efficiency of 98% (Marot-Leblond et al., 2009). While the CandiVagi assay (SR2B) (Marot-Leblond et al., 2009) has not been approved for the medical market in mainland China, there have also been no new products of ICC, especially in developing countries. This study was the first evaluation of the detection performance of ICC in China (developed by Nanjing Liming Biological Products Co., Ltd., China.). No significant differences were seen between the detection performance of the CandiVagi assay (SR2B) (Marot-Leblond et al., 2009) and the ICC in this study.

The limit of detection of the ICC was lower by 100 times than that of GSSE for organism suspensions. In simulated specimens, the limit of detection of ICC was also 10 times lower than that of GSSE. The intensity of the test line of ICC was + at 7×10^8 dilution and + + + at 7×10^{10} dilution, thus we speculated the intensity as + + at 7×10^9 dilution. In fact, our speculation was affirmed by the actual result at 7×10^9 dilution, as recorded in Table 1. Meanwhile, the result was + at 7×10^6 dilution and at 7×10^8 dilution, + + at 7×10^9 dilution and ± at 7×10^4 dilution. We thus speculated ± at 7×10^5 dilution, and judged the 'breakpoint' for the ICC as 7×10^6. The results were positive for six *Candida* spp. and negative for the remaining 16 common pathogens, indicating a better specificity of detection for *Candida* spp. with the ICC. However, the limit of detection of the ICC for each of the

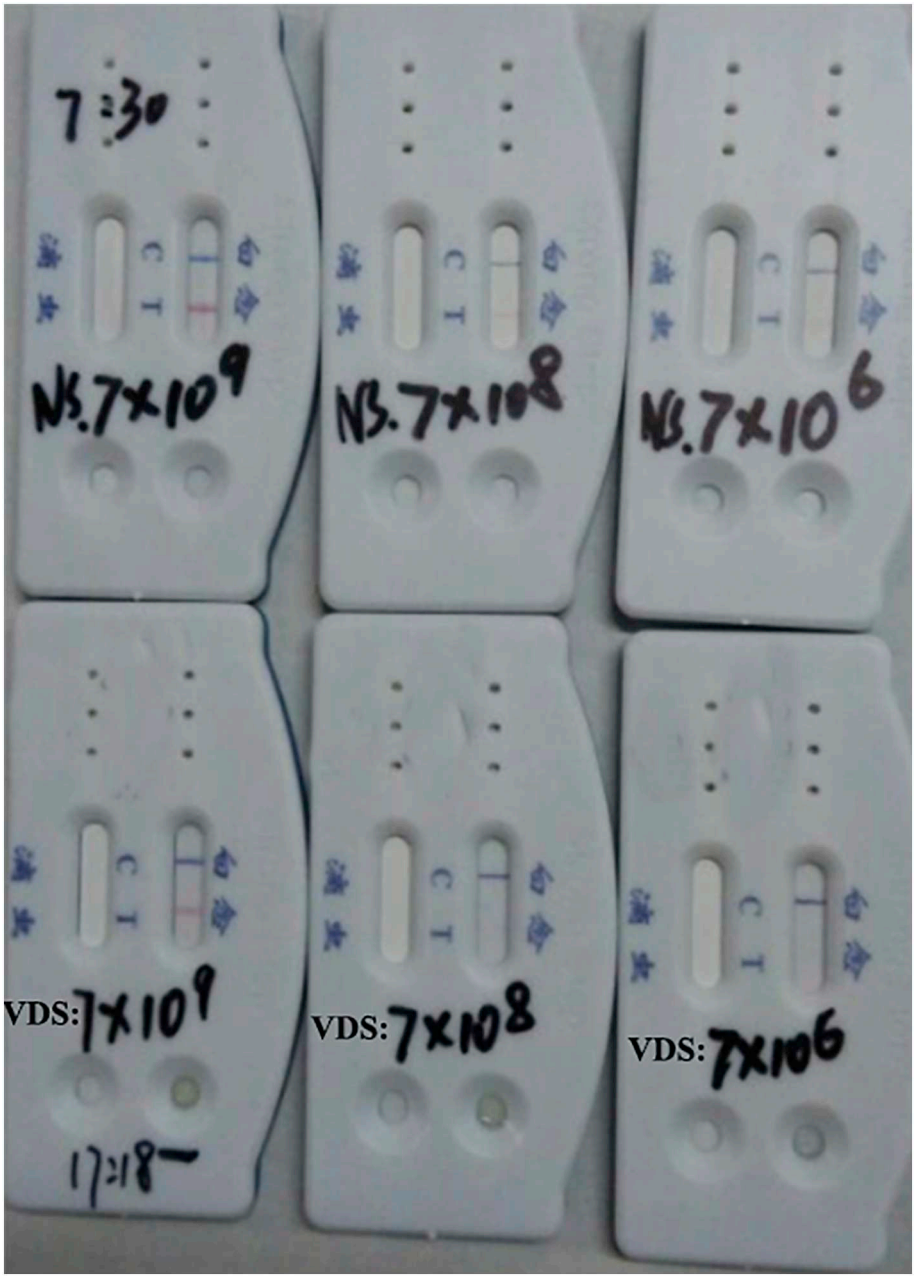

Fig. 2. Double-blind trials of simulated specimens/diluted organism suspension of *C. albicans* by immunochromatography test. NS: normal saline or 0.9% NaCl, VDS: vaginal discharge specimen.

Table 2
Overall performance of ICC taking GSSE as the standard.

ICC	GSSE		Total
	Positive	Negative	
Positive	106	2	108
Negative	7	219	226
Total	113	221	334

Sensitivity = (106/113) × 100% = 93.81%.
Specificity = (219/221) × 100% = 99.10%.
Accuracy = (325/334) × 100% = 97.31%.
Positive predictive value = (106/108) × 100% = 98.14%.
Negative predictive value = (219/226) × 100% = 96.90%.

Table 3
Performance of ICC in 98 VDS of *Candida spp*.

ICC	GSSE		Total
	Positive	Negative	
Positive	59	2	61
Negative	11	26	37
Total	70	28	98

Sensitivity = (59/70) × 100% = 84.39%.
Specificity = (26/28) × 100% = 92.86%.
Accuracy = (85/98) × 100% = 86.74%.
Positive predictive value = (59/61) × 100% = 96.72%.
Negative predictive value = (26/37) × 100% = 70.27%.

Table 4
Comparison between the two semi-quantitative methods for detecting *Candida spp*.

GSSE n		ICC			
		−	+	++	+++
−	28	26	2	0	0
+	23	8	10	3	2
++	20	2	9	7	2
+++	27	1	10	5	11
Total	98	37	28	13	15

six Candida species was not determined, and further investigation is needed in the future.

Overall, the sensitivity, specificity, positive predictive value and negative predictive value of ICC were 93.81%, 99.10%, 98.14% and 96.90%, respectively. However, the results of detection were inconsistent between the two methods for a few specimens (those determined as + and +++ by GSSE). Further comparison of the two methods showed that there were still differences between the semi-quantitative indicators and overall performance indicators of ICC, especially in the sensitivity, accuracy and negative predictive value. However, the differences were less conspicuous in the specificity and positive predictive value, especially for specimens determined as - and ++ by GSSE (Table 3 and Table 4). For immunoassays based on antigens, an excessive amount of antigens may lead to the hook effect (Evans et al., 1986; Matsui et al., 2009), as usually seen with human chorionic gonadotropin (Nerenz et al., 2014). According to Tables 4, 27 specimens were determined as strongly positive (+++) by GSSE and 11 specimens might be plagued by the hook effect. Further investigation of this phenomenon is needed.

In brief, the ICC described in this report has better specificity, sensitivity, positive predictive value and negative predictive value for the detection of *Candida* spp. in VDS. It does not require expensive equipment or special expertise, and therefore should greatly reduce the required labor while ensuring accurate detection when extensively applied in the clinic.

Conflict of interest

All the authors declare that they have no conflict of interest.

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