Vol. 36 / No. 2 / August 2024
Sinta 2 (Decree No: 200/M/KPT/2020)
Online ISSN : 2549-4082 ; Print ISSN : 1978-4279
Available online at https://e-journal.unair.ac.id/BIKK

Berkala Ilmu Kesehatan Kulit dan Kelamin

Original Article
Periodical of Dermatology and Venereology

The Validity of Point-of-Care Test (POCT) Lateral Flow Immunochromatographic Assay (LFIA) *Candida albicans* for the Diagnosis of Vulvovaginal Candidiasis

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ABSTRACT

Background: Vulvovaginal candidiasis is one of the most common causes of complaints of vaginal discharge, which is mostly caused by *Candida albicans*. The common diagnosis of vulvovaginal candidiasis is microscopic and culture examination. However, the availability of examinations is limited at the private clinic, and for culture examinations, the cost is high and results take several days. The point-of-care test (POCT) lateral flow immunochromatographic assay (LFIA) is a tool that can detect *Candida albicans* antigens, but this diagnostic test has never been carried out in Indonesia. **Purpose:** to know the validity of POCT LFIA *Candida albicans* for the diagnosis of vulvovaginal candidiasis caused by *Candida albicans* in women with complaints of vaginal discharge. **Methods:** Cross-sectional observational study with a diagnostic test design, using vaginal discharge samples. Vaginal discharge samples were subjected to POCT LFIA examination with the Medomic *Candida albicans*/ *Trichomonas vaginalis*/ *Gardnerella vaginalis* Antigen Combo Test Kit® and *Candida* spp culture. **Result:** POCT LFIA *Candida albicans* had a sensitivity of 100%, specificity of 89.9%, accuracy of 90.32 %, LR (+) 9, LR (-) 0, PPV 50%, and NPV 100% against *Candida* spp. culture. **Conclusion:** The POCT LFIA *Candida albicans* can be used to diagnose vulvovaginal candidiasis caused by *Candida albicans* in women who complain of vaginal discharge.

Keywords: vulvovaginal candidiasis, *Candida* culture, point of care test, lateral flow immunochromatographic assay.

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| Article info |
Submited: 31-01-2024, Accepted: 03-04-2024, Published: 31-07-2024

BACKGROUND

Vaginal discharge is a symptom that women often complain about regarding their genital organs. Vulvovaginal candidiasis is one of the most common causes of pathological vaginal discharge.[1] Vulvovaginal candidiasis is not a sexually transmitted infection but can occur due to *Candida* spp. Colonization, which has increased in women with immunodeficiency, diabetes mellitus, pregnancy, and the human deficiency virus (HIV).[2] The dominant *Candida* spp. that causes vulvovaginal candidiasis is *Candida albicans*, although it can also be caused by *non-albicans* species, such as *Candida glabrata*, *Candida tropicalis*, *Candida* krusei and *Candida parapsilosis*.[3] Increased colonization of *Candida albicans* can cause mannan and beta-glucans in cell walls, the formation of biofilms, adhesins, aspartyl proteases, and candidalysin, to be recognized by the body as foreign objects so that they can trigger an immune reaction.[4,5]

Vulvovaginal candidiasis occurs in 1-14% women of reproductive age worldwide. The prevalence of vulvovaginal candidiasis can vary depending on the location and research subjects.[6] The diagnosis of vulvovaginal candidiasis is still challenging. Clinical examination, microscopic examination, and culture examination each have weaknesses. In the community, many women diagnose vulvovaginal candidiasis independently without seeing a doctor for several reasons, such as not having time to see a doctor, negative stigma, or difficult access to health services.[7] Incorrect diagnosis of vulvovaginal candidiasis and

DOI : 10.20473/bikk.V36.2.2024.136-141

delays in diagnosis and treatment can increase the risk of recurrent vulvovaginal candidiasis, anti-fungal resistance, and the risk of complications in pregnancy in the form of premature labor or systemic fungal infections in newborns.[8]

Strategies that can be implemented to facilitate diagnosis, prevent misuse of over-the-counter antifungals, and reduce the risk of complications and resistance can be achieved by providing rapid diagnostic tools or what are often called point of care tests (POCT).[9] Point of care tests for vulvovaginal candidiasis are already on the market, but they are not yet available in Indonesia and are difficult to obtain. The POCT for vulvovaginal candidiasis is widely produced by several factories in China and can be purchased and imported into Indonesia, one of which uses the lateral flow immunochromatographic assay (LFIA) method to detect IgG, namely the Medomic *Candida albicans*/ *Trichomonas vaginalis*/ *Gardnerella vaginalis* Antigen Combo Test Kit® (Jiangsu Medomic Medical Technology Co., Ltd., Nanjing, China), which can detect *Candida albicans*, *Trichomonas vaginalis*, and *Gardnerella vaginalis* antigens.[10] Currently there are many diagnostic methods or tools available for the diagnosis of vulvovaginal candidiasis, but some are not officially available in Indonesia, therefore doctors must be aware and a gold standard diagnostic test is required before using a diagnostic tool.[11]

METHODS

This study is a cross-sectional observational study with a diagnostic test design using vaginal discharge samples. The research subjects were adult women who were undergoing treatment at the skin and genital clinic at Dr. Sardjito General Hospital and Gedongtengen Community Health Center Yogyakarta. This study included 31 research subjects who met the inclusion criteria: 18-55 years old woman, had sexual contact, complained of vaginal discharge, and were willing to take part in the research by signing an informed consent. Exclusion criteria were subjects who were pregnant, breastfeeding, currently menstruating, and were in therapy for complaints of vaginal discharge.

Vaginal discharge samples are taken during an inspection. Two samples were taken from the posterior vaginal wall with a sterile cotton swab in a circular motion, wiping the posterior vaginal wall three times. Sampling was carried out by researchers. One sample for culture examination is placed in a sterile tube containing 5 ml of sterile 0.9% NaCl and closed tightly. One sample for the POCT LFIA examination is inserted into the buffer fluid provided in the POCT LFIA kit.

Researchers brought culture examination samples to the microbiology laboratory of the Faculty of Medicine, Duta Wacana Christian University, Yogyakarta, on the same day and stored them at room temperature (25°C). Samples were incolutaded on SDA using a sterile loop in a zig-zag direction and then incubated at 37°C for 2x24 hours. *Candida* spp. colonies-those grown on SDA- were transferred to CHROMagar^{TM} media: and grown for 2x24 hours with incubation at 37 °C. Identified colonies based on the color of the growing colonies and counting the colonies. The examination and reading of culture results are carried out by laboratory analysts.

In diagnostic test analysis, the culture of Candida spp. is positive if the culture examination yields positive results. *Candida albicans* species was identified with a colony number greater than the limit of detection (LOD) POCT LFIA *Candida albicans* (Medomic *Candida albicans/ Trichomonas vaginalis/ Gardnerella vaginalis* Antigen Combo Test Kit®), $4x10^4$ CFU/ml. If the culture test result is negative, falls below the limit of detection (LOD), or the species of Candida is not albicans, the outcome is negative. The examination and reading of culture results were carried out by laboratory analysts, who did not know the results of the POCT examination. The POCT LFIA *Candida albicans* examination is positive if the examination results show two lines, namely the control line "C" and the test line "CA." A negative result if the examination results show one line, namely the control line "C." The researchers sampled and read the POCT LFIA results according to the product manual instructions. This research has been reviewed and approve by the Ethics Committee at Faculty of Medicine, Public Health and Nursing Universitas Gadjah Mada – DR. Sardjitu General Hospital (No.KE/FK/1367/EC/2023).

RESULT

The age range of research subjects with complaints of vaginal discharge was 18-50 years, and the majority were aged 26-35 years, or 13 subjects (41.9%). On culture examination of *Candida* spp., of the 31 samples, 14 samples (45%) were positive for *Candida* spp. (*Candida albicans*; *non-albicans*). Of the 14 positive culture results, there were 12 samples (85.7%) with one species of *Candida* spp. and two samples (14.3%) were cultured for the *Candida* spp. mixture. Culture results based on *Candida* species

obtained five positive samples for *Candida albicans* (36%), four positive samples for *Candida glabrata* (29%), two positive samples for *Candida tropicalis* (14%), one positive sample for *Candida krusei* (7%), one positive sample for *Candida albicans* and *Candida tropicalis* (7%), and one sample was positive for *Candida glabrata* and *Candida tropicalis* (7%). A description of culture results for *Candida* spp. can be seen in Figure 1, and positive *Candida albicans* culture results can be seen in Figure 2. Based on *Candida albicans* or *non-albicans* species, six samples (43%) were found with *Candida albicans* and eight samples (57%) with *Candida non- albicans.*

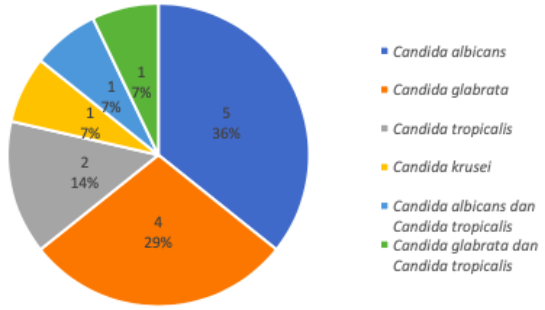

Figure 1. Culture results based on *Candida* species.

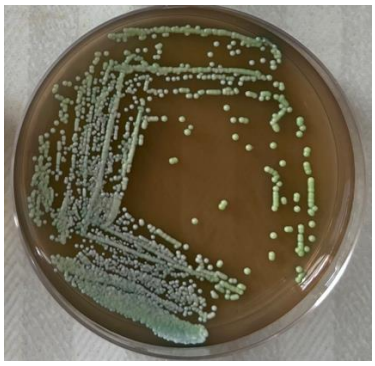

Figure 2. *Candida albicans* in CHROMagar™ media.

In this study, two samples were obtained with culture results for *Candida* spp., a mixture of *Candida albicans* and *Candida tropicalis*; *Candida glabrata* and *Candida tropicalis*. Positive culture findings revealed six samples of Candida albicans and eight samples of *Candida non-albicans.*

Three of the six Candida albicans samples cultured exceeded the LOD POCT LFIA *Candida albicans*. ($1.2x10^7$CFU/ml; $1.02x10^7$CFU/ml; $9.5x10^7$CFU/ml) and three other samples with fewer *Candida albicans* than LOD POCT LFIA *Candida albicans* ($2.5x10^2$CFU/ml; $5.6x10^2$CFU/ml; $2.6x10^2$CFU/ml). The LFIA point of care test used in this study only detected the *Candida albicans* species with an LOD of $4x10^4$ CFU/ml, so three *Candida albicans* culture samples $<4x10^4$ CFU/ml and eight non-albicans *Candida* culture samples yielded negative culture results in the analysis. Culture results of *Candida* spp. 17 samples were negative, so the negative culture results for *Candida albicans* in the analysis were 28 samples.

In the POCT LFIA *Candida albicans* examination, six positive results were obtained from 31 body fluid samples and 25 negative results. Six positive results from the *Candida albicans* POCT LFIA examination, consisting of three *Candida albicans* culture results that were more than the *Candida albicans* POCT LFIA LOD (true positive), two non-albicans *Candida culture* results (false positive), and one negative culture result (false positive). The positive result of POCT LFIA *Candida albicans* can be seen in Figure 3.

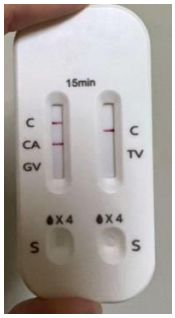

Figure 3. Positive result of the POCT LFIA *Candida* albicans examination.

The result of the validity of POCT LFIA *Candida albicans* can be seen in Table 1. POCT LFIA *Candida albicans* had a sensitivity of 100% (95% CI 29,24% - 100 %), specificity of 89,29% (95% CI 71,77% - 97,73%), accuracy of 90,32 % (95% CI 74,25% - 97,96%), LR (+) 9 (95% CI 3,2 - 27,19), LR (-) 0, PPV 50% (95% CI 25,55% - 74,45%), NPV 100% (95% CI 86,28% - 100%) against *Candida* spp. culture.

Table 1. POCT LFIA *Candida albicans* diagnostic test analysis table against Candida spp. culture

POCT LFIA	Culture		Total
	(+)	(-)	
(+)	3	3	6
(-)	0	25	25
Total	3	28	31
Sensitivity		3/3 = 100%	
Specificity		25/28 = 89.29%	
Accuracy		3+25/31= 90.32%	
LR (+)		1/1-0,89= 9	
LR (-)		1-1/0,89 = 0	
PPV		3/6 = 50%	
NPV		25/25 = 100%	

POCT = Point-of-Care Test; LFIA = Lateral Flow Immunochromatographic Assay ; LR = Likelihood Ratio; PPV = Positive Predictive Value; NPV = Negative Predictive Value

DISCUSSION

In this study, the age range of research subjects with complaints of vaginal discharge was 18-50 years, and the majority were aged 26-35 years, or 13 subjects (41.9%). These results are similar to those of Venugopal *et al*,. who reported complaints of vaginal discharge: the majority were in the 26–35 year age group (34%) because this age is included in the sexually active age group.[12] Sexual activity can affect the balance of vaginal flora and increase the risk of fungal or bacterial infections that can cause vaginal discharge. This age group generally also experiences stress, which can have an impact on the weakness of the immune system. A weak immune system can increase the risk of vaginal infections that cause vaginal discharge.[13]

On culture examination of *Candida* spp., of the 31 samples, 14 samples (45%) were positive for *Candida* spp. (*Candida albicans*; *non-albicans*). These results are similar to a study of the etiology of vaginal discharge in sexually active women at a tertiary health center in North Kerala, India. In this study, it was reported that vaginal discharge with culture results for *Candida* spp. were positive by 40.5%.[14] These results can vary depending on geographical conditions: or example high humidity can create conditions that are more conducive to the growth of *Candida*, which can cause vulvovaginal candidiasis. Demographic factors of research subjects such as age and reproductive status can also influence the results of a study. For example, vulvovaginal candidiasis is more common in women of childbearing and pregnant age because there is an increase in the hormone estrogen.[2,6]

The results of this study show that *Candida albicans* (36%) is the dominant species compared to other non-*Candida albicans* species. However, *Candida albicans*, which is usually reported as 90-95% of the causes of vulvovaginal candidiasis, is now shifting to *non-albicans Candida* species.[15,16] In this study, based on *Candida albicans* or *non-albicans* species, there were six samples (43%) with *Candida albicans* and eight samples (57%) with *Candida non-albicans*. The results of this study are similar to the research of Jimoh *et al*., who reported that *non-albicans Candida* species were found in more than 50% of *Candida* species on vaginal discharge.[17] This is possible due to widespread and inappropriate anti-fungal treatment carried out by the patient herself with inappropriate doses, long-term treatment, and repeated treatment, which can trigger the evolution of treatment resistance, causing *non-albicans Candida* to become more dominant.[15-17]

Candida glabrata (29%) is a *non-albicans Candida* species that is most commonly found in culture examination results of vaginal discharge samples. The distribution of non-albicans *Candida* species varies greatly depending on the population and study location.[6] In this study, there were mixed cultures of *Candida* species in two positive culture samples (14.3%). The results of this study are similar to Mahmoudi *et al*., that found 10.3% of culture results grew more than one species of *Candida*.[18] *Candida albicans* and *Candida glabrata* are the *Candida* species that are most commonly found in culture results. *Candida tropicalis* can form pseudohyphae and biofilms so that it has a high virulence factor to become a single or mixed infection.[19,20]

In vulvovaginal candidiasis infection, there are *Candida* virulence factors that are recognized by the immune system as foreign objects, so that they can trigger a humoral adaptive immune response. *Candida* virulence factors include mannan, glycoprotein, beta-glucan, ergosterol, agglutinin, adhesin, aspartyl protease, and candidalysin. Antigen-antibody reactions can be detected, one way is through the LFIA. Polyclonal or monoclonal antibodies used for diagnostics are usually IgG, which is more stable, sensitive to binding to antigens, easy to isolate, and has minimal cross-reactivity. The presence of a reaction between virulence factors and *Candida albicans* polyclonal or monoclonal antibodies will give positive results in the form of test lines so that they can be used to diagnose vulvovaginal candidiasis. [4,5,10,21]

The validity of the diagnostic tool was assessed using measures of sensitivity and specificity. Sensitivity is the ability of a test to show which individuals from the entire population are actually sick. Specificity is the ability of a test to indicate which individuals are not suffering from the disease and which are not actually sick.[22] Sensitivity and specificity values of at least 80.0% show that the tool being tested has good validity as a diagnostic tool.[23] The POCT LFIA *Candida albicans* in this research have a higher sensitivity but lower specificity when compared to SavvychechTM, which also contains *Candida albicans* polyclonal antibodies, which are reported to have a sensitivity of 79% and a specificity of 96% for culture examination.[7] The sensitivity and specificity of POCT LFIA *Candida albicans* in this study was no better than

CandiVagi™ (SR2B, Avrille, France), this POCT uses an IgM epitope monoclonal antibody β-1,2-mannopyranosyl which has a sensitivity of 96.6% and specificity 98.6%[24], but POCT is difficult to obtain or import in Indonesia.

The weakness of this study is the inclusion criteria for research subjects with complaints of vaginal discharge were less specific for the signs and symptoms of vulvovaginal candidiasis, so the number of positive *Candida albicans* culture examination samples analyzed was small. The advantage of this research is the POCT LFIA *Candida albicans* diagnostic test was analyzed using culture, so the validity value obtained is the result of the gold standard examination of vulvovaginal candidiasis. In the analysis, the results of a positive culture examination had a *Candida albicans* count >4x10^4 CFU/ml, so a positive culture result for *Candida albicans* in the analysis was a subject with vulvovaginal candidiasis, not colonization.

The LFIA *Candida albicans* point of care test can be used as a diagnostic tool for vulvovaginal candidiasis caused by *Candida albicans* in women who complain of vaginal discharge with sensitivity 100% : and specificity 89.29% against *Candida* spp. culture. Suggestions for further research include similar studies with research subjects that use a syndromic approach to diagnose vulvovaginal candidiasis, as well as the development of POCT for the diagnosis of vulvovaginal candidiasis that can detect *Candida albicans* and *non-albicans*.

ACKNOWLEDGEMENT

Researchers would like to express their deepest gratitude to Dr. drg. M.M. Suryani Hutomo, MDSc, as an analyst and Ratna Niansari, S.Si, as a laboratory assistant in the microbiology laboratory at the Faculty of Medicine, Duta Wacana Christian University, Yogyakarta, who assisted researchers in examining the culture of *Candida* spp.

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