Validity Evaluation of In-House Preparation Kit, Vaginal pH Paper Test Combined Amine Tube Test, for the Simple Diagnosis of Bacterial Vaginosis

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Objective: *Determine the positive in-house preparation kit for suggested bacterial vaginosis (BV) for both elevated vaginal pH > 4.5 and positive amine test, as well as evaluate for validity of sensitivity, specificity, positive predictive value, and negative predictive value against Chandeying criteria for confirmed BV.*
Material and Method: *A cross-sectional study among the women who presented with an abnormal vaginal discharge (AVD) or asymptomatic annual cervical cytology screening was done. Each vaginal discharge was divided into two parts of investigation. The first part included the clinical criteria of confirmed BV, based on at least three out of five indicators, the vaginal pH > 4.5, homogeneous and thin discharge (milky discharge), positive sniff/amine test, clue cell > 20% of total vaginal epithelial cells, and scanty or absence lactobacilli. The second part included the in-house preparation kit of suggestive BV, relied on elevated vaginal pH > 4.5 and positive amine tube test.*
Results: *Twenty-six women were enrolled. Of the complaint of AVD/asymptomatic had 2/10 of confirmed BV (12 cases), and 1/13 of confirmed non-BV (14 cases). The in-house preparation kit, compared with the clinical criteria, had sensitivity of 91%, specificity of 71%, positive predictive value of 73%, and negative predictive value of 90%. There were false negative of 1/12 cases (8.3%), and false positive of 4/14 cases (28.5%).*
Conclusion: *The in-house preparation kit favorably compared with the clinical criteria and has the advantage of being simple, rapid, and easily performed in resource poor setting. Further development on sensitivity and specificity of the test is suggested.*

Keywords: *Bacterial vaginosis, In-house, pH paper test, Amine tube test*

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Abnormal vaginal discharge (AVD), a most common symptom of lower genital tract infection (LGTI), was defined as any one those three presentations: 1) excessive vaginal discharge not associated menstruation, pre-, mid-, and post-period; 2) offensive or malodourous discharge; and 3) yellowish or mucopurulent discharge[1]. A study of 240 reproductive age women in Thailand, the bacterial vaginosis (BV) is the most common LGTI, affecting 37.9% of women attending gynecologic clinic with the symptom of AVD, followed by mucopurulent cervicitis (MPC) with 33.7%, non-infective leucorrhoea (NIL) with 29.1%, vaginal candidiasis (VC) with 22.0%, and vaginal trichomoniasis (VT) with 4.1%. Among these, 15.8% had infection with two conditions, and 0.4% with more than two conditions[2].

Symptoms alone are not reliable for the diagnosis of BV. The diagnosis is based on a diagnostic criteria of clinical and laboratory indicators as follows, 1) the vaginal pH > 4.5, 2) homogeneous and thin discharge (milky discharge), 3) positive sniff/amine test, 4) clue cell > 20% of total vaginal epithelial cells, and 5) scanty or absence lactobacilli. The clinical criteria of BV diagnosis can be made if the investigation fulfills three out of four first indicators (Amsel Criteria), or three out of five indicators (Chandeying Criteria)[3,4].

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The pH level can be measured by placing a drop of the discharge on a short-range pH paper. Contamination with cervical mucus has to be avoided, as the normal pH of cervical mucus is alkaline. The sensitivity/specificity/positive predictive value/ negative predictive values of the vaginal pH more than 4.5 for the diagnosis of BV are 95/72/67/96%[2]. Whereas, the amine test is positive when fishy odor, amines or organic acids produced by the alkalinization of anaerobic bacteria, is released when a drop of 10% potassium hydroxide (KOH) is added onto the vaginal discharge, in either the microscopic slide or posterior blade of vaginal speculum. A positive amine test is strongly suggestive of BV and VT[5].

BV preferably needs to be treated only when the symptomatic patient fulfills the diagnostic criteria. However, the issue regarding treatment of asymptomatic BV is for the secondary preventive treatment[6].

The aim of the present study was to determine the positive in-house preparation kit for suggested BV which means both elevated pH > 4.5 and positive amine test, as well as evaluate for validity of sensitivity, specificity, positive predictive value, and negative predictive value, against Chandeying criteria[4], for confirmed BV.

Material and Method

The present study was approved by the Ethics Committee of the Faculty of Medicine, Prince of Songkla University, Thailand, and it was a cross-sectional study conducted in Department of Obstetrics and Gynecology between October 2011 and February 2012.

The women, who presented with either an AVD or asymptomatic annual cervical cytology screening, were enrolled. A brief history was taken inclusive of the presenting complaints, contraceptive use, date of last Pap smear, and last sexual intercourse without barrier method, as well as antibiotic allergy.

A pelvic examination was then performed. A vaginal speculum, without lubricated substance was inserted and the presence of vaginal discharge was evaluated. Vaginal secretions were classified as floccular, thin homogeneous (milky), or curd-like. Vaginal fluid from the posterior vaginal fornix was tested for pH level, both by a commercial pH paper, Merck, short-range pH paper of pH 3.8 (yellow) to 5.4 (blue), and two in-house pH papers. The first one of bromophenol blue, has the range of 3.0 (yellow) to 4.6 (blue violet), while the second one of bromocresol green, has the range of 3.8 (yellow) to 5.4 (blue)[7]. Both prepared by 1 mg of each acid-base indicator substance, dissolved in absolute ethanol 1 ml, soaked the white filter paper with solution, and then let it dry. The standardization analysis was performed with various known pH solutions. On the test procedure, the pH paper was dabbed with the specimen, and miraculously changed the color. The pH test value of dual comparison can be read off by direct comparison of the test strip color.

In addition, the amine tube test, nitrous acid test, was also determined, by dissolving the vaginal specimen with 0.9% NaCl 0.6 ml, put two drops onto a test tube and added on with several drops of 10% HCl until it becomes acidity (amine salt), confirmed by pH paper test and kept cool in an ice bath. Another an ice cold tube, containing of 20% $NaNO_2$ 1 ml, was added with 10% HCl drop by drop until it becomes acidity, and the solvent was slowly poured on amine salt. The brick effervescence due to evolution of nitrogen indicated the presence primary aliphatic amine, considering as the positive nitrous acid test. While the green precipitate, yellowish solid, or oil was the negative nitrous acid test[8,9].

The normal saline and KOH wet mount preparation were prepared and examined microscopically for fungal element, trichomonad, clue cell, polymorphonuclear leucocytes (PMNs): vaginal epithelial cells ratio, and lactobacilli.

The conventional diagnosis of BV was concluded if the client fulfills at least 3 of 5 indicators of 1) the vaginal pH > 4.5; 2) homogeneous and thin discharge (milky discharge); 3) positive sniff/amine test; 4) clue cell > 20% of total vaginal epithelial cells; and 5) scanty or absence lactobacilli[4]. The in-house preparation Kit, pH paper test combined amine tube test, were separately performed. The simple diagnosis of BV will be concluded if vaginal pH > 4.5 plus amine tube test was positive.

Results

Twenty-six women were enrolled, and the mean age was 43.4 ± 9.49 years, range of 24 to 58 years. Of the complaint of AVD/asymptomatic had 2/10 of confirmed BV (12 cases), and 1/13 of confirmed non-BV (14 cases).

The clinical indicators of BV, comparison of the clinical criteria versus in-house preparation kit, are demonstrated in Table 1. The clinical diagnosis of BV/non-BV group had commercial pH test > 4.5 of 12 (100%)/1 (7.1%), scanty or absence of lactobacilli of 12 (100%)/4 (28.5%), clue cell of 11 (91.6%)/

2 (14.2%), homogeneous discharge of 9 (75.0%)/ 2 (14.2%), and amine test positive of 5 (38.4%)/ 2 (14.2%), respectively. The simple in-house indicators of BV/non-BV were pH test > 4.5 of 12 (100%)/ 5 (35.7%), and amine tube test positive of 11 (91.6%)/ 8 (57.1%), respectively.

In comparison with clinical criteria of BV diagnosis, the in-house preparation kit had a false negative of 1/12 cases (8.3%) among the BV group, while it had a false positive of 4/14 cases (28.5%) among non-BV group. In addition, the sensitivity was 91%, specificity of 71%, positive predictive value of 73%, and negative predictive value of 90%, as shown in Table 2.

Discussion

Whereas the primary care setting in a rural area needs simple diagnostic test for female LGTI regarding BV, with the reason of lacking of facilities and support for peripheral laboratory such as well-trained microscopist, light microscopy, wet mount, and smear stain preparation. A good, reasonably priced, easy-to-use rapid diagnostic kit would be a big favor in the ambulatory care.

The clinical criteria had the sensitivity/ specificity of 77.9/58.4%, while FemExam Card 1, with test sites for pH and amine as same the in-house preparation kit had sensitivity/specificity of 71.4/72.8%, both were compared with the Gold standard of Nugent criteria[10]. By calculation, the sensitivity/specificity of FemExam is approximately 96.1/124.6% of Amsel criteria[3], which was similar to Chandeying criteria use in the present study[4].

Thus, in-house preparation kit/FemExam Card 1, the sensitivity was 91/96.1%, and specificity of 71/124.6%. The in-house preparation kit had slightly lower sensitive but much lower specificity than FemExam Card 1. The first priority of development for the further study should include various tests of amine such Hinsberg test, Rimini test, and so on, while the appropriate pH indicator substances should be the following antecedence.

Several studies reported that approximately 50 to 70% of women with BV are asymptomatic[3,11,12]. The present study had a higher prevalence of asymptomatic BV of 10 in 12 cases (83.3%). Is it necessary to treat asymptomatic BV in non-pregnant women? A randomized, double-blind, placebo-controlled

Table 1. Comparison between in-house preparation kit and clinical criteria[2,4]

Diagnostic criteria	Commercial pH test > 4.5 No. (%)	Homogeneous discharge No. (%)	Amine test positive No. (%)	Clue cell > 20% No. (%)	Lactobacilli scanty of absence No. (%)
Confirmed BV (n = 12)	12 (100)	9 (75.0)	5 (38.4)	11 (91.6)	12 (100)
Confirmed non-BV (n = 14)	1 (7.1)	2 (14.2)	1 (7.1)	2 (14.2)	4 (28.5)
In-house preparation kit	pH paper test > 4.5 No. (%)		Amine tube test positive No. (%)		
Suggested BV (n = 11 in 12)	12 (100)		11 (91.6)		
Suggested non-BV (n = 10 in 14)	5 (35.7)		8 (57.1)		

Table 2. Sensitivity, specificity, positive predictive value, and negative predictive value of in-house preparation kit, compared with the clinical criteria [2,4]

		Clinical criteria		
		Criteria positive	Criteria negative	
In-house preparation kit	Test outcome positive	11	4	Positive predictive value = 11/11 + 4 = 0.73
	Test outcome negative	1	10	Negative predictive value = 10/1 + 10 = 0.90
		Sensitivity = 11/11+1 = 0.91	Specificity = 10/4+10 = 0.71	

trial comparing metronidazole gel with placebo among 75 women who had asymptomatic BV showed greater percentage of resolution, but not statistical significance[13]. Certainly, there are indications to treat asymptomatic BV in non-pregnant women who undergo surgical procedures such as abortion and hysterectomy, and by extension to prior to transvaginal procedures such as intrauterine device insertion, and endometrial biopsy[14].

The treatment of asymptomatic BV without bearing gynecologic surgical procedure is controversial. Considerably, whether women with asymptomatic BV are truly without symptomatology or whether poorly recognized or underreported symptom may be present. The authors prefer to provide the clinical counseling for the client to make the self-decision, according to secondary preventive treatment[6].

Concern of optimal treatment, the in-house preparation kit has had false negative/false positive of 1 in 12 (8.3%)/4 in 14 (28.5%), as well as the negative/positive predictive value of 90/73%. Those would imply to the proportion of overtreatment/undertreatment, respectively.

In conclusion, the in-house preparation kit favorably compares with the clinical criteria, and has the advantage of being simple, rapid, and easily performed in a re-source poor setting. Further development on sensitivity and specificity of the test is suggested.

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Potential conflicts of interest

None.

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การประเมินค่าความถูกต้องของชุดเตรียมเองโดยหน่วยงาน ทั้งกระดาษทดสอบความเป็นกรดด่างร่วมกับหลอดทดสอบสารเอมีนในช่องคลอด สำหรับการวินิจฉัยภาวะช่องคลอดอักเสบแบคทีเรียผสมแบบเรียบง่าย

สุชีรา ธนนิมิตร, ภณิดา เกื้อสุวรรณ, เกศรา นิตยบูรณ์, วิไลวรรณ โชติเกียรติ, ณัฐพร จันทร์ดียิ่ง, วีระพล จันทร์ดียิ่ง

วัตถุประสงค์: *เพื่อกำหนดชุดเตรียมเองโดยหน่วยงานได้ผลบวก สำหรับการบอกเป็นนัยถึงภาวะช่องคลอดอักเสบแบคทีเรียผสม ซึ่งหมายความว่าทั้งการทดสอบความเป็นกรดด่างช่องคลอดเพิ่มขึ้นมากกว่า 4.5 และสารเอมีนได้ผลบวก เช่นเดียวกับประเมินค่าความถูกต้องเกี่ยวกับความไว ความจำเพาะ คุณค่าทำนายผลบวก และคุณค่าทำนายผลลบ เทียบกับเกณฑ์จันทร์ดียิ่งในการวินิจฉัยในการยืนยันภาวะช่องคลอดอักเสบแบคทีเรียผสม*

วัสดุและวิธีการ: *เป็นการศึกษาแบบตัดขวางในหญิงที่มีภาวะมูกช่องคลอดผิดปกติ หรือ ตรวจเซลล์มะเร็งปากมดลูก โดยไม่มีอาการ มูกช่องคลอดแบ่งเป็นสองส่วน การตรวจหาส่วนแรก ตรวจด้วยเกณฑ์ทางคลินิกเพื่อยืนยันภาวะช่องคลอดอักเสบแบคทีเรียผสม อิงกับการมีตัวบ่งชี้อย่างน้อย 3 ใน 5 กล่าวคือ หนึ่ง) ความเป็นกรดด่างช่องคลอดมากกว่า 4.5 สอง) มูกเป็นเนื้อเดียวกันและเจือจาง (คล้ายน้ำนม) สาม) ทดสอบสารเคมี/ดมกลิ่นได้ผลบวก สี่) เซลล์เยื่อบุช่องคลอดมีแบคทีเรียเกาะทึบมากกว่าร้อยละ 20 ของเซลล์ทั้งหมด ห้า) เชื้อแลคโตแบซิไลจำนวนน้อยมาก/ไม่มี ส่วนที่สอง ตรวจด้วยชุดเตรียมเองโดยหน่วยงาน อาศัยความเป็นกรดด่างช่องคลอดเพิ่มขึ้น และสารเอมีนได้ผลบวก เป็นการชี้แนะว่าเป็นภาวะช่องคลอดอักเสบแบคทีเรียผสม*

ผลการศึกษา: *มีผู้หญิงจำนวน 26 คน เข้าการศึกษา จำนวนผู้ป่วยภาวะมูกช่องคลอดผิดปกติ/ไม่มีอาการของกลุ่ม ยืนยันภาวะช่องคลอดอักเสบแบคทีเรียผสม (12 ราย) 2/10 ราย ของกลุ่มไม่ยืนยันภาวะช่องคลอดอักเสบแบคทีเรียผสม (14 ราย) 1/13 ราย ชุดเตรียมเองโดยหน่วยงานเมื่อเปรียบเทียบเกณฑ์ทางคลินิกมีความไวร้อยละ 91 ความจำเพาะ ร้อยละ 71 คุณค่าทำนายผลบวก ร้อยละ 73 และคุณค่าทำนายผลลบร้อยละ 90 ส่วนผลลบเทียมเท่ากับ 1 ใน 12 ราย (ร้อยละ 8.3) และ ผลบวกเทียมเท่ากับ 4 ใน 14 ราย (ร้อยละ 28.5)*

สรุป: *ชุดเตรียมเองโดยหน่วยงานเปรียบเทียบไปด้วยกันกับเกณฑ์ทางคลินิก และมีข้อได้เปรียบด้านความเรียบง่าย รวดเร็ว และสามารถดำเนินการง่ายในสถานที่มีทรัพยากรจำกัด แนะนำให้พัฒนาเกี่ยวกับความไวและความจำเพาะของการทดสอบ*