Diagnostic evaluation of the BD Affirm™ VPIII assay as a point-of-care test for the diagnosis of bacterial vaginosis, trichomoniasis and candidiasis in a population of pregnant women from South Africa

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Plagiarism:

DECLARATION

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LIST OF ACRONYMS

STIs – Sexually Transmitted Infections

BV – Bacterial Vaginosis

PPV – Positive predictive value

NPV – Negative predictive value

WHO – World Health Organisation

qPCR – Quantitative Polymerase Chain Reaction

VVC – Vulval Vaginal Candidiasis

HIV – Human Immunodeficiency Virus

TV – *Trichomonas vaginalis*

LoD – limit of detection

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Oral presentation:

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ABSTRACT

OBJECTIVE: Untreated Sexually Transmitted Infections (STIs) and Bacterial vaginosis (BV) pose a serious health risk to mother and child. Limited data exist on the use of the BD Affirm VPIII assay as a point-of-care test. This study compared the BD Affirm VPIII assay to the BD Max™ Vaginal assay (reference test) for the detection of BV, *Trichomonas vaginalis*, and *Candida spp*. The prevalence of single and co-infections are also reported here.

METHODS: The study enrolled 273 pregnant women from King Edward VIII hospital in Durban. Socio-demographic, sexual behaviour and clinical data were collected from all consenting women. The women provided two self-collected vaginal swabs for testing. The swabs were tested using the BD Affirm VPIII assay and the BD Max™ Vaginal assay. The prevalence of BV, trichomoniasis and candidiasis was calculated as the percentage of women who tested positive for BV, *T.vaginalis* and *Candida* infection and 95% confidence intervals (CIs) were calculated for these percentages using the formulas for calculating CIs for proportions. The number of co-infections was calculated using chi-square analysis. The diagnostic accuracy of the BD Affirm™ VPIII assay compared to the BD Max assay was assessed through the calculation of sensitivity, specificity, Negative Predictive Value (NPV) and Positive Predictive Value (PPV) and their respective 95% confidence intervals.

RESULTS: In this study population, 85% of the participants were unmarried; however, 84% reported having a regular partner, and 96.3% did not use a condom regularly. The prevalence of Bacterial Vaginosis, Candidiasis and Trichomoniasis was 49.4%, 57.2% and 10.3%, respectively. A large proportion of women (78.8%) in this study did not have a discharge despite being positive for one or more pathogens. The BD Affirm™ VPIII assay showed a moderate sensitivity (79.8%) and specificity (80.3%) for diagnosing BV in all participants. The assay had an excellent specificity for *Candida* and *T. vaginalis* of 97.4% and 100.0%; respectively, however, it exhibited poor sensitivities of 52.9% and 42.4%, respectively.

CONCLUSION: Our findings show a higher prevalence of Bacterial Vaginosis in antenatal attendees than previously reported, while the prevalence of Candidiasis and Trichomoniasis was in keeping with previous reports. The high number of asymptomatic infections detected is of concern and indicates the need for the re-evaluation of the syndromic management approach, especially in the antenatal population. The BD Affirm™ VPIII assay was found to be unsuitable as a screening test for vaginal infections in pregnancy. The assay performed better as a confirmatory test and may serve useful if used in conjunction with other clinical parameters such as vaginal pH.

CHAPTER ONE - INTRODUCTION

BACKGROUND

According to the World Health Organizations (WHO) 2016 estimates, there are more than 1 million Sexually Transmitted Infections (STIs) acquired every day worldwide. Each year there are an estimated 357 million new STI infections with 1 of 4 STIs: chlamydia (131 million), gonorrhoea (78 million), syphilis (5.6 million) and trichomoniasis (143 million).[1] In the primary care setting, vaginitis is considered one of the most common gynecologic diagnosis. This condition occurs as a result of bacterial vaginosis, vulvovaginal candidiasis or trichomoniasis infection.[2] Bacterial vaginosis, candidiasis and trichomoniasis in pregnancy have been associated with low birth weight, pre-term delivery and premature rupture of membranes.[3,4,5] South Africa uses Syndromic Management guidelines for the diagnosis and treatment of vaginal discharges in pregnant women. Several studies have shown that syndromic management is inadequate for this purpose due to the high number of asymptomatic genital tract infections seen.[6–10] The test and treat approach for vaginal discharges in pregnant women may be the best alternative.[11]

In the past, diagnostic methods for Bacterial vaginosis relied on the use of clinical criteria and microscopy using Amsel's Criteria or Nugent's criteria.[12] Methods for *Candida* included the observation of budding yeast cells and pseudohyphae on wet mount or a positive culture for *Candida*.[13] *Trichomonas* was detected by wet mount, in culture, or via biochemical detection.[14] Nucleic acid amplification-based assays have since been introduced and are widely used for detection of these pathogens. Molecular assays reduce error, are less laborious and have better performance.[15]

In this study, we evaluated the performance of the BD Affirm VPIII Microbial Identification assay against the BD Max™ Vaginal assay for the diagnosis of BV, Candidiasis and Trichomoniasis in a population of pregnant women. The prevalence estimates and co-infections of these pathogens are also reported through this study.

CHAPTER TWO – LITERATURE REVIEW

2.1.1. BACTERIAL VAGINOSIS

Bacterial vaginosis is caused by the abnormal ecology of the vaginal environment. Certain factors cause an alteration in the microbiome, which raises vaginal pH and which results in symptoms from none to very bothersome. [16] Symptomatic women typically present with vaginal discharge and or vaginal odour. The discharge is thin and homogenous and off-white with an unpleasant fishy odour more noticeable during menstruation and after sexual intercourse.[16] BV is characterized by alterations in the vaginal environment: (1) a shift in vaginal flora from normal *Lactobacillus* species to predominantly facultative anaerobes with high bacterial diversity; (2) amines production induced by the anaerobic flora and (3) a rise in vaginal pH to greater than 4.5.[16] BV is often associated with a mixed vaginitis caused by *Candida* and/or *Trichomonas vaginalis* and is often associated with a cervicitis (endocervical discharge and induced bleeding of the cervix).[16]

The microbial imbalance/dysbiosis in the vaginal environment is characterised by a reduction of the normal lactobacilli and an increase in the concentration of anaerobic Gram-negative rods and other organisms. The majority of bacteria detected in women with BV are *Gardnerella vaginalis*, *Bacteroides* species, *Peptostreptococcus* species, *Prevotella* species, *Porphyromonas* species, *Mycoplasma hominis*, *Ureaplasma urealyticum*, *Mobiluncus*, *Megasphaera*, *Sneathia*, and *Clostridiales* species. Also common are *Fusobacterium species* and *Atopobium vaginae*.[16] A study in 2005 by Fredericks et al. identified 35 bacterial phylotypes that were associated with BV, with *G. vaginalis* being the most common. Women with BV had a mean of 12.6 phylotypes per sample. These newly identified organisms by polymerase chain reaction (PCR) were classified in the Clostridiales order and termed "BV associated bacterium (BVAB) 1, 2 and 3" and specific indicators of BV. [17]

The dominant lactobacilli of the normal flora are hydrogen peroxide producing and thereby prevent the overgrowth of anaerobes normally present in the vaginal flora. Loss of lactobacilli causes a rise in pH and subsequent overgrowth of vaginal anaerobes.[16] These anaerobes produce large amounts of proteolytic carboxylase enzymes. These enzymes break down peptides into amines that are volatile and quite malodorous. These amines increase vaginal transudation with squamous epithelial cell exfoliation, and this results in the clinical features seen in BV.[16] The rise in pH also facilitates the adhesion of *G.vaginalis* to the exfoliating epithelial cells. There is increasing evidence that *G.vaginalis* forms a biofilm to which other species adhere. *G.vaginalis* forms 90% of the biofilm with *Atopobium vaginae* making up the rest.[16] Desquamation results in the classic clue cells diagnostic of the disorder.[16]

2.1.2. CONSEQUENCES OF BV

Pregnant women with BV are at a higher risk of preterm delivery as BV causes endometrial bacterial colonisation, postpartum fever, post-hysterectomy vaginal cuff cellulitis, plasma cell endometritis postabortal infection.[16] BV doubles the risk of preterm delivery before gestation week 37. [18]

Alterations in estrogen and progesterone levels in pregnancy induce physiological changes, such as pH changes in the genital tract. [19] These physiological changes result in vaginal mucosa congestion and hypertrophy, which induce the growth of anaerobic bacteria and other pathogenic microorganisms. [19]

Future health implications of BV include increased susceptibility to other STIs. BV is also a risk factor for transmission and acquisition of Human Immunodeficiency Virus (HIV).[20] Bautista et al. in 2016 showed that elevated cervicovaginal inflammatory cytokine levels in pregnancy-related BV might increase one's vulnerability to an STI.[21] Abbai et al. in 2015 showed that BV infections are associated with a higher incidence of *Trichomonas vaginalis* and *Chlamydia trachomatis* infections.[4]

2.1.3. DIAGNOSIS OF BV

BV can be diagnosed using Amsel's criteria designed for bedside diagnosis.[15] This method is simple but requires the use of a microscope. At least three of the four criteria must be present for a diagnosis to be made: (1) the presence of a thin, greyish-white homogenous discharge that coats the vaginal wall; (2) a vaginal pH of more than 4.5; (3) a positive whiff test, characterized as the presence of a fishy odour with the addition of a drop of 10 percent potassium hydroxide to vaginal discharge and (4) the presence of clue cells on a saline wet mount. Clue cells are epithelial cells that have coccobacilli adhering to the edges of the cell.[15]

BV can also be diagnosed using Nugent's criteria or Ison and Hay criteria which involves performing a Gram's stain.[12,22] This method requires more time, trained personnel and resources than the Amsel's criteria as it relies on the actual grading of the microorganisms seen under the microscope.[16] The diagnosis of BV using Nugent's criteria has been the Gold standard for many years. Cytology smears and culture methods are not suitable for diagnosis of bacterial vaginosis as they are inaccurate in their diagnosis.[16]

There are commercially available tests for the diagnosis of BV such as the OSOM BV Blue, BD Affirm™ VPIII and the BD Max™ vaginal panel. The OSOM BV Blue system is a chromogenic diagnostic test based on detecting sialidase activity in vaginal samples. Sialidase is produced by pathogens associated with BV such as *Gardnerella*, *Porphyromonas*, *Prevotella* and *Mobiluncus*. The test can be used as a

point-of-care test and takes only 10 minutes. Bradshaw et al. in 2003 evaluated the use of the OSOM BV Blue test compared to Nugent's criteria and Amsel's criteria.[23] The test performed well against Nugent's criteria and poorly against Amsel's criteria. A disadvantage of the BV Blue test is that it detects activity for bacterial vaginosis only and not for *Trichomonas* or *Candida*.

In 1994, Briselden et al. introduced the BD Affirm VP test as a rapid, objective and automated test for the detection of *T.vaginalis* and clinically significant levels of *G. vaginalis* of $>5 \times 10^5$ CFU/ml; that is comparable to wet mount examination for clue cells and superior to wet mount examination for the detection of trichomonads.[24] It could be used as a point-of-care test and took less than an hour to perform. Sheiness et al. in 1992 reported that the use of these DNA probe tests for BV had increased sensitivity and specificity when used with elevated pH as a diagnostic indicator.[25] Briselden et al. in 1994 further illustrated this by showing that the BD Affirm™ VPIII assay for BV had greater sensitivity and specificity of 95 and 99 percent when used in conjunction with vaginal pH or wet mount than when used alone.[24] A study in Indianapolis, USA in 2003 showed that the Affirm VP test was more sensitive in symptomatic women than conventional wet mount microscopy.[26] Crist et al. in 2011 showed that the BD Affirm™ VPIII outperformed and provided faster results when compared to microscopy and culture methods for the diagnosis of bacterial vaginosis and yeast vaginitis.[27]

Quantitative polymerase chain reaction (qPCR)-based assays such as the BD Max™ Vaginal assay are based on the molecular quantification of *Lactobacillus*, *G. vaginalis*, *Atopobium* and other BV associated bacteria. qPCR is the gold standard in the quantitative analysis of nucleic acids. These tests have good sensitivity and specificity but are expensive to perform. Dhiman et al. showed in 2016 that qPCR has a better sensitivity than Nugent's scoring system for the diagnosis of BV [28] while Sobel et al. in 2015 showed that PCR is superior in sensitivity and specificity when compared to the BD Affirm™ VPIII assay.[13]

2.1.4. PREVALENCE ESTIMATES FOR BV

Table 1. Summary of prevalence data for Bacterial Vaginosis

Author	Organism	Method	Prevalence	Population	Country
South Africa:					
Johnson et al, 2005	BV	various - review article	24-52%	antenatal	South Africa
Mlisana et al, 2012	BV	Nugents criteria	52.7%	clinical trial participants	South Africa
Redelinghuys et al., 2017	BV	Nugents criteria	17.7%	antenatal	South Africa
Abbai et al., 2015	BV	Amsel's	31.0%	clinical trial participants	South Africa
Torrone et al., 2018	BV	Nugent's criteria - a meta-analysis	41.2%	clinical trial participants	South Africa
Joyisa et al., 2019	BV	Nugents criteria	37.3%	antenatal	South Africa
other:					
Mengistie et al, 2014	BV	Nugents criteria	19.4%	antenatal	Ethiopia
Olowe et al., 2014	BV	Wet mount	38.0%	antenatal	Nigeria
Marconi et al., 2015	BV	Nugents criteria	30.1%	primary health care	Brazil
Bitew et al., 2017	BV	Nugents criteria	48.6%	primary health care	Ethiopia

A study conducted by Bitew et al. in 2017 in an Ethiopian primary healthcare clinic reported a prevalence of 48.6%% for BV.[29] Other African studies conducted in Ethiopia and Nigeria found a prevalence of 19.4% and 38.0% for BV in antenatal populations.[30,31]A meta-analysis conducted by Torrone et al. in 2018 on women participating in HIV prevention trials in Sub-Saharan Africa reported a mean prevalence of 41.2% for BV in South Africa.[32] Similarly Mlisana et al. and Abbai et al. in 2012 and 2015 reported high prevalences of BV (52.7% and 31.0% respectively) for women participating in HIV prevention clinical trials.[4,6]

A Sentinel survey conducted in South Africa in 2005 in antenatal clinic attendees found a prevalence ranging from 24% to 52% for BV.[10] The prevalence of BV in the antenatal population in South Africa reported by Redelinghuys in 2017 was 17.7%.[33] A recently published study in Durban, South Africa found a prevalence of 37.3% for BV among antenatal women.[34]

2.2.1. CANDIDIASIS

Vaginal candidiasis is caused by colonisation of the vagina by the yeast *Candida*. The most common species identified in vulvovaginal candidiasis (VVC) is *C. albicans*, with *C. glabrata* being the second most common.[13]

VVC is responsible for 80% to 90% of infections during pregnancy. *Candida* species have been shown to colonise the vagina in at least 30% of all pregnant women.[35] The increased risk of VVC is likely caused by pregnancy-related factors such as a decrease in immunity, increased estrogen and increased glycogen production of the vaginal mucosa. Increased estrogen aids the adherence of yeast to vaginal mucosal cells. In addition, estrogen induces hyphal formation and the secretion of certain enzymes such as aspartyl proteinases and phospholipases. These virulence factors further enhance the colonisation of yeast in vaginal mucosal epithelial cells.[35]

2.2.2. CONSEQUENCES OF CANDIDIASIS

Typical symptoms of VVC include itching, burning, redness, swelling, and discharge. It is characterised by vulvar and vaginal pruritis, external dysuria, white cottage cheese discharge, and vulvovaginal excoriations.[35] Previous studies have shown that candidiasis during pregnancy may be associated with premature rupture of membranes and poor pregnancy outcomes. Aguin et al. (2015) showed a higher preterm birth rate in women with untreated asymptomatic candidiasis compared to women without candidiasis.[35] Although rare, *Candida* infection may become intra-amniotic and cause systemic congenital infection, cerebral candidiasis, or fetal demise.[35]

2.2.3. DIAGNOSIS OF CANDIDIASIS

Laboratory diagnosis of Candida infection can be made by microscopy and culture methods. The sensitivity of microscopy is 60-70% with many false positives and false negatives. Culture on Sabouraud's glucose agar has been the gold standard for many years. However, results can take from 48 - 96hrs. Colonies are seen on culture and confirmed by observation of budding yeast cells on gram stain. A Germ tube test can also be done to confirm the presence of *C .albicans*. The use of Chromogenic agar medium has also allowed the differentiation of *Candida* species while maintaining sensitivity. [13]

The use of DNA homology probes in tests such as the BD Affirm™ VPIII assay offers results within an hour. This test may be an alternative to microscopy and culture methods. The use of PCR for the detection of *Candida* was first applied in 1993, and since then numerous PCR based techniques have been developed for the detection of *Candida*.[36] qPCR was first optimized by Trama et al. in 2005 for *C. albicans, C. glabrata, C. parapsilosis* and *C.tropicalis*.[36] qPCR is now a viable option for rapid detection of *Candida* picking up low copy numbers however these tests may be more expensive and furthermore the detection of low levels of *Candida* may not indicate clinical infection. The FDA approved BD Max™

Vaginal assay is an automated qPCR method that uses a cut off value for *Candida* which is a superior indication of *Candida* vaginitis.

2.2.4. PREVALENCE STATS FOR CANDIDA

Table 2. Summary of prevalence data for Candida

Author	Organism	Method	Prevalence	Population	Country
South Africa:					
Johnson et al, 2005	CA	review article	9-59%	antenatal	South Africa
Garrett et al., 2017	CA	Gram stain	17.6%	primary health care	South Africa
other:					
Olowe et al, 2014	CA	culture and germtube	36.0%	antenatal	Nigeria
Marconi et al, 2015	CA	Gram stain	1.4%	primary health care	Brazil
Mushi et al., 2019	CA	culture and MALDI-TOF	66.6%	antenatal	Tanzania
Konadu et al, 2019	CA	wet mount	1.4%	antenatal	Ghana
Kamga et al, 2019	CA	wet mount	27.8%	antenatal	Cameroon

A study conducted by Olowe et al. in 2014 reported a prevalence of 36.0% for *Candida* using culture in a Nigerian antenatal population.[31] More recent African studies in Tanzania, Ghana, and Cameroon have reported prevalence estimates ranging from 1.4% to 66.6% in antenatal populations.[37–39] These prevalence estimates were obtained by culture and microscopy techniques.

The prevalence of *Candida* reported by Johnson et al. 2005 in a South African sentinel survey was between 9 and 59% for antenatal populations.[10] The studies included in the survey used microscopy and culture techniques for diagnosis. The last reported prevalence of *Candida* in South Africa was 17.6% by Garrett et al. in 2017 in STI clinic attendees.[40]

2.3.1. TRICHOMONIASIS

Trichomoniasis is caused by the flagellate protozoan *Trichomonas vaginalis. T.vaginalis* is considered to be sexually transmittable and sometimes related to low socioeconomic levels.[41] Women infected with *T.vaginalis* can experience a range of symptoms, including itching frothy odorous green-grey vaginal discharge, and dysuria, as well as pelvic inflammatory disease and fallopian tube pathology.[42]

2.3.2. CONSEQUENCES OF TRICHOMONIASIS

In pregnant women, *T. vaginalis* has been associated with an increased incidence of postpartum fever and endometritis, changes in cervical cell morphology and premature rupture of membranes. [14] It has been linked to adverse pregnancy outcomes such as low birth weight and pre-term labour. *T.vaginalis* is also associated with an increased risk of acquiring HIV and increased vaginal shedding of HIV.[42] It has been suggested that infection with this parasite increases maternal-to-infant transmission of HIV. Despite the high prevalence *T. vaginalis* infections, little or no attention is given to the infection during antenatal services as majority of the routine antenatal services focus more on HIV.[43]

2.3.3. DIAGNOSIS OF TRICHOMONIASIS

T.vaginalis is commonly detected using direct wet mount microscopy, culture and nucleic acid amplification tests. Traditionally diagnosis was made by the microscopic observation of motile protozoa in vaginal specimens via wet preparation. Characteristic jerky movements and the presence of flagella contributed to the diagnosis. For the parasite to be viable, the microscopic analysis has to be done timeously. This method although cost-effective has a low sensitivity as there is under-diagnosis.[14]

Broth culture technique has been the gold standard for the diagnosis of *T.vaginalis* for many years. This technique involves the inoculation of a broth such as Diamond's TYI medium and incubation for 2-7 days in carbon dioxide, with an additional subculture. The technique is useful when the testing laboratory is at a distance from the clinic; however, it is more expensive than wet mount and open to bacterial contamination even though antibiotics are added to the medium. Culture systems such as the BioMed InPouch system for *T.vaginalis* were developed, but this test has a slower growth due to aerobic culture conditions.[14] Cell culture has also been used for the cultivation and detection of *T.vaginalis*. This technique offers higher sensitivity than broth culture but is more expensive, more laborious to perform and also prone to bacterial contamination. Some stains such as Acridine orange, Periodic-acid Schiff as well as Pap smears have been used in the past to identify *T.vaginalis* however these stains produce false positives and false negatives and the use of a fixative removes the ability to detect the Trichomonads based on motility.[14]

In recent years nucleic acid amplification tests have been introduced for the detection of *T.vaginalis* such as the Gen-Probe Aptima assay, BD ProbeTec TV assay, Cepheid GeneXpert TV assay, BD Max™ Vaginal assay and the BD Affirm™ VPIII assay. Molecular assays reduce error, are less laborious and have better performance. These tests have performed well against the conventional wet mount and culture systems of the past.

2.3.4. PREVALENCE STATS FOR TV

Table 3. Summary of prevalence data for T.vaginalis

Author	Organism	Method	Prevalence	Population	Country
South Africa:					
Johnson et al, 2005	TV	review article	12-52%	antenatal	South Africa
Altini et al., 2006	TV	review article	30%	antenatal	South Africa
Mlisana et al., 2012	TV	BD Probetec	20.3%	clinical trial participants	South Africa
Abbai et al., 2013	TV	In-pouch	10.0%	clinical trial participants	South Africa
Abbai et al, 2015	TV	PCR	8.3%	clinical trial participants	South Africa
Moodley et al., 2015	TV	BD Probetec	15.3%	antenatal	South Africa
de Waaij et al., 2017	TV	PCR	20.0%	primary health care	South Africa
Jones et al., 2013	TV	PCR	10.0%	community	South Africa
Price et al., 2018	TV	In-pouch	20.0%	antenatal	South Africa
Torrone et al., 2018	TV	NAAT, in-pouch, wet mount	8.6%	clinical trial participants	South Africa
other:					
Olowe et al, 2014	TV	wet mount and culture	2.0%	antenatal	Nigeria
Marconi et al., 2015	TV	Diamonds culture	1.4%	primary health care	Brazil
Kamga et al, 2019	TV	wet mount	1.0%	antenatal	Tanzania

African studies conducted in Nigeria and Tanzania reported a prevalence of 1.0% to 2.0% for *T.vaginalis* in antenatal populations.[31,39] In South Africa prevalence estimates for *T.vaginalis* have been obtained from antenatal women, high-risk women participating in clinical trials and women from the general population. Prevalence estimates for antenatal women ranged from 12% to 52%.[8,10,42,44] The prevalence of *T.vaginalis* for women participating in HIV clinical trials ranged from 8.6% to 20.3% in South Africa.[4,6,32,45]For women from the general population the prevalence estimate was 10% for *T.vaginalis*.[46]

2.4. TREATMENT BASED ON THE SYNDROMIC MANAGEMENT

As per the WHO recommendations and the South African Department of Health maternity guidelines 2015, South Africa follows a syndromic management approach for the treatment of STIs. The figure below describes the treatment regimens based on the observed clinical symptoms.

The illustration below shows the vaginal discharge algorithm currently used. Pregnant women who present with abnormal vaginal discharge are treated for vaginal candidiasis on clinical indication (using clotrimazole) and treated syndromically for gonorrhoea, chlamydia and *T.vaginalis* with triple antibiotics (using Ceftriaxone 250mg, Azithromycin 1g and Metronidazole 2g)[47].

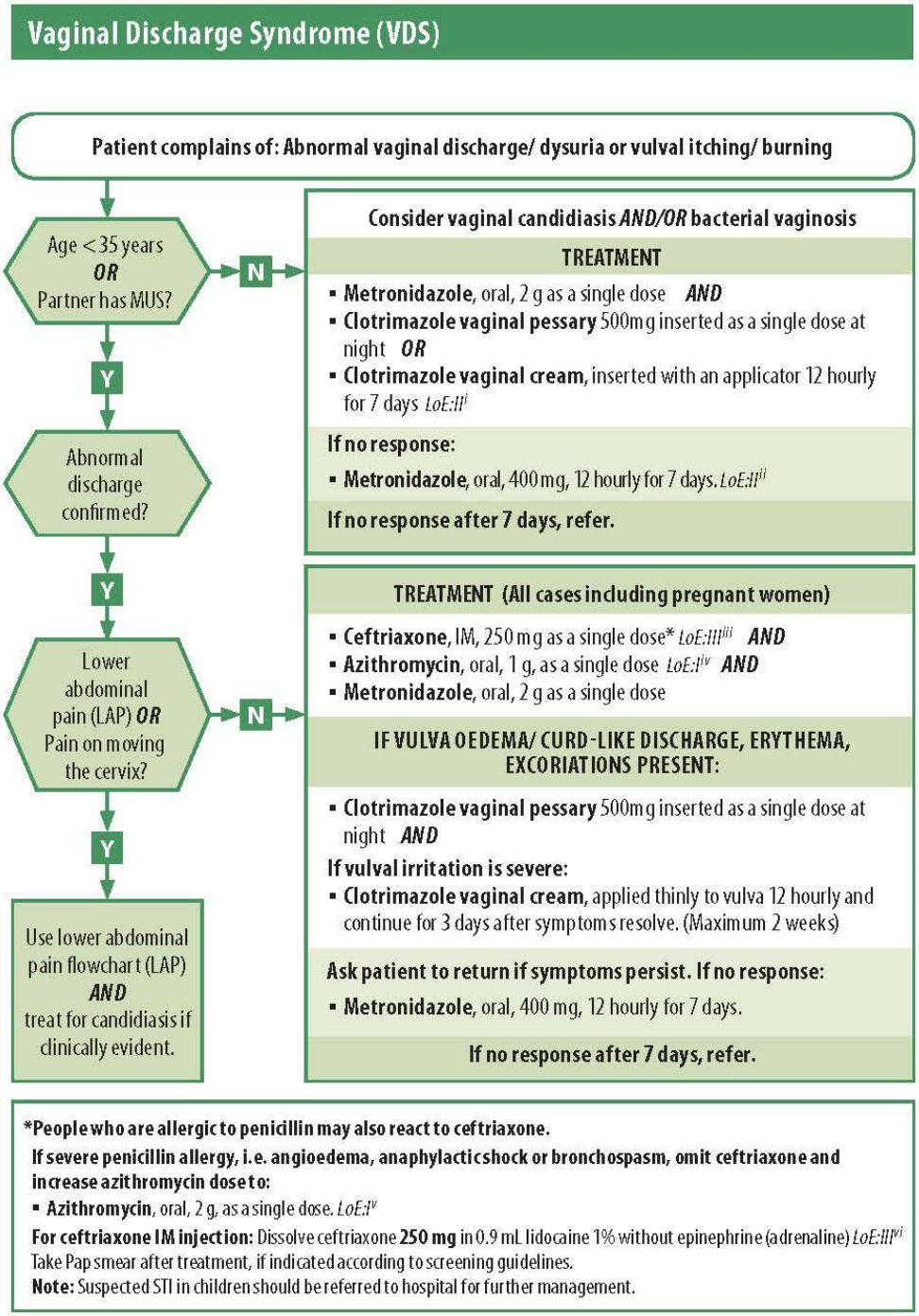


Figure 1. Taken from South African Dept of Health Maternity guidelines 2015

Studies have shown that vaginal discharge is inaccurate in predicting pathological conditions in pregnancy and that asymptomatic trichomoniasis and bacterial vaginosis is a challenge in developing countries.[48] While syndromic management has its advantages of treating patients at the first visit and being easy to follow, it has the following limitations:

(1) it may lead to over-treatment;
(2) increase the potential to develop antimicrobial resistance,
(3) increase costs by the supplying of unnecessary medication[6] and
(4) Most importantly, STIs that show no symptoms are missed.[49]

A study by Mlisana et al. in 2012 in South Africa showed that asymptomatic infections occur in at least half of all STI-infected women[6] and a study by Moodley et al. in 2014 confirmed this in pregnant women[44]. Untreated STIs in pregnancy is associated with pelvic inflammatory disease (PID), tubal factor infertility and ectopic pregnancy.[6] Inaccurate and inconsistent diagnosis of vaginosis and vaginitis in pregnancy apart from the serious risks mentioned causes irritating symptoms that can disrupt the quality of life and which can lead to continued symptoms, repeat visits and unnecessary health care system costs.[50]

Estimates on the proportion of asymptomatic *T.vaginalis* infections are as high as 80%, and the Centres for Disease Control (CDC) recommends screening for *T.vaginalis* among HIV-infected women, particularly HIV-infected pregnant women.[42] Zemouri et al. (2016) conducted a systematic review of published studies from 2000 to 2015 evaluating the diagnostic accuracy of the WHO Vaginal Discharge Flowchart where it was shown that the addition of microscopy to identify TV and BV improved the diagnostic accuracy, resulting in more cases being correctly treated thereby resulting in a reduction in overtreatment and missed cases. [9] A disadvantage of this additional testing is that microscopy is a specialised technique which requires more resources and skill and which may not be feasible in most settings.

Johnson et al. in 2011 showed that Syndromic management programs are unlikely to have a significant impact on the prevalence of asymptomatic STIs in South Africa and showed the need for better screening programs.[51] In a study by van der Eem et al. in 2016 it was shown that nearly half of the women without vaginal discharge and/or vulval irritation on examination tested positive for at least one STI and did not receive adequate treatment.[7] In a 2012 South African Study, Mlisana et al. showed that most of the women in their study who had one or more STIs did not have clinical symptoms. They also highlighted the urgent need for better strategies to manage asymptomatic STIs.[6] Mlisana et al. reported a total of 87.7% of laboratory-diagnosed STIs were asymptomatic.[6] Badman et al. (2016) conducted a study in Papau New Guinea in 2014 where it was shown that more than 70% of pregnant women with a curable STI were

asymptomatic and they had concluded that the STI syndromic management based on clinical presentation is a poor strategy for the detection and treatment of STIs.[11]

The above studies showed that Syndromic management is not adequate for the screening and treatment of vaginal discharge syndrome. The risks are far greater in pregnant women, and a test and treat strategy will be beneficial in this population.

2.5 LABORATORY BASED TESTING FOR VAGINAL INFECTIONS

The BD Max™ Vaginal assay was used as a gold standard in this study to detect the presence of *T.vaginalis*, *Candida species* and Bacterial vaginosis. This assay is an FDA approved assay which incorporates automated DNA extraction and real-time polymerase chain reaction (PCR) for the direct, qualitative detection of pathogens from DNA of vaginal specimens.

Figure 2. The BD Max™ instrument in the SCM Laboratory

The BD Max™ Vaginal assay is an automated diagnostic test approved by the FDA for the detection of Vaginal pathogens associated with Bacterial Vaginosis, Vulvovaginal Candidiasis, and Trichomoniasis from vaginal swabs. The system uses real-time polymerase chain reaction (PCR) to amplify specific targets and uses target-specific hybridisation probes to detect and differentiate the DNA (Illustrated in Figure 3.)

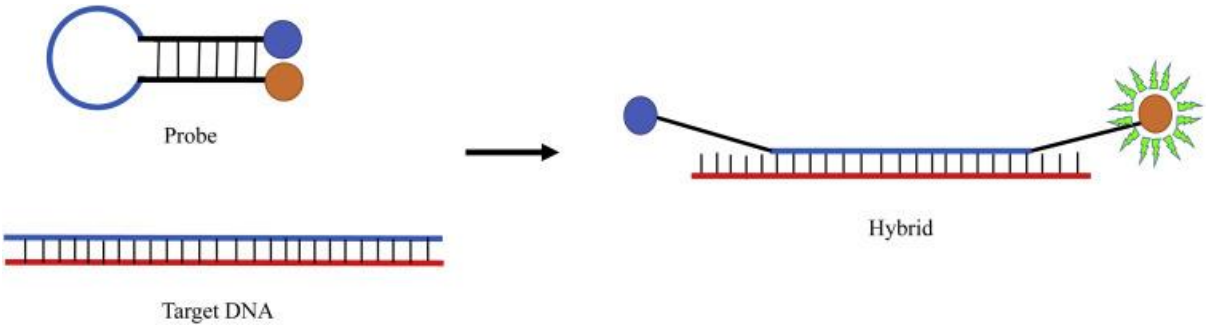


Figure 3. Principle of the Real-Time Technology L.Overbergh, C.Mathieu, Molecular Diagnostics (3rd Edition, 2017

Results are qualitative and are reported as a result for:

(1) Bacterial Vaginosis {by using an algorithm that calculates the ratio of *Lactobacillus crispatus (Limit of Detection (LoD): 55cfu/ml)* and *Lactobacillus jensenii* (LoD: 510 cfu/ml), *Gardnerella vaginalis* (LoD: 962 cfu/ml), *Atopobium vaginae (LoD: 127 cfu/ml)*, BV associated bacteria-2 (LoD: 464 copies/ml) and *Megasphaera-1 (LoD: 2265 copies/ml)*},

(2) *Candida spp* {which includes *C.albicans (LoD:17787 cfu/ml), C.tropicalis (LoD 313 cfu/ml), C.parapsillosis (LoD: 30660 cfu/ml)* and *C.dubliniensis (LoD: 4002 cfu/ml)*},

(3) *Candida glabrata (LoD: 202 cfu/ml),*

(4) *Candida krusei* (LoD: 1035 cfu/ml) and

(5) *Trichomonas vaginalis* (LoD: 22 cells/ml)

In 2017 Gaydos et al. validated the accuracy of the BD Max™ Vaginal assay using self-collected swabs and clinician collected swabs against Nugent's criteria and Amsel's criteria for BV, Chromagar and Sabouraud's agar and sequencing for *Candida*, and wet mount and culture for *T.vaginalis*. This assay had shown a high sensitivity and specificity of the BD Max™ Vaginal assay compared to conventional methods of detection. [52]

2.6. POINT-OF-CARE TESTING FOR VAGINAL INFECTIONS

The BD Affirm™ VPIII Microbial Identification assay uses two distinct single-stranded nucleic acid probes for each organism (a capture probe and a colour development probe) which are complementary to unique genetic sequences of target organisms. The test is based on the principles of nucleic acid hybridisation (seen in Figure 4).

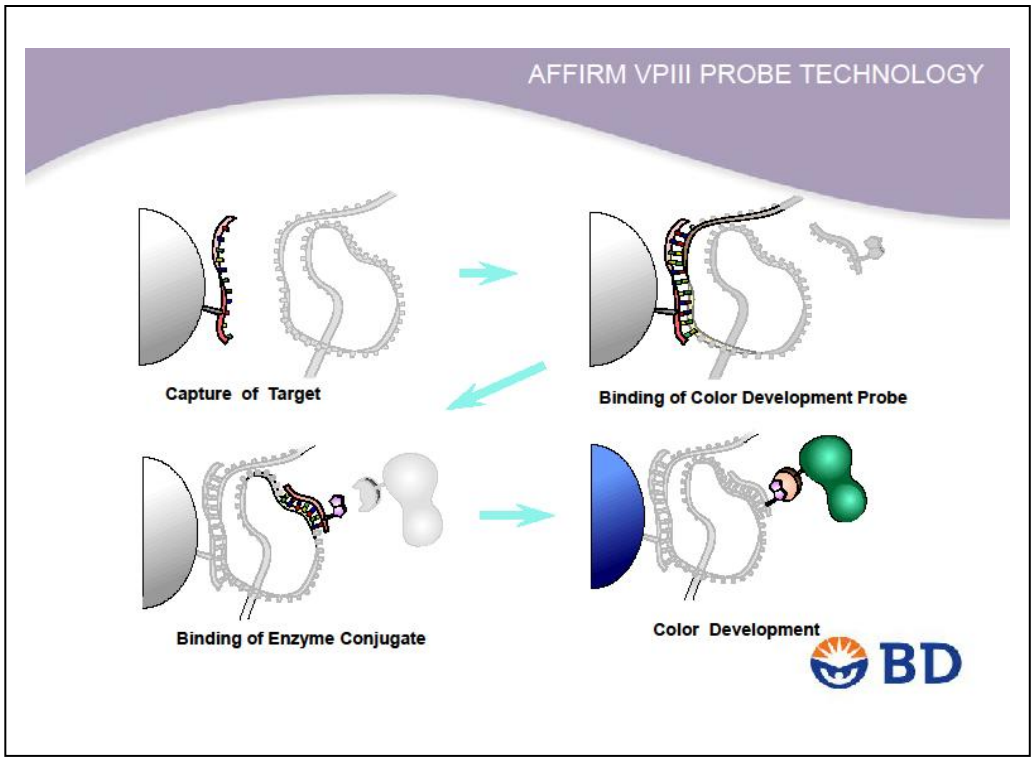


Figure 4.Showing the principle of the BD Affirm™ VPIII *assay (supplied by Becton Dickinson)*

The Probe Analysis Card (PAC) has capture probes embedded on a bead for each target organism. The Reagent Cassette (RC) contains the colour development probes.

During sample preparation, the sample is lysed with Lysis Solution (L) and heated. Heating ruptures the walls of the organism and releases the nucleic acids. Buffer Solution (B) is added, which stabilises the nucleic acid and provides the conditions for the hybridisation process. The sample is added to the first well of the Reagent Cassette (RC) with the PAC, and automated processing is initiated. The BD MicroProbe™ Processor moves the PAC from one well of the Reagent Cassette (RC) to another. Hybridisation occurs on the Probe Analysis Card while it is in the first and second wells of the Reagent Cassette.

The sequence of events:

Well 1 - Hybridization of the analyte to the capture probe occurs on the bead.

Well 2 - Hybridization of colour development probes occurs.

Well 3 - All unbound components and probes are washed away.

Well 4 – Binding of enzyme conjugate to the captured analyte occurs.

Wells 5 and 6 - Unbound conjugate is washed away.

Well 7 - Substrate is converted to a blue-coloured product if bound enzyme conjugate is present on the bead.

Final step – Reading of results of colour development of each of the target organism's beads and controls.

Results are reported positive for:

(1) *T.vaginalis* – LoD: 5 x 10^3 trichomonads

(2) *Gardnerella vaginalis* – LoD: 2 x 10^5 cfu

(3) *Candida* (which includes *C.albicans*, *C.glabrata*, *C.kefyr*, *C.krusei*, *C.parapsillosis* and *C.tropicalis*)- LoD: 1 x 10^4 Candida cells

Figure 5. Showing the BD Affirm™ VPIII instrument in the SCM Laboratory

A Papau New Guinea study had further shown that a novel point-of-care testing strategy for STIs among pregnant women using the test and treat approach was feasible.[11] The study had evaluated the Cepheid GeneXpert CT/NG/TV assay and the BV Blue assay as point-of-care tests in a primary health care setting, using self-collected vaginal swabs. The limitation of this study was that separate tests for *T.vaginalis* and BV were performed and testing for *Candida* was not conducted.[11] Crist et al. in 2011 showed that the BD Affirm™ VPIII assay outperformed and provided faster results than the Amsel's criteria, Nugent's criteria and yeast culture using swab samples, in primary health care patients for the diagnosis of BV and *Candida*.[53]

Cartwright et al in 2013 compared the BD Affirm™ VPIII assay to other Nucleic acid amplification tests (Gen-Probe assay for *T.vaginalis*, CAN-PCR for *Candida* spp, and BV-PCR for Bacterial Vaginosis) using vaginal swabs, in which they concluded that all three tests had to be used in combination to achieve an accuracy better than the BD Affirm™ VPIII assay on its own.[54]

A Study by Briselden et al. showed that the BD Affirm™ VPIII assay was more sensitive than wet mount for the detection of *T.vaginalis* in swab samples.[24]

All the above studies show that there is a need for a point-of-care test such as the BD Affirm™ VPIII assay for diagnosing vaginosis and vaginitis in a clinic setting. The BD Affirm™ VPIII assay has the potential to become the ideal one-assay POC as it tests all three pathogens in one cartridge simultaneously.

2.7. RATIONALE FOR STUDY

Point-of-care (POC) tests can potentially bridge the gap in diagnosis of BV, *T.vaginalis* and *Candida* infection. These tests could lead to an improvement in health outcomes in the antenatal setting. Access to POC diagnostics will lead to evidence-based treatment at the first visit.[55] Additionally, POC tests are rapid, cost-effective and easy to perform in the clinic setting and will ensure the completion of the test and treat cycle.

In this study, we have evaluated the BD Affirm™ VPIII assay as a point of care test. The BD Affirm™ VPIII assay is a multi-analyte, DNA probe-based system which detects *Gardnerella vaginalis* (BV), *T.vaginalis* and *Candida spp* simultaneously from a single sample.

We have decided on this study for the following reasons:

(1) There is limited published data on the performance of the BD Affirm™ VPIII assay against real-time molecular testing methods such as the BD Max™ Vaginal assay (FDA approved).

(2) Most published studies on the BD Affirm™ VPIII assay have recruited symptomatic patients only, in this study, we have sampled all eligible women, including those that do not complain of symptoms.

(3) None of the previous studies on point-of-care tests had evaluated a test for all three vaginal infections: BV, Trichomoniasis and Candidiasis in a single test, nor have they used an antenatal study population.

(4) Prevalence data on BV and STIs in the antenatal population in South Africa is lacking. BV and *Candida* were last reported in 2012 and 2005, respectively. *T.vaginalis* prevalence was last reported in 2018; however, this was in an HIV-infected population only.

2.8. STUDY AIM:

This study aimed to determine the diagnostic accuracy of the BD Affirm™ VPIII test when compared to the BD Max™ Vaginal panel in diagnosing bacterial vaginosis, trichomoniasis and candidiasis in pregnant women.

2.9. HYPOTHESIS:

We hypothesise that the BD Affirm™ VPIII can be used as a screening test for the diagnosis of bacterial vaginosis, trichomoniasis and candidiasis in pregnant women. In addition, we hypothesise that there will be a high prevalence of symptomatic and asymptomatic vaginal infections in the study population.

2.10. STUDY OBJECTIVES:

1. To assess the diagnostic performance of the BD Affirm™ VPIII assay when compared to the BD Max™ Vaginal assay by comparing sensitivity and specificity.

2. To determine the prevalence of bacterial vaginosis, trichomoniasis and candidiasis in patients presenting for antenatal care.

3. To determine the number of asymptomatic cases in the studied population.

CHAPTER THREE - METHODS

3.1. STUDY DESIGN AND SETTING

This was a prospective observational cross-sectional study. The study population was recruited from the King Edward VIII hospital in Durban, KwaZulu Natal. All patients attending the ANC clinic on the study days were invited to participate in the study. Recruitment continued daily until the sample size had been reached. Screening and enrolment was a combined visit. A questionnaire was administered to collect data on the women's demographics, sexual behaviour and clinical information. During the study visit, women were asked to provide two self-collected vaginal swab samples.

Symptomatic participants (before treatment) were characterised as those who complained of an abnormal vaginal discharge/dysuria or vulval itching/burning, and asymptomatic participants were characterized as those who did not complain of any of the above symptoms. All symptomatic women were treated as per the syndromic management guidelines. The guidelines advocate the use of a 2g single dose of metronidazole and clotrimazole vaginal pessary (single dose) or clotrimazole vaginal cream (12 hourly for seven days). Participants who were asymptomatic and who tested positive for infection were not eligible for treatment as per the current syndromic management guidelines.

3.2. ETHICAL CONSIDERATIONS AND CONFIDENTIALITY

Ethical and human participant research approvals were obtained from the Biomedical Research Ethics committee (BE643/17) of the University of KwaZulu-Natal. Furthermore, this was a sub-study of a larger study that had already obtained gatekeeper permission, DoH approval and BREC approval (BE214/17).

All interviews were conducted in private, and all study-related information has been stored securely. All records and specimens have been identified by study ID numbers only to maintain participant confidentiality. Only participants who had given written informed consent were included in the study.

All participant's samples were discarded after testing. Completed study forms have been filed in locked cupboards. Study risks were minimal. None of the participants complained of discomfort with the self-collected swab collection.

3.3. STUDY POPULATION:

The study cohort was made up of 273 pregnant women 18 years and older and willing to provide written informed consent. The study enrolled the first 273 women who met the eligibility criteria. HIV seropositivity was not an exclusionary criterion for the study. The recruitment process took six months to completion between November 2017 and May 2018.

3.4. INCLUSION CRITERIA:

- Age 18 years and older;
- Willing to provide written informed consent;
- Willing to provide swab samples that will be tested for vaginal pathogens.

3.5. EXCLUSION CRITERIA

- Not willing to provide informed consent
- Women who have received antibiotic or antifungal treatment within the week.

For all eligible participants, the following procedures took place at the enrolment visit:

1. Obtaining written informed consent
2. Collection of vaginal swab samples
3. Collection of data on demographics, sexual behaviour and clinical information

3.6. SAMPLE COLLECTION:

Two self-collected vaginal swabs were obtained from each participant for testing, as illustrated in the Study procedure below (Figure 2). Instructions for the collection of the self-collected swabs were given to the study participants (refer to Appendix 4)

The swabs were transported within two hours to the School of Clinical Medicine Laboratory, College of Health Sciences, Medical School campus, University of KwaZulu-Natal. At the laboratory, one vaginal swab was placed in the BD Affirm™ VPIII Specimen collection tube and the other in the BD Max™ Vaginal assay swab diluent tube. Samples for the BD Affirm™ VPIII assay were tested immediately. Samples for the BD Max™ Vaginal assay were stored at 2-8 degrees and batch tested within five days.

3.7. STUDY PROCEDURE:

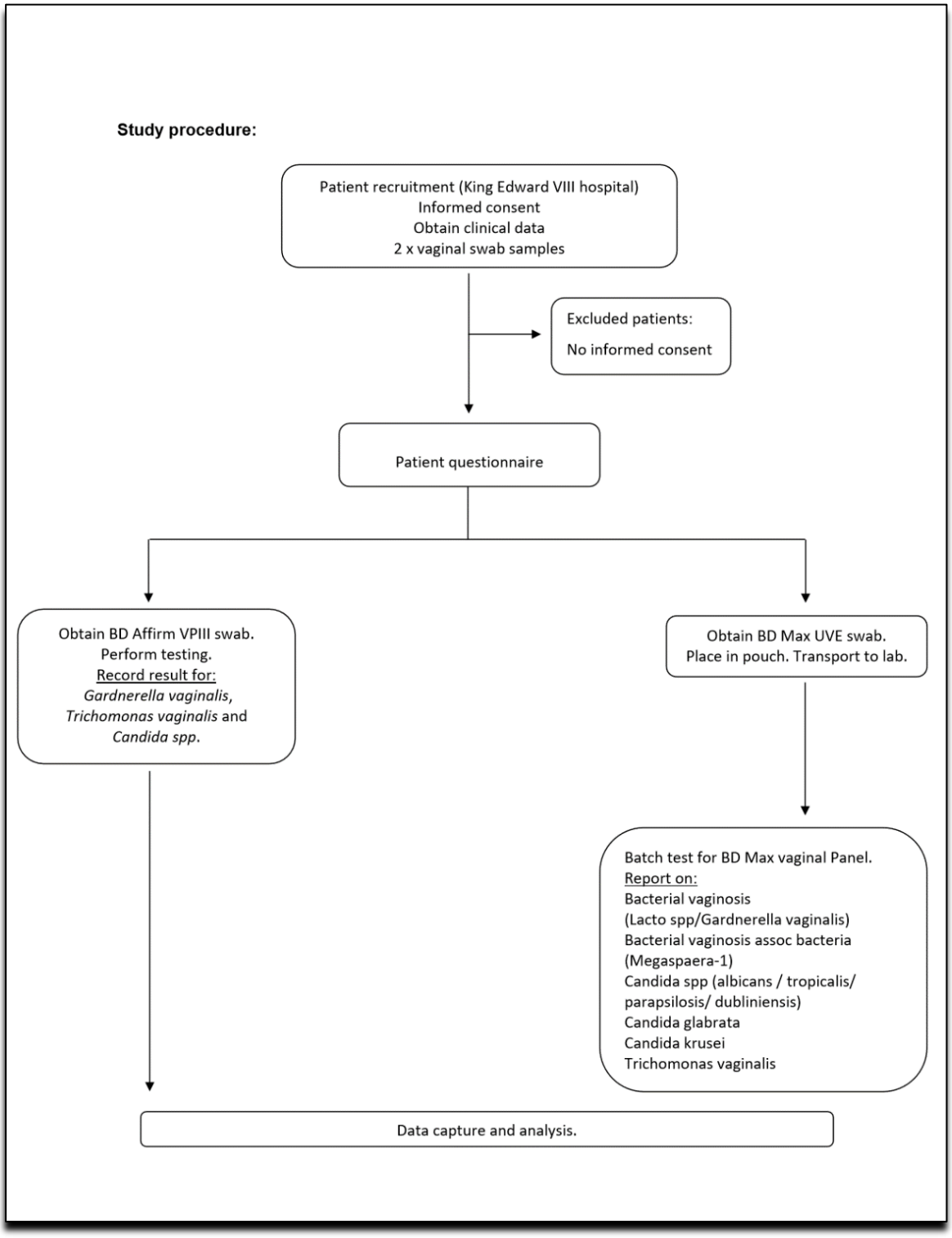


Figure 6.Flow chart of Study procedures

3.8. DATA COLLECTION AND MANAGEMENT

Data at enrollment was collected by the use of a structured paper-based questionnaire administered by the researcher (Refer to Appendix 1). Study participants were given a unique study number sequentially as enrolment occurred. Clinical data were entered into a spreadsheet that contained the laboratory number and demographics information etc. Laboratory results for the BD Affirm™ VPIII assay were handwritten in logs which were later entered into the Excel spreadsheet. The BD Max™ instrument printed reports from which data was entered onto the spreadsheet. All the entered results were cross-checked by a second person.

3.9. LABORATORY TESTING

All samples were tested by both the BD Affirm™ VPIII assay and the BD Max™ Vaginal assay.

3.9.1. BD AFFIRM™ VPIII ASSAY PROCEDURE

The test was conducted using the manufacturer's guidelines:

1. The BD MicroProbe lysis block was switched ON to heat at 85°C, and the kit reagents were left to reach room temperature, and mixed.
2. The Sample collection tube (with the swab in the cap) was opened, and 12 drops of lysis solution (L) was added to the tube using the dropper provided.
3. The swab in the tube was mixed vigorously by swirling and moving up and down against the side of the tube for at least 10 secs.
4. The tube was re-capped and placed into the lysis block to heat for 10 minutes.
5. After removal from the lysis block, 12 drops of Buffer solution (B) was added to the tube (containing the swab) and mixed by flicking the tube ten times.
6. The swabs were after that squeezed against the side of the tubes, removed, discarded, and the tube was re-capped with a Filter tip (FT).
7. The MicroProbe processor was switched ON.
8. A Reagent cassette (RC) was thereafter labelled, opened and loaded for each sample.
9. Thereafter a Probe Analysis Card (PAC) was labelled and placed in Well 1.
10. Four drops of Substrate solution (S) was added to well 7.

11. Each sample tube was matched and placed inverted into the Well 1. The sample was squeezed into each well.
12. The caddy was thereafter placed on the instrument and RUN. Processing time was count down from 32:50.
13. After the run, the PACs were removed, blotted lightly with a paper towel and interpreted.

3.9.2. BD AFFIRM™ VPIII ASSAY INTERPRETATION

The Probe Analysis Cards were inspected visually for colour development next to the target organism:

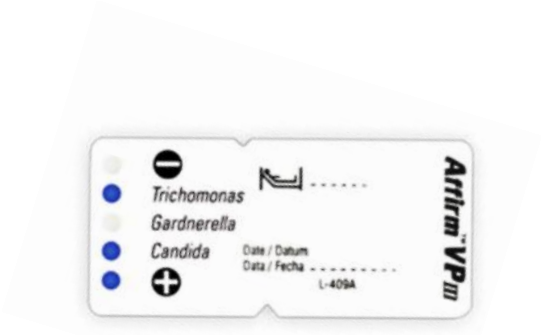


Image by Becton Dickinson

3.9.3. BD MAX™ VAGINAL PANEL PROCEDURE

Samples were processed according to the manufacturer's guidelines.

1. The swabs in sample buffer tubes were vortexed for 1 minute, and the swab was squeezed and discarded.
2. The sample buffer tubes were uncapped and re-capped with a blue septum cap.
3. Sample tubes were placed on the rack together with unitised reagent strips, extraction vials and master mix vials.
4. Samples were logged onto the instrument, and the rack was loaded into the instrument with the PCR cartridge and the run initiated. An average run took 2.5 hrs.

3.9.4. BD MAX™ VAGINAL PANEL INTERPRETATION

The BD Max™ results were interpreted using the guidelines below:

Assay Result Reported (Vaginitis Master Mix)	Interpretation of Results
TV POS	*Trichomonas vaginalis* DNA Detected
TV NEG	No *Trichomonas vaginalis* detected
Cgroup POS	*Candida* group DNA Detected (*Candida albicans* and/or *Candida tropicalis* and/or *Candida parapsilosis* and/or *Candida dubliniensis*)
Cgroup NEG	No *Candida* group detected (*Candida albicans* and/or *Candida tropicalis* and/or *Candida parapsilosis* and/or *Candida dubliniensis*)
Cgla POS	*Candida glabrata* DNA Detected
Cgla NEG	No *Candida glabrata* detected
Ckru POS	*Candida krusei* DNA Detected
Ckru NEG	No *Candida krusei* detected
UNR	Unresolved – inhibitory sample or reagent failure; No target detected and no amplification of Sample Processing Control
IND	Indeterminate result due to BD MAX System failure (with Warning or Error Codes[a])
INC	Incomplete Run (with Warning or Error Codes[a])
Assay Result Reported (Vaginosis Master Mix)	**Interpretation of Results**
BV POS	Vaginosis Panel DNA detected. Detection of marker combinations related to bacterial vaginosis: *Gardnerella vaginalis* and/or *L. crispatus* and/or *L. jensenii* and/or *Atopobium vaginae* and/or BVAB-2 and/or *Megasphaera-1*
BV NEG	Detection of marker combinations related to normal vaginal flora
UNR	Unresolved – inhibitory sample or reagent failure; No target detected and no amplification of Sample Processing Control
IND	Indeterminate result due to BD MAX System failure (with Warning or Error Codes[a])
INC	Incomplete Run (with Warning or Error Codes[a])

Figure 7. Interpretation of the BD Max™ assay (BD Max™ vaginal Panel package insert)

3.9.5. BD MAX™ VAGINAL PANEL REPORT

Date/Time: 12/14/2017 4:27:25 AM
Username: ADMIN
MDS

Run Report: 13

Instrument #: CT0149(1)
Version #: V4.60 A
Laboratory

Run 13: Run 6 Vaginitis Study 15Mar18

Position	Test Name	Sample Tube	Patient ID	Result
SP Status		PCR Status	Accession	
Kit Lot			Master Mix Lot	
A1	BD MAX Vaginal 46	B1271108262018103 1CS73	--	BV NEG, Cgroup POS
Success		Success	V117	Ckru NEG, Cgla NEG
K6733960620180923			--	TV NEG
A2	BD MAX Vaginal 46	B1271108262018103 1DI49	--	BV NEG, Cgroup POS
Success		Success	V118	Ckru POS, Cgla NEG
K6733960620180923			--	TV NEG
A3	BD MAX Vaginal 46	B1271108262018103 1CV09	--	BV NEG, Cgroup POS
Success		Success	V119	Ckru NEG, Cgla NEG
K6733960620180923			--	TV NEG
A4	BD MAX Vaginal 46	B1271108262018103 1CU67	--	BV UNR, Cgroup POS
Success		Success	V120	Ckru NEG, Cgla NEG
K6733960620180923			--	TV NEG
A5	BD MAX Vaginal 46	B1271108262018103 1DJ49	--	BV POS, Cgroup POS
Success		Success	V121	Ckru NEG, Cgla NEG

Figure 8. Shows a BD Max™ run report generated by the instrument

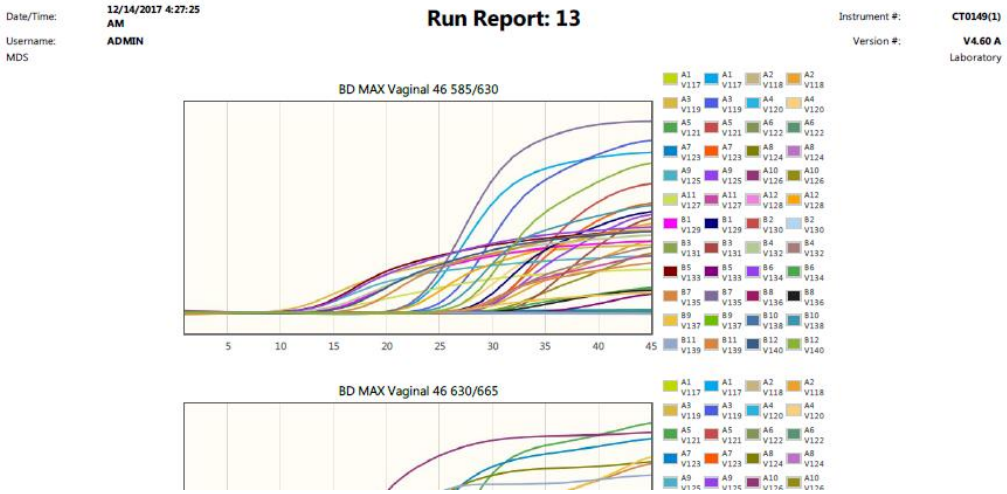


Figure 9. Shows the Real-Time PCR Cycle Thresholds generated on a BD Max™ Vaginal assay *report*

3.10 DATA ANALYSIS

The prevalence of BV, trichomoniasis and candidiasis was calculated as the percentage of women who tested positive for BV, *T.vaginalis* and *Candida* infection and 95% confidence intervals (CIs) were calculated for these percentages using the formulas for calculating CIs for proportions. The number of co-infections was calculated using chi-square analysis. The diagnostic accuracy of the BD Affirm™ VPIII assay compared to the BD Max™ Vaginal assay was assessed through the calculation of sensitivity, specificity, Negative Predictive Value (NPV) and Positive Predictive Value (PPV) and their respective 95% confidence intervals. All analyses were conducted using STATA analysis package.

CHAPTER FOUR – RESULTS

4.1 CHARACTERISTICS OF THE STUDY POPULATION

The percentage of pregnant women who tested positive for any pathogen was 81.6%. *Candida* was the most prevalent pathogen in this study, with a prevalence of 57.2%. The prevalence of Bacterial vaginosis was 49.4%, and *T.vaginalis* was detected in 10.3% of the women.

The characteristics of the study population are described in Table 4. The median age (Q1-Q3) of the study population was 28.0 (24.0-33.0). Overall, the majority of the participants did not experience symptoms of current abnormal vaginal discharge (66.3%). The majority of the study women had a high school education only (66.7%), were unmarried (85.0%), and 60.8% were not cohabiting with their sex partner. In addition, 44.0% of the women reported not knowing if their sex partner had other partners.

With respect to behavioural factors, 83.9% of the women had reported having a regular sex partner, 75.5% of the women had experienced their first sex between 15-20 years of age, and 52.4% had between 2-4 lifetime number of sex partners. In addition, 61.9% had sometimes used a condom, and 68.9% had not used a condom during their last sex act. A large proportion of the women were non-smokers (95.6%) and did not consume alcohol (88.3%). Approximately 90.1% of the women did not perform intravaginal practices.

Clinically, 57.5% of the women were in the third trimester of pregnancy, 78.8% of the women did not have a history of pre-term labour, 74.0% of the women did not experience a past miscarriage, and 90.5% of the women did not experience a past spontaneous abortion. With respect to past infections, 59.0% of the women did not experience symptoms of abnormal discharge in the past, 59.7% of the women had not been previously treated for an STI, and 30.4% were HIV negative.

Table 4: Demographic and behavioural characteristics of the study population according to individual infection status as determined by the BD Max™ Vaginal assay

	BV neg (n=127)	BV pos (n=124)	p-value	TV neg (n=243)	TV pos (n=28)	p-value	Can neg (n=116)	Can pos (n=155)	p-value	Overall (n=273)
age			0.896			0.711			0.113	
Mean±SD(CV%)	28.7±6.57(22.9)	28.4±5.87(20.7)		28.4±6.33(22.3)	28.6±4.99(17.4)		29.1±6.39(21.9)	27.9±6.03(21.6)		28.5±6.20(21.8)
Median(Q1-Q3)	27.0(24.0-34.0)	28.0(24.0-33.0)		28.0(24.0-33.0)	27.0(25.0-33.3)		28.0(24.0-34.0)	27.0(23.5-32.5)		28.0(24.0-33.0)
Min-Max	18.0-43.0	18.0-43.0		18.0-43.0	20.0-38.0		18.0-43.0	18.0-43.0		18.0-43.0
Current abnormal vaginal discharge			0.079			0.128			**< 0.001**	
No	93 (73.2%)	78 (62.9%)		165 (67.9%)	15 (53.6%)		90 (77.6%)	90 (58.1%)		181 (66.3%)
Yes	34 (26.8%)	46 (37.1%)		78 (32.1%)	13 (46.4%)		26 (22.4%)	65 (41.9%)		92 (33.7%)
Highest level of education			**0.008**			0.472			**0.018**	
College/University	40 (31.5%)	29 (23.4%)		66 (27.2%)	5 (17.9%)		37 (31.9%)	34 (21.9%)		71 (26.0%)
Did not attend school	2 (1.6%)	0 (0%)		2 (0.8%)	0 (0%)		2 (1.7%)	0 (0%)		2 (0.7%)
High school	82 (64.6%)	81 (65.3%)		161 (66.3%)	20 (71.4%)		67 (57.8%)	114 (73.5%)		182 (66.7%)
Primary school	3 (2.4%)	14 (11.3%)		14 (5.8%)	3 (10.7%)		10 (8.6%)	7 (4.5%)		18 (6.6%)
Married			0.095			0.581			0.530	
No	101 (79.5%)	109 (87.9%)		207 (85.2%)	23 (82.1%)		97 (83.6%)	133 (85.8%)		232 (85.0%)
Yes	25 (19.7%)	15 (12.1%)		35 (14.4%)	5 (17.9%)		19 (16.4%)	21 (13.5%)		40 (14.7%)
Missing	1 (0.8%)	0 (0%)		1 (0.4%)	0 (0%)		0 (0%)	1 (0.6%)		1 (0.4%)
Regular sex partner			0.146			0.174			0.842	
No	25 (19.7%)	16 (12.9%)		36 (14.8%)	7 (25.0%)		19 (16.4%)	24 (15.5%)		44 (16.1%)
Yes	102 (80.3%)	108 (87.1%)		207 (85.2%)	21 (75.0%)		97 (83.6%)	131 (84.5%)		229 (83.9%)
Co-habiting			0.979			0.668			0.157	
No	76 (59.8%)	74 (59.7%)		149 (61.3%)	16 (57.1%)		65 (56.0%)	100 (64.5%)		166 (60.8%)
Yes	51 (40.2%)	50 (40.3%)		94 (38.7%)	12 (42.9%)		51 (44.0%)	55 (35.5%)		107 (39.2%)
Age of sexual debut			0.245			0.965			0.469	
<15	8 (6.3%)	5 (4.0%)		12 (4.9%)	1 (3.6%)		7 (6.0%)	6 (3.9%)		13 (4.8%)
>25	6 (4.7%)	1 (0.8%)		7 (2.9%)	0 (0%)		4 (3.4%)	3 (1.9%)		7 (2.6%)
15-20	92 (72.4%)	96 (77.4%)		181 (74.5%)	23 (82.1%)		82 (70.7%)	122 (78.7%)		206 (75.5%)
21-25	21 (16.5%)	22 (17.7%)		43 (17.7%)	4 (14.3%)		23 (19.8%)	24 (15.5%)		47 (17.2%)
no. of lifetime sexual partners			0.563			0.161			0.555	
>4	24 (18.9%)	23 (18.5%)		44 (18.1%)	8 (28.6%)		19 (16.4%)	33 (21.3%)		52 (19.0%)
1	40 (31.5%)	32 (25.8%)		72 (29.6%)	4 (14.3%)		35 (30.2%)	41 (26.5%)		78 (28.6%)
2-4	63 (49.6%)	69 (55.6%)		127 (52.3%)	16 (57.1%)		62 (53.4%)	81 (52.3%)		143 (52.4%)
Partner has other partners			**0.007**			0.067			0.779	
Don't know	43 (33.9%)	61 (49.2%)		109 (44.9%)	10 (35.7%)		52 (44.8%)	67 (43.2%)		120 (44.0%)
No	47 (37.0%)	25 (20.2%)		71 (29.2%)	5 (17.9%)		34 (29.3%)	42 (27.1%)		76 (27.8%)
Yes	37 (29.1%)	38 (30.6%)		63 (25.9%)	13 (46.4%)		30 (25.9%)	46 (29.7%)		77 (28.2%)
Condom use			0.980			0.350			**0.048**	
Always	5 (3.9%)	5 (4.0%)		9 (3.7%)	1 (3.6%)		5 (4.3%)	5 (3.2%)		10 (3.7%)

Never	34 (26.8%)	36 (29.0%)		70 (28.8%)	6 (21.4%)		41 (35.3%)	35 (22.6%)		76 (27.8%)
Rarely	8 (6.3%)	8 (6.5%)		18 (7.4%)	0 (0%)		4 (3.4%)	14 (9.0%)		18 (6.6%)
Sometimes	80 (63.0%)	75 (60.5%)		146 (60.1%)	21 (75.0%)		66 (56.9%)	101 (65.2%)		169 (61.9%)
Condom used in last sex act			0.689			0.925			0.091	
No	88 (69.3%)	83 (66.9%)		167 (68.7%)	19 (67.9%)		86 (74.1%)	100 (64.5%)		188 (68.9%)
Yes	39 (30.7%)	41 (33.1%)		76 (31.3%)	9 (32.1%)		30 (25.9%)	55 (35.5%)		85 (31.1%)
Smoking			0.069			**0.025**			0.087	
No	124 (97.6%)	115 (92.7%)		235 (96.7%)	24 (85.7%)		108 (93.1%)	151 (97.4%)		261 (95.6%)
Yes	3 (2.4%)	9 (7.3%)		8 (3.3%)	4 (14.3%)		8 (6.9%)	4 (2.6%)		12 (4.4%)
Alcohol consumption			**0.010**			0.348			0.518	
No	118 (92.9%)	102 (82.3%)		216 (88.9%)	23 (82.1%)		104 (89.7%)	135 (87.1%)		241 (88.3%)
Yes	9 (7.1%)	22 (17.7%)		27 (11.1%)	5 (17.9%)		12 (10.3%)	20 (12.9%)		32 (11.7%)
Intravaginal practice			0.613			1.000			0.145	
No	117 (92.1%)	112 (90.3%)		218 (89.7%)	26 (92.9%)		108 (93.1%)	136 (87.7%)		246 (90.1%)
Yes	10 (7.9%)	12 (9.7%)		25 (10.3%)	2 (7.1%)		8 (6.9%)	19 (12.3%)		27 (9.9%)
Trimester of pregnancy			0.339			0.578			0.093	
1st	12 (9.4%)	16 (12.9%)		27 (11.1%)	5 (17.9%)		16 (13.8%)	16 (10.3%)		32 (11.7%)
2nd	36 (28.3%)	42 (33.9%)		75 (30.9%)	8 (28.6%)		42 (36.2%)	41 (26.5%)		84 (30.8%)
3rd	79 (62.2%)	66 (53.2%)		141 (58.0%)	15 (53.6%)		58 (50.0%)	98 (63.2%)		157 (57.5%)
History of preterm labour			0.621			0.076			0.305	
No	99 (78.0%)	102 (82.3%)		195 (80.2%)	19 (67.9%)		95 (81.9%)	119 (76.8%)		215 (78.8%)
Yes	24 (18.9%)	21 (16.9%)		43 (17.7%)	9 (32.1%)		19 (16.4%)	33 (21.3%)		53 (19.4%)
Missing	4 (3.1%)	1 (0.8%)		5 (2.1%)	0 (0%)		2 (1.7%)	3 (1.9%)		5 (1.8%)
Past miscarriage			0.914			0.916			0.582	
No	96 (75.6%)	93 (75.0%)		180 (74.1%)	21 (75.0%)		88 (75.9%)	113 (72.9%)		202 (74.0%)
Yes	31 (24.4%)	31 (25.0%)		63 (25.9%)	7 (25.0%)		28 (24.1%)	42 (27.1%)		71 (26.0%)
Past spontaneous abortion			0.784			0.324			**0.042**	
No	115 (90.6%)	111 (89.5%)		221 (90.9%)	24 (85.7%)		100 (86.2%)	145 (93.5%)		247 (90.5%)
Yes	12 (9.4%)	13 (10.5%)		22 (9.1%)	4 (14.3%)		16 (13.8%)	10 (6.5%)		26 (9.5%)
Past abnormal discharge			0.644			0.062			0.143	
No	79 (62.2%)	73 (58.9%)		148 (60.9%)	12 (42.9%)		74 (63.8%)	86 (55.5%)		161 (59.0%)
Yes	48 (37.8%)	50 (40.3%)		94 (38.7%)	16 (57.1%)		41 (35.3%)	69 (44.5%)		111 (40.7%)
Missing	0 (0%)	1 (0.8%)		1 (0.4%)	0 (0%)		1 (0.9%)	0 (0%)		1 (0.4%)
Past treatment for an STI			0.536			0.140			0.307	
No	74 (58.3%)	77 (62.1%)		148 (60.9%)	13 (46.4%)		73 (62.9%)	88 (56.8%)		163 (59.7%)
Yes	53 (41.7%)	47 (37.9%)		95 (39.1%)	15 (53.6%)		43 (37.1%)	67 (43.2%)		110 (40.3%)
HIV status			0.653			**0.011**			**0.045**	
Negative	41 (32.3%)	35 (28.2%)		1 (0.4%)	0 (0%)		40 (34.5%)	42 (27.1%)		83 (30.4%)
Positive	26 (20.5%)	26 (21.0%)		79 (32.5%)	3 (10.7%)		18 (15.5%)	41 (26.5%)		59 (21.6%)
Don’t know	0 (0%)	1 (0.8%)		48 (19.8%)	11 (39.3%)		0 (0%)	1 (0.6%)		1 (0.4%)
Missing	60 (47.2%)	62 (50.0%)		115 (47.3%)	14 (50.0%)		58 (50.0%)	71 (45.8%)		130 (47.6%)

4.2 FACTORS SIGNIFICANTLY ASSOCIATED WITH VAGINAL INFECTIONS

The variables which were significantly associated with BV were having a high school level of education (p=0.008), not knowing if their partner had other partners (p=0.007) and not consuming alcohol (p=0.010) (Table 4).

The majority of women who tested positive for *Candida* reported not having a current abnormal vaginal discharge (p<0.001), had a high school level of education only (p=0.018), used condoms sometimes (p=0.048) and did not experience a past spontaneous abortion (p=0.042). Within the *Candida* negative group, the majority of the women were HIV negative (p=0.045) (Table 4).

According to the analysis, smoking and HIV status was significantly associated with *T.vaginalis* infection. The majority of the women who tested positive for *T.vaginalis* were non-smokers (p=0.025). Being HIV positive was strongly associated with *T.vaginalis* (p=0.011) (Table 4).

4.3 PREVALENCE OF SYMPTOMATIC AND ASYMPTOMATIC INFECTIONS

Overall, 33.7% of the women reported having a current abnormal discharge (Table 4). Of the symptomatic women, 37.1% tested positive for BV, 46.4% tested positive for *T.vaginalis,* and 41.9% tested positive for *Candida.* There were only six women who reported having a current abnormal discharge that was negative for BV, *T.vaginalis* and/or *Candida.*

Of the 66.3% of the women who did not have a current abnormal vaginal discharge, 78.8% tested positive for either BV, *T.vaginalis* and/or *Candida*. These women were undiagnosed using the current syndromic management vaginal discharge algorithm.

For Bacterial Vaginosis, 78/124 positives were asymptomatic, showing a high prevalence of 62.9%. More than half of the *Candida* and *T.vaginalis* infections were asymptomatic, i.e. 90/155 cases (58.1%) and 15/28 cases (53.6%) for *Candida* and *T.vaginalis* respectively (Table 4).

4.4 PREVALENCE OF CO-INFECTIONS

Table 5.Prevalence of BV, Candida and T.vaginalis co-infections by the BD Max™ vaginal assay

BV	**neg (n=127)**	**pos (n=124)**	**p-value**	**Overall (n=273)**
T.vaginalis			0.928	
neg	113 (89.0%)	109 (87.9%)		243 (89.0%)
pos	14 (11.0%)	14 (11.3%)		28 (10.3%)
Missing	0 (0%)	1 (0.8%)		2 (0.7%)
Candida			0.214	
neg	51 (40.2%)	59 (47.6%)		116 (42.5%)
pos	76 (59.8%)	64 (51.6%)		155 (56.8%)
Missing	0 (0%)	1 (0.8%)		2 (0.7%)
T.vaginalis	**neg (n=243)**	**pos (n=28)**	**p-value**	**Overall (n=273)**
Candida			0.224	
neg	101 (41.6%)	15 (53.6%)		116 (42.5%)
pos	142 (58.4%)	13 (46.4%)		155 (56.8%)
Missing	0 (0%)	0 (0%)		2 (0.7%)

Of the 124 women who tested BV positive, 14 (11.3%) women also tested *T.vaginalis* positive. More than half (51.6%) of the BV positive women were co-infected with *Candida*. Amongst the 28 women who tested *T.vaginalis* positive, 46.4% were co-infected with *Candida*. Despite the high percentage of co-infections, there was no statistical significance ($p>0.05$) (Table 5).

4.5 LABORATORY FINDINGS

The full set of results of the BD Affirm™ VPIII assay and the BD Max™ Vaginal assay are shown in Appendix 3. In summary, the BD Affirm™ VPIII assay generated 100% valid results for all three pathogens.

For the BD Max™ Vaginal assay, one sample was indeterminate for all three pathogens, and 21 samples were unresolved for BV. These samples were repeated with the same outcome. The unresolved results could be due to the failure of the internal sample controls as a result of inhibitors present in the sample.

Of the 273 pregnant women enrolled in this study, 251 were included in the BV analysis. In addition, 271 were included for the *Candida* and *T.vaginalis* analysis, one was unresolved, and one was indeterminate by the BD Max™ Vaginal assay.

4.5.1 PERFORMANCE OF THE BD AFFIRM™ VPIII ASSAY COMPARED TO THE BD MAX™ VAGINAL PANEL

The diagnostic performance of the BD Affirm™ VPIII in comparison to the BD Max™ Vaginal assay for the three pathogens is described in Table 6.

Of the 124 participants that were positive for BV using the BD Max™ Vaginal assay, only 99 were correctly identified by the BD Affirm™ VPIII assay. This resulted in a sensitivity of 79.84% for the BD Affirm™ VPIII assay in diagnosing BV. In addition, 25 BV positives detected by the reference method were missed by the BD Affirm™ VPIII. Out of the 127 BV negatives diagnosed by the reference method, 102 were correctly identified by the BD Affirm™ VPIII assay resulting in a specificity of 80.31% for the assay. There were 25 false positives diagnosed by the BD Affirm™ VPIII Assay (Table 6).

For *Candida*, a total of 73/155 *Candida* positives identified by the BD Max™ Vaginal assay were negative on the BD Affirm™ VPIII assay resulting in a poor sensitivity of 52.90%. However, 113 out of the 116 negatives were correctly reported as negative by the BD Affirm™ VPIII assay resulting in high specificity of 97.41%. There were 3 false positives for *Candida* (Table 6).

Similarly, for *T.vaginalis* 15 out of the 28 *T.vaginalis* positives by the BD Max™ Vaginal assay were negative with the BD Affirm™ VPIII assay resulting in a poor sensitivity of 46.43%. Despite the poor sensitivity, all of the 243 *T.vaginalis* negatives were correctly classified by the BD Affirm™ VPIII assay. The assay, therefore, had an excellent specificity for *T.vaginalis* of 100% (Table 6).

Table 6. Performance characteristics of the BD Affirm™ VPIII *assay when compared to the* BD Max™ Vaginal assay

		Investigational Test performance: BD Affirm™ VP III					
Identification	**Prevalence : BD Max™ Vaginal assay**	**Prevalence: BD Affirm™ VP III assay**	**Sensitivity**	**Specificity**	**PPV (95% CI)**	**NPV (95% CI)**	**ROC Area**
BV	**49.4 (124/251)[#] (43.2-55.6)**	47.6 (130/273) (41.7-53.6)	79.84 (71.69-86.51)	80.31(72.33-86.84)	79.84 (73.38-85.05)	80.31 (73.99-85.41)	0.80
Candida spp	**57.2 (155/271)[$] (51.2-63.0)**	31.5 (86/273) (26.2-37.3)	52.90 (44.73-60.96)	97.41 (92.63-99.46)	96.47 (89.86-98.83)	60.75 (56.65-64.71)	0.75
T.vaginalis	**10.3 (28/271)* (7.2-14.6)**	4.8 (13/273) (2.8-8.1)	46.43 (27.51-66.13)	100.00 (98.49-100.00)	100.00 (100.00-100.00)	94.19 (91.98-95.81)	0.73

PPV, positive predictive value; NPV, negative predictive value; BV, bacterial vaginosis; *T.vaginalis, Trichomonas vaginalis*

Data are % (n/N) (95% confidence interval) or % (95% confidence interval)

[#] twenty one out of the 273 tests for BV were unresolved by the BD Max Reference method, and one was indeterminate. These were therefore excluded in the analysis
[$] one out of the 273 tests for *Candida* was unresolved by the BD Max Reference method, and one was indeterminate. These were therefore excluded in the analysis
***one out of the 273 tests for *T.vaginalis* was unresolved by the BD Max Reference method, and one was indeterminate. These were therefore excluded in the analysis**

4.5.2 PERFORMANCE OF BD Affirm™ VPIII ASSAY IN SYMPTOMATIC PARTICIPANTS

For BV, in symptomatic women, the BD Affirm™ VPIII assay exhibited an improved sensitivity of 82.61% when compared to the overall sensitivity of 79.84% in all participants (asymptomatic and symptomatic) (Table 7).
Similarly, for Candida, the BD Affirm™ VPIII assay performed better in symptomatic women (sensitivity 66.15%) when compared to all participants (sensitivity 52.90%).
There was no difference in the performance of the test with respect to sensitivity for *T.vaginalis* (46.43%) (Table 7).

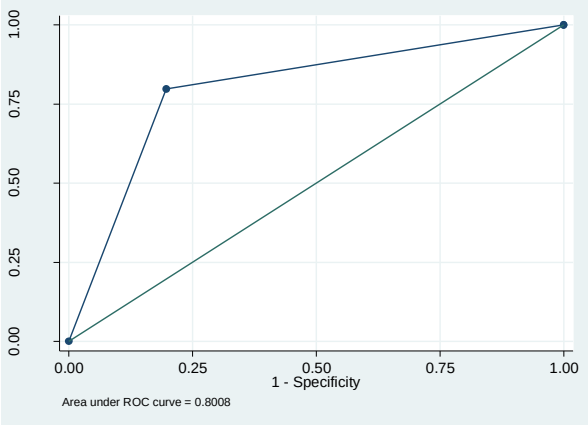
Table 7. Shows the performance of the BD Affirm™ VPIII *assay in symptomatic participants.*

	%Sensitivity (overall)	%Sensitivity (symptomatic)	%Specificity (overall)	%Specificity (symptomatic)	%PPV (overall)	%PPV (symptomatic)	%NPV (overall)	%NPV (symptomatic)
BV	79.84	82.61	80.31	61.76	79.84	74.51	80.31	72.41
Candida spp	52.90	66.15	97.41	96.15	96.47	97.73	60.75	46.81
T.vaginalis	46.43	46.15	100.00	100.00	100.00	100.00	94.19	91.76

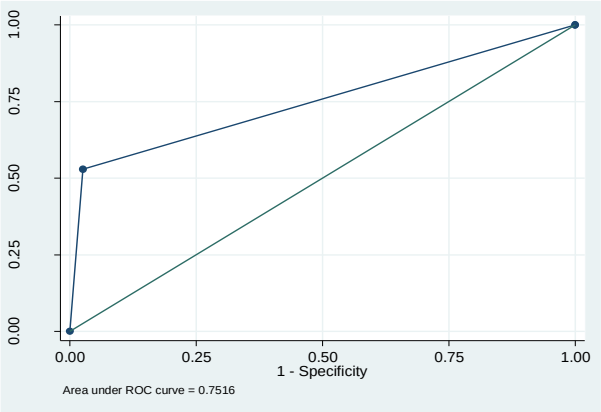
Receiver Operating Characteristics (ROC) curves show the trade-off between sensitivity and specificity for a diagnostic test, ie.test accuracy. The ROC curve analysis for the BD Affirm™ VPIII assay in diagnosing BV was fairly good (area=0.80). The test is, therefore fairly accurate in distinguishing infected from uninfected individuals for BV (Figure 10a).

The ROC curves for *Candida* (Figure 10b) and *T.vaginalis* (Figure 10c) were shown to be fair (area=0.75 and 0.73, respectively). The test is therefore not very accurate in differentiating infected from uninfected patients for these two pathogens and therefore may not be suitable to use alone as a point-of-care test.

a.



b.



c.

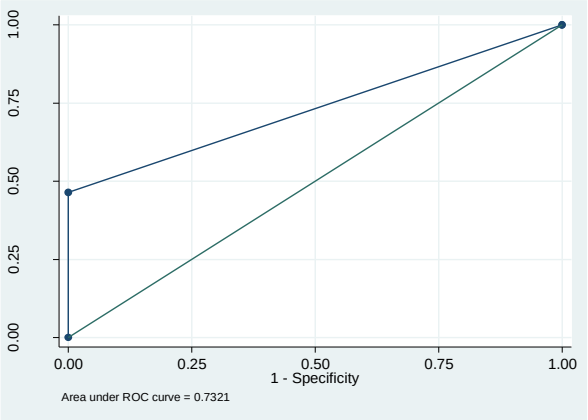


Figure 10. Receiving Operating Characteristic (ROC) curve analysis for (a) BV, (b) Candida species, and (c) T. vaginalis

CHAPTER FIVE

5.1 DISCUSSION

The correct diagnosis and treatment of Bacterial Vaginosis, Candidiasis and Trichomoniasis are vital in pregnancy as poor diagnosis and treatment results in poor pregnancy outcomes. This study observed a higher prevalence of BV (49.4%) in pregnant women in South Africa than previously reported (37.3%).[33] The high prevalence of BV observed in our population could be attributed to the following socio-demographic and behavioural characteristics. The majority of women (55.6%) in the BV positive group had reported having between 2-4 lifetime sex partners. Increased number of lifetime sex partners has been shown to be associated with an increased risk of vaginal infections.[4] In addition, close to 80% of the BV positive women had experienced their first sexual act at the age of 15-20 years. Early age of sexual debut could be associated with vaginal infections. The lack of condom use was also higher in BV positive women. The variables which were significantly associated with BV in our study population were having a high school level of education (p=0.008), not knowing if their partner had other partners (p=0.007) and not consuming alcohol (p=0.010). A study by Yzeiraj-Kalemaj et al. in 2013 found a low level of education to be significantly associated with positive BV status amongst pregnant women, which differs from the findings of the present study.[56] In the current study a higher proportion of women had a high school level of education, and this could have contributed to of a higher prevalence of BV infection in this group. In this study women who reported not knowing if their partners had other partners were at higher risk for BV. Abbai et al. in 2018 reported similar findings.[57] In this study, we found that alcohol consumption was not a risk factor for BV infection. Our findings differ from the study by Francis et al. in 2015.[58] However, the current study consisted of a larger proportion of non-alcohol users which could have contributed to the negative association observed.

The observed prevalence estimates for *Candida spp.* in this study was 57.2%. The high prevalence of *Candida* could be due to the fact that during pregnancy estrogen levels are high and these high levels provides a conducive environment for the growth of *Candida spp*.[38]

The majority of women who tested positive for *Candida spp.* in this study reported not having a current abnormal vaginal discharge (p<0.001), had a high school level of education only (p=0.018), used condoms sometimes (p=0.048) and did not experience a past spontaneous

abortion (p=0.042). Within the *Candida spp.* negative group, the majority of the women were HIV negative (p=0.045). Konadu et al. in 2019 reported a high proportion of *Candida* positive women to be asymptomatic confirming the findings of our study.[38] A previous study conducted by Faraji et al. in 2012 found a significant association between low level of education (non-tertiary) and VVC.[59] This confirms the findings of our study which found a significant association between non-tertiary education and *Candida* infection. In our study majority of women who were positive for *Candida* reported using condoms "sometimes". However, a study conducted by Djohan et al. in 2019 found a protective association between condom use and the risk for VVC.[60] A study by Liu et al. in 2018 found a positive association between VVC and women who have experienced spontaneous abortion.[61]The findings of our study differ since we found that majority of the *Candida* positive women did not report previous spontaneous abortion. In our study population there were very few events of condom use and past experience of spontaneous abortion which could have resulted in a negative association with *Candida* infection. In this study we found a significant association between HIV negative status and *Candida* infection. However, there was a large proportion of missing data for HIV status thereby negating comparisons with previously published studies.

The prevalence of *T.vaginalis* in this study was 10.3%. This was similar to other cohorts of South African pregnant women. [10,32] The majority of the women who tested positive for *T.vaginalis* on this study were non-smokers (p=0.025). This was an expected finding since our study population was pregnant women. Previous studies which found a significant association between smoking and *T.vaginalis* infection were conducted in non-pregnant populations.[62,63] In our study being HIV positive was strongly associated with *T.vaginalis* (p=0.011). Despite a large proportion of missing data on HIV status our results were similar to a study by Lockhart et al. in 2019.[63]

Syndromic management has its advantages of treating patients at the first visit and being easy to follow. However previous studies have shown that the Syndromic Management guidelines are no longer adequate for the treatment of STIs in women, as vaginal discharge alone is a poor predictor and asymptomatic infections are missed.[6,7,9,11,51] In this study, approximately less than half of the women who tested positive for BV, *Candida spp.* and *T.vaginalis* were symptomatic (37.1%, 41.9% and 46.4%, respectively) and this is consistent with previous reports of a high burden of asymptomatic STIs.[6,44]. If a test and treat approach had been used, 78.82% of the women in the current study with undiagnosed infections could have been adequately treated.

Alternate strategies are required, such as the test and treat approach, particularly in pregnant women.

The current guidelines for maternity care in South Africa advocate essential screening tests for the first antenatal visit. This includes tests for Syphilis, Rhesus Blood group, Hemoglobin, HIV, and Urine protein and glucose. In order to improve maternity healthcare in South Africa, it is important that this list is revisited with the addition of POCTs for an infection that impacts on pregnancy outcome. With recent technology, POCT can now be performed at the clinic, the test results are available within 1-2 hours and treatment can be initiated on the same day. An added advantage of POC testing is that it reduces the risk of over-treating, which results in increased microbial resistance, particularly for *T.vaginalis* infection.

POCTs have the potential to bridge the gap in the diagnosis of vaginosis and vaginitis, especially in high priority populations such as in pregnant women. It is well noted that the cost of POCTs is a barrier to use.[64] Therefore, manufacturers of the POCTs need to be engaged by policymakers on reducing the costs of these assays, especially with the promise of large-scale implementation. Previous studies on POCTs for vaginal pathogens have shown that the BD Affirm™ VPIII assay has the potential to become the ideal one-assay POCT for diagnosing vaginosis and vaginitis in a clinic setting.[24,53,54] To date there is no other single cartridge test that detects BV, *Candida spp.* and *T. vaginalis.* The test is therefore superior, with the added advantage of being easy to perform and having a short time-to-detection. The current study compared the BD Affirm™ VPIII assay to a leading Real-Time molecular laboratory-based assay, the BD Max™ Vaginal assay. The BD Max™ Vaginal assay has been FDA approved since 2016 and uses a newer, more updated technology for the detection of BV, *Candida spp.* and *T.vaginalis*.

In our study, the assay showed a moderate sensitivity for BV (79.84%). The moderate sensitivity could be due to the following reasons (1) The BD Max™ Vaginal assay (reference) has a lower limit of detection for *G.vaginalis* than the BD Affirm™ VPIII and would, therefore, pick up more positives; (2) The BD Max™ Vaginal assay uses a ratio to interpret BV positives and this ratio is based on the presence of *G.vaginalis* but also on other organisms associated with BV; (3) The BD Affirm™ VPIII assay measures the DNA directly from the samples (absolute counts) without amplification while the BD Max™ Vaginal assay measures amplified DNA which is more accurate. Amplification methods of detection have been shown to be more sensitive versus absolute counts.[50]

The low specificity for BV by the BD Affirm™ VPIII could be due to the fact that since the development of the assay there have been newer more diverse populations of bacteria implicated in BV rather than *G.vaginalis* alone, such as *Mobiluncus spp, Bacteroides spp, Atopobium spp*. Thompson et al. 2019 also reported a lower specificity for the BD Affirm™ VPIII when compared to the BD Max™ Vaginal assay, suggesting that the Affirm detects *G.vaginalis* only whereas the BD Max™ Vaginal assay detects a combination of microorganisms.[65]

The BD Affirm™ VPIII assay showed a poor sensitivity for *Candida spp* (52.90%). This poor sensitivity could be attributed to the non-amplification nature of the BD Affirm™ VPIII assay. The test did however exhibit an excellent specificity (97.41%) for *Candida spp* since the assay did not report on false positives. Similarly, the BD Affirm™ VPIII assay showed a poor sensitivity for *T.vaginalis.*(46.43%) and an excellent specificity (100%). The poor sensitivity of the BD Affirm™ VPIII assay for *T.vaginalis* could be due to the higher LoD (5×10^3 trichomonads) to be classified as positive whereas the BD Max™ Vaginal assay has a LoD of 22 cells/ml. Poor sensitivity of the BD Affirm™ VPIII assay for *T.vaginalis* detection was also illustrated in 2011 by Andrea et al. when compared to another molecular amplification assay.[66] The poor sensitivity of the BD Affirm™ VPIII test indicates that the test cannot be used as a screening test but the excellent specificity for *Candida spp.* and *T.vaginalis* indicates that the test can be used as a confirmatory test when vaginitis is suspected.

Our study has also shown an improved sensitivity of the BD Affirm™ VPIII assay when only symptomatic participants were analysed. This is consistent with findings by Haywood et al. in 2004 where it was concluded that symptomatic women were more likely to be positive by the BD Affirm™ VPIII test than asymptomatic women using this test.[26]

5.2. CONCLUSIONS

In summary, this study found a high prevalence of BV in the antenatal population, with previously reported rates for *Candida* and *T.vaginalis* infection. The study also found a very high proportion of women with asymptomatic BV and vaginitis attending antenatal care. Lastly, urgent intervention is required to address the inadequacy of syndromic management in this high priority population.

Our analysis showed that the BD Affirm™ VPIII assay has a lower diagnostic accuracy when compared to the BD Max™ Vaginal assay. These findings are similar to the findings of Cartwright et al. in 2013, where the BD Affirm™ VPIII assay was shown to be less sensitive than molecular methods for the detection of BV, *Candida* and *T.vaginalis* .[54] We conclude that the BD Affirm™ VPIII assay is currently unsuitable as a screening test. However, the test may be useful as a confirmatory test when used in conjunction with clinical criteria such as increased pH as previously reported.[24]

5.3. RECOMMENDATIONS

The BD Affirm test was developed in the 1990s, and further enhancements are required for increased performance and acceptance as a point-of-care test. The test could be optimised to include other pathogens implicated in BV, and although easier to perform than a wet mount, the test requires skilled staff as there are many pre-analytical steps which can introduce error. If the system included the dispensing of reagents in the automation, the test would be more widely accepted as a point-of-care test. However, this assay is still the only point of care assay on the market that simultaneously detects BV, *Candida spp.* and *T.vaginalis* in a single cartridge.

5.4. LIMITATIONS

This study had the following limitations. Firstly, the study was only conducted at one antenatal clinic, which is not a representation of the whole antenatal population in South Africa. However, clinic attendees at King Edward VIII Hospital are from the greater Durban area and are therefore representative of a general population. Secondly, the presence of cervical pathogens, such as *Chlamydia trachomatis* and *Neisseria gonorrhoeae*, were not investigated in this study. These pathogens may have contributed to the abnormal vaginal discharge reported by the study women since 6 women reported discharge but did not test positive for any of the three pathogens investigated in this study. Thirdly, the women were not followed up to assess test-of-cure and the implications of infection on pregnancy outcomes. Following up the women until cure would have allowed us to shed some light on the usefulness of syndromic management in this population. However more than 70% of the women in this study were asymptomatic and would have been missed by syndromic management.

Despite these limitations, the strengths of the study are as follows (1) this study has provided data on the prevalence of BV, *Candida* and *T.vaginalis* in an antenatal population in which there is currently lack of data in South Africa and (2) the study has provided evidence that a large proportion of pregnant women who are asymptomatic carry infection, which re-affirms the limitation of the syndromic management approach, and lastly (3) this study was the first to report on the performance of the BD Affirm^{TM} VPIII POCT in an antenatal population both locally and globally.

CHAPTER SIX - REFERENCES

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CHAPTER SEVEN – APPENDICES

APPENDIX 1 – PATIENT QUESTIONNAIRE

Vaginitis Study

ENROLLMENT FORM

Participant Identifier: ☐☐☐☐☐

Visit Date: ☐☐/☐☐/☐☐☐☐

1. How old are you? years Date of Birth:

   If younger than 18 years of age, please do not enroll into study.....End of form

2. Are you currently experiencing any abnormal discharge from your vagina

No	
Yes	

3. What is your highest level of education?

Did not attend school	
Primary school	
High school	
College, University	

4. Are you married (consensual or legal marriage)?

No	
Yes	

5. Do you have a regular sexual partner?

No	
Yes	

6. Do you currently live with your husband/regular partner?

No	
Yes	

7. How old were you when you first had vaginal sex?

<15 years old	
15-20 years old	
21-25 years old	
>25 years old	

8. How many male sexual partners have you had in your life?

1 partner	
2-4 partners	
>4 partners	

9. Does your partner have other partners?

No	
Yes	
Don't Know	

10. How often do you use condoms during sex?

Never	
Sometimes	
Rarely	

11. Did you use a condom during your last sex act?

No	
Yes	

12. Do you smoke?

No	
Yes	

13. Do you drink alcohol?

No	
Yes	

14. Do you wash inside your vagina with substances other than soap and water?

No	
Yes	

15. Which trimester of pregnancy are you in?

1st trimester	
2nd trimester	
3rd trimester	

16. Have you ever given birth to a preterm baby (<37 weeks)?

No	
Yes	

17. Have you ever had a miscarriage in the past?

No	
Yes	

18. Have you had a spontaneous abortion in the past?

No	
Yes	

19. Have you ever had abnormal, smelly discharge from your vagina in the past?

No	
Yes	

20. Have you ever been treated for an infection passed through sex in the past?

No	
Yes	

END OF FORM

APPENDIX 2:

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

07 February 2018

Mrs F Dessai (217076585)
School of Clinical Medicine
College of Health Sciences
dessaif@ukzn.ac.za

Dear Dessai

Protocol: Diagnostic evaluation of the BD Affirm VPIII assay as a point-of-care test for the diagnosis of bacterial vaginosis, trichomoniasis and candidiasis in a population of pregnant women.
Degree: MMedSc **BREC Ref No: BE643/17**

EXPEDITED APPLICATION

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application received on 08 November 2017.

The study was provisionally approved pending appropriate responses to queries raised. Your response received on 01 December 2017 to BREC correspondence dated 29 November 2017 has been noted by a sub-committee of the Biomedical Research Ethics Committee. The conditions have now been met and the study is given **full ethics approval** and may begin as from 07 February 2018.

This approval is valid for one year from **07 February 2018.** To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2015), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be **RATIFIED** by a full Committee at its next meeting taking place on **13 March 2018.**

We wish you well with this study. We would appreciate receiving copies of all publications arising out of this study.

Yours sincerely

Professor V Rambiritch
Deputy Chair: Biomedical Research Ethics Committee

cc supervisor: abbain@ukzn.ac.za
cc co supervisor: sebitloanem@ukzn.ac.za

APPENDIX 3:

Appendix : Summary of the BD Affirm™ VPIII assay and BD Max™ Vaginal assay *results.*

Sample ID	Sym/Asym	Max BV	Affirm G.vag	Max *Candida*	Affirm *Candida*	Max TV	Affirm T.vag
V001	Sym	**pos**	neg	neg	neg	**pos**	neg
V002	Asym	neg	neg	neg	neg	neg	neg
V003	Asym	**pos**	**pos**	**pos**	neg	neg	neg
V004	Asym	neg	**pos**	**pos**	neg	neg	neg
V005	Asym	neg	neg	neg	neg	neg	neg
V006	Sym	neg	neg	**pos**	**pos**	neg	neg
V007	Sym	neg	neg	**pos**	neg	neg	neg
V008	Sym	neg	**pos**	**pos**	**pos**	neg	neg
V009	Asym	**pos**	**pos**	neg	neg	neg	neg
V010	Sym	**pos**	**pos**	**pos**	**pos**	neg	neg
V011	Sym	**pos**	**pos**	**pos**	**pos**	neg	neg
V012	Asym	**UNR**	**pos**	**pos**	**pos**	neg	neg
V013	Asym	**pos**	**pos**	neg	neg	neg	neg
V014	Sym	**pos**	**pos**	neg	neg	neg	neg
V015	Sym	**UNR**	neg	**pos**	**pos**	neg	neg
V016	Sym	neg	**pos**	neg	neg	neg	neg
V017	Sym	**pos**	**pos**	neg	neg	**pos**	**pos**
V018	Asym	**pos**	neg	neg	neg	neg	neg
V019	Asym	**pos**	**pos**	neg	neg	neg	neg
V020	Asym	neg	neg	neg	neg	neg	neg
V021	Asym	**pos**	**pos**	**pos**	**pos**	**pos**	**pos**
V022	Asym	neg	neg	**pos**	neg	neg	neg
V023	Asym	neg	neg	neg	neg	neg	neg
V024	Asym	**pos**	neg	neg	neg	neg	neg
V025	Asym	neg	neg	neg	neg	neg	neg
V026	Asym	neg	neg	**pos**	**pos**	neg	neg
V027	Asym	**pos**	**pos**	neg	neg	neg	neg
V028	Asym	neg	neg	**pos**	**pos**	neg	neg
V029	Sym	neg	neg	**pos**	**pos**	neg	neg
V030	Asym	neg	neg	**pos**	neg	**pos**	neg
V031	Asym	**pos**	**pos**	neg	neg	neg	neg
V032	Asym	**pos**	**pos**	**pos**	neg	neg	neg
V033	Asym	neg	neg	**pos**	neg	neg	neg
V034	Sym	neg	neg	**pos**	neg	neg	neg
V035	Sym	**pos**	**pos**	**pos**	**pos**	neg	neg
V036	Asym	neg	neg	neg	neg	neg	neg

V037	Sym	**pos**	**pos**	neg	neg	neg	neg
V038	Asym	neg	neg	**pos**	neg	**pos**	neg
V039	Asym	neg	neg	**pos**	**pos**	neg	neg
V040	Asym	neg	neg	neg	neg	neg	neg
V041	Asym	**pos**	neg	**pos**	**pos**	neg	neg
V042	Sym	**pos**	**pos**	**pos**	**pos**	neg	neg
V043	Asym	neg	neg	neg	neg	neg	neg
V044	Asym	**pos**	**pos**	neg	neg	**pos**	**pos**
V045	Sym	**pos**	**pos**	neg	neg	neg	neg
V046	Sym	UNR	neg	neg	neg	neg	neg
V047	Asym	**pos**	**pos**	neg	neg	neg	neg
V048	Asym	**pos**	**pos**	neg	neg	neg	neg
V049	Asym	neg	neg	**pos**	neg	neg	neg
V050	Asym	**pos**	**pos**	**pos**	neg	neg	neg
V051	Sym	**pos**	neg	**pos**	**pos**	**pos**	neg
V052	Asym	**pos**	**pos**	**pos**	neg	neg	neg
V053	Sym	**pos**	**pos**	**pos**	**pos**	neg	neg
V054	Asym	neg	**pos**	**pos**	neg	neg	neg
V055	Asym	**pos**	**pos**	neg	neg	**pos**	**pos**
V056	Sym	neg	**pos**	**pos**	neg	neg	neg
V057	Sym	**pos**	**pos**	**pos**	neg	neg	neg
V058	Asym	**pos**	**pos**	neg	neg	neg	neg
V059	Asym	neg	neg	neg	neg	neg	neg
V060	Sym	**pos**	**pos**	**pos**	**pos**	neg	neg
V061	Asym	**pos**	**pos**	**pos**	**pos**	neg	neg
V062	Asym	**pos**	**pos**	neg	neg	neg	neg
V063	Asym	neg	neg	neg	neg	neg	neg
V064	Asym	**pos**	**pos**	neg	neg	neg	neg
V065	Asym	neg	neg	neg	neg	neg	neg
V066	Sym	**pos**	neg	neg	neg	neg	neg
V067	Asym	**pos**	**pos**	neg	neg	neg	neg
V068	Asym	**pos**	**pos**	**pos**	neg	neg	neg
V069	Sym	**pos**	**pos**	neg	neg	neg	neg
V070	Asym	neg	neg	neg	neg	neg	neg
V071	Sym	neg	neg	neg	neg	neg	neg
V072	Sym	neg	**pos**	**pos**	neg	neg	neg
V073	Asym	**pos**	neg	**pos**	**pos**	neg	neg
V074	Sym	**pos**	**pos**	**pos**	neg	neg	neg
V075	Asym	neg	neg	**pos**	neg	neg	neg
V076	Asym	**pos**	**pos**	neg	neg	neg	neg
V077	Sym	**pos**	**pos**	**pos**	**pos**	neg	neg

V078	Asym	neg	**pos**	**pos**	**pos**	neg	neg
V079	Asym	neg	neg	**pos**	neg	**pos**	neg
V080	Asym	**pos**	**pos**	neg	neg	neg	neg
V081	Sym	**pos**	**pos**	**pos**	neg	neg	neg
V082	Asym	neg	neg	neg	neg	neg	neg
V083	Asym	**pos**	**pos**	**pos**	neg	neg	neg
V084	Asym	**pos**	**pos**	**pos**	neg	neg	neg
V085	Asym	neg	neg	neg	neg	neg	neg
V086	Sym	neg	neg	**pos**	**pos**	**pos**	neg
V087	Asym	neg	**pos**	**pos**	neg	neg	neg
V088	Asym	**pos**	**pos**	**pos**	neg	**pos**	neg
V089	Asym	neg	neg	neg	neg	neg	neg
V090	Asym	**pos**	**pos**	neg	neg	neg	neg
V091	Asym	UNR	neg	neg	neg	neg	neg
V092	Asym	**pos**	**pos**	neg	neg	neg	neg
V093	Asym	neg	neg	**pos**	**pos**	neg	neg
V094	Sym	UNR	**pos**	**pos**	**pos**	neg	neg
V095	Asym	neg	**pos**	**pos**	**pos**	neg	neg
V096	Asym	neg	**pos**	neg	neg	neg	neg
V097	Asym	neg	neg	neg	neg	**pos**	**pos**
V098	Sym	**pos**	**pos**	neg	neg	neg	neg
V099	Asym	**pos**	neg	UNR	neg	UNR	neg
V100	Asym	neg	neg	neg	neg	neg	neg
V101	Asym	neg	neg	**pos**	**pos**	neg	neg
V102	Sym	neg	neg	**pos**	**pos**	neg	neg
V103	Asym	**pos**	neg	neg	neg	neg	neg
V104	Asym	**pos**	neg	**pos**	neg	neg	neg
V105	Asym	neg	neg	**pos**	neg	neg	neg
V106	Asym	neg	**pos**	**pos**	**pos**	neg	neg
V107	Asym	neg	neg	**pos**	**pos**	neg	neg
V108	Asym	UNR	**pos**	neg	neg	neg	neg
V109	Asym	**pos**	neg	neg	neg	neg	neg
V110	Asym	UNR	neg	**pos**	**pos**	neg	neg
V111	Sym	**pos**	neg	neg	neg	neg	neg
V112	Sym	**pos**	neg	**pos**	**pos**	neg	neg
V113	Asym	neg	**pos**	neg	neg	neg	neg
V114	Asym	neg	neg	**pos**	**pos**	neg	neg
V115	Asym	neg	neg	neg	neg	neg	neg
V116	Sym	**pos**	**pos**	**pos**	neg	neg	neg
V117	Asym	neg	neg	**pos**	**pos**	neg	neg
V118	Asym	neg	neg	**pos**	**pos**	neg	neg

V119	Asym	neg	neg	**pos**	neg	neg	neg
V120	Sym	UNR	**pos**	**pos**	neg	neg	neg
V121	Asym	**pos**	**pos**	**pos**	neg	neg	neg
V122	Asym	neg	neg	neg	neg	**pos**	**pos**
V123	Asym	**pos**	**pos**	**pos**	neg	neg	neg
V124	Asym	**pos**	**pos**	neg	neg	neg	neg
V125	Asym	neg	neg	**pos**	neg	neg	neg
V126	Asym	**pos**	**pos**	**pos**	neg	neg	neg
V127	Asym	neg	neg	**pos**	neg	neg	neg
V128	Sym	**pos**	neg	**pos**	**pos**	**pos**	neg
V129	Asym	neg	neg	**pos**	**pos**	neg	neg
V130	Asym	neg	neg	neg	neg	neg	neg
V131	Sym	**pos**	**pos**	**pos**	neg	neg	neg
V132	Asym	neg	neg	**pos**	neg	neg	neg
V133	Sym	neg	neg	neg	neg	neg	neg
V134	Sym	neg	neg	**pos**	neg	neg	neg
V135	Asym	neg	neg	**pos**	**pos**	neg	neg
V136	Sym	**pos**	**pos**	**pos**	neg	neg	neg
V137	Sym	**pos**	neg	neg	neg	neg	neg
V138	Asym	**pos**	**pos**	**pos**	neg	neg	neg
V139	Sym	**pos**	**pos**	**pos**	neg	neg	neg
V140	Asym	neg	neg	**pos**	**pos**	neg	neg
V141	Asym	neg	**pos**	**pos**	neg	neg	neg
V142	Sym	**pos**	**pos**	neg	neg	**pos**	neg
V143	Sym	neg	**pos**	**pos**	**pos**	neg	neg
V144	Asym	neg	**pos**	**pos**	neg	neg	neg
V145	Asym	neg	neg	**pos**	neg	neg	neg
V146	Asym	**pos**	**pos**	neg	neg	neg	neg
V147	Asym	neg	neg	neg	neg	neg	neg
V148	Asym	**pos**	**pos**	neg	neg	neg	neg
V149	Asym	neg	neg	**pos**	neg	neg	neg
V150	Asym	**pos**	**pos**	**pos**	neg	neg	neg
V151	Asym	neg	neg	neg	neg	neg	neg
V152	Asym	neg	neg	neg	neg	neg	neg
V153	Sym	**pos**	**pos**	**pos**	neg	neg	neg
V154	Sym	**pos**	**pos**	**pos**	**pos**	**pos**	**pos**
V155	Asym	neg	neg	**pos**	neg	neg	neg
V156	Asym	**pos**	**pos**	neg	neg	neg	neg
V157	Asym	**pos**	**pos**	**pos**	**pos**	neg	neg
V158	Asym	**pos**	**pos**	**pos**	neg	neg	neg
V159	Asym	**pos**	**pos**	neg	neg	neg	neg

V160	Asym	neg	neg	**pos**	neg	neg	neg
V161	Asym	**pos**	**pos**	neg	neg	neg	neg
V162	Asym	**pos**	**pos**	**pos**	neg	neg	neg
V163	Sym	neg	neg	**pos**	**pos**	neg	neg
V164	Asym	**pos**	**pos**	**pos**	**pos**	**pos**	**pos**
V165	Asym	neg	neg	**pos**	neg	neg	neg
V166	Asym	neg	neg	neg	neg	**pos**	neg
V167	Sym	**pos**	**pos**	**pos**	**pos**	neg	neg
V168	Sym	**pos**	**pos**	**pos**	neg	**pos**	neg
V169	Sym	UNR	**pos**	**pos**	**pos**	neg	neg
V170	Sym	**pos**	**pos**	**pos**	**pos**	neg	neg
V171	Asym	**pos**	**pos**	**pos**	**pos**	neg	neg
V172	Sym	**pos**	**pos**	neg	neg	neg	neg
V173	Sym	**pos**	neg	**pos**	neg	neg	neg
V174	Asym	neg	neg	neg	neg	neg	neg
V175	Asym	neg	neg	neg	neg	neg	neg
V176	Sym	**pos**	**pos**	**pos**	**pos**	neg	neg
V177	Asym	**pos**	**pos**	neg	neg	neg	neg
V178	Asym	neg	**pos**	**pos**	**pos**	neg	neg
V179	Sym	**pos**	**pos**	**pos**	**pos**	neg	neg
V180	Asym	neg	neg	neg	neg	**pos**	neg
V181	Asym	neg	neg	neg	neg	neg	neg
V182	Asym	**pos**	neg	**pos**	neg	neg	neg
V183	Asym	neg	neg	neg	neg	neg	neg
V184	Asym	neg	neg	neg	neg	neg	neg
V185	Asym	neg	**pos**	neg	neg	neg	neg
V186	Asym	**pos**	neg	neg	neg	neg	neg
V187	Sym	neg	neg	**pos**	**pos**	neg	neg
V188	Asym	**pos**	**pos**	**pos**	neg	neg	neg
V189	Asym	neg	neg	neg	neg	neg	neg
V190	Asym	UNR	neg	**pos**	**pos**	neg	neg
V191	Sym	UNR	neg	**pos**	**pos**	neg	neg
V192	Sym	UNR	neg	**pos**	**pos**	neg	neg
V193	Sym	neg	**pos**	neg	neg	**pos**	**pos**
V194	Asym	neg	neg	**pos**	neg	neg	neg
V195	Sym	neg	neg	**pos**	**pos**	neg	neg
V196	Sym	neg	neg	**pos**	**pos**	neg	neg
V197	Sym	neg	**pos**	**pos**	**pos**	neg	neg
V198	Sym	neg	neg	neg	neg	neg	neg
V199	Asym	neg	neg	**pos**	neg	neg	neg
V200	Asym	**pos**	**pos**	**pos**	neg	neg	neg

V201	Asym	neg	neg	**pos**	neg	neg	neg
V202	Sym	neg	**pos**	**pos**	**pos**	neg	neg
V203	Asym	**pos**	neg	neg	neg	neg	neg
V204	Asym	neg	neg	**pos**	**pos**	**pos**	**pos**
V205	Sym	neg	neg	**pos**	**pos**	neg	neg
V206	Sym	IND x2	neg	IND	**pos**	IND	neg
V207	Sym	**pos**	**pos**	**pos**	**pos**	neg	neg
V208	Asym	neg	neg	neg	neg	neg	neg
V209	Sym	neg	neg	**pos**	**pos**	neg	neg
V210	Asym	**pos**	neg	**pos**	**pos**	neg	neg
V211	Asym	**pos**	**pos**	**pos**	**pos**	neg	neg
V212	Asym	**pos**	**pos**	**pos**	**pos**	neg	neg
V213	Asym	**pos**	**pos**	neg	neg	neg	neg
V214	Asym	UNR	neg	neg	neg	neg	neg
V215	Asym	**pos**	**pos**	neg	neg	neg	neg
V216	Asym	neg	neg	neg	neg	**pos**	neg
V217	Sym	**pos**	**pos**	neg	neg	**pos**	neg
V218	Asym	**pos**	neg	neg	**pos**	neg	neg
V219	Asym	**pos**	neg	**pos**	**pos**	neg	neg
V220	Asym	**pos**	neg	neg	neg	neg	neg
V221	Asym	neg	neg	neg	neg	neg	neg
V222	Sym	**pos**	**pos**	**pos**	neg	neg	neg
V223	Asym	**pos**	**pos**	neg	neg	neg	neg
V224	Asym	**pos**	**pos**	neg	neg	neg	neg
V225	Asym	neg	neg	**pos**	**pos**	neg	neg
V226	Asym	UNR	neg	neg	neg	neg	neg
V227	Sym	**pos**	**pos**	**pos**	neg	neg	neg
V228	Asym	neg	neg	**pos**	neg	neg	neg
V229	Sym	**pos**	**pos**	**pos**	neg	neg	neg
V230	Sym	**pos**	**pos**	**pos**	neg	neg	neg
V231	Sym	neg	neg	neg	**pos**	neg	neg
V232	Asym	neg	neg	**pos**	neg	neg	neg
V233	Sym	neg	**pos**	**pos**	**pos**	neg	neg
V234	Asym	**pos**	**pos**	neg	neg	neg	neg
V235	Sym	neg	**pos**	**pos**	**pos**	**pos**	**pos**
V236	Sym	neg	neg	**pos**	**pos**	neg	neg
V237	Sym	UNR	**pos**	neg	neg	neg	neg
V238	Asym	UNR	neg	**pos**	neg	neg	neg
V239	Sym	**pos**	**pos**	neg	neg	neg	neg
V240	Sym	**pos**	**pos**	**pos**	neg	neg	neg
V241	Sym	neg	**pos**	neg	neg	**pos**	**pos**

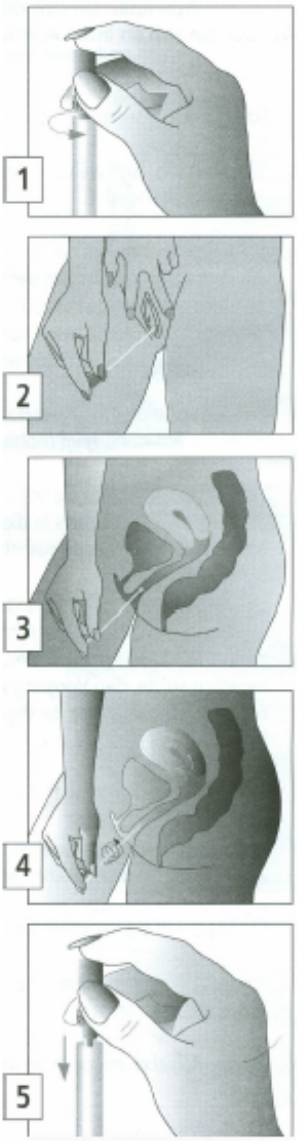
V242	Sym	UNR	neg	**pos**	**pos**	neg	neg
V243	Asym	neg	neg	neg	neg	neg	neg
V244	Asym	**pos**	**pos**	**pos**	neg	neg	neg
V245	Asym	neg	neg	**pos**	**pos**	neg	neg
V246	Asym	**pos**	**pos**	neg	neg	neg	neg
V247	Sym	neg	**pos**	neg	neg	neg	neg
V248	Asym	**pos**	**pos**	**pos**	**pos**	neg	neg
V249	Sym	neg	neg	neg	neg	**pos**	**pos**
V250	Sym	neg	**pos**	**pos**	**pos**	neg	neg
V251	Asym	neg	neg	**pos**	**pos**	neg	neg
V252	Sym	neg	neg	**pos**	**pos**	neg	neg
V253	Sym	UNR	neg	**pos**	neg	neg	neg
V254	Asym	neg	neg	**pos**	**pos**	neg	neg
V255	Asym	**pos**	neg	**pos**	neg	neg	neg
V256	Asym	**pos**	**pos**	neg	neg	neg	neg
V257	Sym	UNR	neg	**pos**	**pos**	neg	neg
V258	Asym	neg	neg	neg	neg	neg	neg
V259	Asym	neg	neg	neg	**pos**	neg	neg
V260	Asym	**pos**	**pos**	neg	neg	neg	neg
V261	Asym	neg	neg	**pos**	neg	neg	neg
V262	Sym	neg	neg	**pos**	**pos**	neg	neg
V263	Asym	UNR	neg	**pos**	**pos**	neg	neg
V281	Asym	neg	neg	neg	neg	neg	neg
V282	Asym	**pos**	**pos**	neg	neg	neg	neg
V283	Asym	**pos**	**pos**	neg	neg	neg	neg
V284	Asym	neg	neg	**pos**	**pos**	neg	neg
V285	Asym	UNR	neg	**pos**	**pos**	neg	neg
V286	Asym	**pos**	**pos**	neg	neg	neg	neg
V287	Sym	**pos**	**pos**	neg	neg	neg	neg
V288	Asym	neg	neg	**pos**	neg	neg	neg
V289	Asym	neg	neg	neg	neg	neg	neg
V290	Asym	**pos**	neg	neg	neg	**pos**	neg

Note. BD Max *Candida* positive results shown were positive for either of the *Candida* groupings (*C.group / C.glabrata* / *C.krusei)*

APPENDIX 4

Instructions for Self-collected vaginal swabs

1. Wash hands with soap and water. Rinse and dry.
2. It is important to maintain a comfortable balance during the collection procedure.
3. Twist the cap to break the seal (Figure 1). Pull the cap with attached swab off the tube. Do not touch the soft tip or lay the swab down. If you touch or drop the swab tip or the swab is laid down, discard the swab and request a new vaginal swab.
4. Hold the swab by the cap with one hand so the swab tip is pointing toward you (Figure 2).
5. With your other hand, gently spread the skin outside the vagina. Insert the tip of the swab into the vaginal opening (Figure 2). Point the tip toward your lower back and relax your muscles.
6. Gently slide the swab no more than two inches into the vagina (Figure 3). If the swab does not slide easily, gently rotate the swab as you push. If it is still difficult, do not attempt to continue. Make sure the swab touches the walls of the vagina so that moisture is absorbed by the swab.
7. Rotate the swab for 10 -15 seconds (Figure 4).
8. Withdraw the swab without touching the skin. Place the swab in the tube and cap securely (Figure 5).
9. Repeat steps 2-8 if a second swab is to be collected.
10. After collection, wash hands with soap and water, rinse, and dry.
11. Return tube(s) with swab(s) as instructed.



Source: https://www.hey.nhs.uk/pathology/departmentofinfection/virology/vaginal-swabs/

Clinical Medicine Laboratory – Vaginitis Study

APPENDIX 5

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28 May 2019

Dear Fazana Dessai

On behalf of the Organizing Committee for Conference on Genomics, Proteomics and Metabolomics: All in the Bioinformatics (CGPMB-2019), I am pleased to send this formal invitation to you as an invited speaker to the CGPMB-2019. This conference will take place in Umfolozi Hotel Casino and Convention Center, Empangeni, KwaZulu-Natal, from 27-28 July, 2019 (https://cpgmb.weebly.com/).

I am also pleased to inform you that based on the available finances; you will be fully covered for conference registration fees and accommodation during the conference. You are further cordially invited to the gala dinner on the 28 July 2019. The dress code will be semi-formal or traditional.

Please submit abstract and registration forms as per instructions provided at the website before 21 June 2019. Kindly ignore registration fee in the form. Your talk and lecture are reflected in the program of the conference (in progress) and can be found at https://cpgmb.weebly.com/.

We would be delighted if you were able to accept the invitation and I look forward to welcoming you to Empangeni in July.

Yours sincerely
S Khajamohiddin
Khajamohiddin Syed

Organized by

Prof Khajamohiddin Syed
Department of Biochemistry and Microbiology
University of Zululand, KwaDlangezwa 3886
KwaZulu-Natal, South Africa

APPENDIX 6:

From: EXBO Abstracts
To: Fazana Dessai
Subject: News About Your Submission
Date: Thursday, 15 August 2019 12:41:54

Document number: 10311
Submission title: Prevalence and detection of Bacterial Vaginosis, Trichomonas vaginalis and Candida spp in a population of pregnant women in South Africa

EPoster Abstract Acceptance

On behalf of the 8th FIDSSA Congress 2019, 7 Ã¢â‚¬â€œ 9 November, the Scientific Committee is delighted to inform you that your abstract has been accepted as an ePoster presentation.

Kindly visit the Congress website, www.fidssacongress.co.za, for programme updates.

Congress registration link, http://fidssacongress.co.za/registration-membership/

Abstracts will be judged throughout the Congress and the Best Presentation Awards will be announced during the Celebration Dinner, on Saturday 09 November.

Kindly confirm with the Congress Office by return email before 25 August, whether you accept this notification and confirm the presenting authors' details.

Please note, all abstract presenters are required to register for the Congress, Early Bird Registration Fees will apply.

Should you have any queries please do not hesitate to contact the congress office.

We look forward to welcoming you to the 8th FIDSSA Congress 2019.

Kind Regards

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For and on behalf of the 8th FIDSSA Congress 2019
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APPENDIX 7:

Original research article

INTERNATIONAL JOURNAL OF STD & AIDS

International Journal of STD & AIDS
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Diagnostic evaluation of the BD Affirm VPIII assay as a point-of-care test for the diagnosis of bacterial vaginosis, trichomoniasis and candidiasis

Fazana Dessai[1], Makandwe Nyirenda[2,3], Motshedisi Sebitloane[4] and Nathlee Abbai[1]

Abstract

Untreated sexually transmitted infections and bacterial vaginosis (BV) pose a serious health risk to mother and child. Limited data exist on the use of the BD Affirm™ VPIII assay as a point-of-care test (POCT). The performance of the BD Affirm™ VPIII assay was compared with the BD Max™ vaginal assay for the diagnosis of BV, *Trichomonas vaginalis* (TV) and *Candida* spp. A total of 273 pregnant women were enrolled in this study and had provided two self-collected vaginal swabs. Sensitivity, specificity, positive predictive value, negative predictive value and prevalence were calculated. The prevalence of BV, candidiasis and trichomoniasis was 49.4, 57.2 and 10.3%, respectively. The BD Affirm™ VPIII assay showed a moderate sensitivity (79.8%) and a moderate specificity (80.3%) for diagnosing BV in all participants. The BD Affirm™ VPIII assay had an excellent specificity for *Candida* spp. and TV of 97.4 and 100%, respectively; however, the assay exhibited poor sensitivities of 52.9 and 46.4%, respectively. This study was the first to report on the performance of the BD Affirm™ VPIII assay as a POCT in an antenatal population. The assay was found to be unsuitable as a screening test for vaginal infections in pregnancy.

Keywords

Affirm, max, pregnant, antenatal, South Africa, bacterial vaginosis, *Trichomonas*, *Trichomonas vaginalis*, *Candida*, prevalence

Date received: 14 August 2019; accepted: 26 November 2019

Introduction

Abnormal vaginal discharge can be caused by bacterial vaginosis (BV), trichomoniasis and candidiasis. In pregnancy these have been associated with low birth weight, preterm delivery and premature rupture of membranes.[1–3] Diagnosing vaginal infections may be complicated especially in pregnancy as the presence of a discharge is a normal occurrence.[4]

During pregnancy, alterations in oestrogen and progesterone levels induce physiological changes, such as a change in pH values, in the lower genital tract. Such physiological changes will result in vaginal mucosa congestion and hypertrophy, which promotes the growth of anaerobic bacteria positively associated with BV and other pathogenic microorganisms within the vagina.[5] BV is a microbial imbalance of the vaginal environment which is characterized by a reduction of normal lactobacilli and an increase in anaerobic bacteria such as *Gardnerella vaginalis*, *Bacteroides spp.*, *Peptostreptococcus spp.*, *Prevotella spp.* *Porphyromonas spp.*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, *Mobiluncus*, *Megasphaera*, *Sneathia*, *Clostridiales spp.*, *Fusobacterium spp.* and

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Email: dessaif@ukzn.ac.za

Atopobium vaginae.[6] BV results in an elevated vaginal pH and an increase in malodorous vaginal discharge.[2] For pregnant women, BV doubles the risk of preterm delivery before gestation week 37.[7] The most recent data collected on antenatal prevalence of BV in South Africa occurred in 2012 (17.6% prevalence).[8]

Candida vaginitis is responsible for 80 90% of lower genital tract infections during pregnancy.[9] It is characterized by vulvar and vaginal pruritus, external dysuria, white cottage cheese-type discharge and vulvovaginal excoriations.[9] There is some evidence that candidiasis during pregnancy may be associated with an increased risk of pregnancy complications such as premature rupture of membranes and poor pregnancy outcome.[9] The prevalence of *Candida* in the antenatal population in South Africa was last reported as 9 59% in 2005.[10]

Trichomonas vaginalis (TV) infections have been associated with poor reproductive outcomes such as low birth weight and premature birth.[11] TV is a flagellate protozoan considered to be sexually transmittable and sometimes related to low socioeconomic levels.[12] In South Africa, TV was last reported to be 20.0% in pregnant women.[11] Despite the high prevalence of TV infections in pregnancy, there is no routine screening during antenatal care as most services focus more on HIV and syphilis.[12]

South Africa follows the World Health Organization (WHO) recommendations and the South African Department of Health maternity guidelines for the treatment of vaginal discharge in pregnancy. Clotrimazole is indicated for *Candida* and Metronidazole for *Trichomonas* and BV.[13] A series of evaluations of the syndromic management flowcharts was commissioned by the WHO and UNAIDS in the 1990s. The main conclusions from these studies reported that the flowchart for vaginal discharge is neither sensitive nor specific.[14] A study in 2016 which evaluated the syndromic management guidelines in South Africa concluded that there was a need for better strategies to manage asymptomatic vaginal infections.[15] Moodley et al.[16] reported on a high prevalence of asymptomatic vaginal infections in pregnant women and recommended that the development and validation of point-of-care tests (POCTs) for vaginal infections be expedited.

In the past, diagnostic methods for BV relied on the use of clinical criteria and microscopy using Amsel's criteria or Nugent's criteria. Methods for *Candida* spp. included the observation of budding yeast cells and pseudohyphae on wet mount or a positive culture for *Candida* spp. TV was detected by wet mount, in culture, or via biochemical detection. These methods require immediate testing, specialized microbiological media and are user dependent. Nucleic acid amplification-based assays have since been introduced and are widely used for detection of these pathogens.[17]

Few studies have focused on the diagnosis of vaginal pathogens in a South African antenatal setting. Previous studies have shown that the BD Affirm™ VPIII assay (Becton Dickinson, Sparks, MD, USA) has the potential to become the ideal one-assay POCT for diagnosing vaginosis and vaginitis in a clinic setting.[18–20] Crist et al.[20] showed that the BD Affirm™ VPIII assay outperformed and provided faster results than the Amsel's criteria, Nugent's criteria and yeast culture using swab samples in primary health care patients for the diagnosis of BV and *Candida*. Gaydos et al. validated the accuracy of the BD Max™ assay (Becton Dickinson, Sparks, MD, USA) using self-collected swabs and clinician-collected swabs against Nugent's criteria and Amsel's criteria for BV, CHROMagar, Sabouraud agar and sequencing for *Candida*, with wet mount and culture for TV. This study had shown a high sensitivity and specificity of the BD Max™ assay compared to conventional methods of detection.[21] In 2016, the BD Max™ vaginal panel was approved by the Food and Drug Administration (FDA) as a diagnostic test for vaginal infections. Despite its advantages, the BD Max™ assay is an expensive assay when compared to conventional methods.

Cartwright et al.[18] compared the BD Affirm™ VPIII assay to other nucleic acid amplification tests (Gen-Probe assay for TV, CAN-PCR for *Candida* spp. and BV-PCR for BV) using vaginal swabs, in which they concluded that all three tests had to be used in combination to achieve an accuracy better than the BD Affirm™ VPIII assay on its own.

A study by Briselden and Hillier[19] showed that the BD Affirm™ VPIII assay was more sensitive than wet mount for the detection of TV in swab samples.

All the above studies show that there is a need for a POCT such as the BD Affirm™ VPIII assay for diagnosing vaginosis and vaginitis in a clinic setting.

In this study, we evaluated the performance of the BD Affirm™ VPIII Microbial Identification assay against the BD Max™ vaginal assay for the diagnosis of BV, candidiasis and trichomoniasis in a population of pregnant women. The prevalence estimates of these pathogens are also reported through this study.

Materials and methods

Subjects

Study participants were recruited from the antenatal clinic at King Edward VIII Hospital, Durban, South Africa from November 2017 to June 2018. All participants were older than 18 years of age and willing to

provide written informed consent. Enrolled participants completed a questionnaire and were asked to provide two self-collected vaginal swabs for analysis.

Symptomatic participants (before treatment) were characterized as those who complained of an abnormal vaginal discharge/dysuria or vulval itching/burning and asymptomatic participants were characterized as those who did not complain of any of the above symptoms. All women were treated as per the syndromic management guidelines. The guidelines advocate the use of a 2 g single dose of metronidazole and clotrimazole vaginal pessary (single dose) or clotrimazole vaginal cream (12-hourly for seven days). This study was approved by the Biomedical Research Ethics committee of the University of KwaZulu-Natal (BE643/17).

Specimen collection and testing

Two self-collected swabs were obtained. The BD Affirm™ VPIII swab was placed dry in a sample collection tube (BD Affirm™ VPIII collection set) and the BD Max™ Vaginal assay swab was placed in a BD Max™ UVE sample buffer tube.

The samples were transported at ambient temperature within 1 h of sample collection. The BD Affirm™ VPIII swab was processed within 2 h in the laboratory following the manufacturer's guidelines. Samples for the BD Max™ vaginal assay were refrigerated until tested. Testing was done within five days as recommended by the manufacturer.

BD Max™ vaginal assay

The BD Max™ vaginal assay was used as the comparator assay in this study. The assay is an automated diagnostic test approved by the FDA for the detection of vaginal pathogens associated with BV, vulvovaginal candidiasis and trichomoniasis from vaginal swabs. The system uses real time polymerase chain reaction to amplify specific targets and uses target-specific hybridization probes to detect and differentiate the DNA. Results are qualitative and are reported as a result for (1) BV (by calculating a ratio of *Lactobacillus crispatus* and *Lactobacillus jensenii*, *G. vaginalis*, *Atopobium vaginae*, BV-associated bacteria-2 and *Megasphaera-1*), (2) *Candida* spp. (which includes *C. albicans*, *C. tropicalis*, *C. parapsilosis* and *C. dubliniensis*), (3) *Candida glabrata*, (4) *Candida krusei* and (5) TV. Indeterminate is defined as a BD Max™ system failure. Unresolved is defined as inhibitory sample or reagent failure or no amplification in the internal control. All indeterminate and unresolved samples were retested.

BD Affirm™ VPIII Microbial Identification assay

The BD Affirm™ VPIII assay is a semi-automated assay that uses single-stranded nucleic acid probes, a capture probe and a colour development probe which are complementary to unique genetic sequences of target organisms. The sample is treated with a lysis solution and heated on a lysing heater, thereafter buffer is added and the sample transferred to the MicroProbe Processor which is loaded with a Reagent Cassette and a Probe Analysis Card. Results are interpreted visually by colour development and are reported for (1) *G. vaginalis*, (2) *Candida* (which includes *C. albicans*, *C. glabrata*, *C. kefyr*, *C. krusei*, *C. parapsilosis* and *C. tropicalis*) and (3) TV. The BD Affirm VPIII Microbial Identification assay was the investigational kit in this study.

Data management and analysis

Study data was collected using a paper-based questionnaire. Electronic data were captured on Microsoft Excel. The diagnostic accuracy of the BD Affirm™ VPIII assay compared to the BD Max™ assay was assessed through calculation of the sensitivity, specificity, negative predictive value and positive predictive value and their respective 95% confidence intervals using STATA software (StataCorp, TX). To further illustrate overall accuracy, receiver operating characteristic (ROC) curve analysis was done using STATA. The prevalence of BV, TV and candidiasis was reported as proportions with confidence intervals using the BD Max™ Vaginal assay data. A p-value of ≤ 0.05 was considered as significant.

Results

Baseline characteristics

Table 1 describes the baseline characteristics of the study population ($n = 273$) according to individual infection status as determined by the BD Max™ Vaginal assay.

The variables that were significantly associated with BV were having a high school level of education ($p = 0.008$), not knowing if their partner had other partners ($p = 0.007$) and not consuming alcohol ($p = 0.010$). According to the analysis, smoking and HIV status were significantly associated with TV infection. The majority of the women who tested positive for TV were non-smokers ($p = 0.025$). Being HIV-positive was strongly associated with TV ($p = 0.011$). The majority of women who tested positive for *Candida* reported not having a current abnormal vaginal discharge ($p < 0.001$), had a high school level of education only ($p = 0.018$), used condoms sometimes

Table 1. Demographic and behavioural characteristics of the study population.

	BV neg (n = 127)	BV pos (n = 124)	p-value	BV overall (n = 273)	TV neg (n = 243)	TV pos (n = 28)	p-value	TV overall (n = 273)	Can neg (n = 116)	Can pos (n = 155)	p-value	Can overall (n = 273)
Age (years)			0.896				0.711				0.113	
Mean ± SD (CV%)	28.7 ± 6.57 (22.9)	28.4 ± 5.87 (20.7)		28.5 ± 6.20 (21.8)	28.4 ± 6.33 (22.3)	28.6 ± 4.99 (17.4)		28.5 ± 6.20 (21.8)	29.1 ± 6.39 (21.9)	27.9 ± 6.03 (21.6)		28.5 ± 6.20 (21.8)
Median (Q1–Q3)	27.0 (24.0–34.0)	28.0 (24.0–33.0)		28.0 (24.0–33.0)	28.0 (24.0–33.0)	27.0 (25.0–33.3)		28.0 (24.0–33.0)	28.0 (24.0–34.0)	27.0 (23.5–32.5)		28.0 (24.0–33.0)
Min–Max	18.0–43.0	18.0–43.0		18.0–43.0	18.0–43.0	20.0–38.0		18.0–43.0	18.0–43.0	18.0–43.0		18.0–43.0
Current abnormal vaginal discharge			0.079				0.128				**< 0.001**	
No	93 (73.2%)	78 (62.9%)		181 (66.3%)	165 (67.9%)	15 (53.6%)		181 (66.3%)	90 (77.6%)	90 (58.1%)		181 (66.3%)
Yes	34 (26.8%)	46 (37.1%)		92 (33.7%)	78 (32.1%)	13 (46.4%)		92 (33.7%)	26 (22.4%)	65 (41.9%)		92 (33.7%)
Highest level of education			**0.008**				0.472				**0.018**	
College/University	40 (31.5%)	29 (23.4%)		71 (26.0%)	66 (27.2%)	5 (17.9%)		71 (26.0%)	37 (31.9%)	34 (21.9%)		71 (26.0%)
Did not attend school	2 (1.6%)	0 (0%)		2 (0.7%)	2 (0.8%)	0 (0%)		2 (0.7%)	2 (1.7%)	0 (0%)		2 (0.7%)
High school	82 (64.6%)	81 (65.3%)		182 (66.7%)	161 (66.3%)	20 (71.4%)		182 (66.7%)	67 (57.8%)	114 (73.5%)		182 (66.7%)
Primary school	3 (2.4%)	14 (11.3%)		18 (6.6%)	14 (5.8%)	3 (10.7%)		18 (6.6%)	10 (8.6%)	7 (4.5%)		18 (6.6%)
Married			0.095				0.581				0.530	
No	101 (79.5%)	109 (87.9%)		232 (85.0%)	207 (85.2%)	23 (82.1%)		232 (85.0%)	97 (83.6%)	133 (85.8%)		232 (85.0%)
Yes	25 (19.7%)	15 (12.1%)		40 (14.7%)	35 (14.4%)	5 (17.9%)		40 (14.7%)	19 (16.4%)	21 (13.5%)		40 (14.7%)
Missing	1 (0.8%)	0 (0%)		1 (0.4%)	1 (0.4%)	0 (0%)		1 (0.4%)	0 (0%)	1 (0.6%)		1 (0.4%)
Regular sex partner			0.146				0.174				0.842	
No	25 (19.7%)	16 (12.9%)		44 (16.1%)	36 (14.8%)	7 (25.0%)		44 (16.1%)	19 (16.4%)	24 (15.5%)		44 (16.1%)
Yes	102 (80.3%)	108 (87.1%)		229 (83.9%)	207 (85.2%)	21 (75.0%)		229 (83.9%)	97 (83.6%)	131 (84.5%)		229 (83.9%)
Co-habiting			0.979				0.668				0.157	
No	76 (59.8%)	74 (59.7%)		166 (60.8%)	149 (61.3%)	16 (57.1%)		166 (60.8%)	65 (56.0%)	100 (64.5%)		166 (60.8%)
Yes	51 (40.2%)	50 (40.3%)		107 (39.2%)	94 (38.7%)	12 (42.9%)		107 (39.2%)	51 (44.0%)	55 (35.5%)		107 (39.2%)
Age of sexual debut (years)			0.245				0.965				0.469	
<15	8 (6.3%)	5 (4.0%)		13 (4.8%)	12 (4.9%)	1 (3.6%)		13 (4.8%)	7 (6.0%)	6 (3.9%)		13 (4.8%)
>25	6 (4.7%)	1 (0.8%)		7 (2.6%)	7 (2.9%)	0 (0%)		7 (2.6%)	4 (3.4%)	3 (1.9%)		7 (2.6%)
15–20	92 (72.4%)	96 (77.4%)		206 (75.5%)	181 (74.5%)	23 (82.1%)		206 (75.5%)	82 (70.7%)	122 (78.7%)		206 (75.5%)
21–25	21 (16.5%)	22 (17.7%)		47 (17.2%)	43 (17.7%)	4 (14.3%)		47 (17.2%)	23 (19.8%)	24 (15.5%)		47 (17.2%)
No. of lifetime sexual partners			0.563				0.161				0.555	
>4	24 (18.9%)	23 (18.5%)		52 (19.0%)	44 (18.1%)	8 (28.6%)		52 (19.0%)	19 (16.4%)	33 (21.3%)		52 (19.0%)
1	40 (31.5%)	32 (25.8%)		78 (28.6%)	72 (29.6%)	4 (14.3%)		78 (28.6%)	35 (30.2%)	41 (26.5%)		78 (28.6%)
2–4	63 (49.6%)	69 (55.6%)		143 (52.4%)	127 (52.3%)	16 (57.1%)		143 (52.4%)	62 (53.4%)	81 (52.3%)		143 (52.4%)
Partner has other partners			**0.007**				0.067				0.779	
Don't know	43 (33.9%)	61 (49.2%)		120 (44.0%)	109 (44.9%)	10 (35.7%)		120 (44.0%)	52 (44.8%)	67 (43.2%)		120 (44.0%)
No	47 (37.0%)	25 (20.2%)		76 (27.8%)	71 (29.2%)	5 (17.9%)		76 (27.8%)	34 (29.3%)	42 (27.1%)		76 (27.8%)
Yes	37 (29.1%)	38 (30.6%)		77 (28.2%)	63 (25.9%)	13 (46.4%)		77 (28.2%)	30 (25.9%)	46 (29.7%)		77 (28.2%)
Condom use			0.980				0.350				**0.048**	
Always	5 (3.9%)	5 (4.0%)		10 (3.7%)	9 (3.7%)	1 (3.6%)		10 (3.7%)	5 (4.3%)	5 (3.2%)		10 (3.7%)
Never	34 (26.8%)	36 (29.0%)		76 (27.8%)	70 (28.8%)	6 (21.4%)		76 (27.8%)	41 (35.3%)	35 (22.6%)		76 (27.8%)
Rarely	8 (6.3%)	8 (6.5%)		18 (6.6%)	18 (7.4%)	0 (0%)		18 (6.6%)	4 (3.4%)	14 (9.0%)		18 (6.6%)
Sometimes	80 (63.0%)	75 (60.5%)		169 (61.9%)	146 (60.1%)	21 (75.0%)		169 (61.9%)	66 (56.9%)	101 (65.2%)		169 (61.9%)
Condom used in last sex act			0.689				0.925				0.091	
No	88 (69.3%)	83 (66.9%)		188 (68.9%)	167 (68.7%)	19 (67.9%)		188 (68.9%)	86 (74.1%)	100 (64.5%)		188 (68.9%)
Yes	39 (30.7%)	41 (33.1%)		85 (31.1%)	76 (31.3%)	9 (32.1%)		85 (31.1%)	30 (25.9%)	55 (35.5%)		85 (31.1%)
Smoking			0.069				**0.025**				0.087	
No	124 (97.6%)	115 (92.7%)		261 (95.6%)	235 (96.7%)	24 (85.7%)		261 (95.6%)	108 (93.1%)	151 (97.4%)		261 (95.6%)
Yes	3 (2.4%)	9 (7.3%)		12 (4.4%)	8 (3.3%)	4 (14.3%)		12 (4.4%)	8 (6.9%)	4 (2.6%)		12 (4.4%)

(continued)

Table 1. Continued.

	BV neg (n = 127)	BV pos (n = 124)	p-value	BV overall (n = 273)	TV neg (n = 243)	TV pos (n = 28)	p-value	TV overall (n = 273)	Can neg (n = 116)	Can pos (n = 155)	p-value	Can overall (n = 273)
Alcohol consumption			**0.010**				0.348				0.518	
No	118 (92.9%)	102 (82.3%)		241 (88.3%)	216 (88.9%)	23 (82.1%)		241 (88.3%)	104 (89.7%)	135 (87.1%)		241 (88.3%)
Yes	9 (7.1%)	22 (17.7%)		32 (11.7%)	27 (11.1%)	5 (17.9%)		32 (11.7%)	12 (10.3%)	20 (12.9%)		32 (11.7%)
Intravaginal practice			0.613				1.000				0.145	
No	117 (92.1%)	112 (90.3%)		246 (90.1%)	218 (89.7%)	26 (92.9%)		246 (90.1%)	108 (93.1%)	136 (87.7%)		246 (90.1%)
Yes	10 (7.9%)	12 (9.7%)		27 (9.9%)	25 (10.3%)	2 (7.1%)		27 (9.9%)	8 (6.9%)	19 (12.3%)		27 (9.9%)
Trimester of pregnancy			0.339				0.578				0.093	
1st	12 (9.4%)	16 (12.9%)		32 (11.7%)	27 (11.1%)	5 (17.9%)		32 (11.7%)	16 (13.8%)	16 (10.3%)		32 (11.7%)
2nd	36 (28.3%)	42 (33.9%)		84 (30.8%)	75 (30.9%)	8 (28.6%)		84 (30.8%)	42 (36.2%)	41 (26.5%)		84 (30.8%)
3rd	79 (62.2%)	66 (53.2%)		157 (57.5%)	141 (58.0%)	15 (53.6%)		157 (57.5%)	58 (50.0%)	98 (63.2%)		157 (57.5%)
History of preterm labour			0.621				0.076				0.305	
No	99 (78.0%)	102 (82.3%)		215 (78.8%)	195 (80.2%)	19 (67.9%)		215 (78.8%)	95 (81.9%)	119 (76.8%)		215 (78.8%)
Yes	24 (18.9%)	21 (16.9%)		53 (19.4%)	43 (17.7%)	9 (32.1%)		53 (19.4%)	19 (16.4%)	33 (21.3%)		53 (19.4%)
Missing	4 (3.1%)	1 (0.8%)		5 (1.8%)	5 (2.1%)	0 (0%)		5 (1.8%)	2 (1.7%)	3 (1.9%)		5 (1.8%)
Past miscarriage			0.914				0.916				0.582	
No	96 (75.6%)	93 (75.0%)		202 (74.0%)	180 (74.1%)	21 (75.0%)		202 (74.0%)	88 (75.9%)	113 (72.9%)		202 (74.0%)
Yes	31 (24.4%)	31 (25.0%)		71 (26.0%)	63 (25.9%)	7 (25.0%)		71 (26.0%)	28 (24.1%)	42 (27.1%)		71 (26.0%)
Past spontaneous abortion			0.784				0.324				**0.042**	
No	115 (90.6%)	111 (89.5%)		247 (90.5%)	221 (90.9%)	24 (85.7%)		247 (90.5%)	100 (86.2%)	145 (93.5%)		247 (90.5%)
Yes	12 (9.4%)	13 (10.5%)		26 (9.5%)	22 (9.1%)	4 (14.3%)		26 (9.5%)	16 (13.8%)	10 (6.5%)		26 (9.5%)
Past abnormal discharge			0.644				0.062				0.143	
No	79 (62.2%)	73 (58.9%)		161 (59.0%)	148 (60.9%)	12 (42.9%)		161 (59.0%)	74 (63.8%)	86 (55.5%)		161 (59.0%)
Yes	48 (37.8%)	50 (40.3%)		111 (40.7%)	94 (38.7%)	16 (57.1%)		111 (40.7%)	41 (35.3%)	69 (44.5%)		111 (40.7%)
Missing	0 (0%)	1 (0.8%)		1 (0.4%)	1 (0.4%)	0 (0%)		1 (0.4%)	1 (0.9%)	0 (0%)		1 (0.4%)
Past treatment for an STI			0.536				0.140				0.307	
No	74 (58.3%)	77 (62.1%)		163 (59.7%)	148 (60.9%)	13 (46.4%)		163 (59.7%)	73 (62.9%)	88 (56.8%)		163 (59.7%)
Yes	53 (41.7%)	47 (37.9%)		110 (40.3%)	95 (39.1%)	15 (53.6%)		110 (40.3%)	43 (37.1%)	67 (43.2%)		110 (40.3%)
HIV status			0.653				**0.011**				**0.045**	
Negative	41 (32.3%)	35 (28.2%)		83 (30.4%)	1 (0.4%)	0 (0%)		83 (30.4%)	40 (34.5%)	42 (27.1%)		83 (30.4%)
Positive	26 (20.5%)	26 (21.0%)		59 (21.6%)	79 (32.5%)	3 (10.7%)		59 (21.6%)	18 (15.5%)	41 (26.5%)		59 (21.6%)
Don't know	0 (0%)	1 (0.8%)		1 (0.4%)	48 (19.8%)	11 (39.3%)		1 (0.4%)	0 (0%)	1 (0.6%)		1 (0.4%)
Missing	60 (47.2%)	62 (50.0%)		130 (47.6%)	115 (47.3%)	14 (50.0%)		130 (47.6%)	58 (50.0%)	71 (45.8%)		130 (47.6%)

BV: bacterial vaginosis; TV: *Trichomonas vaginalis*. Significant p-values are represented in bold.

($p = 0.048$) and did not experience a past spontaneous abortion ($p = 0.042$). Within the *Candida*-negative group, the majority of the women were HIV-negative ($p = 0.045$).

Laboratory findings. Of the 273 pregnant women enrolled in this study, 251 were included in the BV analysis, 21 were unresolved and one indeterminate by the BD Max™ Vaginal assay. In addition, 271 were included for the *Candida* and TV analysis, one was unresolved and one was indeterminate by the BD Max™ Vaginal assay.

The percentage of pregnant women who tested positive for any pathogen was 81.6% (n = 223/273) (not shown). *Candida* was the most prevalent pathogen in this study with a prevalence of 57.2% (n = 155/271). The prevalence of BV was 49.4% (n = 124/251) and TV was detected in 10.3% (n = 28/271) of the women (Table 2).

BD Affirm™ VPIII compared to the BD Max™ Vaginal assay for diagnosing BV

The diagnostic performance of the BD Affirm™ VPIII in comparison to the BD Max™ Vaginal assay for the three pathogens is described in Table 2.

The BD Affirm™ VPIII assay showed a moderate sensitivity (79.8%) and a moderate specificity (80.3%) for diagnosing BV in all participants. However, the sensitivity was higher (82.6%) for BV in symptomatic women compared to asymptomatic women (Table 3).

BD Affirm™ VPIII compared to the BD Max™ Vaginal assay for diagnosing Candida spp. and TV

The BD Affirm™ VPIII assay had an excellent specificity for *Candida* spp. and TV of 97.4 and 100.00%, respectively; however, the assay exhibited poor sensitivities of 52.9 and 46.4%, respectively (Table 2).

According to the ROC curve analysis for BV (Table 2), the test was fairly good (area = 0.80) in distinguishing infected from uninfected individuals. The ROC curves for *Candida* spp. (Table 2) and TV (Table 2) were shown to be fair (area = 0.75 and 0.73, respectively).

This study showed that the BD Affirm™ VPIII assay has a lower diagnostic accuracy when compared to the BD Max™ Vaginal assay (nucleic acid amplification test).

Discussion

The correct diagnosis and treatment of BV, candidiasis and trichomoniasis is vital in pregnancy as poor diagnosis and treatment results in poor pregnancy

Table 2. Performance characteristics of the BD Affirm™ VPIII.

		Investigational test performance: BD Affirm™ VPIII						
Identification	Prevalence: BD Max™ Vaginal assay	Prevalence: BD Affirm™ VPIII	Sensitivity	Specificity	PPV (95% CI)	NPV (95% CI)	ROC Area
BV	49.4 (124/251)[a] (43.2–55.6)	47.6 (130/273) (41.7–53.6)	79.84 (71.69–86.51)	80.31 (72.33–86.84)	79.84 (73.38–85.05)	80.31 (73.99–85.41)	0.80
Candida spp.	57.2 (155/271)[b] (51.2–63.0)	31.5 (86/273) (26.2–37.3)	52.90 (44.73–60.96)	97.41 (92.63–99.46)	96.47 (89.86–98.83)	60.75 (56.65–64.71)	0.75
T. vaginalis	10.3 (28/271)[c] (7.2–14.6)	4.8 (13/273) (2.8–8.1)	46.43 (27.51–66.13)	100.00 (98.49–100.00)	100.00 (100.00–100.00)	94.19 (91.98–95.81)	0.73

BV: bacterial vaginosis; NPV: negative predictive value; PPV: positive predictive value; ROC: receiver operating characteristic.
Data are % (n/N) (95% confidence interval) or % (95% confidence interval).
[a]Twenty-one out of the 273 tests for BV were unresolved by the BD Max™ Vaginal assay Reference method and one was indeterminate. These were therefore excluded in the analysis.
[b]One out of the 273 tests for *Candida* was unresolved by the BD Max™ Vaginal assay Reference method and one was indeterminate. These were therefore excluded in the analysis.
[c]One out of the 273 tests for *T. vaginalis* was unresolved by the BD Max™ Vaginal assay Reference method and one was indeterminate. These were therefore excluded in the analysis.

Table 3. Performance of the BD Affirm VPIII assay in all participants compared to symptomatic participants.

	%Sensitivity (overall)	%Sensitivity (symptomatic)	%Specificity (overall)	%Specificity (symptomatic)	%PPV (overall)	%PPV (symptomatic)	%NPV (overall)	%NPV (symptomatic)
BV	79.84	82.61	80.31	61.76	79.84	74.51	80.31	72.41
Candida spp.	52.90	66.15	97.41	96.15	96.47	97.73	60.75	46.81
T. vaginalis	46.43	46.15	100.00	100.00	100.00	100.00	94.19	91.76

BV: bacterial vaginosis; NPV: negative predictive value; PPV: positive predictive value.

outcomes. We have observed a higher prevalence of BV (49.4%) in pregnant women in South Africa than previously reported (17.6%).[8] However, these studies were conducted approximately seven years ago (2012). The high prevalence of BV-observed in our population could be attributed to the following socio-demographic and behavioural characteristics. The majority of women (55.6%) in the BV positive group had reported having between two and four lifetime sex partners. Increased number of lifetime sex partners has been shown to be associated with increased risk of vaginal infections.[2] In addition, close to 80% of the BV-positive women had experienced their first sexual act at the age of 15 20 years. Early age of sexual debut could be associated with vaginal infections. The lack of condom use was also higher in the BV-positive women. The current study provides updated prevalence estimates of this syndrome in a population of South African pregnant women, thereby adding to the body of recent literature. The observed prevalence estimates for *Candida* spp. (57.2%) and TV (10.3%) in our population were similar to other cohorts of South African pregnant women.[10,22] In this study, less than half of the women who tested positive for BV, *Candida* spp. and TV were symptomatic (37.1, 41.9 and 46.4%, respectively) and this is consistent with previous reports of a high burden of asymptomatic sexually transmitted infections (STIs).[16,23] If a test-and-treat approach had been used, 78.8% of women in the current study with undiagnosed infections could have been adequately treated.

Previous studies have shown that the syndromic management guidelines are no longer adequate for the treatment of STIs in women, as vaginal discharge alone is a poor predictor.[14,15,23,24] Alternate strategies are required, such as the test-and-treat approach, particularly in pregnant women.

POCTs have the potential to bridge the gap in the diagnosis of vaginosis and vaginitis, especially in high priority populations such as in pregnant women. It is well noted that the cost of POCTs is a barrier to their use.[25] Therefore, manufacturers of the POCTs need to be engaged by policy makers on reducing the costs of these assays, especially with the promise of large-scale implementation.

Previous studies on POCTs for vaginal pathogens have shown that the BD Affirm™ VPIII assay has the potential to become the ideal one-assay POCT for diagnosing vaginosis and vaginitis in a clinic setting.[18–20] To date, there is no other single cartridge test that detects BV, *Candida* spp. and TV. The test is therefore superior, with the added advantage of being easy to perform and having a short time-to-detection. The current study compared the BD Affirm™ VPIII to a leading Real-Time molecular laboratory-based assay the BD Max™ Vaginal assay. The BD Max™ vaginal assay has been FDA approved since 2016 and uses a newer more updated technology for the detection of BV, *Candida* spp. and TV. This study showed that the BD Affirm™ VPIII assay has a lower diagnostic accuracy when compared to the BD Max™ Vaginal assay (nucleic acid amplification test). These findings are similar to the findings of Cartwright et al.[18]

In our study the assay showed a moderate sensitivity for BV but a poor sensitivity for *Candida* spp. and TV. The higher sensitivity for BV could be due to the fact that the BD Affirm™ VPIII assay detect *G. vaginalis*, which has been shown to be prevalent in more than 80% of BV cases.[26] The poor sensitivity for *Candida* spp. and TV could be due to the fact that the assay, although a DNA-based test, is a non-amplified test and the comparator assay (the BD Max™ Vaginal assay) is an amplification-based assay.

The test exhibited an excellent specificity for *Candida* spp. and TV (97.41 and 100.00%, respectively) but showed only a moderate specificity for BV (80.31%). The poor sensitivity of the BD Affirm™ VPIII assay indicates that the test cannot be used as screening test but the excellent specificity for *Candida* spp. and TV indicates that the test can be used as a confirmatory test when vaginitis is suspected. The low specificity for BV could be due to the fact that since the development of the assay there have been newer more diverse populations of bacteria implicated in BV rather than *G. vaginalis* alone, such as *Mobiluncus* spp., *Bacteroides* spp. and *Atopobium* spp.

Thompson et al.[27] also reported a lower specificity for the BD Affirm™ VPIII assay when compared to the BD Max™ Vaginal assay, suggesting that the Affirm detects *G. vaginalis* only whereas the BD

Max™ Vaginal assay detects a combination of microorganisms.

The BD Affirm™ VPIII assay was developed in the 1990s and further enhancements are required for increased performance and acceptance as a POCT. The test could be optimized to include other pathogens implicated in BV and although easier to perform than a wet mount, the test requires skilled staff as there are many pre-analytical steps which can introduce error. If the system included the dispensing of reagents in the automation, the test would be more widely accepted as a POCT. This assay is still the only POCT which tests both vaginosis and vaginitis in a single device.

Limitations

This study had the following limitations. The study was only conducted at one antenatal clinic which is not a representation of the whole antenatal population in South Africa. However, patients attending the hospital reside in the greater Durban Metropolitan region. Due to the cross-sectional design of the study, the women were not followed up to assess test-of-cure and the implications of infection on pregnancy outcomes. Following up the women until cure would have allowed us to shed some light on the usefulness of syndromic management in this population. The study used the BD Max™ Vaginal assay as the comparator assay which has been shown to have sensitivities and specificities less than 100% when compared to conventional methods.

Despite these limitations, the strengths of the study are as follows: (1) this study has provided data on the prevalence of BV, *Candida* spp. and TV in an antenatal population in which there is currently a lack of data in South Africa and (2) the study has also provided evidence that a large proportion of pregnant women who are asymptomatic carry infection, which re-affirms the limitation of the syndromic management approach, and lastly (3) this study was the first to report on the performance of the BD Affirm™ VPIII assay POCT in an antenatal population in South Africa.

Conclusions

In summary, this study found a high prevalence of BV in the antenatal population, and was consistent with previously reported rates for *Candida* spp. and TV infection. The BD Affirm™ VPIII assay is unsuitable as a screening test for vaginal infections as evidenced in this study. However, this assay is still the only commercially available POCT that simultaneously detects BV, *Candida* spp. and TV in a single cartridge. With further enhancements, it has the potential to become an ideal screening POCT.

Lastly, the study found a very high proportion of women with asymptomatic BV and asymptomatic vaginitis attending antenatal care. Urgent intervention is required to address the inadequacy of syndromic management in this high priority population.

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