A Novel Study to Detect and Quantify Microorganisms Associated with Bacterial Vaginosis from Urine Samples

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PREFACE

The experimental work described in this thesis was conducted at the School of Clinical Medicine Laboratory, Nelson R. Mandela School of Medicine, University of KwaZulu-Natal, Durban, South Africa, from February 2018 to February 2019, under the supervision of Dr Nathlee Abbai.

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PERMISSION TO SUBMIT

As the candidate's supervisors, we have read the thesis and have given our approval for submission for examination

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ABSTRACT

Bacterial vaginosis (BV) is the most common vaginal condition found in women of reproductive age. The lack of published data on the detection of BV-associated pathogens from urine, a non-invasive sample, lends novelty to the present study. This study aimed to detect and quantify *Gardnerella vaginalis, Prevotella bivia*, *Atopobium vaginae* and *Lactobacilllus crispatus* from urine, as an alternative non-invasive method to vaginal swabs from pregnant women using droplet digital PCR (ddPCR). A total of n=100 DNA samples (50 paired urine and swabs) were tested. The samples were stratified as BV negative and positive using the BD MAX Vaginal panel assay (Becton Dickinson). Total DNA was extracted from urine (10 ml) and swabs (1 ml) using the PureLink Microbiome Kit (ThermoFisher Scientific). The concentration of extracted DNA for urine and swab samples was determined using the Nanodrop Spectrophotometer (ThermoScientific). Droplet digital PCR was used to determine the absolute quantification of the pathogens using commercially available primer and probe sets. *G. vaginalis* was observed as the most abundant microorganism, followed by *A. vaginae* and *P. bivia* in the BV positive samples. When comparing abundance of microorganisms across urine and swab, it was shown that there was no significant difference across both sample types in the BV negative group. A significant difference in the BV positive group ($p=0.004$) was only observed for *A. vaginae.* Good correlation between the urine and swab was observed for *G. vaginalis* ($R=0.63$, $p<0.0001$), *L. crispatus* ($R=0.71$, $p<0.0001$) and *P. bivia* ($R=0.50$, $p<0.0001$). However, a weak correlation across both sample types was observed for *A. vaginae* ($R= 0.21$, $p=0.001$). We observed that urine has the potential to serve as an alternative sample collection method to detect BV-associated bacteria. In addition, the data generated in this study provides a basis for the development of ddPCR as a diagnostic tool for BV.

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ABBREVIATIONS

˚C	Degree Celsius
<	Less than
>	Greater than
µl	Microliter
A. vaginae	*Atopobium vaginae*
BD	Becton Dickinson
BREC	Biomedical Research Ethics Committee
BV	Bacterial Vaginosis
Ch1	Channel 1
CNV	Copy number variation
CVL	Cervicovaginal lavage
ddPCR	Droplet digital PCR
DNA	Deoxyribonucleic acid
dUTP	Deoxyuridine triphosphate
e.g.	Example
FISH	Fluorescence in-situ hybridization
G. vaginalis	*Gardnerella vaginalis*
HIV	Human Immunodeficiency Virus
ID	Identification number
L. crispatus	*Lactobacillus crispatus*
Min	Minute
ml	Millilitre
n	Number
ng	Nano gram
P. bivia	*Prevotella bivia*
PCR	Polymerase Chain Reaction
PID	Pelvic inflammatory disease
pH	Potential Hydrogen
PROM	Premature rupture of membranes
qPCR	Quantitative PCR
STIs	Sexually transmitted infections
USA	United states of America
UTI	Urinary tract infection

CHAPTER ONE

1. INTRODUCTION

Bacterial vaginosis, also known as BV, is a clinical condition which is distinguished by alterations of the vaginal microbiota, where the predominant normal lactobacilli species are replaced by diverse communities of anaerobic and facultative bacteria (1-3). These bacteria include: *Gardnerella vaginalis, Atopobium* species, *Prevotella, Mobiluncus, Mycoplasma* and numerous other pathogens (2-6).

The prevalence of BV in women (both pregnant and non-pregnant population) depends on the clinical setting, sociodemographic factors, diagnostic criteria, gestational age, vitamin D deficiency, smoking and numerous other factors (3, 7, 8). Within Africa, South Africa has the highest prevalence of BV (1). The prevalence of BV in pregnant women ranges from 14% to 21% in Western countries and 13.6–18% in Asian countries (9). At baseline, 31.03% of the non-pregnant women were diagnosed as BV positive in Durban, South Africa (10).

The common clinical symptom of BV is the occurrence of an abnormal malodorous vaginal discharge. Implications of untreated BV include pelvic inflammatory disease (PID) and increased susceptibility to sexually transmitted infections (STIs) and human immunodeficiency virus (HIV) (3, 7). These studies have further shown that the prevalence of HIV infection appears to correlate with increasing severity of BV (7, 11, 12). Untreated BV is also associated with severe pregnancy outcomes that include late miscarriages, preterm labour, premature rapture of membranes (PROM), post-partum endometritis, low birth weight infants and a host of other complications (6, 13, 14).

Bacterial vaginosis is classically diagnosed using the gold standard, Nugent Scoring System (5-7, 14, 15). Other methods of diagnosis include Amsel Criteria and a host of nucleic acid amplification tests (qualitative and quantitative polymerase chain reaction (PCR)) (3, 5, 6, 14). However, the limitation of current diagnostic methods is the use of vaginal swabs. Vaginal swabs are not ideal for use as a diagnostic specimen during pregnancy since it is considered to be an invasive method of sample collection. In contrast, urine is a non-invasive sample.

Recent studies have reported on the use of PCR to diagnose BV, however, these studies have used vaginal swabs to detect the BV pathogens using PCR (16-20). In majority of health care clinic settings, pregnant women are routinely screened for the presence of glucose and leukocyctes in their urine. As a result of urine being collected at every visit, it makes urine easy to be collected for BV testing. The aim of this study was to detect and quantify microorganisms associated with BV by droplet digital PCR (ddPCR) from urine and vaginal swab samples of pregnant women. This study will be the first to provide data on the detection and quantification of BV pathogens from urine collected from South African pregnant women.

CHAPTER TWO

2. THE LITERATURE REVIEW

2.1. Definition of Bacterial Vaginosis

BV is the most common lower genital tract disorder in women of reproductive age (1). The common symptoms of BV include: vaginal pain, itching, abnormal vaginal discharge and/or a burning sensation (3). Bacterial vaginosis is defined as a vaginal condition in which the dominant healthy Lactobacilli are replaced by a variable mixture of anaerobic and facultative bacteria (2). The exact composition of the bacterial microbiota cannot be identified in woman as it varies from each women, however, the common BV-associated pathogens include *Gardnerella*, *Atopobium*, *Prevotella*, *Megasphaera*, *Sneathia* and *Mycoplasma* (2-6).

2.2. Epidemiology of BV

The aetiology of BV and the reason for the differing prevalence across the world is unclear. In a systematic review by Kenyon *et al* 2013 (1), the global epidemiology of BV was attempted to be summarized using the data from peer-reviewed publications that used the Nugent scoring criteria to diagnose the patient with BV. In Sub-Saharan Africa, BV prevalence was the highest in the Southern and Eastern parts of Africa. In Africa, South Africa had the highest prevalence of BV. The prevalence of BV in Durban was 52% and in rural KwaZulu-Natal and Khayelitshia the prevalence of BV was around 58% (1). Gambia had the second highest prevalence of BV at 37% followed by Uganda at 34%. The lowest prevalence of BV was 12% in Maputo, Mozambique (1). During pregnancy, the reported prevalence of BV, ranges from 4.9% to 49% (9). The prevalence of BV in women (both pregnant and non-pregnant population) depends on the clinical setting, sociodemographic factors, diagnostic criteria, gestational age and numerous other factors (8). The prevalence of BV in pregnant women ranges from 14% to 21% in Western countries and 13.6–18% in Asian countries (9). In South Africa, the prevalence of BV was reported to be 17.6% in a population of pregnant women (21).

2.3. Risk factors for BV

There are numerous risk factors that are associated with BV. Many of these factors fall under the lifestyle category. Sexual activity plays a major contributory role in BV. The lack of condom use, multiple sexual partners or new sexual partners can result in BV acquisition. Sexual intercourse alters the normal vaginal microbiome and introduces new vaginal microorganisms. Other reported risk factors include intrauterine devices, black ethnicity, vaginal douching; smoking, lack of male circumcision, poverty, poor education, low vitamin D levels and genetic variation of a wide range of host genes (3, 7, 22)

2.4. Consequences of BV in pregnant women

Bacterial vaginosis is a lower vaginal tract condition that causes numerous problems pre and post pregnancy. In pregnancy, BV can result in late miscarriages, preterm labour, premature rupture of membranes (PROM), post-partum endometritis, low birth weight infants and a host of other complications (6, 13, 14). BV is also associated with the increased risk of acquisition of human immunodeficiency virus (HIV) and sexually transmitted infections (STIs) such as gonorrhoea, trichomoniasis and herpes simplex virus type 2 (7). Several studies show that women with BV have a higher incidence of HIV infection. The prevalence of HIV infection also correlates with increasing severity of BV (3, 7, 11, 12).

2.5 Microorganisms associated with BV

As mentioned earlier, the common BV-associated pathogens include *Gardnerella*, *Atopobium*, *Prevotella*, *Megasphaera*, *Sneathia* and *Mycoplasma* (2-6). Some of these microorganisms are described in greater detail below:

2.5.1. Gardnerella Vaginalis

2.5.1.1. Morphological characterization

In 1953, *Gardnerella vaginalis* (*G. vaginalis*), was first isolated by Dr. S. Leopold (23). *G. vaginalis* belongs to the family bifidobacteriaceae (3). *G. vaginalis* has cells which are small, non-motile, non-encapsulated, non-spore-forming, pleomorphic rods and has an average dimension of 0.4 by 1.5 µm. When Gram-stained, the cells appear as Gram variable as a result of its peptidoglycan layer (Fig 1a). *G. vaginalis,* is a fastidious microorganism and requires the presence of carbon dioxide for growth (24).

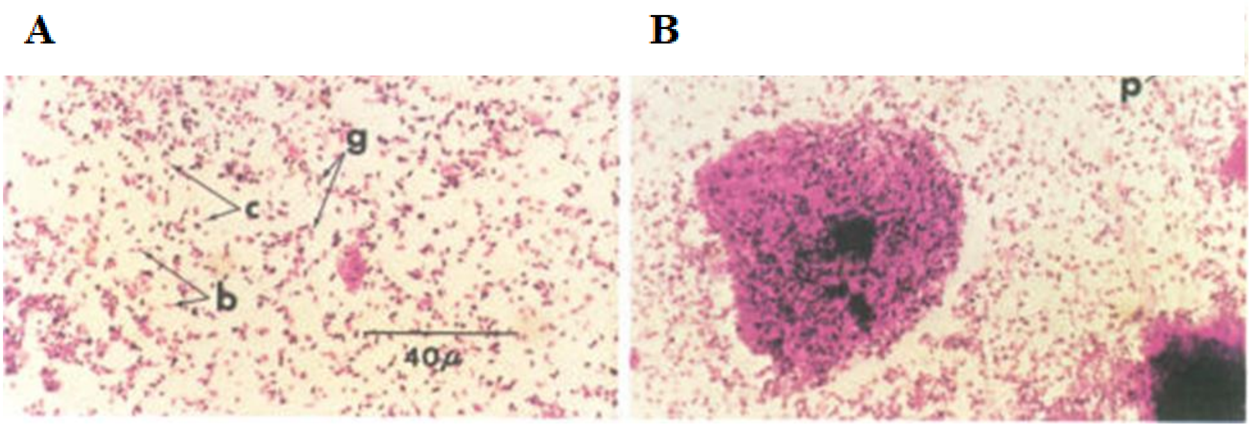


Fig. 1 (a) displays the Gram stain of *G. vaginalis*. It appears to be Gram variable and pleomorphic rods are observed. (b) Displays a clue cell. Clue cells are the clustering and adherence of *G. vaginalis* on the vaginal epithelial cell (25).

2.5.1.2. Role in BV

Gardnerella has been one of the most consistent microorganisms associated with BV. It has been reported that vaginal mucosa polymicrobial biofilms are associated with BV (3, 4, 26). *Gardnerella* adheres to the vaginal epithelial cells (known as a clue cell) and forms a biofilm on the walls of the vagina (Fig. 1b). This mechanism permits the tolerance of *Gardnerella* to lactic acid and hydrogen peroxide which are produced by lactobacilli. *G. vaginalis* can also be found in a healthy vaginal microbiome (in lower concentrations), thereby suggesting that *G. vaginalis* does not always form a biofilm. Researchers have hypothesized that different *G. vaginalis* strains may contain different virulence potentials (4). Recently, a comparative analysis of 17 clinical isolates of *G. vaginalis* suggested that the species can be subdivided into 4 clades and that there may be multiple species of *G. vaginalis*. Ahmed et al found that the degree of diversity among the strains was exceptionally high for a single species (27).

2.5.2. Atopobium vaginae

2.5.2.1. Morphological characterization

Atopobium vaginae (*A. vaginae*) was first associated with bacterial vaginosis in 2004 (28). However, *Atopobium* was discovered in 1999 by Rodriguez as a common commensal of the woman's vagina (29). *A. vaginae* belong to the family *Coriobacteriaceae* and are represented by anaerobic, small, elongated, Gram positive cocci bacteria (3). *A. vaginae* are cocci that appear singly, in pairs or in short chains (Fig. 2). *Atopobium* is not used as one of the key microorganisms in the Nugent scoring system, as the variable cells can be camouflaged and overlooked during microscopic examination. This microorganism is a fastidious bacterium and grows slowly in agar media thereby making it difficult to culture (3).

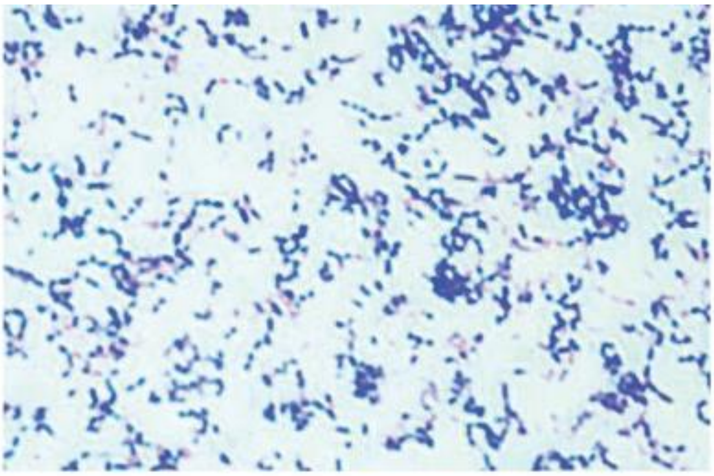


Fig. 2 Micrograph of a Gram stain showing Gram-positive bacteria, with *A. vaginae* visible as single cells, in pairs or short chains (30).

2.5.2.2. Role in BV

Atopobium has only been reported about 10 years ago to be involved in BV. Swidsinski *et al* (2013) found vaginal biopsies with vaginal biofilm to be positive for *G. vaginalis* and *A. vaginae* when using fluorescent probes (31). *Gardnerella* is capable of adhering strongly to the vaginal epithelial cells and forms vaginal biofilms. *G. vaginalis* initiates the colonization of the vaginal epithelium and creates the environment for other BV-associated pathogens to attach, an example is *A. vaginae.* Molecular studies demonstrated that the probable role of *Atopobium* in BV could be in the establishment of the biofilm together with *G. vaginalis. A. vaginae* has been reported to be found in 80-90% of relapse cases of BV (32). Therefore, *A. vaginae* is considered one of the important microorganisms associated with BV. However, whether *A. vaginae* plays a more central role in the pathogenesis of BV has not been established (3). A study conducted by Bradshaw et al (2006) reported that *A. vaginae* is strongly associated with recurrent BV and that it is rarely detected without *G. vaginalis* (33, 34).

2.5.3. Prevotella species

2.5.3.1. Morphological characterization

Prevotella species belong to the family *Prevotellaceae*. These bacteria are anaerobic, Gram negative, pleomorphic, non-motile rods that were previously classified as *Bacteriodes* (3). Studies have shown that *Prevotella* make up the "*Bacteriodes* morphotype" which is used to determine Nugent scores and that species of the genus *Bacteriodes* are rare. In comparison to other species of *Prevotella, Prevotella bivia* is considered to be significantly associated with BV (3).

2.5.3.2. Role in BV

Prevotella species produce polyamines during metabolic activity which increase the vaginal pH. The increase in vaginal pH enhances the growth for other anaerobic BV-associated pathogens. The production of ammonia by this bacteria was shown to enhance the growth of *G. vaginalis*. A synergistic relationship exists between both *G. vaginalis* and *Prevotella* as the amino acid produced by *G. vaginalis* is utilized by *Prevotella.* Therefore, *Prevotella* species have a positive association with bacterial vaginosis (3).

2.6. Microorganisms associated with a healthy vagina

2.6.1. Lactobacillus species

2.6.1.1. Morphological characterization

Lactobacillus species belong to the family *Lactobacillaceae*. *Lactobacillus* are Gram positive, facultative anaerobic, rod shaped and non-spore forming bacteria (Fig. 3). In around 70% of women, a *Lactobacillus* species is dominant, although that has been found to vary between American women of European origin and those of African origin, the latter group tending to have more diverse vaginal microbiota. Similar differences have also been identified in comparisons between Belgian and Tanzanian women (35). There are four major types of *Lactobacillus* species that play a role in BV:

Lactobacillus crispatus, *Lactobacillus iners*, *Lactobacillus gasseri*, or *Lactobacillus jensenii*. *Lactobacillus crispatus* (*L. crispatus*) is reported to be the most dominant of the four species to be found in a healthy vaginal microbiome (36). *Lactobacillus gasseri,* and *Lactobacillus jensenii* also play a role in keeping the vaginal environment healthy and free from pathogens. However, it has been reported that a *Lactobacillus iners* (*L. iners*) dominated vaginal community seems to be less stable than the other species and is more frequently associated with vaginal dysbiosis (Fig. 4). *L. iners* is reported to be a transitional bacteria since it can be found in the healthy vaginal microbiome as well as in a non-healthy vaginal microbiome (i.e. BV positive patient) (37)

L. cripatus, is the dominant component in the healthy vaginal microbiota in women all across the world. *Lactobacillus* maintains a healthy vagina as it produces a substantial amount of lactic acid and hydrogen peroxide that sustains an acidic vaginal environment of a pH of 3.5-4.5. A maintained acidic environment inhibits the overgrowth of pathogenic microorganisms such as the BV causing bacteria (*G. vaginalis, A. vaginae* etc.) (2, 36, 37).

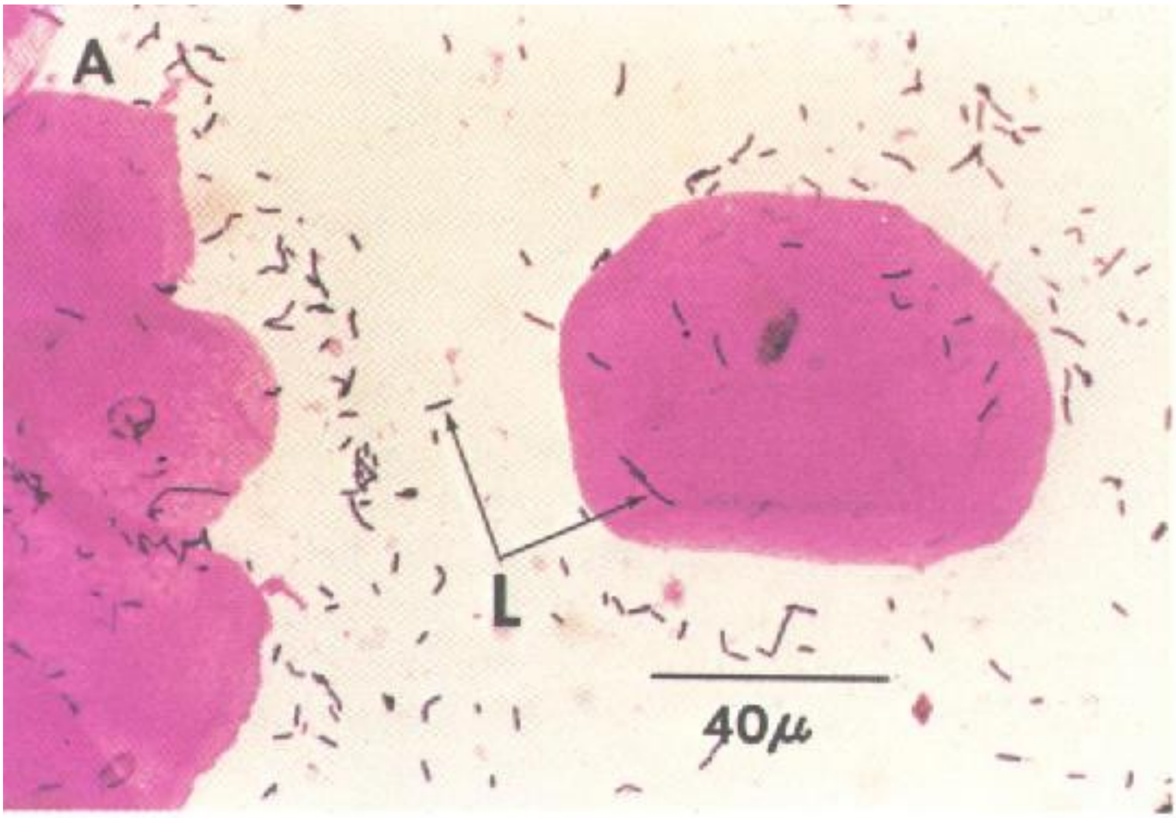


Fig. 3: Depicts large Gram positive rod shaped *Lactobacillus* species (25).

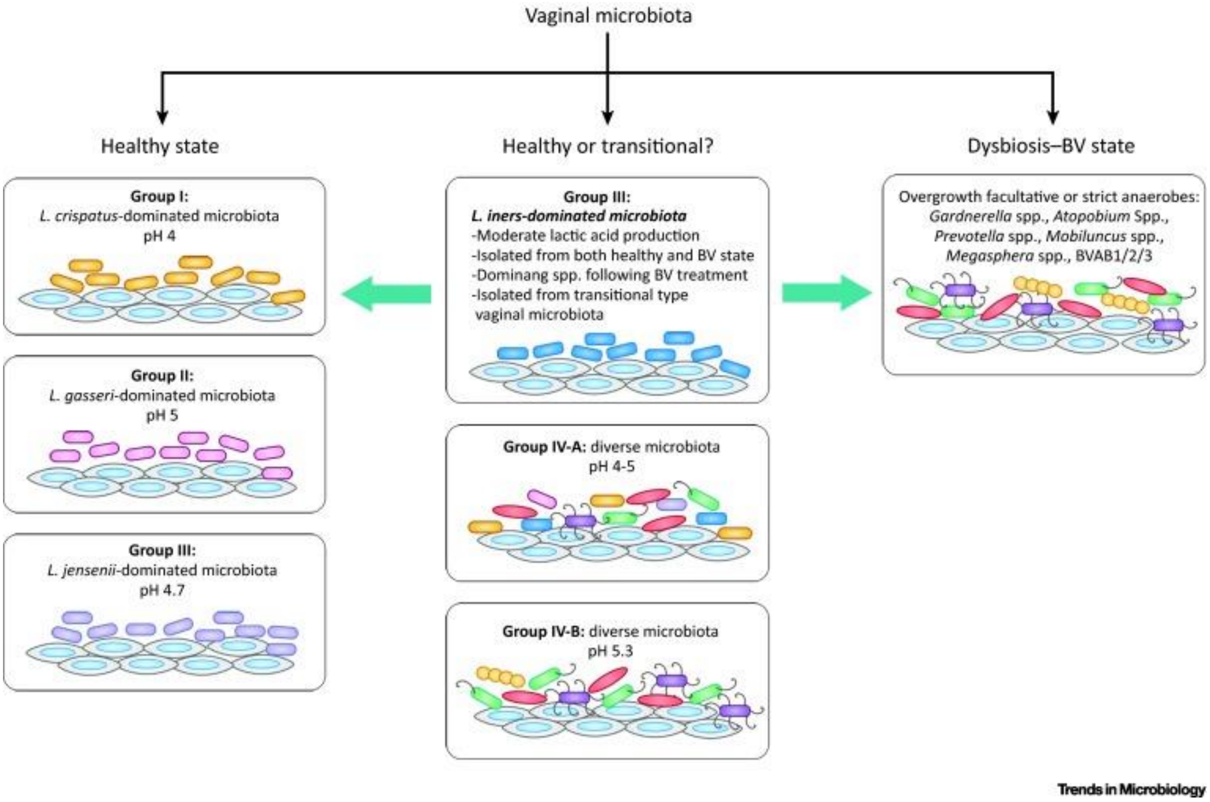


Fig. 4 Schematic diagram illustrating the different species of Lactobacilli and the role they play in the vaginal microbiota. *L. crispatus, L. gasseri* and *L. jensenii* maintain the healthy vaginal state whilst *L. iners* is a transitional bacteria. *L. iners* can be found in both the healthy and non-healthy vaginal microbiota (37).

2.7. Diagnosis of BV: Non-molecular biology based methods

The clinical diagnosis of BV is identified by the presence of at least three out of the four Amsel criteria which include: a raised vaginal pH (above 4.5), an increased amount of vaginal discharge (milky in colour), the presence of clue cells when vaginal fluid smears are examined by a microscope and an amine odour after 10% potassium hydroxide has been added (3, 5, 38). The advantages of the Amsel criteria are as follows: method of detection does not require intensive training; it is cost effective as well as easy and fast to perform. However, this method is not considered ideal for BV diagnosis due to its low specificity (5).

An alternative clinical diagnosis for the detection of BV is Gram staining of vaginal smears. This method is evaluated according to the Nugent scoring system ("gold standard"). This system scores vaginal smears from 0 to 10, depending upon the numbers of three bacterial morphotypes which are seen on the slide that contains the smear. These bacteria include *Gardnerella vaginalis/Bacteriodes, Mobiluncus* and Lactobacilli (3, 5, 6, 15). A score of 0–3 represents normal vaginal microbiota (BV negative), a score of 4–6 represents intermediate vaginal microbiota, and a score of 7–10 is considered as diagnostic for BV (BV positive) (7, 15). Although this method of detection is fast and easy to perform, it has the following disadvantages: the vaginal smears used remain subjective, this method is user dependent and it requires a trained laboratory personnel to prepare and read the slides. Thus, it is not a suitable choice of method to be implemented in primary health care facilities.

2.8. Diagnosis of BV: Molecular Biology based methods

The main microorganisms associated with BV are obligate anaerobes that are either difficult to recover or unrecoverable using culture-based methods. This makes the true evaluation of the vaginal microbiome using microbial culture methods challenging. The knowledge on the microbial ecosystem of BV and the association of different bacteria with BV, has increased due to advances in molecular techniques. These molecular techniques include: conventional PCR, real-time PCR, multiplex PCR, broad-range bacterial 16s rDNA PCR, and fluorescence *in situ* hybridization (FISH) (3, 6).

Polymerase Chain Reaction assays of vaginal-fluid samples provides information on the bacterial compositions in the female vagina from women with and without BV. Development of PCR-based assays can be used to detect and quantify genital tract organisms (e.g. mycoplasmas cannot be detected using Gram stain). This is useful since *Mycoplasma hominis* is suggested to be associated with BV and cannot be identified using microbial culture methods. As per the BD MAX assay which is an FDA approved multiplex PCR assay for the quantitative detection of BV, the following microorganisms form part of the panel: *Lactobacillus crispatus* 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. A study done by Dhiman *et al* 2016 (39), showed that PCR has a higher sensitivity for the diagnosis of BV when compared to Nugent scoring. The predisposition of specific bacteria towards the development of BV or the pathological outcomes of BV can be determined using PCR. PCR-based assays offers the ability to be less subjective when diagnosing BV as compared to alternative methods. This is because PCR assays can be developed to provide quantitative analysis (copy number) for specific types of organisms in the genital tract (3, 14).

2.9. Droplet Digital Polymerase Chain Reaction

2.9.1 What is ddPCR?

Droplet digital PCR is an advanced quantitative PCR assay that relies on water-oil emulsion droplet technology. It is an ultrasensitive technology that provides absolute nucleic acid quantification (40, 41).

2.9.2 The process of ddPCR

Figure 5 shows the schematic workflow of ddPCR. As shown in figure 5, the sample and the test reagents are loaded into the eight channel droplet generator cartridge. Thereafter, a vacuum is applied to draw the sample and oil. In approximately 2 minutes the eight samples are converted into eight sets of 20 000 droplets. The surfactant-stabilized droplets are transferred to a 96-well PCR plate. The plate is then inserted into a conventional thermocycler for droplet PCR amplification. The droplets are assigned colours to indicate if they are negative or positive which is based on their fluorescence amplitude (40-42). Each droplet in a sample is plotted on a graph of fluorescence intensity versus droplet number. All positive droplet are scored as positive (above threshold intensity) and each is assigned a value of 1. All negative droplets (below the threshold) are scored as negative and assigned a value of zero. This counting technique provides a digital signal from which to calculate the starting targeting DNA concentration by a statistical analysis of the numbers of positive and negative droplets in a given sample. ddPCR from a multiplex experiment in which two targets are PCR amplified can also be viewed as a 2 dimensional plot in which FAM fluorescence is plotted versus HEX fluorescence for each droplet.

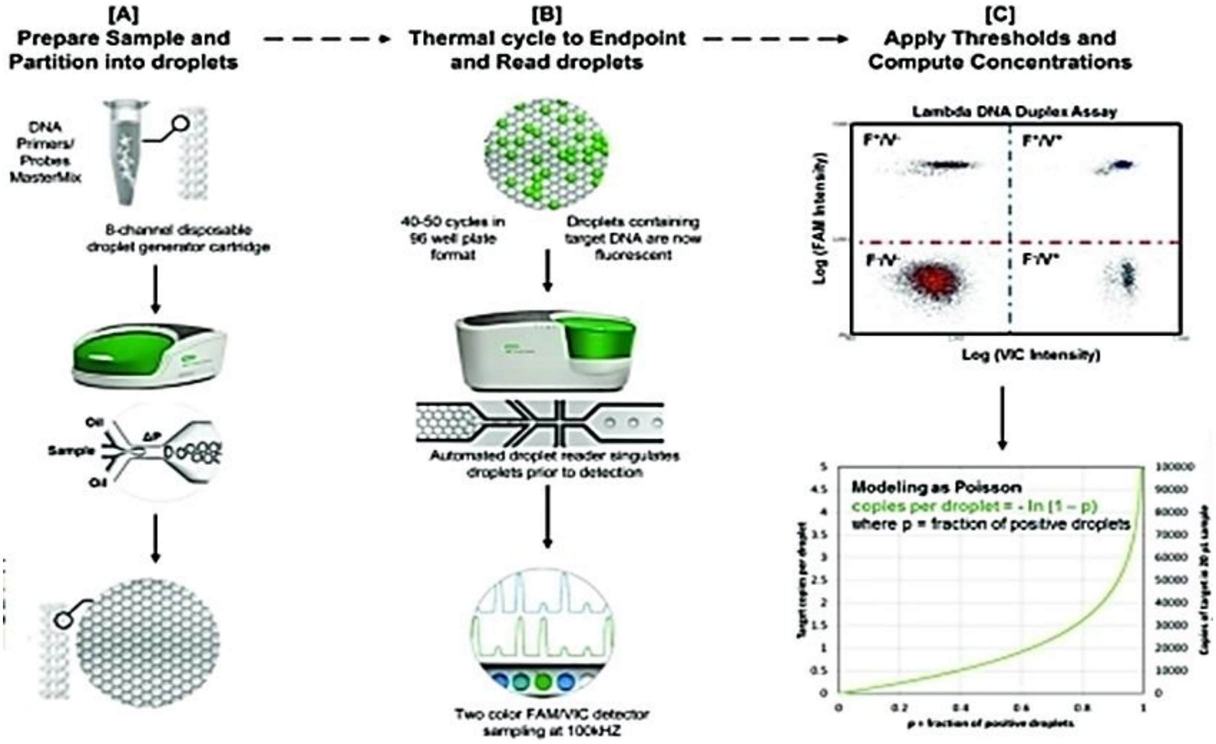


Fig. 5: Schematic representation of the workflow of droplet digital PCR (42).

2.9.3. Application of ddPCR

The application for ddPCR covers various areas of biology. The first application that ddPCR is used for is liquid biopsy. Liquid biopsy is a non-invasive test that detects cancer cells from DNA which is shed from tumours in the blood. Target circulating tumour DNA are present at low levels in a complex background of cell-free DNA. The high level of sensitivity permits ddPCR to accurately detect and quantify rare sequences in the presence of abundant targets. The second application in which ddPCR is used is for copy number variation (CNV). Over 10% of the human genome is composed of CNVs that contain sequences larger than 1 kb. Droplet digital PCR has been demonstrated to be well suited for high-throughput studies that profile CNV (43). Droplet digital PCR can provide accurate CNV quantification in single wells. The precision and sensitivity of ddPCR technology allows the system to distinguish small changes much more readily. Furthermore, the accuracy of ddPCR is less sensitive to changes in amplification efficiency, consequently creating large inaccuracies in qPCR measurements.

A third application for the use of ddPCR is gene expression. The precision of ddPCR provides higher resolution in gene expression measurements. Droplet digital PCR allows scientists to accurately and precisely quantify the finer changes in expression levels less than twofold. Droplet digital PCR can also be used to increase the accuracy and efficiency of next generation sequencing (44). A final application that ddPCR can be used for is the microbiome analysis and pathogen detection. This is currently the most widespread use of ddPCR in microbiology. The analysis of microbiome and pathogen detection requires the detection and quantitation of low-abundance microorganisms in a complex background. Droplet digital PCR is used in pathogen detection for detecting and monitoring viral loads, microbial drug resistance as well as for the detection of pathogens in food (44).

2.9.4. Advantages and disadvantages of using ddPCR

When compared to qPCR, ddPCR has the following advantages: absolute quantification without the need for a standard curve, less affected by sample inhibitors, not affected by poor amplification efficiency, provides more precision and improved detection of low-copy number variants (44). Droplet digital PCR reduces the overall cost, uses lower sample and reagent volumes when compared to other molecular methods whilst still maintaining the overall sensitivity and precision. Several other benefits of using ddPCR include: absolute quantification (provides an absolute count of target DNA copies per input sample) without the need of generating a standard curve, lower equipment cost as well as reduced consumable costs. Other benefits of ddPCR include: High throughput (simple and easy to use workflow with 96 sample throughput which does not require multiple dilution steps) and it also eliminates PCR efficiency bias (error rates are reduced by removing the reliance on amplification efficiency of qPCR) (42).

Despite the several advantages outlined above, ddPCR may also be accompanied with some disadvantages that include: limited reaction mixture volume, smaller dynamic range, molecular dropout, less accurate quantification of larger amplicons and lower throughput when compared to qPCR.

2.10. Current study: Rationale, aims and objectives

2.10.1. Study Rationale

Currently, there is a lack of published data on the diagnosis of BV from urine samples of pregnant women in our setting, thereby making this study novel. The detection of BV-associated pathogens from urine offers a much more comfortable, convenient and less biased sampling method when compared to vaginal swabs (downstream processing is dependent on amount of material on swab) especially for pregnant women. In majority of the health care clinic settings, pregnant women are screened for glucose and leukocyctes in urine (45). Therefore, it makes urine easy to be collected for BV testing. In addition, there is also limited information on the use of ddPCR for absolute quantification of BV-associated microorganisms. The data generated from this study will add to the growing body of literature regarding the methods of diagnosis for BV. If urine is found to be a feasible sample for diagnosis of BV, this could inform the development of newer testing platforms for this syndrome which could lead to the development of ddPCR using urine as a diagnostic test for BV.

2.10.2. Aims of the study

The aim of this study is to detect and quantify microorganisms associated with bacterial vaginosis by droplet digital PCR from urine and vaginal swab samples of pregnant women.

2.10.3. Study Objectives

The first objective of this study was to select paired urine and swab samples from pregnant women who were diagnosed as BV positive and BV negative**.** The second objective was to extract DNA from urine and swabs using a commercially available kit. The third objective was to detect and quantify *Gardnerella vaginalis, Atopobium vaginae, Prevotella bivia* (pathogenic bacterium) and *Lactobacillus crispatus* (normal vaginal microbiota) from the paired DNA samples by droplet digital PCR. These microorganisms were selected since they are strongly associated with BV. The fourth objective was to compare the abundance of each BV pathogen in the urine and swab samples. Lastly, the final objective was to rank the abundance of each BV pathogen.

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Ethical statement

Full ethics approval for the present study was granted by the Biomedical Research Ethics Committee (BREC) of the University of KwaZulu-Natal (BE276/18) (Appendix 2).

3.2 Study setting and population

This study is a sub-study of a larger study. For the larger study, pregnant women 18 years and older, willing to provide written informed consent, willing to provide biological samples and willing to be tested for vaginal pathogens were recruited from the King Edward VIII hospital in Durban, South Africa between November 2017 and April 2018 (Appendix 2). Data on socio-demographic, behavioral and clinical information was collected from the study participants using a structured questionnaire. Each woman provided a self-collected vaginal swab and urine sample. The vaginal swabs were collected before the urine sample. The vaginal swabs were eluted in 10% phosphate buffered saline (PBS) and the recovered suspension was stored at -20˚C until further use. Urine samples were subjected to DNA extraction on the day of collection and the recovered DNA was stored at -20˚C until further use. The BV status of the enrolled women was determined using the BD MAX Vaginal Panel assay (Becton Dickinson) on the self-collected swabs. 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. Results are qualitative and are reported as a result for Bacterial Vaginosis {by using an algorithm that calculates the ratio of *Lactobacillus crispatus* (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)}.We did not use the Nugent scoring since we found that this technique did not work with self-collected vaginal swabs as collected in this study due to inadequate sample material on the majority of the swabs.

Women who presented with symptoms of abnormal vaginal discharge 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).

3.3 Sample selection

For this sub-study, a total of n=50 paired urine and swab DNA samples was used (25 per group). The study sample set included n=25 BV negative samples and n=25 BV positive samples for both urine and swab. Women for this sub-study were selected at random according to their BV status (BV negative or positive) based on the BD MAX results.

3.4 Laboratory testing

3.4.1. DNA isolation

Total DNA was extracted from urine (10 ml) and swabs (1 ml) using the PureLink Microbiome Kit (ThermoFisher Scientific). The concentration of extracted DNA for urine and swab samples was determined using the Nanodrop Spectrophotometer (ThermoScientific).

3.4.2. Droplet Digital PCR

Droplet digital PCR is an advanced quantitative PCR assay that relies on water-oil emulsion droplet technology, and offers the benefits of minimal sample and reagent volumes when compared to other methods, whilst still maintaining the overall sensitivity and precision. Furthermore, ddPCR determines absolute quantification i.e. absolute count of target DNA copies per input sample without the need of generating a standard curve (40, 41).

TaqMan probes (Thermo Fisher) were used to quantify *Gardnerella vaginalis* (Assay ID: Ba04646236_s1), *Prevotella bivia* (Assay ID: Ba04646278_s1), *Atopobium vaginae* (Assay ID: Ba04646222_s1) and *Lactobacillus crispatus* (Assay ID: Ba04646245_s1). A total of 2.5µl of extracted DNA from either urine or swabs was used in the 20µl ddPCR reaction with the 2x digital PCR supermix for probes (No dUTP). Droplets were generated using the manual droplet generator (Bio-Rad), Droplet Generation Oil for Probes (Bio-Rad) and the PCR mix containing the sample. A total of 40µl of droplets were used for the PCR reaction, with the following conditions; 95°C for 10 min, 40 cycles of 94°C for 30 sec and 60°C for 1 min, and 98°C for 10 min. Samples were read on the QX200 Droplet Reader (Bio-Rad) using the QuantaSoft Software and acquired on Ch1 for FAM. Analysis was performed on the QuantaSoft Software using manual thresholding.

3.5. Statistical analysis

The analysis was conducted in two stages, namely the descriptive and inferential statistics. Since the data showed some deviation from normality and with some extreme values, the nonparametric tests were used to compare any differences in measurements between groups. Wilcoxon tests were used to assess the differences between two groups, whereas the Kruskal test applicable to more than two groups. The distributions within the groups were also visually displayed as boxplots. All the analysis was done with the aid of a freely available Statistical Computing software called R, version 3.6.1. The correlation between urine and vaginal swab groups were evaluated using Spearman’s correlation (GraphPad, USA) and significance was tested at $p< 0.05$.

CHAPTER FOUR

4. RESULTS

4.1 Characteristics of study population

Table 1 describes the characteristics of the pregnant women investigated in this study. In this population, a higher proportion of the women did not experience symptoms of abnormal vaginal discharge at enrolment (66% vs 34%). The majority of the women had attained a high school level of education (64%) and were unmarried (84%). With respect to sexual behaviour, most women had reported having a regular sexual partner (90%), experiencing sexual debut between the ages of 15-20 years (84%), having between 2-4 lifetime sexual partners and not practicing condom use during their last sex act (46%). Most women were in the third trimester of pregnancy (56%) and had no previous history of STIs (52%). When stratified according to BV status, having a regular sex partner and trimester of pregnancy was found to be statistically significant with being BV positive. Women who reported having a regular sex partner had a prevalent BV infection when compared to women who did not have a regular sex partner (p= 0.05). A higher proportion of women in the third trimester of pregnancy were BV positive when compared to women in the first and second trimester of pregnancy (p=0.04).

Table 1: Characteristics of the study population according to BV status

	BV Positive	**BV Negative**	**Total**	**P value**
Total	**25**	**25**	**50**	
Age				0.602
mean(SD)	29.2 (5)	28.4 (6.7)	28.8 (5.9)	
Current abnormal discharge				0.136
No	14 (56)	19 (76)	33 (66)	
Yes	11 (44)	6 (24)	17 (34)	
Level of education				0.377
Primary school	1 (4)	0 (0)	1 (2)	
High school	14 (56)	18 (72)	32 (64)	
College/University	10 (40)	7 (28)	17 (34)	
Married				0.702
No	22 (88)	20 (80)	42 (84)	
Yes	3 (12)	5 (20)	8 (16)	
Has a regular sex partner				0.05
No	0 (0)	5 (20)	5 (10)	
Yes	25 (100)	20 (80)	45 (90)	

Cohabiting with partner				0.39
No	13 (52)	16 (64)	29 (58)	
Yes	12 (48)	9 (36)	21 (42)	
Age of first sex				0.83
<15	1 (4)	1 (4)	2 (4)	
15-20	22 (88)	20 (80)	42 (84)	
21-25	2 (8)	3 (12)	5 (10)	
>25	0 (0)	1 (4)	1 (2)	
Number of lifetime sex partners				0.816
1	6 (24)	7 (28)	13 (26)	
2-4	11 (44)	12 (48)	23 (46)	
>4	8 (32)	6 (24)	14 (28)	
Partner has other partners				0.186
No	5 (20)	9 (36)	14 (28)	
Yes	12 (48)	6 (24)	18 (36)	
Don't know	8 (32)	10 (40)	18 (36)	
Condom use				0.597
Never	6 (24)	5 (20)	11 (22)	
Rarely	3 (12)	1 (4)	4 (8)	
Sometimes	15 (60)	16 (64)	31 (62)	
Always	1 (4)	3 (12)	4 (8)	
Condom use at last sex act				0.37
No	15 (60)	18 (72)	33 (66)	
Yes	10 (40)	7 (28)	17 (34)	
Smokes				1
No	24 (96)	24 (96)	48 (96)	
Yes	1 (4)	1 (4)	2 (4)	
Consumes alcohol				0.189
No	20 (80)	24 (96)	44 (88)	
Yes	5 (20)	1 (4)	6 (12)	
Intravaginal practices				0.11
Yes	4 (16)	0 (0)	4 (8)	
No	21 (84)	25 (100)	46 (92)	
Trimester of pregnancy				0.04
1st	6 (24)	1 (4)	7 (14)	

2nd	9 (36)	6 (24)	15 (30)	
3rd	10 (40)	18 (72)	28 (56)	
Past preterm delivery				0.189
No	20 (80)	23 (95.8)	43 (87.8)	
Yes	5 (20)	1 (4.2)	6 (12.2)	
Past miscarriage				0.48
No	19 (76)	21 (84)	40 (80)	
Yes	6 (24)	4 (16)	10 (20)	
Abnormal discharge in the past				0.254
No	12 (48)	16 (64)	28 (56)	
Yes	13 (52)	9 (36)	22 (44)	
Previous treatment of Sexually transmitted infections				1
No	13 (52)	13 (52)	26 (52)	
Yes	12 (48)	12 (48)	24 (48)	

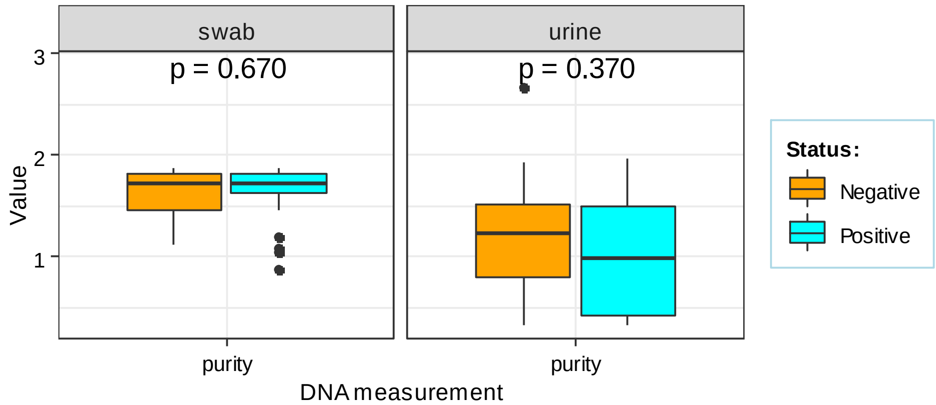
4.2 Quantification of extracted DNA by spectrophotometry

DNA was successfully extracted from all samples processed. The concentration of urine-extracted DNA occurred in the range 1.2 – 585 ng/µl with accompanying A_{260}/A_{280} ratios in the range 0.32-1.93. The swab-extracted DNA concentrations ranged from 2.6-134.6 ng/µl with A_{260}/A_{280} ratios of 0.88-1.87. All patient samples that were tested produced valid results for the ddPCR reactions. The quality controls used in the ddPCR runs had produced the desired outputs and ddPCR was also able to detect the concentration of the DNA in both the urine and swab samples despite having relatively low A_{260}/A_{280} purity ratios (Appendix 1)

When comparing the median DNA concentration and purity values in the urine versus the swab sample samples across BV positive and BV negative samples, it was observed that there was no significant difference between both sample types across the BV states ($p>0.05$) (Table 2, Fig 6). This indicates that there was no bias in the sample quality or integrity for the future downstream reactions.

Table 2: The ranges of concentration and purity in swab and urine samples DNA

		Purity			Concentration		
DNA_in	status	Q1	Q2	Q3	Q1	Q2	Q3
swab	Negative	1.4625	1.715	1.81	7	12.95	18.95
swab	Positive	1.62	1.72	1.82	7.1	11.8	26.2
urine	Negative	0.7975	1.23	1.5175	4.75	9.65	14.2
urine	Positive	0.42	0.99	1.49	4.9	9.9	15.5



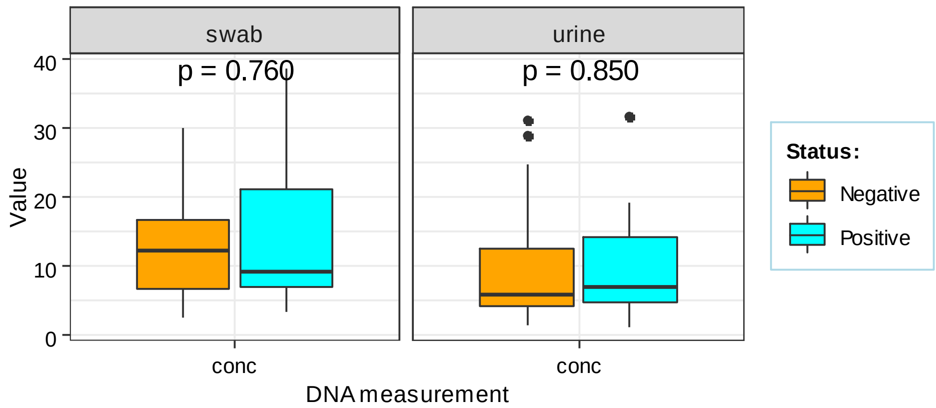


Fig. 6: Comparison of the DNA concentration and purity values in the swab and urine samples across the BV states.

4.3 Droplet Digital PCR

All samples produced valid results for the ddPCR reactions. A complete set of raw data for each pathogen is represented as Appendix 1. Sample numbers 1-25 were classified as BV negative and sample numbers 26-50 were classified as BV positive.

The ddPCR was able to detect and quantify *G. vaginalis* across the BV states from urine and swab DNA. As expected a higher copy of this bacterium was present in the BV positive samples when compared to BV negative samples. Similarly, for *A. vaginae* and *P. bivia* higher copy numbers of these bacteria were found in BV positive samples when compared to the negative samples.

According to the ddPCR analysis, *L. crispatus* was shown to be present in higher abundance in the BV negative samples when compared to the BV positive samples (Appendix 1).

4.3.1. Quantitative comparisons of BV-associated pathogens in urine versus vaginal swab samples

The median copy numbers of each bacterium quantified from urine was compared to the copy numbers of each bacterium for the swab samples. This comparison was made across the BV states. According to Fig. 7 for the BV negative samples, there was no significant differences in the median copy number of each bacterium between the urine and swab samples ($p>0.05$). However, in the BV positive samples a higher median copy for *A. vaginae* was observed in the swab samples when compared to the urine samples ($p=0.004$).

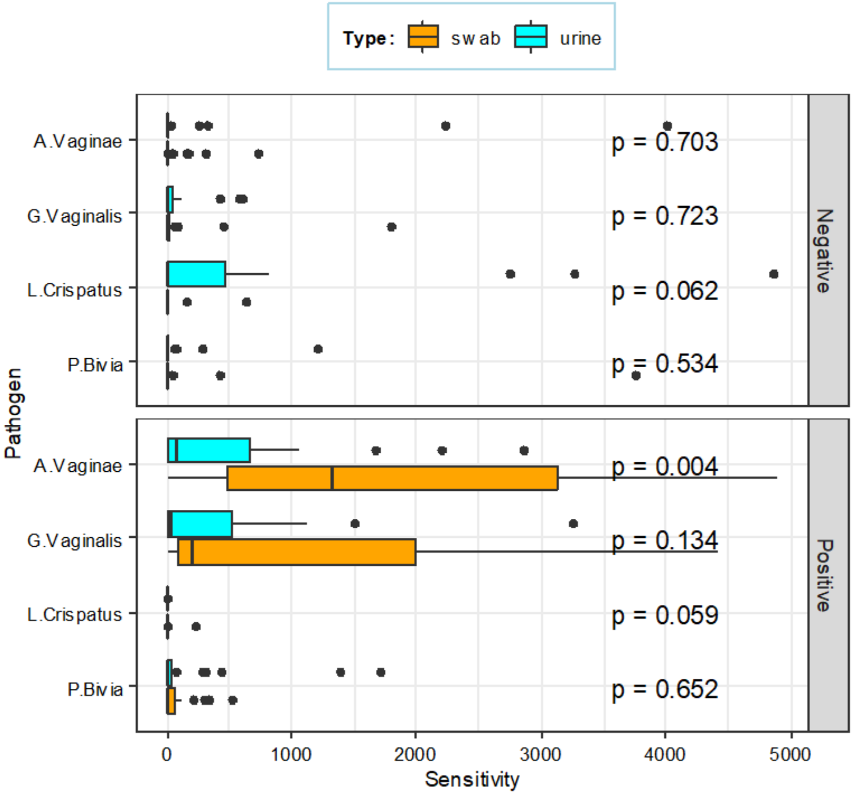


Fig. 7: Comparison of the median copy numbers of each bacterium in urine sample when compared to the swab sample

A spearman's correlation for the swab and urine samples for each pathogen was performed. A good correlation between the two sample types was noted for *G. vaginalsis*, *P. bivia* and *L. crispatus* (Fig. 8). This data is in accordance with Fig. 7 which showed that there is no significant difference in the copy numbers across both sample types for the pathogens above. However, for *A. vaginae* a weak correlation between urine and swab samples was noted (Fig. 8). This can be expected, since a significant difference in the bacterial abundance between urine and swab samples was observed for *A. vaginae* in the BV positive group (Fig. 7).

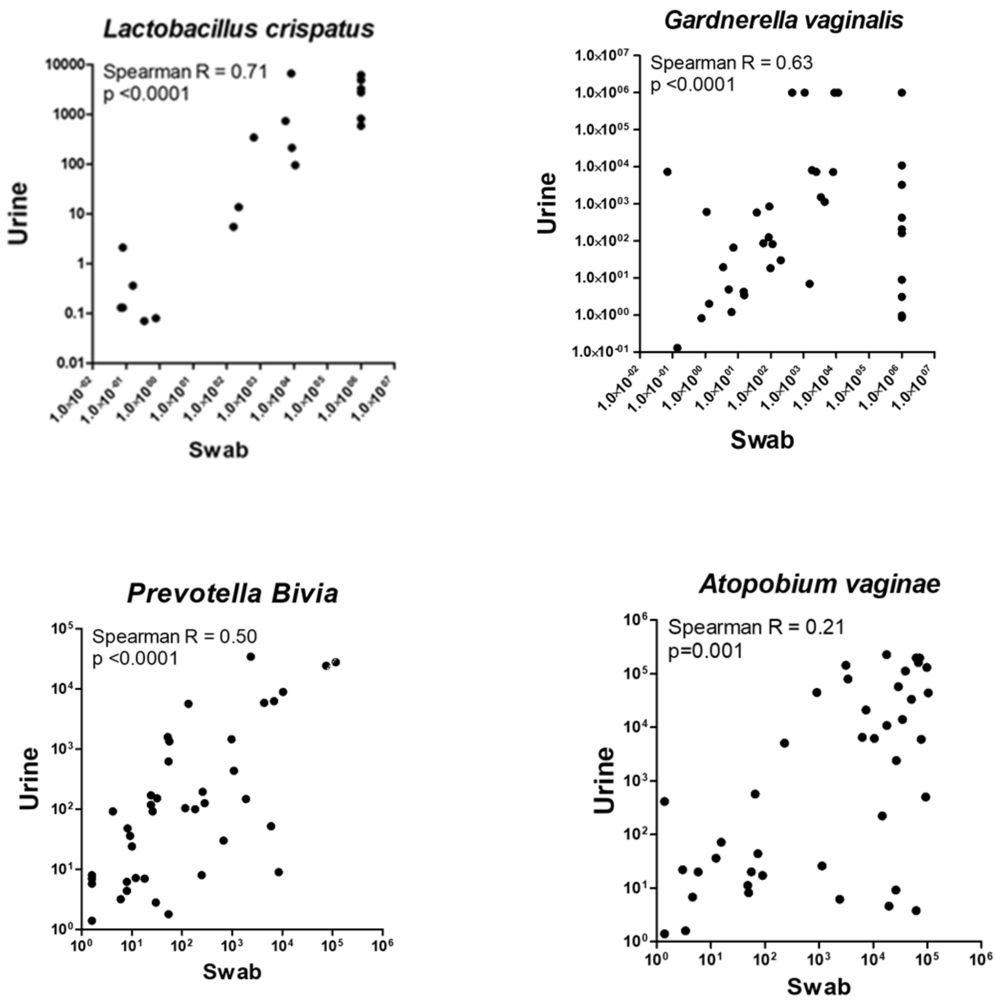


Fig. 8: The graph shows the correlation of urine versus swab samples for the detection and quantification of *L. crispatus*. A strong correlation between the sample types, R= 0.71, *p*<0.0001 was observed. The plot shows the correlation of urine versus swab samples for *G. vaginalis*. A strong correlation between urine and swab samples, R= 0.63, *p*<0.0001 was observed. The graph on the bottom left displays the correlation of urine versus swab samples for *P. bivia* is shown here. A strong correlation between urine and swab samples, R=0.50, *p*< 0.0001 was observed; followed by the correlation of urine versus swab samples for *A. vaginae*. A weak correlation between urine and swab samples, R= 0.21, *p*=0.001 was observed.

4.3.2 Comparison of the abundance of each bacterium across the BV states

For the swab samples, it was observed that a higher copy number of *A. vaginae* and *G. vaginalis* was present in the BV positive samples when compared to the BV negative samples (p <0.001), respectively (Fig. 9). For *L. crispatus*, a higher copy number of this bacterium was present in the BV negative samples when compared to the BV positive samples (p=0.029) (Fig. 9).

Similarly, for the urine samples, it was observed that a higher copy number of *A. vaginae* and *G. vaginalis* was present in the BV positive samples when compared to the BV negative samples (p= 0.041 and p=0.029), respectively. As observed with the swab samples, a higher copy number of *L. crispatus*, was present in the BV negative samples when compared to the BV positive samples (p=0.004) (Fig. 9). There was no significant association between the copy numbers of *P. bivia* in both urine and swab samples across the BV states (p= 0.321 and p= 0.327), respectively.

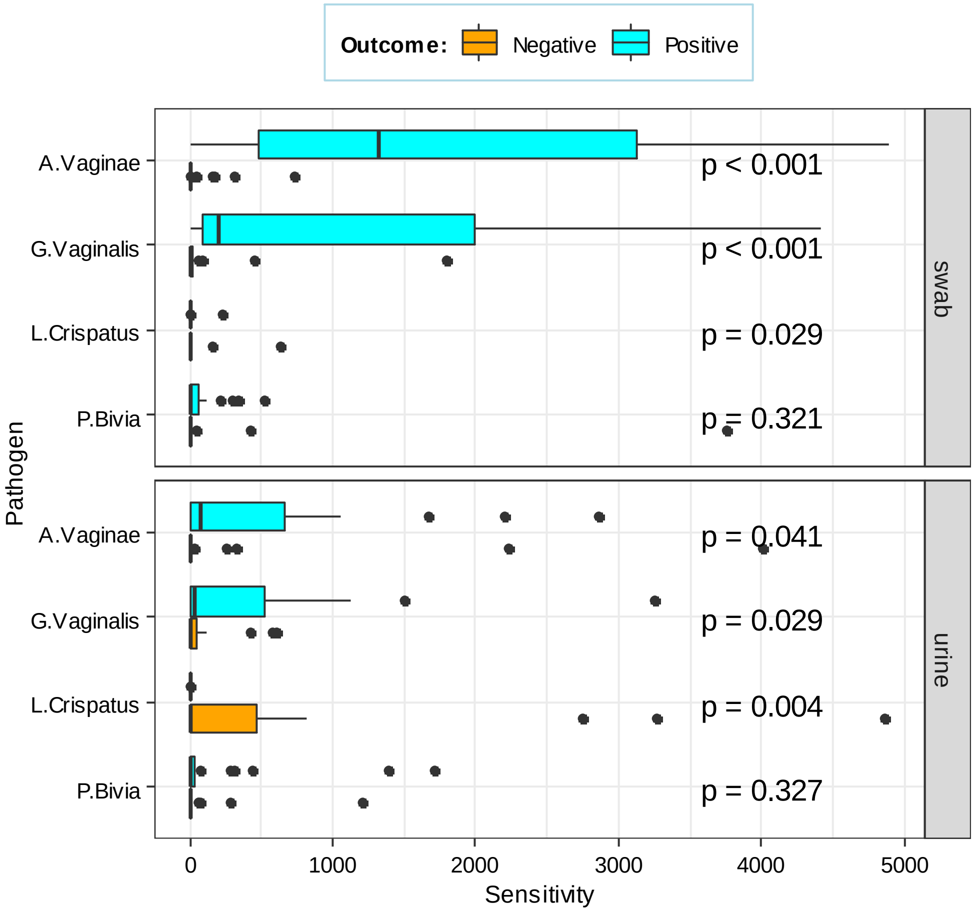


Fig. 9: Comparison of the abundances of the different bacteria across the BV states in the urine and swab samples.

4.3.3 Comparison of the abundance of each bacterium in BV negative and BV positive samples

G. vaginalis was observed as the most abundant microorganism, followed by *A. vaginae* and *P. bivia* in the BV positive women, whereas *L. crispatus* was the most abundant in the BV negative women. A higher abundance of *A. vaginae* and *G. vaginalis* was observed in BV positive when compared to BV negative women as expected. This association was found to be statistically significant (p<0.001). However, there was no statistically significant association between the abundance of *P. bivia* across the BV states (p=0.228). As expected, a higher abundance of *L. crispatus* was shown to be present in the BV negative samples when compared to the BV positive samples (p= 0.002) (Fig. 10).

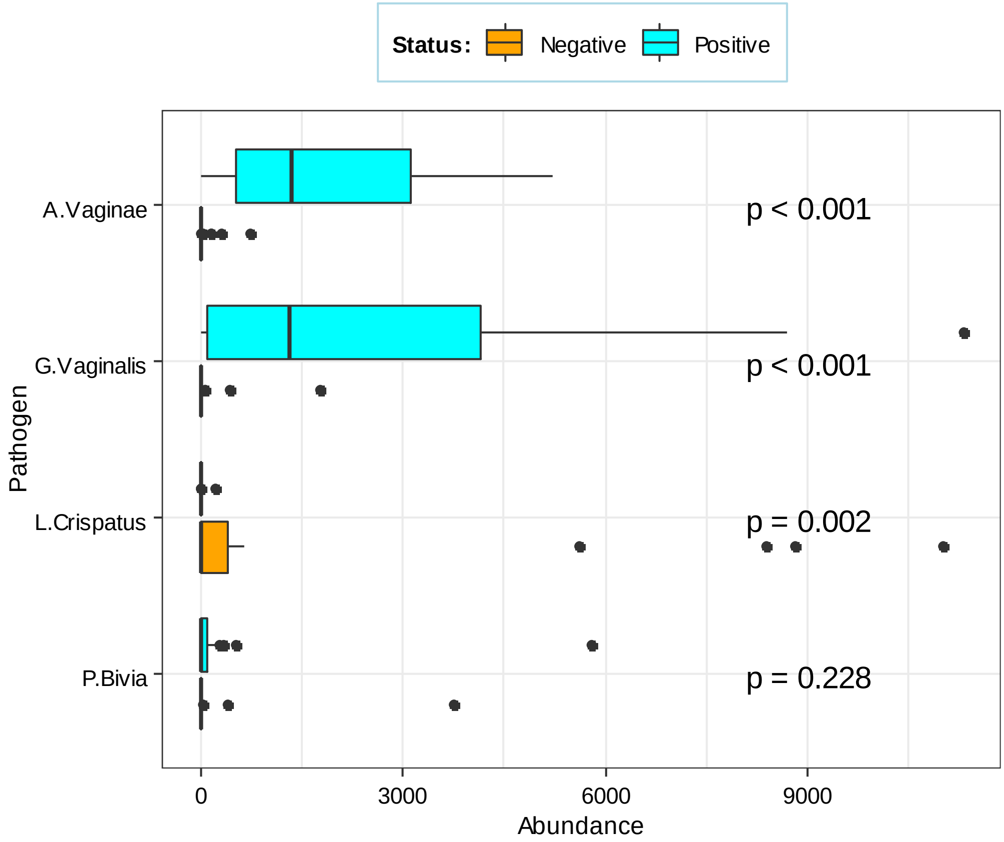


Fig. 10: The abundance of each bacterium in relation to BV status

4.3.4. Comparison of bacterial abundance related to clinical symptoms of discharge

The median copy numbers of the individual bacteria was compared to the clinical symptom of discharge. A higher abundance of *G. vaginalis* was observed in women who presented with abnormal vaginal discharge when compared to women who reported not having any discharge. This association was found to have a borderline significance (p=0.062). Similarly, a higher abundance of *P. bivia* was observed in women with discharge (p=0.032). There was no significant association between clinical symptoms and *A. vaginae* and *L. crispatus* (p>0.05) (Fig. 11).

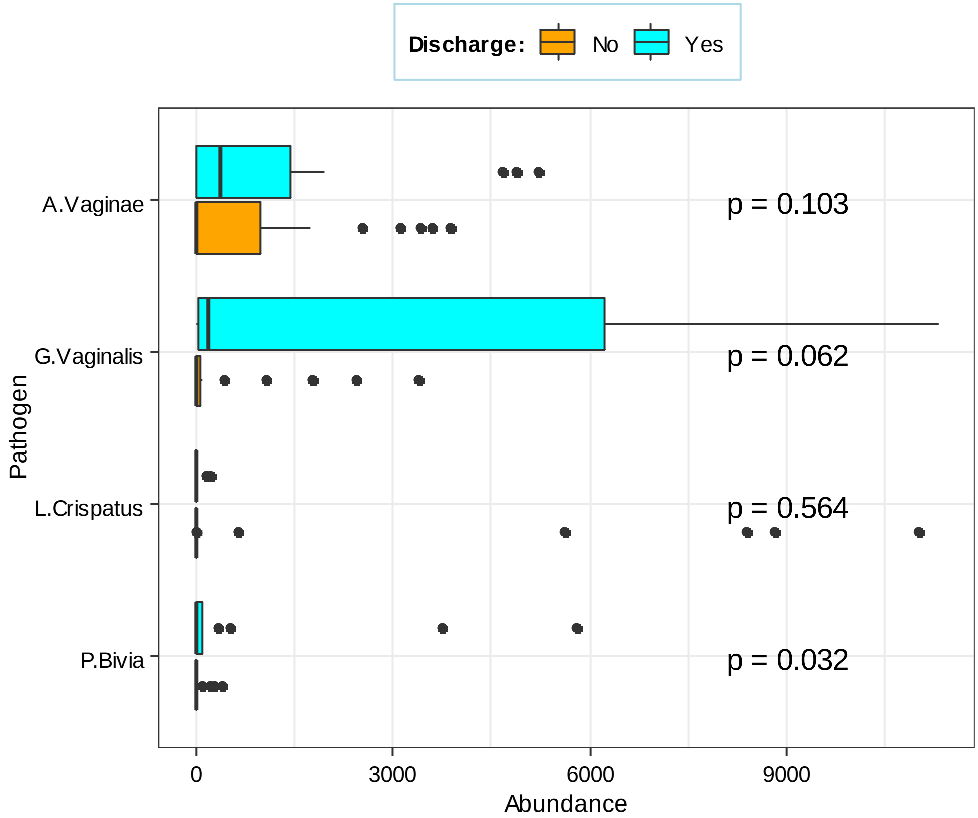


Fig. 11: Comparison of bacterial abundances in relation to abnormal vaginal discharge in swab samples.

4.3.5. Comparison of bacterial abundance related to trimester of pregnancy

According to Fig. 12, there is no significant association between the abundance of *A. vaginae, G. vaginalis* and *P. bivia* in women across the different trimesters of pregnancy (p>0.05) (Fig. 12). However, a higher abundance of *L. crispatus* was observed in women in the third trimester of pregnancy when compared to women in the first and second trimester of pregnancy. This association was found to be statistically significant (p= 0.021).

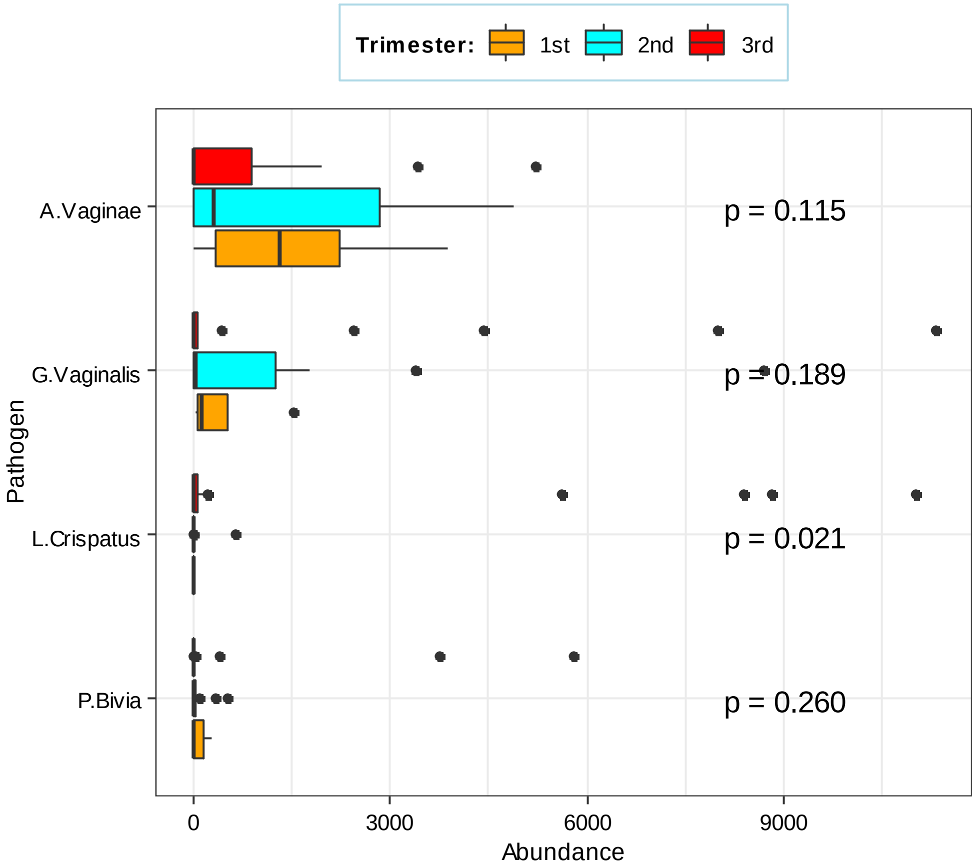


Fig. 12: Abundance of the individual bacteria across the different trimesters of pregnancy

CHAPTER FIVE

5. DISCUSSION

Bacterial vaginosis is a clinical condition that is classically diagnosed using a vaginal swab. Nugent Scoring is performed to detect if a patient is BV positive, intermediate or negative (15, 45). However, science is moving towards molecular techniques to detect BV since molecular techniques are able to detect the BV-associated pathogens more accurately (5, 14, 18, 33). BV plays a negative role in pregnant women as it may result in severe consequences to the unborn infant. The collection of vaginal swabs to detect pathogens during pregnancy is not practical and is extremely uncomfortable for the mother. Urine may be considered as an ideal sample choice for the detection of pathogens (especially from pregnant women) since it is non-invasive and easy to collect.

This study provides the first evidence for the detection and quantification of BV-associated bacteria (*G. vaginalis*, *P. bivia, A. vaginae* and *L. crispatus*) in urine samples collected from South African pregnant women. In addition, this study provides the first report on the use of droplet digital PCR (ddPCR) for the detection and quantification of these specific bacteria.

The present study employed the use of a high-throughput ddPCR system to determine the absolute quantification of DNA copy numbers without the need to generate a standard curve. This technology has been reported to provide higher precision and sensitivity than real-time PCR and is less labour intensive (40, 41). To date, ddPCR has been used for the quantitative detection of numerous pathogens (46-49). Currently, there is a lack of data on the use of ddPCR for quantification of BV-associated pathogens. This study was able to successfully use ddPCR to detect these BV-associated pathogens in both urine and vaginal swab samples regardless of the purity of the DNA. Thereby, making ddPCR a reliable technology to detect BV-associated pathogens. Droplet digital PCR was able to successfully detect and quantify *G. vaginalis, A. vaginae*, *P. bivia and L. crispatus* across the BV states (BV negative and positive) from urine and vaginal swab DNA samples. As expected, a higher copy of *G. vaginalis, A. vaginae* and *P. bivia* was present in the BV positive samples when compared to BV negative samples.

This study showed *G. vaginalis* to be the most abundant BV microorganism when compared to *A. vaginae* and *P. bivia* in the samples tested. This correlates with previous study findings that have reported *Gardnerella* to be the leading BV-associated pathogen (3, 26, 32, 50). The levels of abundance of *G. vaginalis* is used as the indicative microorganism for BV (32). In a study conducted by Beverly et al (2005), the results demonstrated that quantitative PCR for *G. vaginalis*, significantly correlates with the Nugent Gram stain method for BV diagnosis (51). It was found that women who were diagnosed as BV positive according to the Nugent score, had significantly higher numbers of *G. vaginalis* ($p< 0.0001$) in the cervicovaginal lavage (CVL) when compared to women without BV (51). Similarly, in the current study, a high load of *G .vaginalis* was detected in all the BV positive samples (both urine and vaginal swabs). This result was expected as *G. vaginalis* is one of the key BV microorganisms that is searched for when performing Nugent scoring (15, 32, 51). If there is sufficient quantity of *G. vaginalis* present in the vaginal smear, the patient is diagnosed as BV positive in the Nugent scoring method (15).

The findings from the present study indicated that urine is an appropriate sample for detection of *G. vaginalis* since a good correlation was obtained between urine and swabs (R=0.63, p<0.0001). The findings of this study are supported by other studies conducted internationally. In a study conducted by Swidsinski et al (2013), the authors reported on the presence of *G. vaginalis* in first-void urine samples obtained from German pregnant women (31). More recently, Datcu et al (2014) also demonstrated the potential to detect *G. vaginalis* from a urine sample collected from women in the general population of Greenland (45).

In this study, *A. vaginae* was the second most abundant bacterium detected amongst the BV-associated pathogens. This was expected as *Atopobium* species have been discovered to be found in the vaginal microbiota of women with BV (52). A study conducted by Bradshaw et al (2006) also reported on the presence of *A. vaginae* in Australian women diagnosed with BV in Melbourne, Australia (33). The current study found that there was a significant difference of *A. vaginae* in the swab sample compared to the urine sample and a poor correlation (R=0.21, *p*=0.001) between both sample types was noted.

Prevotella was reported as the least abundant amongst the other two pathogens in this study. Machado et al (2015) noted that a symbiotic relationship between *G. vaginalis* and *P. bivia* exists (5). This demonstrates that the presence of a *G. vaginalis* biofilm stimulates the growth of *P. bivia in vitro (5)*, thereby, explaining the reason for the lower mean concentration of *P. bivia* when compared to the other investigative pathogens.

The current study found a strong correlation (R=0.50, *p*= <0.0001) between the urine and swabs samples for the detection of *P. bivia.* Similar results were found in a study conducted by Datcu et al (2014) in women from the general population of Greenland, thereby confirming our findings on the detection of *Prevotella* from urine (45). After an extensive survey of the literature, there were no published findings on the detection of *A. vaginae* and *P. bivia* from urine samples collected from pregnant women, thereby limiting the discussion of the current study. However, the current study now provides data that fills this gap in knowledge.

According to the ddPCR analysis, *L. crispatus* was shown to be present at a higher abundance in the BV negative samples when compared to the BV positive samples. This result was expected as *L. crispatus* is associated with the healthy vaginal microbiota (2, 37, 53). In this study, *L. crispatus* was shown to be present in both urine and swabs samples from women who were classified as BV negative. A good correlation between the two sample types was observed (R= 0.71, *p*<0.0001). The result correlates with literature as *L. crispatus* is associated with BV negative diagnosis. *L. crispatus* produces lactic acid and other compounds that are inhibitors of BV-associated bacterial species. Women colonized by *L. crispatus* in their vagina, are less likely to develop BV (37, 53). Obtaining data on bacteria that are positively and negatively associated with BV will assist with future studies that aim to determine the bacterial load cut-off in urine for optimal BV prediction. This provides the rationale for the inclusion of *L. crispatus* in the current study.

To date, published studies that have reported on the detection of BV-associated bacteria from urine have performed labour intensive molecular assays such as fluorescent *in situ* hybridization (FISH) and conventional real-time PCR (requiring the generation of a standard curve) (31, 45). The current study has the strength in that it employed the use of a high-throughput ddPCR system to determine absolute quantification of DNA copy numbers without the need to generate a standard curve. This technology has been reported to provide higher precision and sensitivity than real-time PCR assays and is less labour intensive (40, 41).

The results obtained in this study indicate the successful detection and quantification of all investigative BV-associated bacteria from urine samples using ddPCR. The detection of BV-associated pathogens from urine offers a much more comfortable and less biased sampling method when compared to self-collected vaginal swabs (downstream processing is dependent on amount of material on swab). Moreover, ddPCR was able detect the bacteria in urine that was collected at any-time (study did not request first-void urine only) thereby indicating the potential of ddPCR to detect exact copy numbers regardless of when the urine was collected. Droplet digital PCR was able to detect these BV-associated pathogens regardless of the purity of the DNA. The lack of published data on the detection of BV pathogens from urine samples collected from pregnant women in the South African and the African setting lends novelty to the present study. In addition, there is currently no published work on the use of ddPCR for absolute quantification of BV-associated microorganisms.

The present study had the following limitations: firstly every known vaginal bacterium known to cause BV was not attempted to be investigated (bacterial vaginosis-associated bacterium 2, *Eggerthella*-like bacterium, *Sneathia*, *Megasphaera* type 1, *Mobiluncus*) due to funding restraints, and lastly, the present study was a sub-study which only included 100 patient samples.

However, the strengths of this study are as follows, the stratification according to BV status was performed using an FDA approved automated assay for the detection of BV (i.e. BD MAX vaginal panel assay), which is highly sensitive, specific and is not subjective. As mentioned previously, the current study used ddPCR which is highly sensitive to determine absolute quantification of DNA copy number without the need to generate a standard curve. This assay has been reported to be a more sensitive method than real-time PCR and is less labour intensive. In addition, ddPCR can be used to detect and quantify target sequences with DNA that has been degraded and contain inhibitory substances. The data obtained from this study can be used as preliminary data to develop larger studies on this technology.

CHAPTER SIX

6. CONCLUSION

Bacterial vaginosis (BV) affects approximately 42.1% of young South African women between the ages of 15-24 years. (54). The prevalence of BV in South African pregnant women is approximately 17.6% (21) and in Durban, the prevalence of BV in women is 52% (1). For pregnant women, BV results in late miscarriages, preterm labour, premature rupture of membranes (PROM), post-partum endometritis, low birth weight infants and a host of other complications (6, 13, 14). The preferred method for diagnosis of BV is nucleic acid amplification as it results in more accurate detection and diagnosis. The current nucleic acid methods that is used for detection of BV is the FDA approved BD MAX Vaginal panel assay and conventional real-time PCR. However, the disadvantages of using these platforms is that it only uses vaginal swabs for detection, the platforms are relatively expensive and are dependent on the sample integrity which means that if there is less material on the vaginal swab the platforms will be unable to detect the pathogens. Whereas, ddPCR, provides absolute quantification without the need of a standard curve, the amplification is not affected by sample inhibitors or poor amplification efficiency and the technology is able to detect low-copy number variants. Furthermore, ddPCR is cost effective, uses lower sample and reagent volumes compared to other molecular methods whilst still maintaining the overall sensitivity and precision. In this study, ddPCR was also able to detect the BV associated pathogens from low concentration and low purity DNA samples.

Droplet digital PCR proves more advantageous, as it was able to successfully detect the pathogenic BV-causing investigative microorganisms from urine DNA samples in this study. The use of urine as an alternative method of sample collection as opposed to vaginal swab samples is highly favoured as it is less invasive and provides easy collection. In majority of the health care clinical settings, pregnant women are screened for glucose and leukocyctes in urine (45). Therefore, urine is already collected which makes urine easy to be collected for BV testing. Thus, the use of urine as an alternative sample collection method is more feasible. Collecting a vaginal swab from pregnant women who are at an advanced gestation age can be extremely uncomfortable and causes discomfort. Urine samples can be easily collected without any discomfort experienced.

In this study, a positive correlation was detected between urine and vaginal swab samples for all investigative BV-associated pathogens thereby indicating that urine can successfully be used as an alternative method to diagnose BV. Similarly, a study conducted by Datcu et al (2014) showed a linear correlation between vaginal and urine samples (17). The study further demonstrated that as a result of the linear correlation it can be assumed that the bacterial DNA loads quantified in the urine sample possibly represents vaginal secretions washed out by the urine rather than presence of bacterial associated with UTIs (17). Similarly, this assumption could be applied to the present study findings observed with the urine samples. This further emphasizes the validity of possibly using urine as an alternative sample collection method as opposed to vaginal swabs. A future research direction will be to develop ddPCR using urine as a diagnostic test for BV.

CHAPTER SEVEN

7. REFERENCES

1. Kenyon C, Colebunders R, Crucitti T. The global epidemiology of bacterial vaginosis: a systematic review. American journal of obstetrics and gynecology. 2013;209(6):505-23.

2. Nasioudis D, Linhares I, Ledger W, Witkin S. Bacterial vaginosis: a critical analysis of current knowledge. BJOG: An International Journal of Obstetrics & Gynaecology. 2017;124(1):61-9.

3. Onderdonk AB, Delaney ML, Fichorova RN. The human microbiome during bacterial vaginosis. Clinical microbiology reviews. 2016;29(2):223-38.

4. Hardy L, Jespers V, Van den Bulck M, Buyze J, Mwambarangwe L, Musengamana V, et al. The presence of the putative *Gardnerella vaginalis* sialidase A gene in vaginal specimens is associated with bacterial vaginosis biofilm. PloS one. 2017;12(2):e0172522.

5. Machado A, Castro J, Cereija T, Almeida C, Cerca N. Diagnosis of bacterial vaginosis by a new multiplex peptide nucleic acid fluorescence in situ hybridization method. PeerJ. 2015;3:e780.

6. Malaguti N, Bahls LD, Uchimura NS, Gimenes F, Consolaro MEL. Sensitive detection of thirteen bacterial vaginosis-associated agents using multiplex polymerase chain reaction. BioMed research international. 2015;2015.

7. Bitew A, Abebaw Y, Bekele D, Mihret A. Prevalence of bacterial vaginosis and associated risk factors among women complaining of genital tract infection. International Journal of Microbiology. 2017;2017.

8. Trabert B, Misra DP. Risk factors for bacterial vaginosis during pregnancy among African American women. American journal of obstetrics and gynecology. 2007;197(5):477. e1-. e8.

9. Ishaque S, Yakoob MY, Imdad A, Goldenberg RL, Eisele TP, Bhutta ZA. Effectiveness of interventions to screen and manage infections during pregnancy on reducing stillbirths: a review. BMC Public Health. 2011;11(3):S3.

10. Abbai NS, Reddy T, Ramjee G. Prevalent bacterial vaginosis infection–a risk factor for incident sexually transmitted infections in women in Durban, South Africa. International journal of STD & AIDS. 2016;27(14):1283-8.

11. Cohen CR, Duerr A, Pruithithada N, Rugpao S, Hillier S, Garcia P, et al. Bacterial vaginosis and HIV seroprevalence among female commercial sex workers in Chiang Mai, Thailand. AIDS (London, England). 1995;9(9):1093-7.

12. Taha TE, Gray RH, Kumwenda NI, Hoover DR, Mtimavalye L, Liomba GN, et al. HIV infection and disturbances of vaginal flora during pregnancy. Journal of acquired immune deficiency syndromes and human retrovirology: official publication of the International Retrovirology Association. 1999;20(1):52-9.

13. Ajayi V, Sadauki H, Randawa A. Bacterial vaginosis is a common vaginal infection among first-time antenatal clinic attendees: evidence from a tertiary health facility in North-West Nigeria. J Prev Inf Cntrl. 2016;2:2.

14. Zariffard MR, Saifuddin M, Sha BE, Spear GT. Detection of bacterial vaginosis-related organisms by real-time PCR for *Lactobacilli, Gardnerella vaginalis* and *Mycoplasma hominis*. FEMS Immunology & Medical Microbiology. 2002;34(4):277-81.

15. Nugent RP, Krohn MA, Hillier SL. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. Journal of clinical microbiology. 1991;29(2):297-301.

16. Cartwright CP, Lembke BD, Ramachandran K, Body BA, Nye MB, Rivers CA, et al. Development and validation of a semiquantitative, multitarget PCR assay for diagnosis of bacterial vaginosis. Journal of clinical microbiology. 2012;50(7):2321-9.

17. Datcu R, Gesink D, Mulvad G, Montgomery-Andersen R, Rink E, Koch A, et al. Vaginal microbiome in women from Greenland assessed by microscopy and quantitative PCR. BMC infectious diseases. 2013;13(1):480.

18. Fredricks DN, Fiedler TL, Thomas KK, Oakley BB, Marrazzo JM. Targeted PCR for detection of vaginal bacteria associated with bacterial vaginosis. Journal of clinical microbiology. 2007;45(10):3270-6.

19. Menard J-P, Fenollar F, Henry M, Bretelle F, Raoult D. Molecular quantification of *Gardnerella vaginalis* and *Atopobium vaginae* loads to predict bacterial vaginosis. Clinical Infectious Diseases. 2008;47(1):33-43.

20. Shipitsyna E, Roos A, Datcu R, Hallén A, Fredlund H, Jensen JS, et al. Composition of the vaginal microbiota in women of reproductive age–sensitive and specific molecular diagnosis of bacterial vaginosis is possible? PloS one. 2013;8(4):e60670.

21. Redelinghuys MJ, Ehlers MM, Dreyer AW, Lombaard H, Kock MM. P3.035 Prevalence of Genital Mycoplasmas and Bacterial Vaginosis in Pregnant Women in Gauteng, South Africa. Sexually Transmitted Infections. 2013;89(Suppl 1):A159-A.

22. Fairley CK, Fethers KA, Bradshaw CS, Hocking JS, Gurrin LC. Sexual Risk Factors and Bacterial Vaginosis: A Systematic Review and Meta-Analysis. Clinical Infectious Diseases. 2008;47(11):1426-35.

23. Leopold S. Heretofore undescribed organism isolated from the genitourinary system. United States Armed Forces Medical Journal. 1953;4(2):263-6.

24. Harwich MD, Alves JM, Buck GA, Strauss JF, Patterson JL, Oki AT, et al. Drawing the line between commensal and pathogenic *Gardnerella vaginalis* through genome analysis and virulence studies. BMC genomics. 2010;11(1):375.

25. Spiegel CA, Amsel R, Holmes K. Diagnosis of bacterial vaginosis by direct gram stain of vaginal fluid. Journal of clinical microbiology. 1983;18(1):170-7.

26. Janulaitiene M, Paliulyte V, Grinceviciene S, Zakareviciene J, Vladisauskiene A, Marcinkute A, et al. Prevalence and distribution of *Gardnerella vaginalis* subgroups in women with and without bacterial vaginosis. BMC infectious diseases. 2017;17(1):394.

27. Ahmed A, Earl J, Retchless A, Hillier SL, Rabe LK, Cherpes TL, et al. Comparative genomic analyses of 17 clinical isolates of *Gardnerella vaginalis* provide evidence of multiple genetically isolated clades consistent with subspeciation into genovars. Journal of bacteriology. 2012;194(15):3922-37.

28. Burton JP, Devillard E, Cadieux PA, Hammond J-A, Reid G. Detection of Atopobium vaginae in postmenopausal women by cultivation-independent methods warrants further investigation. Journal of clinical microbiology. 2004;42(4):1829-31.

29. Jovita MR, Collins MD, Sjödén B, Falsen E. Characterization of a novel *Atopobium* isolate from the human vagina: description of *Atopobium vaginae* sp. nov. International Journal of Systematic and Evolutionary Microbiology. 1999;49(4):1573-6.

30. Polatti F. Bacterial vaginosis, *Atopobium vaginae* and nifuratel. Current clinical pharmacology. 2012;7(1):36-40.

31. Swidsinski A, Verstraelen H, Loening-Baucke V, Swidsinski S, Mendling W, Halwani Z. Presence of a polymicrobial endometrial biofilm in patients with bacterial vaginosis. PloS one. 2013;8(1):e53997.

32. Hardy L, Jespers V, Abdellati S, De Baetselier I, Mwambarangwe L, Musengamana V, et al. A fruitful alliance: the synergy between *Atopobium vaginae* and *Gardnerella vaginalis* in bacterial vaginosis-associated biofilm. Sexually Transmitted Infection. 2016;92(7):487-91.

33. Bradshaw C, Tabrizi S, Fairley C, Morton A, Rudland E, Garland S. The association of *Atopobium vaginae* and *Gardnerella vaginalis* with bacterial vaginosis and recurrence after oral metronidazole therapy. The Journal of infectious diseases. 2006;194(6):828-36.

34. Morton AN, Bradshaw CS, Fairley CK, Rudland E, Garland SM, Tabrizi SN. The Association of *Atopobium vaginae* and *Gardnerella vaginalis* with Bacterial Vaginosis and Recurrence after Oral Metronidazole Therapy. The Journal of Infectious Diseases. 2006;194(6):828-36.

35. Ma B, Forney LJ, Ravel J. Vaginal microbiome: rethinking health and disease. Annual review of microbiology. 2012;66:371-89.

36. Abdelmaksoud AA, Koparde VN, Sheth NU, Serrano MG, Glascock AL, Fettweis JM, et al. Comparison of *Lactobacillus crispatus* isolates from *Lactobacillus*-dominated vaginal microbiomes with isolates from microbiomes containing bacterial vaginosis-associated bacteria. Microbiology. 2016;162(Pt 3):466.

37. Petrova MI, Reid G, Vaneechoutte M, Lebeer S. *Lactobacillus iners*: friend or foe? Trends in microbiology. 2017;25(3):182-91.

38. Amsel R, Totten PA, Spiegel CA, Chen KC, Eschenbach D, Holmes KK. Nonspecific vaginitis: diagnostic criteria and microbial and epidemiologic associations. The American journal of medicine. 1983;74(1):14-22.

39. Dhiman N, Yourshaw C, Chintalapudi M, Turner C, Murphy E. Diagnostic evaluation of a multiplex quantitative real-time PCR assay for bacterial vaginosis. J Women's Health Care. 2016;5(293):2167-0420.1000293.

40. Hindson BJ, Ness KD, Masquelier DA, Belgrader P, Heredia NJ, Makarewicz AJ, et al. High-throughput droplet digital PCR system for absolute quantitation of DNA copy number. Analytical chemistry. 2011;83(22):8604-10.

41. Hindson CM, Chevillet JR, Briggs HA, Gallichotte EN, Ruf IK, Hindson BJ, et al. Absolute quantification by droplet digital PCR versus analog real-time PCR. Nature methods. 2013;10(10):1003.

42. Pinheiro LB, Coleman VA, Hindson CM, Herrmann J, Hindson BJ, Bhat S, et al. Evaluation of a droplet digital polymerase chain reaction format for DNA copy number quantification. Analytical chemistry. 2011;84(2):1003-11.

43. Boettger LM, Handsaker RE, Zody MC, McCarroll SA. Structural haplotypes and recent evolution of the human 17q21. 31 region. Nature genetics. 2012;44(8):881.

44. Kuypers J, Jerome KR. Applications of digital PCR for clinical microbiology. Journal of clinical microbiology. 2017;55(6):1621-8.

45. Datcu R, Gesink D, Mulvad G, Montgomery-Andersen R, Rink E, Koch A, et al. Bacterial Vaginosis Diagnosed by Analysis of First-Void-Urine Specimens. Journal of Clinical Microbiology. 2014;52(1):218-25.

46. Hayden R, Gu Z, Ingersoll J, Abdul-Ali D, Shi L, Pounds S, et al. Comparison of droplet digital PCR to real-time PCR for quantitative detection of cytomegalovirus. Journal of clinical microbiology. 2013;51(2):540-6.

47. Kelley K, Cosman A, Belgrader P, Chapman B, Sullivan DC. Detection of methicillin-resistant Staphylococcus aureus by a duplex droplet digital PCR assay. Journal of clinical microbiology. 2013;51(7):2033-9.

48. King JL, Smith AD, Mitchell EA, Allen MS. Validation of droplet digital PCR for the detection and absolute quantification of Borrelia DNA in Ixodes scapularis ticks. Parasitology. 2017;144(4):359-67.

49. Talarico S, Safaeian M, Gonzalez P, Hildesheim A, Herrero R, Porras C, et al. Quantitative detection and genotyping of Helicobacter pylori from stool using droplet digital PCR reveals variation in bacterial loads that correlates with cagA virulence gene carriage. Helicobacter. 2016;21(4):325-33.

50. Baruah FK, Sharma A, Das C, Hazarika NK, Hussain JH. Role of Gardnerella vaginalis as an etiological agent of bacterial vaginosis. Iranian journal of microbiology. 2014;6(6):409.

51. Beverly ES, Chen HY, Wang QJ, Zariffard MR, Cohen MH, Spear GT. Utility of Amsel criteria, Nugent score, and quantitative PCR for *Gardnerella vaginalis, Mycoplasma hominis,* and

Lactobacillus spp. for diagnosis of bacterial vaginosis in human immunodeficiency virus-infected women. Journal of clinical microbiology. 2005;43(9):4607-12.
52. Ling Z, Liu X, Luo Y, Wu X, Yuan L, Tong X, et al. Associations between vaginal pathogenic community and bacterial vaginosis in Chinese reproductive-age women. PLoS One. 2013;8(10):e76589.
53. Abdelmaksoud AA, Koparde VN, Sheth NU, Serrano MG, Glascock AL, Fettweis JM, et al. Comparison of *Lactobacillus crispatus* isolates from *Lactobacillus*-dominated vaginal microbiomes with isolates from microbiomes containing bacterial vaginosis-associated bacteria. Microbiology. 2016;162(3):466-75.
54. Torrone EA, Morrison CS, Chen P-L, Kwok C, Francis SC, Hayes RJ, et al. Prevalence of sexually transmitted infections and bacterial vaginosis among women in sub-Saharan Africa: An individual participant data meta-analysis of 18 HIV prevention studies. PLoS medicine. 2018;15(2):e1002511.

CHAPTER EIGHT

8. APPENDICES

APPENDIX 1: Raw data from the ddPCR results and DNA extraction as well as the Schematic of ddPCR controls for selected microorganism

Correlation between sample numbers sent for ddPCR and participant ID numbers.

BV Negative		BV Positive	
Sample Number	**Sample number on vaginitis study**	**Sample Number**	**Sample number on vaginitis study**
S1	V29	**S26**	V35
S2	V30	**S27**	V42
S3	V33	**S28**	V44
S4	V39	**S29**	V124
S5	V40	**S30**	V47
S6	V43	**S31**	V50
S7	V49	**S32**	V51
S8	V56	**S33**	V53
S9	V63	**S34**	V55
S10	V65	**S35**	V57
S11	V70	**S36**	V74
S12	V71	**S37**	V76
S13	V72	**S38**	V81
S14	V75	**S39**	V009
S15	V78	**S40**	V010
S16	V79	**S41**	V011
S17	V82	**S42**	V013
S18	V85	**S43**	V017
S19	V86	**S44**	V018
S20	V89	**S45**	V019
S21	V93	**S46**	V024
S22	V95	**S47**	V001
S23	V96	**S48**	V003
S24	V101	**S49**	V027
S25	V102	**S50**	V083

Table showing the DNA purity and DNA concentration values for urine and swab samples

Sample ID	Sample type (Urine/ Swabs)	BV status	Purity (A260/A280)	Concentration (ng/µl)
V001	Swab	BV positive	1.65	36.8
V003	Swab	BV positive	1.72	7.2
V009	Swab	BV positive	1.75	26.2
V010	Swab	BV positive	1.87	134.6
V011	Swab	BV positive	1.07	8.5
V013	Swab	BV positive	1.68	5.7
V017	Swab	BV positive	1.46	6.2
V018	Swab	BV positive	1.77	14.5
V019	Swab	BV positive	1.65	7.1
V024	Swab	BV positive	1.82	53.8
V027	Swab	BV positive	1.58	17.6
V035	Swab	BV positive	1.20	14.8
V042	Swab	BV positive	1.84	38.7
V044	Swab	BV positive	0.88	3.5
V047	Swab	BV positive	1.82	16.0
V050	Swab	BV positive	1.05	3.5
V051	Swab	BV Positive	1.68	37.4

V055	Swab	BV positive	1.84	9.3
V057	Swab	BV positive	1.63	4.3
V074	Swab	BV positive	1.84	24.5
V076	Swab	BV positive	1.76	8.8
V081	Swab	BV positive	1.62	7.0
V083	Swab	BV positive	1.81	30.9
V124	Swab	BV positive	1.83	7.6
V053	Swab	BV positive	1.87	11.8
V029	Swab	BV negative	1.71	16.4
V030	Swab	BV negative	1.55	7.8
V033	Swab	BV negative	1.72	6.9
V039	Swab	BV negative	1.80	13.3
V040	Swab	BV negative	1.45	4.9
V043	Swab	BV negative	1.46	7.3
V049	Swab	BV negative	1.77	4.9
V056	Swab	BV negative	1.87	23.4
V063	Swab	BV negative	1.81	13.9
V065	Swab	BV negative	1.81	29.9
V070	Swab	BV negative	1.56	16.3

V071	Swab	BV negative	1.35	3.2
V072	Swab	BV negative	1.82	76.4
V075	Swab	BV negative	1.84	12.6
V078	Swab	BV negative	1.83	21.3
V079	Swab	BV negative	1.29	17.6
V082	Swab	BV negative	1.54	2.6
V085	Swab	BV negative	1.26	21.2
V086	Swab	BV negative	1.87	19.4
V089	Swab	BV negative	1.78	16.1
V093	Swab	BV negative	1.72	10.5
V095	Swab	BV negative	1.47	10.8
V096	Swab	BV negative	1.61	6.0
V101	Swab	BV negative	1.11	63.6
V102	Swab	BV negative	1.35	3.6
V001	Urine	BV positive	0.88	17.9
V003	Urine	BV positive	1.08	3.0
V009	Urine	BV positive	0.32	7.3
V010	Urine	BV positive	0.41	1.7
V011	Urine	BV positive	0.32	585.0

V013	Urine	BV positive	0.40	162.3
V017	Urine	BV positive	1.84	19.2
V018	Urine	BV positive	1.17	2.1
V019	Urine	BV positive	1.90	15.4
V024	Urine	BV positive	1.25	1.2
V027	Urine	BV positive	1.97	6.0
V035	Urine	BV positive	0.85	4.8
V042	Urine	BV positive	0.99	13.9
V044	Urine	BV positive	1.45	10.5
V047	Urine	BV positive	1.78	10.2
V050	Urine	BV positive	0.43	6.9
V051	Urine	BV Positive	0.42	9.9
V055	Urine	BV positive	0.72	31.8
V057	Urine	BV positive	0.94	5.2
V074	Urine	BV positive	1.49	15.5
V076	Urine	BV positive	0.41	461.2
V081	Urine	BV positive	0.37	4.9
V083	Urine	BV positive	1.27	6.1
V124	Urine	BV positive	1.51	4.2

V053	Urine	BV positive	1.55	14.2
V029	Urine	BV negative	1.57	14.2
V030	Urine	BV negative	0.66	5.1
V033	Urine	BV negative	0.33	520.7
V039	Urine	BV negative	1.24	5.5
V040	Urine	BV negative	1.16	81.2
V043	Urine	BV negative	0.36	10.5
V049	Urine	BV negative	0.77	28.9
V056	Urine	BV negative	1.48	81.0
V063	Urine	BV negative	0.42	4.9
V065	Urine	BV negative	0.88	1.9
V070	Urine	BV negative	0.76	11.9
V071	Urine	BV negative	1.41	5.2
V072	Urine	BV negative	1.53	24.9
V075	Urine	BV negative	0.32	4.7
V078	Urine	BV negative	1.82	12.6
V079	Urine	BV negative	1.83	12.6
V082	Urine	BV negative	1.05	3.1
V085	Urine	BV negative	1.11	3.9

V086	Urine	BV negative	1.93	3.5
V089	Urine	BV negative	1.09	8.8
V093	Urine	BV negative	1.44	4.0
V095	Urine	BV negative	1.44	11.4
V096	Urine	BV negative	26.6	1.65
V101	Urine	BV negative	1.29	6.0
V102	Urine	BV negative	1.22	31.1

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Absolute Quantification Report

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

Not for diagnostic use

Sample Details	
Project Number:	KRISP0042
Sample Type:	Urine and Vaginal swabs
Number of Samples:	100
Reporting Lab:	KRISP
Requested by:	Dr Nathlee Abbai
Sample Date:	14-06-2018
Report Date:	03-07-2018

Sample	Pathogen	Copies Per ul DNA	Copies Per 20uL Reaction	Poisson Confidence Max	Poisson Confidence Min	Positive Droplets	Negative Droplets	Accepted Droplets	Threshold Set
S1	Lacto Crispatus	0,35	7	0,76	0,12	5	16882	16887	1000
U1	Lacto Crispatus	0,07	1,4	0,34	0,02	1	16440	16441	1000
S1	G Vag	0	0	0,22	0	0	15812	15812	1600
U1	G Vag	0	0	0,2	0	0	17464	17464	1600
S2	Lacto Crispatus	0,07	1,4	0,33	0	1	16903	16904	1000
U2	Lacto Crispatus	0,13	2,6	0,42	0,06	2	17823	17825	1000
S2	G Vag	3,5	70	4,6	2,6	49	16293	16342	1600
U2	G Vag	19,6	392	21,8	17,4	306	18248	18554	1600
S3	Lacto Crispatus	1000000	20000000	1000000	10000	14976	0	14976	1000
U3	Lacto Crispatus	3260	65200	3340	3230	13863	922	14785	1000
S3	G Vag	6,3	126	7,8	5	80	14849	14929	1600
U3	G Vag	1,2	24	1,9	0,7	19	18374	18393	1600
S4	Lacto Crispatus	8800	176000	9800	8100	14438	8	14446	1000
U4	Lacto Crispatus	212	4240	220	209	3009	15208	18217	1000
S4	G Vag	5,2	104	6,6	4,1	73	16330	16403	1600
U4	G Vag	4,9	98	6,1	3,9	77	18409	18486	1600

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Sample	Pathogen	Copies Per ul DNA	Copies Per 20uL Reaction	Poisson Confidence Max	Poisson Confidence Min	Positive Droplets	Negative Droplets	Accepted Droplets	Threshold Set
S5	Lacto Crispatus	5630	112600	5860	5440	14282	120	14402	1000
U5	Lacto Crispatus	737	14740	753	729	8553	9821	18374	1000
S5	G Vag	0	0	0,22	0	0	16299	16299	1600
U5	G Vag	0,29	5,8	0,69	0,09	4	16051	16055	1600
S6	Lacto Crispatus	11000	220000	14800	9200	11715	1	11716	1000
U6	Lacto Crispatus	94,8	1896	99,9	92,2	1347	16049	17396	1000
S6	G Vag	0	0	0,2	0	0	17537	17537	1600
U6	G Vag	0	0	0,2	0	0	18035	18035	1600
S7	Lacto Crispatus	0	0	0,44	0	0	7990	7990	1000
U7	Lacto Crispatus	0,07	1,4	0,35	0,02	1	16214	16215	1000
S7	G Vag	59,5	1190	63,7	55,4	792	15264	16056	1600
U7	G Vag	85,9	1718	90,9	81	1163	15351	16514	1600
S8	Lacto Crispatus	0,14	2,8	0,68	0,01	1	8322	8323	1000
U8	Lacto Crispatus	0	0	0,23	0	0	15607	15607	1000
S8	G Vag	86	1720	90,9	81,1	1192	15714	16906	1600
U8	G Vag	125	2500	131	119	1670	14855	16525	1600
S9	Lacto Crispatus	8400	168000	9100	8100	15241	12	15253	1000
U9	Lacto Crispatus	6620	132400	7040	6420	9099	33	9132	1000
S9	G Vag	0,07	1,4	0,32	0	1	17615	17616	1600
U9	G Vag	0	0	0,26	0	0	13511	13511	1600
S10	Lacto Crispatus	1000000	20000000	1000000	10000	10113	0	10113	1000
U10	Lacto Crispatus	2750	55000	2820	2710	8748	939	9687	1000
S10	G Vag	15,5	310	17,5	13,5	225	16995	17220	1600
U10	G Vag	3,4	68	4,5	2,5	43	14992	15035	1600
S11	Lacto Crispatus	0	0	0,19	0	0	18108	18108	1000
U11	Lacto Crispatus	0,06	1,2	0,3	0,02	1	19016	19017	1000

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Absolute Quantification Report

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INYUVESI YAKWAZULU-NATALI

Not for diagnostic use

Sample	Pathogen	Copies Per ul DNA	Copies Per 20uL Reaction	Poisson Confidence Max	Poisson Confidence Min	Positive Droplets	Negative Droplets	Accepted Droplets	Threshold Set
S11	G Vag	0,14	2,8	0,44	0,02	2	17087	17089	1600
U11	G Vag	0,13	2,6	0,42	0,02	2	17753	17755	1600
S12	Lacto Crispatus	0,16	3,2	0,51	0,07	2	14738	14740	1000
U12	Lacto Crispatus	0,36	7,2	0,95	0,19	3	9802	9805	1000
S12	G Vag	1,1	22	1,7	0,7	18	18967	18985	1600
U12	G Vag	603	12060	618	589	7080	10564	17644	1600
S13	Lacto Crispatus	0,32	6,4	0,76	0,18	4	14636	14640	1000
U13	Lacto Crispatus	0	0	0,44	0	0	7966	7966	1000
S13	G Vag	1000000	20000000	1000000	10000	17680	0	17680	1600
U13	G Vag	1000000	20000000	1000000	10000	14447	0	14447	1600
S14	Lacto Crispatus	1000000	20000000	1000000	10000	14884	0	14884	1000
U14	Lacto Crispatus	588	11760	602	581	7118	10986	18104	1000
S14	G Vag	0	0	0,22	0	0	15988	15988	1600
U14	G Vag	2,4	48	3,3	1,7	36	17519	17555	1600
S15	Lacto Crispatus	640	12800	656	632	6815	9423	16238	1000
U15	Lacto Crispatus	341	6820	351	336	4709	13991	18700	1000
S15	G Vag	1794	35880	1830	1760	12383	3443	15826	1600
U15	G Vag	8100	162000	8700	7600	17160	17	17177	1600
S16	Lacto Crispatus	1000000	20000000	1000000	10000	16926	0	16926	1000
U16	Lacto Crispatus	4860	97200	5000	4800	18867	308	19175	1000
S16	G Vag	0,76	15,2	1,28	0,41	12	18551	18563	1600
U16	G Vag	0,82	16,4	1,4	0,42	11	15838	15849	1600
S17	Lacto Crispatus	0	0	0.26	0	0	13546	13546	1629
U17	Lacto Crispatus	0	0	0.27	0	0	12885	12885	1629
S17	G Vag	1.1	22	2	0.5	8	8735	8743	2000
U17	G Vag	0	0	0.31	0	0	11357	11357	2000

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Absolute Quantification Report

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

Not for diagnostic use

Sample	Pathogen	Copies Per ul DNA	Copies Per 20uL Reaction	Poisson Confidence Max	Poisson Confidence Min	Positive Droplets	Negative Droplets	Accepted Droplets	Threshold Set
S18	Lacto Crispatus	0	0	0.23	0	0	15550	15550	1629
U18	Lacto Crispatus	0	0	0.26	0	0	13307	13307	1629
S18	G Vag	7.2	144	9	5.7	73	11822	11895	2000
U18	G Vag	66	1320	71	60	543	9432	9975	2000
S19	Lacto Crispatus	0.09	1.8	0.43	0.02	1	12993	12994	1629
U19	Lacto Crispatus	0	0	0.22	0	0	16241	16241	1629
S19	G Vag	0	0	0.31	0	0	11328	11328	2000
U19	G Vag	0.19	3.8	0.62	0.03	2	12067	12069	2000
S20	Lacto Crispatus	1000000	20000000	1000000	10000	13413	0	13413	1629
U20	Lacto Crispatus	6160	123200	6420	6030	14913	80	14993	1629
S20	G Vag	1.3	26	2.2	0.7	12	10563	10575	2000
U20	G Vag	2	40	3	1.3	24	13931	13955	2000
S21	Lacto Crispatus	0	0	0.24	0	0	14483	14483	1629
U21	Lacto Crispatus	0.07	1.4	0.36	0.02	1	15790	15791	1629
S21	G Vag	15	300	17.1	12.9	193	15052	15245	2000
U21	G Vag	4.3	86	5.6	3.2	49	13409	13458	2000
S22	Lacto Crispatus	0	0	0.33	0	0	10614	10614	1629
U22	Lacto Crispatus	0.32	6.4	0.76	0.18	4	14673	14677	1629
S22	G Vag	449	8980	462	436	4542	9771	14313	2000
U22	G Vag	1000000	20000000	1000000	10000	10395	0	10395	2000
S23	Lacto Crispatus	0	0	0.25	0	0	13865	13865	1629
U23	Lacto Crispatus	0.25	5	0.65	0.13	3	14325	14328	1629
S23	G Vag	37.2	744	41	33.5	379	11785	12164	2000
U23	G Vag	583	11660	602	564	3662	5713	9375	2000
S24	Lacto Crispatus	1000000	20000000	1000000	10000	13597	0	13597	1629
U24	Lacto Crispatus	817	16340	836	807	7223	7204	14427	1629

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Sample	Pathogen	Copies Per ul DNA	Copies Per 20uL Reaction	Poisson Confidence Max	Poisson Confidence Min	Positive Droplets	Negative Droplets	Accepted Droplets	Threshold Set
S24	G Vag	0	0	0.26	0	0	13623	13623	2000
U24	G Vag	0.18	3.6	0.58	0.03	2	12964	12966	2000
S25	Lacto Crispatus	159	3180	168	154	1115	7719	8834	1629
U25	Lacto Crispatus	5.5	110	7	4.8	59	12608	12667	1629
S25	G Vag	1000000	20000000	1000000	10000	16186	0	16186	2000
U25	G Vag	422	8440	449	395	951	2205	3156	2000
S26	Lacto Crispatus	0	0	0.5	0	0	7087	7087	1629
U26	Lacto Crispatus	0	0	0.28	0	0	12571	12571	1629
S26	G Vag	4420	88400	4570	4280	10811	259	11070	2000
U26	G Vag	1129	22580	1178	1082	2294	1425	3719	2000
S27	Lacto Crispatus	0.11	2.2	0.54	0.03	1	10372	10373	1629
U27	Lacto Crispatus	0	0	0.27	0	0	12935	12935	1629
S27	G Vag	8000	160000	8500	7400	15544	18	15562	2000
U27	G Vag	7200	144000	8100	6500	4206	9	4215	2000
S28	Lacto Crispatus	0.77	15.4	1.45	0.53	8	12144	12152	1629
U28	Lacto Crispatus	0.08	1.6	0.36	0.02	1	15458	15459	1629
S28	G Vag	1084	21680	1106	1062	9837	6506	16343	2000
U28	G Vag	1000000	20000000	1000000	10000	5502	0	5502	2000
S29	Lacto Crispatus	0	0	0.32	0	0	11118	11118	1629
U29	Lacto Crispatus	0	0	0.23	0	0	15070	15070	1629
S29	G Vag	3390	67800	3470	3310	13584	809	14393	2000
U29	G Vag	1500	30000	1560	1450	3720	1435	5155	2000
S30	Lacto Crispatus	0	0	0.34	0	0	10251	10251	1629
U30	Lacto Crispatus	0	0	0.28	0	0	12720	12720	1629
S30	G Vag	2440	48800	2490	2390	11477	1650	13127	2000
U30	G Vag	7300	146000	8300	6500	3321	7	3328	2000

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Sample	Pathogen	Copies Per ul DNA	Copies Per 20uL Reaction	Poisson Confiden ce Max	Poisson Confidence Min	Positive Droplets	Negative Droplets	Accepted Droplets	Threshold Set
S31	Lacto Crispatus	0	0	0.37	0	0	9587	9587	1629
U31	Lacto Crispatus	0.1	2	0.47	0.03	1	12055	12056	1629
S31	G Vag	1000000	20000000	1000000	10000	13859	0	13859	2000
U31	G Vag	3250	65000	3390	3120	4295	290	4585	2000
S32	Lacto Crispatus	0	0	0.38	0	0	9235	9235	1629
U32	Lacto Crispatus	0	0	0.27	0	0	13032	13032	1629
S32	G Vag	1538	30760	1571	1505	9452	3507	12959	2000
U32	G Vag	6.9	138	9.6	4.8	33	5578	5611	2000
S33	Lacto crispatus	0	0	0.28	0	0	12507	12507	1604
U33	Lacto crispatus	0	0	0.3	0	0	11661	11661	1604
S33	G vag	1000000	20000000	1000000	10000	14330	0	14330	1559
U33	G vag	10900	218000	14700	9900	10800	1	10801	1559
S34	Lacto crispatus	0	0	0.25	0	0	14128	14128	1604
U34	Lacto crispatus	0.08	1.6	0.38	0.02	1	14678	14679	1604
S34	G vag	1000000	20000000	1000000	10000	12917	0	12917	1559
U34	G vag	1000000	20000000	1000000	10000	13393	0	13393	1559
S35	Lacto crispatus	0	0	0.24	0	0	14470	14470	1604
U35	Lacto crispatus	0.08	1.6	0.36	0.02	1	15540	15541	1604
S35	G vag	201	4020	209	197	2488	13362	15850	1559
U35	G vag	30.1	602	33	28.6	415	16006	16421	1559
S36	Lacto crispatus	227	4540	236	222	2487	11690	14177	1604
U36	Lacto crispatus	13.6	272	15.6	12.6	172	14807	14979	1604
S36	G vag	1000000	20000000	1000000	10000	14730	0	14730	1559
U36	G vag	1000000	20000000	1000000	10000	13948	0	13948	1559
S37	Lacto crispatus	0	0	0.24	0	0	14609	14609	1604
U37	Lacto crispatus	0.07	1.4	0.33	0.02	1	16867	16868	1604

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krisp **Absolute Quantification Report** UNIVERSITY OF KWAZULU-NATAL INYUVESI YAKWAZULU-NATALI

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Sample	Pathogen	Copies Per ul DNA	Copies Per 20uL Reaction	Poisson Confidence Max	Poisson Confidence Min	Positive Droplets	Negative Droplets	Accepted Droplets	Threshold Set
S37	G vag	1000000	20000000	1000000	10000	15768	0	15768	1559
U37	G vag	0.86	17.2	1.47	0.62	11	15102	15113	1559
S38	Lacto crispatus	0	0	0.27	0	0	12847	12847	1604
U38	Lacto crispatus	0	0	0.23	0	0	15251	15251	1604
S38	G vag	1000000	20000000	1000000	10000	14167	0	14167	1559
U38	G vag	161	3220	169	158	1915	13028	14943	1559
S39	Lacto crispatus	0.08	1.6	0.39	0.02	1	14463	14464	1604
U39	Lacto crispatus	0.13	2.6	0.43	0.06	2	17562	17564	1604
S39	G vag	1000000	20000000	1000000	10000	14359	0	14359	1559
U39	G vag	3.1	62	4.2	2.6	37	14229	14266	1559
S40	Lacto crispatus	0	0	0.24	0	0	14445	14445	1604
U40	Lacto crispatus	0.09	1.8	0.41	0.02	1	13828	13829	1604
S40	G vag	1000000	20000000	1000000	10000	15155	0	15155	1559
U40	G vag	9	180	10.6	8.2	131	17029	17160	1559
S41	Lacto crispatus	0	0	0.76	0	0	4638	4638	1604
U41	Lacto crispatus	0	0	0.26	0	0	13808	13808	1604
S41	G vag	8700	174000	9900	8200	8045	5	8050	1559
U41	G vag	1000000	20000000	1000000	10000	12362	0	12362	1559
S42	Lacto crispatus	0	0	0.25	0	0	13907	13907	1604
U42	Lacto crispatus	0	0	0.34	0	0	10326	10326	1604
S42	G vag	0.07	1.4	0.35	0.02	1	16151	16152	1559
U42	G vag	7300	146000	7800	7070	12368	25	12393	1559
S43	Lacto crispatus	0	0	0.23	0	0	15028	15028	1604
U43	Lacto crispatus	0	0	0.22	0	0	15700	15700	1604
S43	G vag	11300	226000	15100	10300	15218	1	15219	1559
U43	G vag	1000000	20000000	1000000	10000	16512	0	16512	1559

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krisp **Absolute Quantification Report**

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

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Sample	Pathogen	Copies Per ul DNA	Copies Per 20uL Reaction	Poisson Confidence Max	Poisson Confidence Min	Positive Droplets	Negative Droplets	Accepted Droplets	Threshold Set
S44	Lacto crispatus	0	0	0.21	0	0	16518	16518	1604
U44	Lacto crispatus	0	0	0.25	0	0	14126	14126	1604
S44	G vag	113	2260	119.3	109.7	1225	12154	13379	1559
U44	G vag	82	1640	87	79.5	1062	14704	15766	1559
S45	Lacto crispatus	0	0	0.23	0	0	15096	15096	1604
U45	Lacto crispatus	0	0	0.23	0	0	15317	15317	1604
S45	G vag	90	1800	95	87.4	1224	15401	16625	1559
U45	G vag	850	17000	871	840	6923	6533	13456	1559
S46	Lacto crispatus	0	0	0.23	0	0	15621	15621	1604
U46	Lacto crispatus	0.16	3.2	0.51	0.07	2	14861	14863	1604
S46	G vag	6.1	122	7.5	5.4	78	15112	15190	1559
U46	G vag	0	0	0.27	0	0	13251	13251	1559
S47	Lacto crispatus	0	0	0.32	0	0	11046	11046	1604
U47	Lacto crispatus	0.14	2.8	0.44	0.06	2	17163	17165	1604
S47	G vag	98.2	1964	103.8	95.4	1186	13619	14805	1559
U47	G vag	18.3	366	20.5	17.1	253	16167	16420	1559
S48	Lacto crispatus	0	0	0.23	0	0	15275	15275	1604
U48	Lacto crispatus	0	0	0.22	0	0	16042	16042	1604
S48	G vag	1000000	20000000	1000000	10000	14924	0	14924	1559
U48	G vag	207	4140	215	203	2695	13975	16670	1559
S49	Lacto crispatus	0	0	0.27	0	0	13010	13010	1604
U49	Lacto crispatus	0.08	1.6	0.39	0.02	1	14378	14379	1604
S49	G vag	1000000	20000000	1000000	10000	15086	0	15086	1559
U49	G vag	0.97	19.4	1.6	0.72	13	15793	15806	1559
S50	Lacto crispatus	0.08	1.6	0.39	0.02	1	14316	14317	1604
U50	Lacto crispatus	2.1	42	3	1.7	25	14182	14207	1604

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Sample	Pathogen	Copies Per ul DNA	Copies Per 20uL Reaction	Poisson Confidence Max	Poisson Confidence Min	Positive Droplets	Negative Droplets	Accepted Droplets	Threshold Set
S50	G vag	1000000	20000000	1000000	10000	14988	0	14988	1559
U50	G vag	1000000	20000000	1000000	10000	14947	0	14947	1559
pos cont	Lacto Crispatus	0,26	5,2	0,61	0,15	4	18109	18113	1000
pos cont	G Vag	157	3140	163	151	2542	17844	20386	1600
pos cont	Lacto Crispatus	0.08	1.6	0.39	0.02	1	14475	14476	1629
pos cont	G Vag	169	3380	176	161	1920	12445	14365	2000
pos cont	Lacto crispatus	0	0	0.23	0	0	15434	15434	1604
pos cont	G vag	200	4000	211	194	1322	7146	8468	1559
NTC	G vag	0	0	0.21	0	0	16832	16832	1559
NTC	G vag	0	0	0.2	0	0	17890	17890	1559
NTC	Lacto Crispatus	0	0	0,18	0	0	19193	19193	1000
NTC	G Vag	0	0	0,17	0	0	21280	21280	1600
NTC	Lacto Crispatus	0	0	0.23	0	0	15432	15432	1629
NTC	Lacto crispatus	0	0	0.26	0	0	13716	13716	1604
NTC	Lacto crispatus	0	0	0.25	0	0	14286	14286	1604
NTC	Lacto crispatus	0	0	0.24	0	0	14573	14573	1604

Comments:
Results look fine overall. It was noticed that certain samples had >20 million copies per 20ul indicating that their concentrations were high. It is recommended that these samples be diluted in future prior to testing.

Test performed by:	Dr Jennifer Giandhari
Position:	Medical Scientist

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KwaZulu-Natal Research Innovation and Sequencing Platform

Laboratory Result Report

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INYUVESI YAKWAZULU-NATALI

Project Information:

Project number:	KRISP0065
Test(s) requested:	Absolute Quantification – Droplet Digital PCR (ddPCR)
Pathogens:	- *Atopobium vaginae* - *Prevotella bivia*
Sample receipt date:	19 October 2018
Result report date:	24 October 2018

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Sample Information:

Sample type:	Extracted DNA
Sample suitability:	Appropriate
Sample quality:	Good

Report to:

Client name:	Prof. Nathlee Abbai
Contact details:	abbain@ukzn.ac.za 031 260 4439

Laboratory Result Report

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Results:

Vaginal swab samples – *P. Bivia*

Sample	Concentration per uL	Copies Per 20uL	PoissonConf Max	PoissonConf Min	Positive droplets	Negative droplets	Accepted Droplets	Threshold
S1 - BV negative	48.7	974	52.7	44.6	553	13093	13646	2342
S2 – BV negative	0.41	8.2	0.89	0.14	5	14410	14415	2342
S3 - BV negative	0.5	10	1.01	0.19	6	14251	14257	2342
S4 - BV negative	14.2	284	16.3	12.1	173	14285	14458	2342
S5 - BV negative	0	0	0.21	0	0	17154	17154	2342
S6 - BV negative	0.15	3	0.5	0.02	2	15193	15195	2342
S7 - BV negative	0.21	4.2	0.55	0.05	3	17058	17061	2342
S8 - BV negative	1.3	26	2.4	0.7	10	8756	8766	2342
S9 - BV negative	0.6	12	1.2	0.2	5	10420	10425	2342
S10 - BV negative	12.3	246	14.6	9.9	102	9734	9836	2342
S11 - BV negative	0	0	0.27	0	0	12836	12836	2342
S12 - BV negative	0	0	0.26	0	0	13351	13351	2342
S13 - BV negative	3750	75000	3850	3660	14359	617	14976	2342
S14 - BV negative	0.08	1.6	0.39	0	1	14586	14587	2342
S15 - BV negative	2.8	56	3.8	2	37	15485	15522	2342
S16 - BV negative	1.6	32	2.4	1.1	24	17326	17350	2342

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S17 - BV negative	0.3	6	1.1	0.1	2	6780	6782	2342
S18 - BV negative	1.2	24	2	0.6	13	12906	12919	2342
S19 - BV negative	0.4	8	0.88	0.14	5	14579	14584	2342
S20 - BV negative	0.08	1.6	0.39	0	1	14292	14293	2342
S21 - BV negative	9.1	182	10.7	7.5	124	15973	16097	2342
S22 - BV negative	0	0	0.28	0	0	12696	12696	2342
S23 - BV negative	6.7	134	8	5.5	114	19835	19949	2342
S24 - BV negative	423	8460	437	410	3810	8805	12615	2010
S25 - BV negative	0.9	18	1.6	0.5	10	12857	12867	2342
S26 - BV positive	0	0	0.24	0	0	14607	14607	2342
S27 - BV positive	5800	116000	6040	5580	13654	99	13753	2342
S28 - BV positive	118	2360	125	112	1160	10955	12115	2342
S29 - BV positive	0.46	9.2	0.94	0.18	6	15411	15417	2342
S30 - BV positive	1.2	24	1.9	0.6	12	12225	12237	2342
S31 - BV positive	0	0	0.23	0	0	15474	15474	2342
S32 - BV positive	0.17	3.4	0.54	0.03	2	13845	13847	2342
S33 - BV positive	518	10360	534	503	4445	8026	12471	2342
S34 - BV positive	218	4360	227	209	2371	11654	14025	2342
S35 - BV positive	94	1880	99	89	1204	14483	15687	2342
S36 - BV positive	5.8	116	7.2	4.6	78	15780	15858	2342
S37 - BV positive	0.08	1.6	0.36	0	1	15399	15400	2342
S38 - BV positive	33.8	676	37.1	30.5	401	13763	14164	2342

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S39 - BV positive	1.5	30	2.3	0.9	19	15004	15023	2342
S40 - BV positive	2.7	54	3.7	2	40	17218	17258	2342
S41 - BV positive	343	6860	354	331	3428	10133	13561	2342
S42 - BV positive	0	0	0.25	0	0	13928	13928	2342
S43 - BV positive	2.7	54	3.8	1.9	35	14992	15027	2342
S44 - BV positive	12.9	258	14.9	10.9	161	14578	14739	2342
S45 - BV positive	2.6	52	3.6	1.8	33	15005	15038	2342
S46 - BV positive	0	0	0.26	0	0	13651	13651	2342
S47 - BV positive	54.3	1086	59	49.6	510	10802	11312	2342
S48 - BV positive	297	5940	308	286	2857	9965	12822	2342
S49 - BV positive	0.4	8	0.94	0.12	4	11775	11779	2342
S50 - BV positive	0.08	1.6	0.4	0	1	14165	14166	2342
Positive control	100	2000	105	94	1227	13884	15111	2342
Negative control	0	0	0.24	0	0	14403	14403	2342

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Vaginal swab samples – *A. vaginae*

Sample	Concentration per uL	Copies Per 20uL	Poisson Conf Max	Poisson Conf Min	Positive droplets	Negative droplets	Accepted Droplets	Threshold
S1 - BV negative	738	14760	759	718	5265	6030	11295	2010
S2 - BV negative	11.5	230	13.5	9.5	125	12701	12826	2010
S3 - BV negative	0.08	1.6	0.39	0	1	14266	14267	2010
S4 - BV negative	0.29	5.8	0.76	0.07	3	12331	12334	2010
S5 - BV negative	0	0	0.24	0	0	14531	14531	2010
S6 - BV negative	0	0	0.26	0	0	13640	13640	2010
S7 - BV negative	0	0	0.21	0	0	16736	16736	2010
S8 - BV negative	172	3440	182	163	1293	8207	9500	2010
S9 - BV negative	0	0	0.25	0	0	13998	13998	2010
S10 - BV negative	4.5	90	5.8	3.4	51	13359	13410	2010
S11 - BV negative	0.17	3.4	0.55	0.03	2	13607	13609	2010
S12 - BV negative	0.15	3	0.49	0.02	2	15527	15529	2010
S13 - BV negative	3.7	74	4.9	2.7	45	14271	14316	2010
S14 - BV negative	0	0	0.23	0	0	15495	15495	2010
S15 - BV negative	315	6300	326	304	3186	10366	13552	2010
S16 - BV negative	0.23	4.6	0.61	0.05	3	15305	15308	2010
S17 - BV negative	0	0	0.24	0	0	14472	14472	2010
S18 - BV negative	3.3	66	4.3	2.4	43	15508	15551	2010

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S19 - BV negative	0.07	1.4	0.33	0	1	17038	17039	2010
S20 - BV negative	0.62	12.4	1.12	0.29	9	17131	17140	2010
S21 - BV negative	2.8	56	3.9	2	38	15673	15711	2010
S22 - BV negative	156	3120	163	148	1763	12460	14223	2010
S23 - BV negative	45.4	908	49.4	41.5	518	13153	13671	2010
S24 - BV negative	2.5	50	3.4	1.8	36	16827	16863	2010
S25 - BV negative	0.78	15.6	1.37	0.39	10	15140	15150	2010
S26 - BV positive	890	17800	910	870	8090	7155	15245	2010
S27 - BV positive	5230	104600	5410	5080	16732	198	16930	2010
S28 - BV positive	3610	72200	3690	3530	14667	717	15384	2010
S29 - BV positive	2550	51000	2610	2500	13655	1760	15415	2010
S30 - BV positive	888	17760	908	869	8363	7421	15784	2010
S31 - BV positive	1742	34840	1776	1710	12938	3808	16746	2010
S32 - BV positive	2.4	48	3.5	1.5	23	11279	11302	2010
S33 - BV positive	4890	97800	5050	4750	13949	222	14171	2010
S34 - BV positive	3120	62400	3190	3050	12337	937	13274	2010
S35 - BV positive	1341	26820	1369	1314	10047	4724	14771	2010
S36 - BV positive	1974	39480	2012	1937	13344	3064	16408	2010
S37 - BV positive	1310	26200	1336	1286	11572	5656	17228	2010
S38 - BV positive	4680	93600	4820	4560	17454	332	17786	2010
S39 - BV positive	3120	62400	3190	3060	17056	1289	18345	2010

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S40 - BV positive	120	2400	126	114	1480	13751	15231	2010
S41 - BV positive	370	7400	382	357	3344	9061	12405	2010
S42 - BV positive	0.07	1.4	0.34	0	1	16292	16293	2010
S43 - BV positive	1456	29120	1488	1426	9466	3866	13332	2010
S44 - BV positive	527	10540	544	511	4095	7244	11339	2010
S45 - BV positive	668	13360	687	650	5282	6907	12189	2010
S46 - BV positive	0.24	4.8	0.63	0.06	3	14913	14916	2010
S47 - BV positive	56.8	1136	61.8	51.8	500	10109	10609	2010
S48 - BV positive	3880	77600	4020	3760	8002	306	8308	2010
S49 - BV positive	978	19560	1002	955	7297	5627	12924	2010
S50 - BV positive	3410	68200	3490	3340	15416	900	16316	2010
Positive control	335	6700	350	320	1894	5754	7648	2010
NTC	0	0	0.2	0	0	17298	17298	2010

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Urine samples – *P. bivia*

Sample	Concentration per uL	Copies Per 20uL	Poisson Conf Max	Poisson Conf Min	Positive droplets	Negative droplets	Accepted Droplets	Threshold
U1 - BV negative	72.8	1456	77.6	68	887	13893	14780	2183
U2 - BV negative	2.4	48	3.3	1.6	30	14887	14917	2183
U3 - BV negative	1.2	24	2	0.7	15	14250	14265	2183
U4 - BV negative	6.3	126	8.1	4.8	58	10778	10836	2183
U5 - BV negative	0.1	2	0.48	0	1	11624	11625	2183
U6 - BV negative	0	0	0.31	0	0	11403	11403	2183
U7 - BV negative	4.6	92	5.9	3.6	63	15923	15986	2183
U8 - BV negative	4.6	92	5.9	3.6	63	15914	15977	2183
U9 - BV negative	0.36	7.2	0.95	0.09	3	9800	9803	2183
U10 - BV negative	0.4	8	1.07	0.1	3	8780	8783	2183
U11 - BV negative	0	0	0.29	0	0	12005	12005	2183
U12 - BV negative	0.18	3.6	0.58	0.03	2	12986	12988	2183
U13 - BV negative	1214	24280	1243	1186	7509	4156	11665	2183
U14 - BV negative	0.29	5.8	0.76	0.07	3	12279	12282	2183
U15 - BV negative	67.2	1344	72	62.4	753	12812	13565	2183
U16 - BV negative	7.6	152	9.4	6.1	85	13065	13150	2183
U17 - BV negative	0.16	3.2	0.53	0.02	2	14312	14314	2183
U18 - BV negative	8.5	170	10.4	6.8	88	12160	12248	2183

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U19 - BV negative	0.22	4.4	0.59	0.05	3	15892	15895	2183
U20 - BV negative	0.35	7	0.76	0.12	5	16792	16797	2183
U21 - BV negative	5	100	6.4	3.9	65	15132	15197	2183
U22 - BV negative	62.4	1248	66.9	58	768	14090	14858	2183
U23 - BV negative	282	5640	292	272	2956	10921	13877	2183
U24 - BV negative	0.45	9	0.92	0.18	6	15601	15607	2183
U25 - BV negative	0.35	7	0.82	0.11	4	13473	13477	2183
U26 - BV positive	0.8	16	1.5	0.4	10	13946	13956	2183
U27 - BV positive	1392	27840	1425	1359	7740	3419	11159	2183
U28 - BV positive	1712	34240	1753	1672	8101	2467	10568	2183
U29 - BV positive	1.8	36	2.8	1	16	10630	10646	2183
U30 - BV positive	5.9	118	7.4	4.6	67	13388	13455	2183
U31 - BV positive	0.17	3.4	0.55	0.03	2	13598	13600	2183
U32 - BV positive	0	0	0.3	0	0	11564	11564	2183
U33 - BV positive	444	8880	457	431	4586	10001	14587	2183
U34 - BV positive	293	5860	306	280	2005	7100	9105	2183
U35 - BV positive	7.4	148	8.7	6	114	18133	18247	2183
U36 - BV positive	5.2	104	6.5	4.1	72	16301	16373	2183
U37 - BV positive	0.07	1.4	0.36	0	1	15787	15788	2183
U38 - BV positive	1.5	30	2.1	0.9	22	17833	17855	2183
U39 - BV positive	0.14	2.8	0.46	0.02	2	16478	16480	2183

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U40 - BV positive	0.09	1.8	0.45	0	1	12477	12478	2183
U41 - BV positive	313	6260	322	303	4035	13258	17293	2183
U42 - BV positive	1.4	28	2.4	0.7	11	9371	9382	2183
U43 - BV positive	31.1	622	33.8	28.4	505	18841	19346	2183
U44 - BV positive	9.7	194	11.2	8.2	159	19228	19387	2183
U45 - BV positive	79.3	1586	84	74.7	1119	16040	17159	2183
U46 - BV positive	1	20	1.7	0.6	13	15088	15101	2183
U47 - BV positive	21.8	436	24.1	19.4	333	17828	18161	2183
U48 - BV positive	2.6	52	3.6	1.8	32	14453	14485	2183
U49 - BV positive	0.31	6.2	0.82	0.07	3	11471	11474	2183
U50 - BV positive	0.4	8	1.2	0.1	3	8129	8132	2183
Positive control	72.8	1456	77.6	68.1	902	14130	15032	2183
Negative control	0	0	0.25	0	0	13946	13946	2183

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Urine samples – *A. vaginae*

Sample	Concentration per uL	Copies Per 20uL	Poisson Conf Max	Poisson Conf Min	Positive droplets	Negative droplets	Accepted Droplets	Threshold
U1 - BV negative	11.1	222	13.4	9	95	10065	10160	1008
U2 - BV negative	254	5080	263	245	3179	13212	16391	1008
U3 - BV negative	0	0	0.2	0	0	17317	17317	1008
U4 - BV negative	1	20	1.7	0.6	14	16095	16109	1008
U5 - BV negative	0	0	0.2	0	0	17822	17822	1008
U6 - BV negative	0.08	1.6	0.36	0	1	15535	15536	1008
U7 - BV negative	0.07	1.4	0.34	0	1	16494	16495	1008
U8 - BV negative	4010	80200	4120	3910	13636	467	14103	1008
U9 - BV negative	0.09	1.8	0.41	0	1	13777	13778	1008
U10 - BV negative	0.86	17.2	1.45	0.46	12	16419	16431	1008
U11 - BV negative	0.08	1.6	0.37	0	1	15096	15097	1008
U12 - BV negative	1.1	22	1.9	0.6	11	11852	11863	1008
U13 - BV negative	2.2	44	3.2	1.5	28	14814	14842	1008
U14 - BV negative	0.21	4.2	0.56	0.05	3	16597	16600	1008
U15 - BV negative	326	6520	336	315	3916	12282	16198	1008
U16 - BV negative	0.34	6.8	0.9	0.08	3	10407	10410	1008
U17 - BV negative	0.07	1.4	0.36	0	1	15751	15752	1008
U18 - BV negative	28.4	568	31.2	25.6	397	16264	16661	1008

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U19 - BV negative	0.07	1.4	0.32	0	1	17574	17575	1008
U20 - BV negative	1.8	36	2.6	1.2	27	17411	17438	1008
U21 - BV negative	1	20	1.6	0.5	14	16826	16840	1008
U22 - BV negative	7220	144400	7620	6860	17117	37	17154	1008
U23 - BV negative	2230	44600	2280	2180	8656	1532	10188	1008
U24 - BV negative	0.41	8.2	0.85	0.16	6	17040	17046	1008
U25 - BV negative	3.6	72	5.1	2.5	29	9406	9435	1008
U26 - BV positive	544	10880	559	529	4964	8449	13413	1008
U27 - BV positive	2197	43940	2243	2153	12218	2233	14451	1008
U28 - BV positive	9900	198000	11300	8800	17351	4	17355	1008
U29 - BV positive	1666	33320	1699	1633	11761	3770	15531	1008
U30 - BV positive	11400	228000	15100	9500	15577	1	15578	1008
U31 - BV positive	698	13960	716	681	6356	7843	14199	1008
U32 - BV positive	0.56	11.2	1.04	0.25	8	16935	16943	1008
U33 - BV positive	6570	131400	6970	6210	9800	37	9837	1008
U34 - BV positive	9900	198000	12200	8600	9341	2	9343	1008
U35 - BV positive	120	2400	127	113	1055	9837	10892	1008
U36 - BV positive	5600	112000	5850	5370	10765	93	10858	1008
U37 - BV positive	0.46	9.2	1.08	0.14	4	10276	10280	1008
U38 - BV positive	25.2	504	28.2	22.2	269	12437	12706	1008
U39 - BV positive	0.19	3.8	0.6	0.03	2	12572	12574	1008

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U40 - BV positive	0.31	6.2	0.83	0.07	3	11235	11238	1008
U41 - BV positive	1053	21060	1077	1030	8463	5843	14306	1008
U42 - BV positive	20.7	414	23	18.3	292	16483	16775	1008
U43 - BV positive	2860	57200	2910	2800	15380	1488	16868	1008
U44 - BV positive	311	6220	321	301	3550	11742	15292	1008
U45 - BV positive	1000000	20000000	1000000	10000	12916	0	12916	1008
U46 - BV positive	0	0	0.29	0	0	12195	12195	1008
U47 - BV positive	1.3	26	2	0.8	18	16555	16573	1008
U48 - BV positive	299	5980	310	289	3207	11063	14270	1008
U49 - BV positive	0.23	4.6	0.75	0.04	2	10065	10067	1008
U50 - BV positive	8100	162000	8800	7400	10363	11	10374	1008
Positive control	939	18780	970	909	3873	3170	7043	1008
Negative control	0	0	0.34	0	0	10224	10224	1696

Quality Control	Internal negative controls – PASSED Internal positive controls - PASSED
Method	ddPCR (BioRad)

Test performed by:	Dr Lavanya Singh
Position:	Medical Scientist

For Research Use Only

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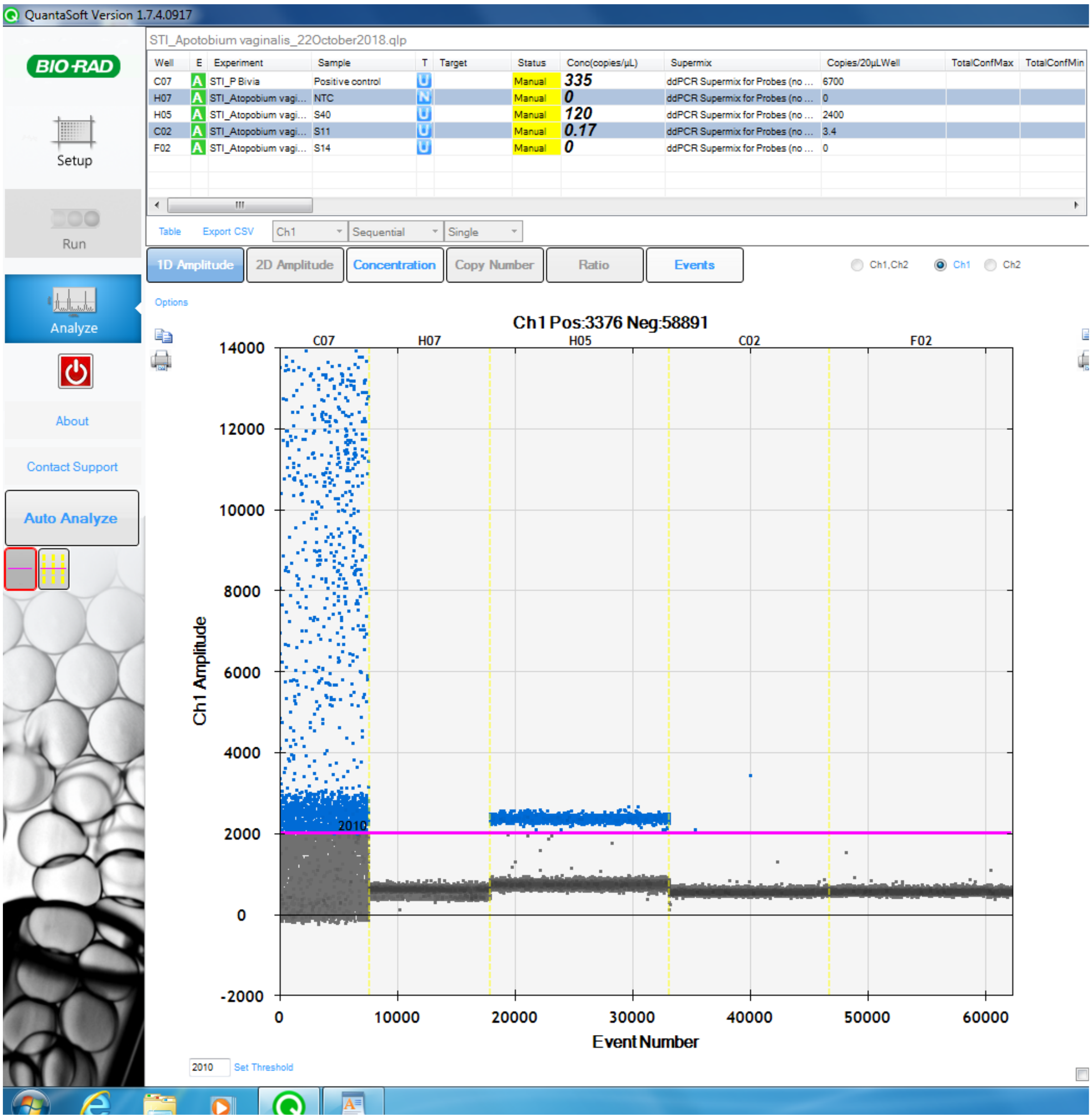
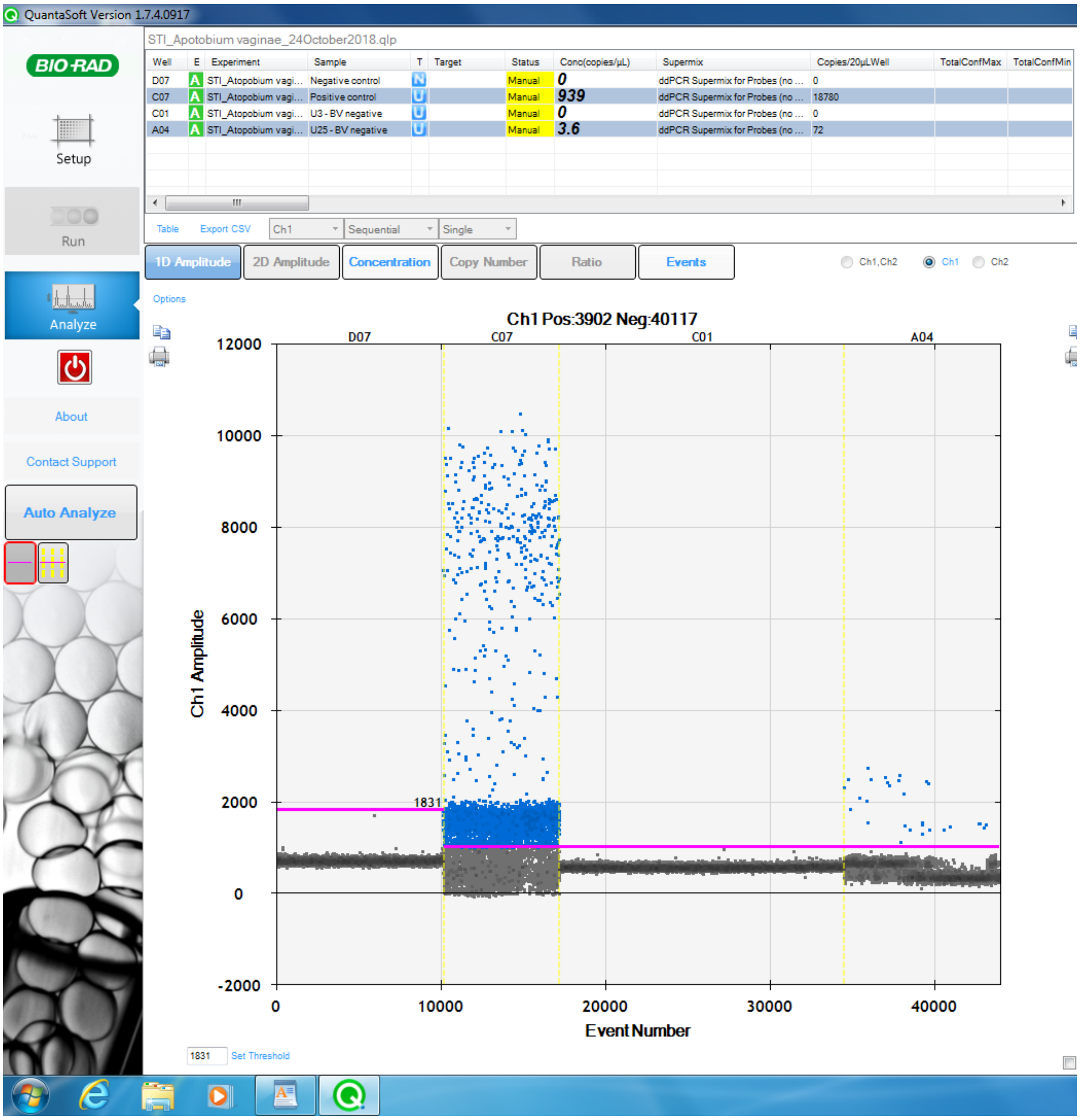
Schematic of ddPCR controls for selected microorganism




QuantaSoft Version 1.7.4.0917
BIO-RAD
Setup
Run
Analyze
About
Contact Support
Auto Analyze
STI_Apotobium vaginae_24October2018.qlp
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D07 A STI_Atopobium vagi... Negative control N Manual 0 ddPCR Supermix for Probes (no ... 0
C07 A STI_Atopobium vagi... Positive control U Manual 939 ddPCR Supermix for Probes (no ... 18780
C01 A STI_Atopobium vagi... U3 - BV negative U Manual 0 ddPCR Supermix for Probes (no ... 0
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Export CSV
Ch1
Sequential
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1D Amplitude
2D Amplitude
Concentration
Copy Number
Ratio
Events
Ch1,Ch2
Ch1
Ch2
Options
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D07
C07
C01
A04
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Event Number
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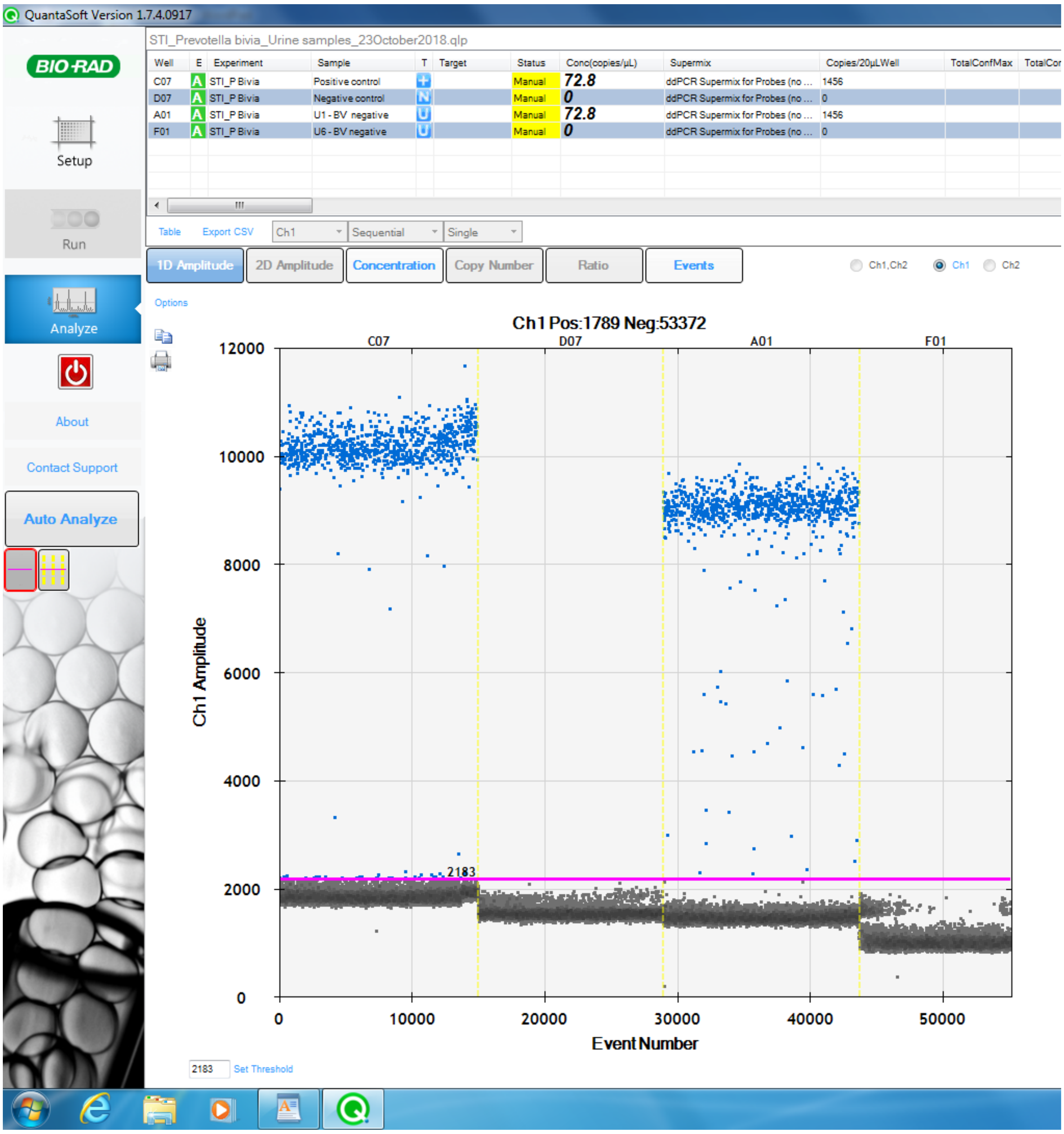
Set Threshold



QuantaSoft Version 1.7.4.0917
BIO-RAD
Setup
Run
Analyze
About
Contact Support
Auto Analyze
STI_Prevotella bivia_Urine samples_23October2018.qlp
Well | E | Experiment | Sample | T | Target | Status | Conc(copies/µL) | Supermix | Copies/20µLWell | TotalConfMax
C07 | A | STI_P Bivia | Positive control | Manual | 72.8 | ddPCR Supermix for Probes (no ... | 1456
D07 | A | STI_P Bivia | Negative control | Manual | 0 | ddPCR Supermix for Probes (no ... | 0
A01 | A | STI_P Bivia | U1 - BV negative | Manual | 72.8 | ddPCR Supermix for Probes (no ... | 1456
F01 | A | STI_P Bivia | U6 - BV negative | Manual | 0 | ddPCR Supermix for Probes (no ... | 0
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Export CSV
Ch1
Sequential
Single
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2D Amplitude
Concentration
Copy Number
Ratio
Events
Ch1,Ch2
Ch1
Ch2
Options
Ch1 Pos:1789 Neg:53372
C07
D07
A01
F01
Ch1 Amplitude
Event Number
2183
Set Threshold

APPENDX 2: Supporting documents

Includes informed consent form, enrollment form, initial BREC approval letter and BREC amendments approval letter

Informed Consent Form

INFORMED CONSENT DOCUMENT

Laboratory based detection of microflora associated with vaginitis in pregnant women presenting at a KwaZulu-Natal hospital

Version 1.0

PRINCIPAL INVESTIGATOR: Dr. Nathlee Abbai
PHONE: 031 260 4439

INFORMED CONSENT

You are being invited to take part in a study called: **Laboratory based detection of microflora associated with vaginitis in pregnant women presenting at a KwaZulu-Natal hospital**

This study is for pregnant women, 18 years and older. Approximately 354 women will be in this study. Before you decide if you want to join this study, we want you to learn about the study. The study staff will talk with you about the study and answer your questions. You may decide not to join or to withdraw from the study at any time.

YOUR PARTICIPATION IS VOLUNTARY

This consent form gives information about the study procedures that will be discussed with you. Your participation is voluntary; you do not have to have the procedures if you do not want to participate in this study. Once you understand the study tests, and if you agree to take part, you will be asked to sign your name on this form. You will be offered a copy of this form to keep.

PURPOSE OF THE STUDY

Bacterial vaginosis (BV) represents the main cause of abnormal vaginal discharge in women of reproductive age. For pregnant women, BV doubles the risk of preterm delivery i.e. delivery before gestation week 37. *Candida* vaginitis is responsible for 80% to 90% of infections during pregnancy. *Trichomonas vaginalis* (TV) infections have been associated with poor reproductive outcomes such as low birth weight and premature birth. Several studies in pregnant women have showed trichomoniasis as the cause of premature rupture of membranes, premature labour, low birth weight infants.

For this study, you will need to provide 3 vaginal swab samples and 1 urine sample. We will test your samples for vaginal infections.

Once the study is over, your samples will be stored and used to test for other infections that may be passed through sexual activity. If you agree to have your samples used for testing in another study please indicate below:

I agree to have my samples used for future testing

Yes ☐ No ☐

Participant Name (print) | Participant Signature | Date

WHAT DO I HAVE TO DO IF I DECIDE TO TAKE PART IN THE STUDY?

If you decide to be in this study, we will be able to start the procedures today. Today's study procedures will take approximately **30-60mins**.
You will be asked to:

- Confirm you are able to join the study and that you understand the study requirements.
- You will be asked questions about your yourself and medical history
- You will be asked to provide 3 vaginal swabs and 1 urine sample.

RISKS AND/OR DISCOMFORTS

Risks of sample collection: You may feel discomfort during the swab sample collection. We will ensure that your sample is collected by a trained person.

Other Possible Risks: You may become embarrassed or worried when discussing your sexual behavior. We will make every effort to make you feel comfortable and protect your privacy and confidentiality whilst you are part of this study. Your visits will take place in private.

CONFIDENTIALITY

We will keep your information confidential. Your personal information may be disclosed if required by law.
Your records may be reviewed by:

- Biomedical Research Ethics Committee of the University of KwaZulu-Natal
- Study staff

The researchers will do everything they can to protect your privacy.

PROBLEMS OR QUESTIONS
The Biomedical Research Ethics Committee of the University of KwaZulu-Natal has approved this study.

BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
Research Office, Westville Campus
Govan Mbeki Building
University of KwaZulu-Natal
Private Bag X 54001, Durban, 4000
KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2602486 - Fax: 27 31 2604609
Email: BREC@ukzn.ac.za

SIGNATURES

If you have read this consent form, or had it read and explained to you, and you understand the information, and you voluntarily agree to participate, please sign your name or make your mark below.

______________________	______________________	______________
Participant Name (print)	Participant Signature	Date
Study Staff Conducting Consent Discussion (print)	Study Staff Signature	Date
*Witness Name (print)	*Witness Signature	*Date

* Witness name, signature and date are required on this consent form only when the consenting participant is not able to read (illiterate)

Enrollment Form

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

Initial BREC Approval Letter

UNIVERSITY OF KWAZULU-NATAL™
INYUVESI YAKWAZULU-NATALI

24 May 2018

Ms D Naicker (214502981)
School of Clinical Medicine
College of Health Sciences
deshantanaicker@gmail.com

Dear Ms Naicker

Protocol: A novel study to detect and quantify the most abundant microorganism associated with bacterial vaginosis from urine samples. Degree: MMedSc BREC Ref No: BE276/18

EXPEDITED APPLICATION: APPROVAL LETTER

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application received on 17 April 2018.

The study was provisionally approved pending appropriate responses to queries raised. Your response received on 17 May 2018 to BREC letter dated 14 May 2018 has been noted by a sub-committee of the Biomedical Research Ethics Committee. The conditions have been met and the study is given **full ethics approval**. Please ensure that site permissions are obtained and forwarded to BREC for approval before commencing research at a site.

This approval is valid for one year from **24 May 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 12 June 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
Chair: Biomedical Research Ethics Committee

cc postgraduate administrator: konar@ukzn.ac.za SCMpgrad@ukzn.ac.za
Supervisor: abbain@ukzn.ac.za

Biomedical Research Ethics Committee
Professor V Rambiritch (Chair)
Westville Campus, Govan Mbeki Building
Postal Address: Private Bag X54001, Durban 4000
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BREC Amendments Approval Letter

UNIVERSITY OF
KWAZULU-NATAL
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YAKWAZULU-NATALI
RESEARCH OFFICE
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Tel: 27 31 2604769 - Fax: 27 31 2604609
Email: BREC@ukzn.ac.za
Website: http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx

16 October 2018

Ms D Naicker (214502981)
School of Clinical Medicine
College of Health Sciences
deshantanaicker@gmail.com

Dear Ms Naicker

Protocol: A novel study to detect and quantify the most abundant microorganism associated with bacterial vaginosis from urine samples.
Degree: MMedSc
BREC Ref No: 276/18

NEW TITLE: A novel study to detect and quantify microorganisms associated with bacterial vaginosis from urine samples.

We wish to advise you that your correspondence received on 10 October 2018 submitting an application for amendments to change the title to the above and other amendments for the above study has been **noted and approved** by a subcommittee of the Biomedical Research Ethics Committee.

The committee will be notified of the above approval at its next meeting to be held on 13 November 2018.

Yours sincerely

Prof V Rambiritch
Chair: Biomedical Research Ethics Committee

cc postgraduate administrator: konar@ukzn.ac.za SCMpgrad@ukzn.ac.za
Supervisor: abbain@ukzn.ac.za