Bacterial Vaginosis BV-Assay White Paper

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Bacterial Vaginosis (BV) is the most common vaginal disorder worldwide and is associated with an increased risk of pre-term birth and acquisition of STDs. According to one study, 40% of the cases of spontaneous preterm labor and preterm birth are thought to be associated with BV (1). BV is the most common cause of vaginal discharge in women of childbearing age, accounting for 40 to 50 percent of cases (2-4). In the US it is estimated the prevalence of BV was 29 percent in the general population of women aged 14 to 49 years and 50 percent in African-American women (5). This included both symptomatic and asymptomatic infection. Worldwide, BV is common among women of reproductive age, with variations according to the population studied (6). Clinically BV represents a complex change in the vaginal flora characterized by a reduction in concentration of the “good bacteria” normally dominant hydrogen-peroxide producing lactobacilli and an increase in concentration of other organisms, “bad bacteria” especially anaerobic gram negative rods (7-10). Using microbiology and molecular diagnostic techniques bacteria that are typically detected in BV cases are Gardnerella vaginalis, Atopobium vaginae, Prevotella species, Porphyromonas species, Bacteroides species, Peptostreptococcus species, Fusobacterium species, Mycoplasma hominis, Mycoplasma genitalium, Ureaplasma urealyticum, and Mobiluncus species (6). Hence these bacteria became targets for detection for BV diagnosis along with the association of a loss of vaginal lactobacilli and a concomitant rise in the numbers of anaerobic and facultative anaerobic bacteria. Gardner and Dukes initially described this condition and identified Gardnerella vaginalis (then referred to as Haemophilius vaginalis) as the causative agent (11). However, in their initial study and in subsequent follow up work they were unable to fulfill Koch’s postulates for disease causation as inoculation of healthy individuals with G. vaginalis did not result in development of vaginitis in all of the patients tested. This disorder is also characterized by a high rate of relapse. It is hypothesized that this feature of BV is related to the ability of some of the BV-associated anaerobes, like Gardnerella vaginalis, to grow as a tenacious biofilm that adheres to the vaginal epithelium (12). Additionally several of these anaerobes, such as G. vaginalis and M. curtisi, have low in vitro susceptibility to metronidazole, the drug most commonly prescribed to treat BV (13), thereby contributing to relapse if not all of the anaerobes are eradicated.

Several different bacteria have been suspected as the causative agent of BV. The leading candidate historically is Gardnerella vaginalis, however this organism has also been found and cultured from women who do not have BV, and as such is not specific for disease (13). Because of the etiologic enigma of Bacterial Vaginosis, physicians and clinicians have had to diagnose BV based upon clinical criteria rather than on cultivation based tests and other standard microbial tests used when one organism is suspect. One such method for diagnosis of BV is through the use of Amsel’s criteria (14), where at least three of the following four conditions must be met:

1) the presence of a thin, homogeneous, milky vaginal discharge;
2) a vaginal-fluid pH that is above 4.5;
3) a positive whiff test (generation of a fishy odor when 10% potassium hydroxide is added to vaginal fluid); and
4) the presence of clue cells (sloughed epithelial cells coated with adherent bacteria) upon microscopic examination of vaginal fluid.
A second clinical method for diagnosing BV is through use of the Nugent score (15) which attempts to distinguish normal vaginal flora (lactobacilli are typically gram-positive rods) from BV-associated organisms (gram-negative organisms) by performing Gram’s stain on vaginal fluid. Neither of these methods is perfect or absolute; both tend to be highly subjective and depend on the skill and experience of the clinician processing the sample. Additionally, use of the Nugent score is based on an assumption of what the normal flora should be, which might not be totally accurate or complete. Therefore, the field of women’s health needs an alternative approach to both diagnosis BV, as well as to monitor for onset and cause. About 50% of women with diagnosable cases of BV lack any symptoms (13, 16), illustrating a further challenge in developing therapeutics and diagnostic strategies. It is only when comprehensive monitoring of these microbial populations began was there an understanding of the dynamics and interplay that exists between various populations of bacteria in this environment. It is these interactions between bacteria that lay the foundation for disease manifestation and progression.

The human microbiome project is a NIH-sponsored study that has attempted to characterize the normal microbial flora found on the human body (17-19). One of the NIH’s major studies called the “Vaginome study” used next-generation sequencing (NGS) of 16s rRNA genes present in a vaginal sample. The 16s rRNA gene is an essential component of prokaryotic translation machinery. This makes it a useful genetic target for microbiome-type studies, as all bacteria have a copy of this gene. Because of this, there is enough conservation to allow for primer design that will allow for amplification of this target from various genetic backgrounds, yet there is also enough diversity present to allow for the ability to identify organisms down to the genera and species level (20). In this way even extremely fastidious organisms or those currently unculturable can also be identified. This type of an approach also dispenses with the assumption of what a healthy or normal microflora is composed of which could limited the study to investigating samples for known organisms. AIBioTech employs a similar type of sequencing strategy to create our Gynocologene panels which provides an ideal diagnostic test for BV. There is currently no single biomarker that is 100% specific for BV. Only by evaluating the entire composition of the vaginal microflora does one get an idea about the relative levels of various types of microorganisms that cause, or contribute to, the onset of BV.

**Gynocologene**

AIBioTech has created an assay which is a custom designed proprietary NGS approach which includes the 16s rRNA gene as a diagnostic for BV. This assay is amenable to any other disorder of the female reproductive tract that is caused or influenced by microorganisms, including STIs and cervical cancer acquisition/diagnosis, as well as any novel disorders not currently attributable to known microorganisms. Since this method derives the sequence of any organism’s DNA present, it is also able to identify novel and previously unculturable organisms. This approach serves to identify any genetic determinant in a mixture of nucleic acids. The assay not only monitors the prokaryotic 16s rRNA copies that exist in patient samples, it also targets the 18s rRNA gene (analogous to the 16s rRNA gene in prokaryotes) to identify parasitic and fungal pathogens, e.g. Trichomonas and Candida spp. respectively,
and targets the Human papilloma viral genetic determinants that are linked to increased risk of cervical cancer. It is in this manner, that this NGS-based diagnostic method has the capacity to be exhaustive. One other major advantage of this approach as a diagnostic over current methods, namely the PAP smear, is that this method is much less invasive. Longitudinal sequencing of the microflora derived from healthy and asymptomatic individuals helps to identify potential colonization by organisms that may contribute or promote BV if given the opportunity to overgrow or out compete the resident lactobacilli. Additionally, while the genera of lactobacilli has members that are viewed as being negatively linked to a diagnosis of BV, some lactobacilli species, e.g. L. iners and L. gasseri, have been reported to be positively associated with BV (21). Thus, by comprehensively monitoring the microbial components of this varied and complex environmental niche, the physician will be ideally suited to proactively diagnose and prescribe medications that could specifically target organisms known to be associated with a poor prognosis and hopefully prevent BV from occurring, or re-occurring. While there is no single etiologic agent that has been identified as the causative agent for BV, the data from the vaginal microbiome project has in part supported the hypothesis that the onset of BV is due to a decrease in the numbers of lactobacilli and a concomitant rise in the numbers of anaerobes. This over growth of a group of microorganisms, that could be viewed in part as opportunistic, can also be viewed as a measure of disorder, as more organisms are present the environment gets increasingly more disordered as compared with the current view of a “normal” vaginal environment that is dominated by health-promoting lactobacilli. Therefore, in the absence of a single etiologic agent, diagnoses of BV based upon the overall entropy found in a patient’s flora; a flora dominated by a single organism would be viewed as much more structured and ordered than a flora comprised of several hundred different species all existing at a fairly high cell density. Assignment of a patient’s entropy score is based upon the total number of different organisms present in a sample, and the relative abundance of DNA sequences attributable to each organism.

Vaginal health is a complex and multi-faceted medical area. There are many discreet types of syndromes/maladies that can afflict women, including but not limited to Bacterial Vaginosis, which is a clinical syndrome caused by an alteration of the vaginal microflora. This disorder is not caused by a single pathogen; therefore to truly diagnosis BV, treat it effectively, and monitor its resolution one must monitor all the bacteria present in this environment. Physicians need access to cutting edge tools and technologies that will allow for this type of comprehensive monitoring. Currently, next-generation sequencing is the only technology capable of achieving that end, and our proposed diagnostic will arm physicians with a powerful tool to assist in their diagnoses.

References:


