Multiplex Detection of Bacteria Associated with Normal Microbiota and with Bacterial Vaginosis in Vaginal Swabs by Use of Oligonucleotide-Coupled Fluorescent Microspheres

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Bacterial vaginosis (BV) is a recurrent condition that is associated with a range of negative outcomes, including the acquisition of human immunodeficiency virus and other sexually transmitted diseases, preterm births, and pelvic inflammatory disease. In contrast to the Lactobacillus-dominated normal vaginal microbiota, BV is characterized by a lack of lactobacilli and an abundance of anaerobic and gram-negative organisms, including Gardnerella vaginalis and Atopobium vaginae. To date, the laboratory diagnosis of BV has relied upon the fulfillment of criteria determined by microscopic observation of Gram-stained vaginal swabs. We describe a molecular-based method for the diagnosis of BV using microscopy as a gold standard.

Bacterial vaginosis (BV), which is defined by a reduction in the vaginal Lactobacillus populations and an increase in the number of microbial species present, including Gardnerella spp., Atopobium spp., and others, is increasingly recognized as an important risk factor for adverse reproductive health outcomes, such as miscarriage and premature birth, and sexually transmitted diseases, including human immunodeficiency virus infection (25). Despite intense investigation, the etiology and clinical course of BV have not been well defined, probably because the normal variation of the vaginal microbial communities between individuals and their dynamics over time are complex and poorly understood. Consequently, the accurate diagnosis of BV, as well as the elaboration of effective prevention and treatment strategies, remains a major challenge (26, 44).

The current “gold standard” for the diagnosis of BV in the laboratory setting relies on microscopic profiling of microbial types in Gram-stained vaginal swab smears by using the criteria of Nugent et al. (29) or Ison and Hay (20). These scoring systems are based on the relative abundance of Lactobacillus morphotypes (large gram-positive rods) compared to the abundance of the bacterial morphotypes suggestive of BV (gram-negative or gram-variable coccobacilli and curved rods and gram-positive cocci). Although this method provides reliable information and is particularly well suited for use in resource-poor settings, it requires well-trained, highly experienced individuals to interpret the results and is simplistic in its reflection of the variety of organisms present in the microbial communities present in healthy individuals with a normal vaginal flora and patients with BV. Potential problems with the subjective interpretation of the Gram stain result, along with an increasing appreciation of the fact that the complexity of the vaginal microbiota can be missed by these classical methods, have resulted in a search for other methods for the diagnosis of BV that can also identify the different species present (5, 25).

In-depth studies of the makeup of vaginal microbial communities are quite common, including studies that use the older culture-based approaches (31) and more recent molecular studies that involve the construction of a clone library on the basis of the 16S rRNA gene (30) or the chaperonin-60 (cpn60) (16) target. While the information generated by these studies is critical to obtaining a greater understanding of the
variability and ecological dynamics of vaginal microbial communities, they remain far too labor-intensive at present for the screening of large numbers of samples or for the routine laboratory diagnosis of BV.

Most recently described molecular approaches for the diagnosis of BV are based on quantitative PCR (qPCR) of the 16S rRNA gene or other targets in bacteria extracted from vaginal samples. For example, one recent study found that the simul-

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a O, phosphorothioate-C; E, phosphorothioate-G; F, phosphorothioate-A; I, inosine; Y, C or T; R, A or G; K, T or G; S, C or G.
b The Luminex capture probes had a 5-amino-C12 linker at the 5′ end.

The goal of the present study was the design of a flow cytometric bead array for the rapid profiling of microbial communities extracted from vaginal samples and its evaluation as a potential technique for the rapid diagnosis of BV in the laboratory setting.

MATERIALS AND METHODS

Studypopulation. Samples were collected from African women as part of a larger study, ongoing since the 1980s, examining the factors associated with resistance to human immunodeficiency virus infection in a cohort of commercial sex workers in Nairobi, Kenya (22). Other samples were collected from young women from North America within the scope of a research program aimed at developing methodologies for studying the vaginal microbiota (36). The study procedures were reviewed and approved by the Health Research Ethics Boards of the University of Manitoba and the University of Nairobi.

Vaginal swabs and microscopy. For the samples from Africa, swabs of the midvaginal canal were collected at the time of speculum examination and were rolled onto a glass slide, air dried, stained with Gram stain reagents, and scored by using the criteria of Nugent et al. (29). The advantages of the cnp60 universal target (UT) as an alternative to the 16S rRNA gene for the identification of bacterial species are increasingly recognized and include the increased resolution available at the species level and its presence at a single copy per genome, improving the reliability of quantitation (7).
by using a combination of primer design software, as described previously (7). The Beacon Designer program (version 7.0; Premier Biosoft International, Palo Alto, CA), which is written to design primer and probe sets for quantitative PCR assays, was used to design oligonucleotide capture probes targeting each of nine species chosen to represent the vaginal microbiota (Table 1). For probe design, the program was set to design a TaqMan nuclease assay with an amplicon length of 100 to 500 bp, PCR primers with a melting temperature of 60°C ± 1°C. The primers suggested for use by the software were not used; rather, amplicons for all bacterial target species were generated using the cpn60 universal PCR primers, as described below. The corresponding probes were set to hybridize within 250 bp of either primer, with the optimum length being 29 to 33 bp, and to have a melting temperature of 5°C higher than that of the primers. Only antisense probes were selected. To select probes that were likely to be specific for the bacterial target of interest (Table 1), unique signatures were identified in the target cpn60 UT sequence by using SigOligo software, as described previously (7, 8). Probes that were suggested for use by the Beacon Designer program were selected on the basis of their locations within the identified signatures, and then the probes were screened against the chaperonin-60 sequence database (cpnDB; http://cpndb.cbrc.nra.ca) by using a modified BLAST algorithm (32) designed for short, nearly exact matches.

**Coupling of oligonucleotide capture probe to fluorescent beads.** Oligonucleotide capture probes were modified by the addition of a 5-amino-C12 linker at the 5′ end to facilitate their covalent coupling to the carboxylated fluorescent microspheres. An aliquot of 400 μl (5 × 10^6 beads) of a fluorescent bead suspension (Luminex Corporation, Austin, TX) was centrifuged at 14,000 × g for 1 min and resuspended in 50 μl of 0.1 M 2-(N-morpholino)ethanesulfonic acid (pH 4.5; Sigma, St. Louis, MO). To this suspension was added 1 nmol of capture oligonucleotide and 2.5 μl of freshly prepared aqueous 10-mg/ml 1-ethyl-3-(dimethylamino)propyl) carbodiimide-HCl (EDC; Pierce Chemical Co., Rockford, IL), and the suspension was incubated at room temperature in the dark for 30 min. Another 2.5-μl aliquot of EDC was then added, and the suspension was incubated as described above. The bead suspension was washed with 1 ml of 0.02% Tween 20, centrifuged at 14,000 × g for 1 min, and resuspended in 1 ml of 0.1% sodium dodecyl sulfate. The beads were centrifuged again and resuspended in 100 μl of TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA). The concentration of beads was determined with a Coulter counter (Beckman Coulter, Fullerton, CA). The beads were stored at 4°C. For the preparation of the bead master mix, each of the different bead suspensions was mixed at a concentration of 100 beads/μl in TE buffer.

**Generation of amplification for analysis with the Luminex instrument.** Plasmid templates or vaginal swab DNA extracts were used as the templates for the PCR with modified universal cpn60-targeted primers (17). To maximize the representation of each of universal primers was used, as described previously (18). For analysis with the Luminex instrument, the two universal PCR primers that amplify the 5′ end of the target of interest, an amplicon was generated by using the cpn60 universal PCR primer set reflecting the detection of a diagnosis. The PCR product was treated with 20 U of T7 exonuclease (New England Biolabs, Pickering, Ontario, Canada) at room temperature for 40 min. Seventeen microliters of the single-stranded PCR product was denatured at 95°C for 10 min, with which 33 μl of the fluorescent bead master mix was added. Hybridization of the oligonucleotide-coupled fluorescent beads to the single-stranded DNA product and a statistically significant negative control sample were chosen for each of the nine capture probes reflected the detection of the target of interest, an amplicon was generated by using the

**Diagnostic sensitivity and specificity of the assay with the Luminex instrument compared with those of microscopy.** To compare the method with the Luminex instrument to the gold standard of microscopy by using the methods of Nugent et al. (29) or Ison and Hay (20), we considered samples that were positive by the criterion presented above, that is, for the sample to contain Gardnerella vaginalis or A. vaginae, or both, to be from women with BV and samples in which no organism was detected to be from individuals with a normal vaginal flora. Analysis with the Luminex instrument was performed in a blinded manner, such that the microscopy results were unknown prior to the analysis with the Luminex instrument and correlations were done afterwards. Sensitivity and specificity, along with 95% confidence intervals, were calculated by standard procedures (2).

**Determination of oligonucleotide capture probe target specificity.** In order to determine whether the oligonucleotide capture probes specifically hybridized to the target of interest, we constructed an artificial vaginal microbiota sample (referred to as the “mixed vaginal panel”), which consisted of equal numbers of copies of cloned cpn60 UT PCR products from 20 species relevant to the vaginal tract microbiota (Table 1). Each probe was evaluated with the mixed vaginal panel and the cpn60 UT amplicon generated as described above (test sample) or with the mixed vaginal panel without the target cpn60 UT (control sample) as the template DNA. The signals generated by the test and control samples were compared to the signal generated from the no-template control. Probes that generated the strongest statistically significant positive signal from the test sample and a statistically significant negative control sample were chosen for each of the target species.

**qPCR.** The bacteria in vaginal swab DNA extracts were quantified by qPCR with species-specific cpn60 UT-targeted PCR primers (Table 1) and a standard curve consisting of serial dilutions of cloned cpn60 UT plasmids, as described previously (7). Briefly, 2 μl of template DNA was added to a solution of 1× SYBR green master mix (Applied Biosystems, Foster City, CA) and 500 nM of each primer in a final volume of 25 μl. The reactions were quantified with an ABI 7500 Fast system (Applied Biosystems). Quantification of L. iners used 1× LC480 SYBR green master mix (Roche) in a final volume of 20 μl and a LightCycler 480 quantitative PCR apparatus (Roche). The results were expressed as the number of genomes detected per qPCR (7).

![Downloaded from http://jcm.asm.org/](http://jcm.asm.org/)
artificial mixed vaginal panel as the template. Three to five probes were evaluated for each of the target organisms. As shown in Fig. 1, the probes that were designed had various performance characteristics in this artificial context, even though they all showed little sequence similarity to anything other than the target template in cpnDB. Nevertheless, it was possible to choose at least one acceptable probe for each of the target organisms. The probes that were chosen for each of the target bacteria on the basis of this analysis are shown in Table 1.

Nineplex assay with plasmid templates. To determine whether the simultaneous detection of all targets postamplification was feasible, equivalent volumes of the amplicon generated from each target template were mixed and analyzed by use of a nineplex Luminex bead master mix. While the signal amplitude varied for each of the probes, from approximately 300 MFI for the L. jensenii probe to about 1,200 MFI for the G. vaginalis probe, it was possible to detect all of the targets simultaneously in this artificial context (Fig. 2).

Quantification of target template amount by the Luminex assay. Since the amount of template DNA should correlate with the amount of product generated, at least over a limited range of concentrations, we determined the effect of the amount of input template DNA on the MFI for four targets: L. iners, Peptostreptococcus anaerobius, G. vaginalis, and A. vaginae. Typical standard curves generated with the L. iners probe are shown in Fig. 3. With a complex template (mixed vaginal panel), the correlation between the input template amount and the signal amplitude was excellent over the range of $5 \times 10^5$ to 50 copies of each target ($r^2$ values, 0.97 for L. iners, 0.97 for G. vaginalis, 0.98 for A. vaginae, and 0.99 for P. anaerobius). Single-target templates typically had poorer correlations over the same range of template amounts ($r^2$ values, 0.97 for L. iners, 0.89 for G. vaginalis, 0.82 for A. vaginae, and 0.95 for P. anaerobius). For both single-target and complex templates, the MFI did not increase linearly with the target template amount beyond $10^9$ copies of template DNA (not shown).

qPCR and Luminex assay analysis of vaginal swabs from individuals from Africa. Vaginal swabs from a cohort of African sex workers were analyzed by microscopy and were scored for BV by using the criteria of Nugent et al. (29). DNA was extracted from the same samples and was used to detect and quantify individual species by qPCR targeting G. vaginalis, A. vaginae, L. iners, L. crispatus, and L. gasseri. Primer sets targeting the cpn60 UT of G. vaginalis, L. crispatus, and L. gasseri were published previously (Table 1); and similar primer sets targeting A. vaginae and L. iners were designed for this study. Validation of the applicability of these primer sets is presented in Fig. S6 in the supplemental material. The efficacy of the DNA extraction technique with the Instagene matrix was investigated by spiking vaginal swabs with serial 10-fold dilutions of Bifidobacterium animalis cells and determining the recovery of B. animalis DNA in the extract by qPCR with cpn60-directed primers specific for B. animalis. This experiment confirmed that B. animalis DNA was recovered in the expected proportions by using Instagene matrix (data not shown). A fiveplex Luminex assay was employed with the same samples and probes for detection of the same organisms. Nine samples considered to be from individuals with a normal vaginal flora (score, 1) and nine samples considered to be from individuals with a diagnosis of BV (score, 3) were included in the analysis. There was, in general, good agreement between the results of the analysis by use of the Luminex instrument (defined as positive or negative, where a positive result is an MFI that is significantly greater than that for the negative control at $P < 0.001$; see Materials and Methods) and the results of qPCR analysis (Fig. 4). The few exceptions served to illustrate the detection limits of the method with the Luminex instrument with complex templates; samples with less than $10^9$ genomes in the qPCR were typically negative by analysis by the Luminex assay, although there were some exceptions in which samples with higher levels were negative (e.g., the sample from individual 31 for L. iners). None of the samples that were scored as being from individuals with a normal vaginal flora by microscopy were positive by use of the Luminex instrument for either of the BV-associated organisms, G. vaginalis and A. vaginae, but all were positive for one or more of the Lactobacillus species by use of the Luminex instrument and qPCR (Fig. 4). Among the nine samples that were scored as being from individuals with BV by microscopy, all were positive for G. vaginalis and/or A. vaginae by use of the Luminex instrument. Seven of the samples from individuals with BV and seven of the samples from individuals with a normal vaginal flora contained some level of L. iners that was detectable by use of the Luminex instrument and/or qPCR, but all of the samples from the Luminex instrument for selected individuals are shown in Fig. 5. As was observed in the samples from Africa, there was generally good agreement between the results of qPCR and those obtained by use of the Luminex instrument for the five targets sought by qPCR (G. vaginalis, A. vaginae, L. iners, L. crispatus, and L. gasseri); samples that were positive by qPCR but negative by use of the Luminex instrument tended to have low levels of the target organism (data not shown). By considering only the results for samples that were positive by both qPCR and with the Luminex instrument, there was a statistically significant positive correlation at the 0.05 significance level between the MFIIs and the quantities determined by qPCR for all targets analyzed except L. crispatus (Table 3). This is consistent with the positive correlations between the number of target template input copies and the MFIIs at lower copy numbers observed by using plasmid templates (Fig. 3).

Comparison of the results obtained with the Luminex instrument and by microscopy for individuals over multiple time points yielded some insight into the composition and dynamics of the vaginal microbiota. For example, many of the samples that scored normal by microscopy showed that one individual (individual 1) or more individuals (individuals 13 and 14) were infected with Lactobacillus spp. by analysis with the Luminex...
FIG. 1. Template specificity for the Luminex capture probes evaluated in this study. For each candidate probe targeting each organism, templates consisted of water (white bars), the mixed vaginal panel including the target organism (gray bars), or the mixed vaginal panel excluding the target organism (black bars). The MFI of each probe for each of the target organisms was measured in triplicate, and the results are expressed as means ± standard deviations. The probes that were ultimately chosen for inclusion in the Luminex array are indicated by an asterisk. In addition to the eight targets shown here, three probes that targeted *Lactobacillus vaginalis* (data not shown) were evaluated, leading to the selection of probe Lvg496p (Table 1).
In contrast, samples from individuals with BV tended to have a more diverse vaginal microbiota with few or no lactobacilli but with *A. vaginae*, *G. vaginalis*, *Prevotella bivia*, and, in some cases, *L. iners* being detected (individuals 5, 11, 16, and 25). The composition of the vaginal microbiota of certain individuals changed over the time course of this analysis, illustrating the shifts in the composition of the microbiota that are detectable by the use of this method. For example, individual 11 changed from having BV to having a normal vaginal flora over the course of the analysis, and this was reflected in the profile of the vaginal microbiota that was revealed by analysis with the Luminex instrument. For this individual, the MFI observed for *G. vaginalis* came to a peak and then waned, while the signal obtained for *L. iners* increased steadily, consistent with the observed transition from BV to a normal microbiota over this time course. The opposite transition was observed for individual 16; at the first three time points that individual scored as having a normal vaginal flora and displayed a steadily decreasing MFI for *L. iners* (Fig. 5), while at the final time point the individual was scored as having BV and displayed a mixed microbiota that included *A. vaginae*, *G. vaginalis*, *P. bivia*, and *L. iners*.

**Sensitivity and specificity of use of Luminex instrument for diagnosis of BV.** The results of the analysis with the Luminex instrument with samples from women from Africa and North America (Fig. 4; Fig. 5) showed that there was reasonable agreement between the diagnosis of BV by traditional microscopy and the detection of *G. vaginalis* and/or *A. vaginae* by the method with the Luminex instrument described here. By using a definition of BV as the detection of one or both of these organisms, the specificity and the sensitivity of the method with the Luminex instrument compared to those of microscopy were calculated. The sensitivity of the method with the Luminex instrument for the diagnosis of BV was lower for the samples from North America (0.86) than for the samples from Africa (1.00), and the overall sensitivity of the method was 0.90 (Table 4). The method was also highly specific; samples in which neither of the two organisms was detected were likely to have been considered to be from individuals with a normal vaginal flora by microscopy (Table 4).

**DISCUSSION**

To our knowledge, this is the first time that a Luminex instrument-based analysis has been applied to the evaluation of vaginal microbial communities. We describe a novel method for characterizing the vaginal microbiota based on the amplification of the *cpn60* UT of all bacteria present in a vaginal swab using universal PCR primers (18), followed by the detection of individual species using oligonucleotides coupled to fluorescent beads and flow cytometry. This method is conceptually similar to previously described methods for the detection and typing of cultured isolates or environmental DNA (6, 10, 39), but it offers the additional advantage of the species specificity that is achievable by the use of *cpn60*-based probes (7). This method also compares favorably to whole-genome hybridization, as described by Nikolaitchouk et al. (28), which provides a similar snapshot of the vaginal microbiota but which is necessarily limited to the analysis of cultured isolates.

We have targeted nine bacterial species for oligonucleotide capture probe development and have shown that it is feasible to design probes specific for all of the targeted species. Our choice of bacterial targets was based on a literature survey of isolates that have been cultured from the vaginal microbiota. Given the increasing amount of information regarding the
FIG. 4. Paired Luminex and qPCR analysis of samples from individuals from Africa who were classified as having a normal vaginal flora (A) or as having BV (B) by the criteria of Nugent et al. (29). Samples were analyzed by a fiveplex Luminex method for the detection of *G. vaginalis*, *A. vaginae*, *L. iners*, and *L. crispatus*, as well as *L. gasseri* (data not shown). *, statistically significantly positive Luminex assay results ($P < 0.001$).
We used the Luminex method to characterize the vaginal microbiota in a cohort of African sex workers. We found that individuals with a normal vaginal microbiota, as defined by microscopy, typically contained one or more of the *Lactobacillus* species analyzed and undetectable levels of *A. vaginae* and *G. vaginalis*. In contrast, samples from individuals with BV were much more likely to contain *A. vaginae* and/or *G. vaginalis* DNA. African individuals with BV often had detectable levels of *L. iners* but not *L. crispatus*. These results are consistent with those presented in other reports describing the association of *A. vaginae* and *G. vaginalis* with BV (4, 11–13, 15, 24, 35, 41).

We used an expanded nineplex analysis of a longitudinal series of samples from adolescents from North America and compared the results of the Luminex assay with those of microscopy and qPCR. In general, the agreement among the methods was excellent; the microbiota of individuals who scored normal by microscopy was usually dominated by one or more of the lactobacilli for which assays were conducted, and individuals with BV generally had more complex microbiota, with *A. vaginae* and *G. vaginalis* commonly being detected. We noted that some of the samples from individuals with BV contained both *G. vaginalis* and *P. bivia* (individuals 5 and 16) and that these samples had low levels of lactobacilli. Furthermore, samples containing *L. crispatus* or *L. gasseri* had undetectable levels of *G. vaginalis* and *P. bivia*, while *L. iners* could be found in either samples from individuals with a normal vaginal flora or samples from individuals with BV. These observations are consistent with the symbiotic relationship between *G. vaginalis* and *P. bivia* that has been reported (34) and with the ability of certain vaginal lactobacilli to inhibit the growth of *G. vaginalis* and *P. bivia* (1). However, despite the symbiotic relationship between *P. bivia* and *P. anaerobius* (33), the latter organism was not found in any of the samples.

Considering the samples from women from both Africa and North America, we determined the sensitivity and the specificity of this multiplex bead-based method for the diagnosis of BV using microscopy as a gold standard. By using only the presence of *A. vaginae* or *G. vaginalis*, or both, as the defining criterion for BV, the method described here performed very well compared to the performance of microscopy and provided additional information regarding the species composition of each sample. The difference in sensitivity between the two sample groups that was measured may relate to the different scoring systems that were used, since the samples from Africa were evaluated by using the criteria of Nugent et al. (29), while the samples from North America were evaluated by using the criteria of Ison and Hay (20). While the overall sensitivity of the Luminex method was not as high as that of the qPCR-based method described by Menard et al. (24), we expect that this method could be improved upon by adding other BV-associated targets to the Luminex array and/or by increasing the number of replicates analyzed for each individual. Since BV is a polymicrobial syndrome with unique individual profiles
A. vaginae, or both. Samples were scored BV negative if neither of these two organisms were detected. Only organism detected was L. vaginalis, which was scored as having BV at time zero, yet the microbiota of the cohort from Africa and the samples from North America. By using the criteria of Nugent et al., (29), a score of 1 to 3 was considered normal, while a score of 7 to 10 was considered diagnostic of BV. By using the criteria of Ison and Hay (20), a score of 1 or 2 was considered normal, while a score of 3 was considered diagnostic of BV. Samples were scored BV positive by the Luminex assay if the MFI was significantly greater ($p < 0.001$, one-tailed Student’s t-test) than the MFI for the controls for either G. vaginalis or A. vaginae, or both. Samples were scored BV negative if neither of these two organisms were detected.

(15), it is important to inform the choice of probes by sequence analysis of the DNA from a range of individuals with BV. Some of the samples showed the limitations of the current Luminex array. For example, individual 31, who was from North America, was scored as having BV at time zero, yet the only organism detected was L. crispatus; it is likely that organisms not represented in the current array were responsible for the diagnosis of BV. Even though two of the probes targeted organisms that are known to be associated with the vaginal microbiota (L. vaginalis and P. anaerobius), use of those probes did not yield positive results for any of the samples, suggesting that these organisms were not present in quantities in the sample sufficient to be detected by this method. In addition, all samples were negative by use of another probe targeting Bifidobacterium bifidum (data not shown), an organism associated with the vaginal microbiota (23). Consistent with this finding, we did not find these organisms in any appreciable abundance by ultradepth- pyrosequencing analysis of the vaginal microbiota of the cohort from Africa and the samples from Winnipeg, Manitoba, Canada (37; J. Schellenberg et al., unpublished). Future Luminex arrays that target the vaginal microbiota will benefit from culture-independent analysis in order to guide the choice of probes. For example, there is an abundance of uncultivated Megasphaera-like organisms that are unique to the vaginal microbiota and that would be good targets for probe development (46); we have observed similar Megasphaera-like species in cpn60-based culture-independent studies of the vaginal microbiota in individuals from Africa (Schellenberg et al., unpublished).

In summary, we describe a multiplex method for the detection and generation of semiquantitative data for organisms within the vaginal microbiota based on the amplification of the cpn60 UT and hybridization to species-specific oligonucleotide-coupled fluorescent beads. This method is rapid and simple, and it provides a means of identifying and quantifying the key species present in a vaginal sample at a given point in time. Since BV is often a transient, recurring condition that is difficult to treat (44), diagnostic methods that facilitate the longitudinal profiling of the vaginal microbiota are desirable. By the method that we describe, it was possible to detect the presence of up to nine species in a single PCR; and further multiplexing, which would take advantage of the full capabilities of the Luminex instrument, is certainly possible. The ease of specific oligonucleotide capture probe design and validation, along with the ability to multiplex the detection and quantification of bacterial species, makes this approach very attractive for characterization of the vaginal microbiota. Future expansion and improvement of the Luminex array with information obtained from in-depth molecular studies will allow the detailed profiling of vaginal microbial communities, as well as the rapid and reproducible diagnosis of BV in the clinical setting.

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