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 easy determination of the species profile within the vaginal microbiota based on the amplification of the chaperonin-60 genes of all bacteria present in the swab and hybridization of the amplicon to species-specific oligonucleotide-coupled fluorescent beads that are identified by flow cytometry with a Luminex instrument. We designed a nineplex Luminex array for characterization of the vaginal microbiota and applied it to the analysis of vaginal swabs from individuals from Africa and North America. Using the presence of *A. vaginae* or *G. vaginalis*, or both, as the defining criterion for BV, we found that the method was highly specific and sensitive for the diagnosis of BV using microscopy as a gold standard.