AutoGenomics

INFINITI® Bacterial Vaginosis QUAD Assay

Directional Package Insert (DPI)

For *In Vitro* Diagnostic Use

CE

FOR EXPORT ONLY

Manufactured by AutoGenomics, Inc., 2980 Scott Street, Vista, CA USA 92081

Authorized EU Agent: Medical Device Safety Service GmbH (MDSS)
Schiffgraben 41, 30175 Hannover, Germany

Doc EM-34044E (English) Rev. D (CO 2612) July 2013
Tel: 760-477-2248 www.autogenomics.com

AutoGenomics

INTENDED USE

The INFINITI Bacterial Vaginosis (BV) QUAD Assay is an *in vitro* diagnostic test for the detection and identification of six pathogens in genomic deoxyribonucleic acid (DNA) obtained from human endocervical and cervical specimens. The INFINITI BV QUAD is designed to identify the following pathogens: *Atopobium vaginae, Bacteroides fragilis, Gardnerella vaginalis, Mobiluncus curtisii, Mobiluncus mulieris,* and *Prevotella bivia.* The INFINITI BV QUAD is a qualitative assay for use in clinical laboratories upon prescription by the attending physician.

BACKGROUND INFORMATION

Bacterial Vaginosis (BV) is the most common disorder that prompts women to seek gynecological care. The most common patient symptoms are discharge, odor and irritation. BV affects up to 23% of women of reproductive age. The bacteria associated with BV are classified as Fastidious Vaginal Microorganisms (FVMs), that are either difficult to culture or have yet to be cultured. As a consequence, the use of molecular techniques to detect FVMs is critical for the clinician to make an accurate BV diagnosis.

BV is characterized by disturbed vaginal microflora where the predominant lactobacilli are depleted and there is an overgrowth of *Gardnerella vaginalis* (GV) and other anaerobic bacteria such as *Bacteroides fragillis* (BF), *Mobiluncus mulieris* (MM) and *Mobiluncus curtisii* (MC). This replacement is still unknown but may be due to the change in vaginal pH which favors the growth of pathogenic bacteria. Risk factors for BV include douching, menstruation, and antibiotic use which raise the pH and favor the growth of BV-assorted microorganisms. Accurate diagnosis and treatment of BV is especially critical for pregnant women, as the disease is associated with a five-fold increased risk for late miscarriage and pre-term birth. BV associated organisms that are part of the endogenous vaginal flora, such as *Prevotella, Peptostreptococus, Gardnerella vaginalis, Mycoplasma and Mobiluncus* which may reside in the rectum, become dominant.

Gardnerella vaginalis (GV)

Gram-variable facultative anaerobic bacteria. Produces the toxin Vaginolysin that triggers inflammation. The ability for GV to form a biofilm on the vaginal epithelium may protect it from antibiotic therapy causing the persistence of BV.

Atopobium vaginae (AV)

Gram-positive anaerobic bacteria. Like GV, the presence at a high concentration is highly sensitive and specific for the diagnosis of BV and is associated with disease recurrence. Some isolates exhibit reduced susceptibility to metronidazole and triggers an inflammatory response from vaginal epithelial cells.

Mobiluncus

M. mulieris (MM) and *M. curtisii* (MC) are slowly growing fastidious organisms that are flagellated and demonstrate a cork-screw motility on a wet mount of vaginal fluid. With microscopy the prevalence of *Mobiluncus* in BV has been reported to be as high as 77%. DNA probes have the advantage of identifying which of the species is present.

TEST PRINCIPLE/ASSAY OVERVIEW

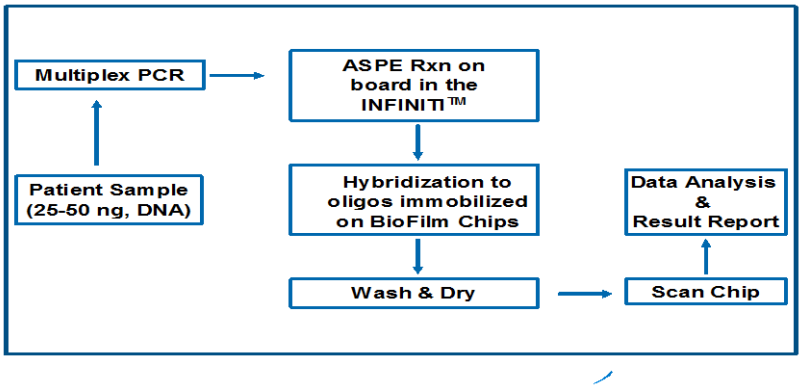
The INFINITI BV QUAD utilizes AutoGenomics' proprietary film-based microarray technology combined with process automation, reagent management and software technology for multiplex detection of the presence of BV pathogens in deoxyribonucleic acid (DNA) obtained from cervical specimens.

The INFINITI BV QUAD is based on the following processes:

a) DNA extraction from endocervical and urine specimens
b) PCR amplification of purified DNA
c) Fluorescent label incorporation using analyte specific primer extension (ASPE)
d) Hybridization of the labeled ASPE primers to a microarray followed by washing.
e) Scanning of the microarray
f) Signal detection and analysis

Steps (c) through (f) are automated by the INFINITI Analyzer and INFINITI PLUS Analyzer.

A schematic overview of the assay is shown below.



DEVICE DESCRIPTION

The INFINITI BV QUAD is an *in vitro* diagnostic device that consists of reagents and instrumentation which includes polymerase chain reaction (PCR) primers, microarrays, a thermal cycler, an imager, and software designed to detect the presence of the following pathogens: *Atopobium vaginae* (AV), *Bacteroides fragillis* (BF), *Gardnerella vaginalis* (GV), *Mobiluncus curtisii* (MC), *Mobiluncus mulieris* (MM), and *Prevotella bivia* (PB).

The INFINITI BV QUAD is comprised of the BioFilmChip® Microarray, the Intellipac® Reagent Module, and the PCR Amplification Mix. The instrumentation for the INFINITI BV QUAD is the AutoGenomics INFINITI Analyzer with the Qmatic™ Operating Software. The INFINITI Analyzer is CE marked.

The **BioFilmChip Microarray** consists of a polyester film coated with proprietary multi-layer components designed for DNA analysis. The layers have been designed to provide a versatile surface to enhance test performance. The INFINITI BV QUAD uses a microarray chip which has capture probes spotted on the surface of the film. Four (4) samples can be run on one microarray. Twelve (12) microarrays are housed in a magazine.

The **Intellipac Reagent Module** which acts as a communication link contains four reservoirs that house the test reagents and has an integrated memory chip. Reagent information such as lot number, expiration date, and volume usage are stored in the memory chip. The Intellipac Reagent Module communicates with the INFINITI Analyzer and provides the reagent information which appears on the assay report and printout. The Intellipac Reagent Management Module provides test reagent for 96 samples.

The **PCR Amplification Mix** consists of the reagents needed for the PCR amplification step of the assay. Each box of the PCR Amplification Mix provides 4 x 500µL vials of PCR Amplification

The **INFINITI Analyzer and INFINITI PLUS Analyzer** automates the INFINITI BV QUAD Assay and integrates all the discrete processes of sample (PCR reaction product) handling, reagent management, hybridization, detection, and results analysis. The assays are processed automatically and read by the built-in confocal microscope. Results are analyzed and presented as positive or negative for the presence of BV pathogens.

The Analyzers are provided with the Operator's Manuals. The Operator's Manual provides detailed description and operating principle of the Analyzers and instructions for use.

The INFINITI Analyzer and INFINITI PLUS Analyzer are CE marked

WARNINGS AND PRECAUTIONS

Handling Requirements

- **For *in vitro* diagnostic use. To be used by qualified laboratory personnel.**
- This test is to be used only with endocervical samples.
- To minimize the risk of cross contamination, sample preparation, PCR reaction set up and PCR product analysis should be performed according to approved guidelines such as CLSI (Molecular Diagnostic Methods for Genetic Diseases: Approved Guideline).
- All patient specimens are potentially hazardous and care should be taken when handling materials of human origin. No test method can offer complete assurance that HCV, HIV or other infectious agents are absent.
- Clean the lab and equipment with fresh 10% bleach or equivalent to prevent contamination.
  Follow the CLSI Guidelines (Molecular Diagnostics Methods for Infectious Diseases; Approved Guidelines; MM3-A).
- Do not pool/mix reagents from different lots.
- Do not use a kit or reagent past its expiration date.
- Store kits and reagents according to the product label.

Laboratory Procedures

- Follow normal precautions for handling laboratory reagents. Do not mix reagents from different containers or from different lots.
- Follow safe laboratory procedures: do not pipette by mouth; wear protective clothing (e.g., disposable gloves laboratory coats) and eye protection; do not eat, drink or smoke in the laboratory work areas; wash hands thoroughly after handling samples and reagents.

Waste Handling
- Dispose of unused reagents, specimens and waste according to applicable country, federal, state and local regulations
- Material Safety data Sheets (MSDS) are available upon request from AutoGenomics Customer Service

Sample Preparation
- Refer to the instructions provided with the sample processing kit.
- The PCR product cannot be stored prior to loading it onto the microarray. Use immediately**.**

INFINITI Analyzer and INFINITI PLUS Analyzer
- **Read the Operator's Manuals before operating the instruments.** Pay particular attention to "Notes".
- Follow the Caution and Safety Warning in the Operator's Manual.
- Refer to the Installation Requirements Section when installing the instrument.
- Refer to the Errors Section when errors are encountered while operating the instrument.
- Refer to the Help Section when problems are encountered

STORAGE / STABILITY

BioFilmChip Microarray: 12 months Refrigerated (2 to 8°C)

Intellipac Reagent: 12 months Refrigerated (2 to 8°C)

Note: Remove the Intellipac from the Analyzer and store refrigerated as soon as possible. Do not use after Intellipac has been opened for four weeks.

Amplification Mix: 18 months Frozen (-30°C to -15°C)

Note: Specific product expiration date is printed on the product label

REAGENTS REQUIRED AND PROVIDED BY AUTOGENOMICS

ASSAY REAGENTS (SUFFICIENT FOR 192 TESTS)
- Catalog Number 04-1140-02 INFINITI BV QUAD Magazine – BioFilmChip® Microarray
  4 magazines per package; 48 tests per magazine
- Catalog Number 04-2140-02 INFINITI BV QUAD Intellipac® Reagent Management Module
  2 modules per package; 96 tests per module, 4 x 1.1 mL of ASPE mix which contains:
  - dNTPs
  - Labeled -dCTP
  - Analyte Specific Primers
  - Extension reaction Buffer
- Catalog Number 04-3140-02 INFINITI BV QUAD Amp Mix: 4 x 500µL vials, 48 tests per vial, of PCR reaction master mix containing
  - dNTPs
  - Multiplex Primer Mix
  - $MgCl_2$
  - PCR Reaction Buffer

REAGENTS REQUIRED BUT NOT PROVIDED WITH THE ASSAY REAGENTS

- AutoGenomics Catalog Number 12-0470-00 INFINITI Sample processing Kit
- Internal Control 1 Catalog Number 12-0170-00
- AutoGenomics Catalog Number 12-0030-00 Solution BF2 (Hybridization Buffer): 6 x 30ml bottles. The hybridization buffer consists of:
  SSC
  Sodium Azide Preservative 0.08%
  EDTA
  10X Blocking Buffer
- AutoGenomics Catalog Number 12-0220-00 BF DNA Template Control
- AutoGenomics Catalog Number 12-0230-00 MM DNA Template Control
- AutoGenomics Catalog Number 12-0240-00 AV DNA Template Control
- AutoGenomics Catalog Number 12-0250-00 GV DNA Template Control
- AutoGenomics Catalog Number 12-0260-00 MC DNA Template Control
- AutoGenomics Catalog Number 12-0270-00 PB DNA Template Control
- **FOR INFINITI Analyzer***:* AutoGenomics Product Number 12-0020-00 Solution BF1
  OR
  FOR INFINITI PLUS Analyzer*:* Product Number 12-0330-00: Buffer Solution BF1

REAGENTS REQUIRED BUT NOT PROVIDED BY AUTOGENOMICS

- Molecular Grade Water (DNAse and RNAse free)
- Platinum Taq DNA Polymerase (Invitrogen Catalog Number 10966-018)

EQUIPMENT

The following equipment is required but not provided with the assay reagents

- Pipettors
- Mini Centrifuge
- Pipette tips
- Microfuge tube Racks
- Thermocycler
- Vortex
- 0.2 ml thin wall tubes for PCR
- 1.5 ml microcentrifuge tubes
- 8-well Flat Strip Caps (Genesee Scientific, Catalog No. 22-623)
- AutoGenomics Product Number 11-0060-00: INFINITI Waste tray Stir Bars
- AutoGenomics Product Number 11-0020-00: INFINITI Waste Tray Liners
- AutoGenomics Product Number 11-0080-00: INFINITI Pipette Tips

- **FOR INFINITI Analyzer***:*
  - AutoGenomics Product Number 10-0010-99: INFINITI Analyzer
  - AutoGenomics Product Number 11-0030-00: 24-Well Plates with Lids
  - AutoGenomics Product Number 11-0050-00: INFINITI Temp Cycle Plate

- **FOR INFINITI PLUS Analyzer***:*
  - AutoGenomics Product Number 10-0020-99: INFINITI PLUS Analyzer
  - AutoGenomics Product Number 11-0100-00: 48-Well Plates and Product Number 11-0110-00: 48 Well Plate Lid

ASSAY PROCEDURE

Specimen Processing

It is recommended that the INFINITI Sample Processing Kit (Catalog Number 12-0470-00) be used for sample processing. Follow the instructions provided with the processing kit.

Recommended Controls

It is recommended that positive controls and a no template control (i.e. molecular grade water) are included in each test run. The no template control serves as a contamination control. If this control is positive, then samples should be tested again taking appropriate measures to prevent contamination.

The following positive controls, available from AutoGenomics, are recommended for use with the INFINITI BV QUAD (refer to section on REAGENTS REQUIRED for Catalog Number)

(a) BF DNA Template Control
(b) MM DNA Template Control
(c) AV DNA Template Control
(d) GV DNA Template Control
(e) MC DNA Template Control
(f) PB DNA Template Control

Note: Please use proper PCR technique to prevent contamination of reagents with positive controls. Sealing the 24WP containing sample DNA and "no template control" samples with caps **before** adding the positive controls is recommended to prevent cross contamination.

Amplification Reaction

Note:

- Keep Taq DNA polymerase on ice.
- Completely thaw reagents at room temperature then immediately place on ice.
- Vortex the amplification mix for 2 to 5 seconds then centrifuge briefly to bring the contents to the bottom of the tube.
- To avoid potential contamination, a separate area is recommended for assembly of the PCR reaction. Decontaminate pipettes and all work surfaces with freshly prepared 10% bleach.
- Filter tips and gloves must be used when handling samples and controls.
- Make sure there is no abnormal evaporation of the PCR product. After PCR is complete, visually inspect for any volume change. All amplification reaction volumes should be about 15 µL. Otherwise, do not proceed with the assay.

Note:

- For the INFINTI Analyzer use the 24WP.
- For the INFINTI PLUS Analyzer use the 48WP.

1. Prepare the PCR master mix

Amplification mix	9.75 µL
Platinum Taq polymerase	0.25 µL
Internal Control 1	1.00 µL
Total volume of PCR master mix	11.0 µL

Note: Calculate the amount of each reagent needed based on the number of reactions.

2. Gently vortex the PCR master mix then dispense 11.0 µL of master mix into wells of the well plate.

3. Add 4.0 µL of sample or control DNA to each well

PCR master mix	11.0 µL
Sample	4.0 µL
Total volume of amplification reaction	15.0 µL

Note: **This is a QUAD assay**. When loading samples, always load the samples in multiples of fours and in consecutive order. For example, if loading 8 samples, load wells A1 to A8. **Do not** load the B wells**.**

4. Place the well plate, sealed with 8-well flat strip caps, in a thermocycler and immediately commence the amplification reaction using the following program.

Step No.	Temperature °C	Time (sec)	No. of Cycles
1	94	120	1
2	94 66-58 (-0.8/cycle) 72	10 60 40	10x
3	94 58 72	10 60 40	30x
4	4	hold	1

Note: After each cycle in step 2, the temperature is decreased by 0.8°C. When an Eppendorf Mastercycler EP was used with the ramp rate set at 75%, the total cycling time was one (1) hours and 44 minutes (± 5 min). If using other thermocycler models, we recommend adjusting the ramp rate in order to obtain an equivalent total cycling time.

Sample Loading

1) Carefully remove the 8-well flat strip caps to avoid splashing.
2) Load the well plate in the appropriate orientation (with well A1 in the back left corner) into the Analyzer
   - **INFINITI Analyzer:** Load the assembled 24WP with the associated lid (Catalog # 11-0030-00).
   - **INFINITI PLUS Analyzer:** Load the assembled 48WP with a clean 48WP lid (see instructions in the INFINITI PLUS Analyzer Operator's Manual) (Catalog # 11-0110-00, reusable).
3) Load the following: assay specific magazines, Intellipac, INFINITI Static Free Pipette tips, and buffer.
   - **FOR INFINITI Analyzer***:*
     Buffer Solution 1 and 2 should be placed in the INFINITI bottle holders. Buffer Solution 1 goes in the left holder (near the magazine) and Buffer Solution 2 in the right holder (near the Intellipac).
   - **FOR INFINITI PLUS Analyzer***:*
     Buffer Solution 2 should be placed in the INFINITI bottle holders. Buffer Solution 2 goes in the right holder (near the Intellipac).

Operation of the Analyzers

Follow the instructions in the Operator's Manuals

INFINITI Analyzer Operator's Manual (Part Number EM-34000)
INFINITI PLUS Analyzer Operator's Manual (Part Number EM-34041)

QUALITY CONTROL

- Maintain calibration of thermocycler according to manufacturer's specifications.
- Maintain calibration of INFINITI or INFINITI PLUS Analyzer according to AutoGenomics' specifications.
- Maintain calibration of pipettes according to manufacturer's specifications.

LIMITATIONS

The results obtained from the INFINITI BV QUAD should be used and interpreted only in the context of the overall clinical diagnosis. AutoGenomics is not responsible for any clinical decisions that are made. Sensitivity of the INFINITI BV QUAD may be less than the comparator method for Mobiluncus curtisii (MC). INFINITI assay does not detect all types of *Atopobium vaginae* (AV). It is specific for AV GenBank DSM15829; ATCC strain BAA-55. Blood in the samples may interfere with the correct calls of *Mobiluncus mulieris* (MM) and *Mobiluncus curtisii* (MC).

INTERPRETATION OF RESULTS

Results from the INFINITI BV QUAD are reported to the user as "Positive" or "Negative" for the presence of pathogens detected.

The Internal Control is intended to identify specimens that contain polymerase inhibitors. INFINITI BV QUAD results are interpreted as follows:

Analyte Result	Internal Control (IC) Result	INTERPRETATION
All Analytes Negative	Positive	Negative
All Analytes Negative	Negative	Invalid Test Result
Any Analyte Positive	Positive or Negative	Valid Test Result

If the analyzer detects a problem or an error (e.g., assay parameters not met), no results will be reported. Instead, a description of the problem or error code will be displayed. The Trouble Shooting section of the INFINITI Analyzer Operator's Manual provides an explanation of the errors. The assay needs to be repeated.

DISPOSAL

Waste materials for the INFINITI BV QUAD are common waste materials generated in clinical laboratories, and should be handled/ disposed of in accordance with the policies/procedures in place in the clinical laboratory.

PERFORMANCE CHARACTERISTICS

Analytical Specificity

Studies related to specificity were conducted during assay development. PCR primer specificity was determined by amplicon size on a gel and sequencing the amplicon. ASP primer specificity was determined by the correct calls made by the assay using known genomic samples. Capture probe

specificity was determined by hybridizing different oligos and demonstrating that correct oligo hybridizes to the known spot.

Analytical Specificity/Cross-Reactivity study demonstrated no cross-reactivity of the INFINITI BV QUAD with the following microbial DNA.

Escherichia coli	*Streptococcus agalactiae*	*Bacteriodes uniformis*
Klebsiella pneumoniae	*Enterococcus (Streptococcus) faecalis*	*Fusobacterium nucleatum*
Pseudomonas aeruginosa	*Streptococcus pyogenes*	*Candida albicans*
Haemophilus influenzae	*Staphylococcus aureus*	*Enterococcus cassioflavus*
Proteus vulgaris	*Staphylococcus epidermidis*	*Candida parapsilosis*
Enterobacter cloacae	*Corynebacterium striatum*	*Candida tropicalis*
Acinetobacter baumannii	*Peptostreptococcus anaerobis*	*Candida krusei*
Moraxella (Branhamella) catarrhalis	*Clostridium sordelli*	*Candida glabrata*
Neisseria meningitidis	*Clostridium difficile*	*Lactobacillus jensenii*
Neisseria gonorrhoeae		

Analytical Sensitivity (Level of Detection)
Analytical sensitivity studies performed using plasmid samples established the limit of detection (copies/test) of the INFINITI BV QUAD. The limit of detection is the lowest concentration of DNA that gives all correct calls. Analytical sensitivity studies were performed using plasmid samples to establish the limit of detection (copies/test) of the INFINITI BV QUAD. The limit of detection is the lowest concentration of DNA that gives all correct calls. The study established the lower limit of detection for *Bacteroides fragillis* (BF) and *Mobiluncus mulieris* (MM) as 200 copies/test, for *Atopobium vaginae* (AV) 100 copies/test, for *Mobiluncus curtisii* (MC) 20 copies/test, and for *Gardnerella vaginalis* (GV) and *Prevotella bivia* (PB) 10 copies/test. The upper limit of detection was determined to be above $2x10^5$ copies/test. The INFINITI BV QUAD operating sample volume range is from 5 to 9µL. The sample volume range is the upper and lower sample volume limits where the INFINITI BV QUAD gave all correct calls.

Sample Carry-over
Sample carryover studies using plasmid samples demonstrated that there is no sample carry-over with the INFINITI BV QUAD. BF, AV, MC, MM, GV, and PB Plasmid DNA samples and TE/Salmon testes buffer were run alternatively to determine if one sample will carry over to the next. No carry-over contamination was observed.

Potential Interference from Blood and other Substances
A substance interference study was performed to determine if the INFINITI BV QUAD will be affected by the presence of products commonly used in the vaginal tract.

Chemical substances that did not interfere with the assay at a concentration of 5% were vaginal cream, vaginal ointment, douche, and contraceptive gel were used to spike plasmid samples (at concentration typically found in the vaginal tract) before testing with the INFINITI BV QUAD.

Whole blood at a concentration of 5% did not interfere with BF, GV, AV or PB; however it did interfere with MM and MC. The addition of whole blood reduced the signals of MM and MC by 97% and 98.6% respectively.

Since whole blood was shown to significantly interfere with the MM and MC, bloody specimens may interfere with assay performance.

Precision and Reproducibility

Precision and reproducibility of the INFINITI BV QUAD was evaluated using plasmid DNA controls at the LOD performed by QC during performance testing for release. The reproducibility study was designed to determine the following:

- Reproducibility of three lots of INFINITI BV QUAD for each analyte (BF, AV, MC, MM, GV, and PB).
- Reproducibility of the INFINITI BV QUAD with three INFINITI Analyzers

All calls were correct. This demonstrated reproducibility of the INFINITI BV QUAD, using three instruments.

Clinical Validation Studies

Clinical Comparison Studies of the INFINITI BV QUAD was conducted using twenty-seven previously characterized clinical samples with 6 possible results per sample (positive or negative calls for 6 pathogens). Each sample was tested independently at 2 sites (i.e., 2 operators & 2 INFINITI Analyzers) for a total of 54 tests.

Table 1: Summary of Comparison Data for Pathogen calls for INFINITI BV QUAD.

	Site 1 N=27	Site 2 N=27
% correct pathogen calls	92.6% 150/162	95.1% 154/162
Overall % correct pathogen calls	93.8% 304/324	

Table 2: Sensitivity and Specificity for INFINITI BV QUAD.

N=54 tests	BF	GV	MM	MC*	AV**	PB***
Sensitivity	100%	100%	100%	67%	8%	100%
Specificity	100%	100%	100%	100%	100%	87%
Positive Predictive Value	100%	100%	100%	100%	100%	22%
Negative Predictive Value	100%	100%	100%	96%	79%	100%

*Sensitivity of the INFINITI BV QUAD may be less than the comparator method for MC.

**The INFINITI BV QUAD does not detect all AV strains that are detectable by 16S rRNA PCR. The INFINITI assay detects type specific AV (GenBank DSM15829; ATCC strain BAA-55).

***The INFINITI assay may be more sensitive for PB than the comparator method. The analysis of AGI's INFINITI data for PB showed the signals were low, near the limit of detection. This could indicate low amount of microorganism in the sample which were not picked up using the comparator method.

AutoGenomics

REFERENCES

1. Spiegel, C., Bacterial Vaginosis, Clinical Microbiology Reviews, Oct. 1991; Vol. 4, No. 4, p. 485-502.
2. Medical Diagnostic Laboratories, L.L.C., "Bacterial Vaginosis Panel"
3. Medical Diagnostic Laboratories, L.L.C., "Atopobium vaginae""