October 21, 2026

Biofire Diagnostics, LLC
Erin Moseley
Manager, Senior Regulatory Affairs Specialist
515 Colorow Dr.
Salt Lake City, Utah 84108

Re: K262136
Trade/Device Name: BIOFIRE SPOTFIRE Vaginitis (VG) Panel
Regulation Number: 21 CFR 866.3975
Regulation Name: Device that detects nucleic acid sequences from microorganisms associated with vaginitis and bacterial vaginosis
Regulatory Class: Class II
Product Code: PQA, NSU, OOI, OUY
Dated: June 24, 2026
Received: June 25, 2026

Dear Erin Moseley:

We have reviewed your section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (the Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database available at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Before making any change significantly affecting the safety or effectiveness of the device, you must submit a new premarket notification in accordance with 21 CFR 807.81. Additional information about changes that may require a new premarket notification are provided in the FDA guidance documents entitled "Deciding When to Submit a 510(k) for a Change to an Existing Device" (https://www.fda.gov/media/99812/download) and "Deciding When to Submit a 510(k) for a Software Change to an Existing Device" (https://www.fda.gov/media/99785/download).

Your device is also subject to, among other requirements, the Quality Management System Regulation (QMSR) (21 CFR Part 820), which includes, but is not limited to, ISO 13485 clause 7.3 (Design controls), ISO 13485 clause 8.3 (Nonconforming product), ISO 13485 clause 8.5.2 (Corrective action), and ISO 13485 clause 8.5.3 (Preventative action). Please note that regardless of whether a change requires premarket review, the QMSR requires device manufacturers to review and approve changes to device design and production (ISO 13485 clause 7.3 and ISO 13485 clause 7.5) and document changes and approvals in the Medical Device File (ISO 13485 clause 4.2.3).

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR Part 803) for devices or postmarketing safety reporting (21 CFR Part 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the Quality Management System Regulation (QMSR) (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR Part 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR Parts 1000-1050.

All medical devices, including Class I and unclassified devices and combination product device constituent parts are required to be in compliance with the final Unique Device Identification System rule ("UDI Rule"). The UDI Rule requires, among other things, that a device bear a unique device identifier (UDI) on its label and package (21 CFR 801.20(a)) unless an exception or alternative applies (21 CFR 801.20(b)) and that the dates on the device label be formatted in accordance with 21 CFR 801.18. The UDI Rule (21 CFR 830.300(a) and 830.320(b)) also requires that certain information be submitted to the Global Unique Device Identification Database (GUDID) (21 CFR Part 830 Subpart E). For additional information on these requirements, please see the UDI System webpage at https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/unique-device-identification-system-udi-system.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the

Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

HIMANI BISHT -S

Himani Bisht, Ph.D.
Assistant Director
Division of Microbiology Devices
OHT7: Office of In Vitro Diagnostics
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 07/31/2026
See PRA Statement below.

510(k) Number *(if known)*
K262136

Device Name
BIOFIRE SPOTFIRE Vaginitis (VG) Panel

Indications for Use *(Describe)*
The BIOFIRE SPOTFIRE Vaginitis (VG) Panel is an automated multiplexed polymerase chain reaction (PCR) test intended for use with BIOFIRE SPOTFIRE System for the simultaneous qualitative detection and identification of nucleic acids from multiple pathogens associated with bacterial vaginosis (BV), vulvovaginal candidiasis (VVC), and trichomoniasis. The BIOFIRE SPOTFIRE Vaginitis (VG) Panel is intended for use on clinician-collected or self-collected (in a clinical setting) vaginal swabs obtained from individuals with symptoms of vaginitis/vaginosis.

The following analytes are identified and differentiated using the BIOFIRE SPOTFIRE Vaginitis (VG) Panel:

Bacteria	Yeast	Parasites
Bacterial Vaginosis (BV) Analytes (not reported individually)	Candida albicans	Trichomonas vaginalis
Fannyhessea (Atopobium) vaginaea	Candida glabrata	
Bacterial Vaginosis-Associated Bacterium 2 (BVAB2)	Candida krusei	
Lactobacillus crispatus	Candida parapsilosis	
Lactobacillus gasseri/paragasseri	Candida tropicalis	
Lactobacillus jensenii/mulieris	Candida dubliniensis	
Sneathia vaginalis (amnii)/ sanguinegens		

The BIOFIRE SPOTFIRE Vaginitis (VG) Panel is indicated as an aid in the diagnosis of specific agents of vaginitis/vaginosis and results should be used in conjunction with other clinical and laboratory findings. The BIOFIRE SPOTFIRE Vaginitis (VG) Panel is not intended to monitor treatment for vaginitis/vaginosis.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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BIOFIRE® SPOTFIRE® Vaginitis (VG) Panel

510(k) Summary
BioFire Diagnostics, LLC

Introduction:

The purpose of this Dual 510(k)/CLIA waiver submission is to obtain clearance for the BIOFIRE® SPOTFIRE® Vaginitis (VG) Panel. The SPOTFIRE VG Panel is compatible with the BIOFIRE® SPOTFIRE® System (K213954), an automated, polymerase chain reaction (PCR)-based in vitro diagnostic system for infectious disease testing.

According to the requirements of 21 CFR 807.92, the information included with this submission provides sufficient detail to understand the basis for a determination of substantial equivalence.

Submitted by:

BioFire Diagnostics, LLC (bioMérieux)
515 Colorow Drive
Salt Lake City, UT 84108

Contact: Erin Moseley
Telephone: 385-414-4985
Email: Erin.Moseley@biomerieux.com

Date submitted: June 24, 2026

Device Name and Classification:

Trade name: BIOFIRE® SPOTFIRE® Vaginitis (VG) Panel

Regulation Number for Device Classification: 21 CFR 866.3975

Classification Name: Device That Detects Nucleic Acid Sequences From Microorganisms Associated With Vaginitis And Bacterial Vaginosis

Product code: PQA

Additional product codes: NSU, OUY, OOI

Predicate Device: BD MAX Vaginal Panel - K201017

Intended Use:

The BIOFIRE® SPOTFIRE® Vaginitis (VG) Panel is an automated multiplexed polymerase chain reaction (PCR) test intended for use with BIOFIRE® SPOTFIRE® System for the simultaneous qualitative detection and identification of nucleic acids from multiple pathogens associated with bacterial vaginosis (BV), vulvovaginal candidiasis (VVC), and trichomoniasis. The BIOFIRE® SPOTFIRE® Vaginitis (VG) Panel is intended for use on clinician-collected or self-collected (in a clinical setting) vaginal swabs obtained from individuals with symptoms of vaginitis/vaginosis.

The following analytes are identified and differentiated using the BIOFIRE SPOTFIRE Vaginitis (VG) Panel:

Bacteria	Yeast	Parasites
Bacterial Vaginosis (BV)	*Candida albicans* *Candida glabrata* *Candida krusei* *Candida parapsilosis* *Candida tropicalis* *Candida dubliniensis*	*Trichomonas vaginalis*
Analytes (not reported individually)		
Fannyhessea (Atopobium) vaginae Bacterial Vaginosis-Associated Bacterium 2 (BVAB2) *Lactobacillus crispatus* *Lactobacillus gasseri/paragasseri* *Lactobacillus jensenii/mulieris* *Sneathia vaginalis (amnii)/sanguinegens*		

The BIOFIRE® SPOTFIRE® Vaginitis (VG) Panel is indicated as an aid in the diagnosis of specific agents of vaginitis/vaginosis and results should be used in conjunction with other clinical and laboratory findings. The BIOFIRE® SPOTFIRE® Vaginitis (VG) Panel is not intended to monitor treatment for vaginitis/vaginosis.

Device Description:

SPOTFIRE VG Panel

The SPOTFIRE VG Panel simultaneously identifies eight different targets: BV (encompasses multiple bacterial targets), six yeasts, and one parasite from clinician collected and self-collected vaginal swab specimens, in molecular transport media collected from individuals with signs and symptoms of vaginitis. The SPOTFIRE VG Panel is compatible with the BIOFIRE® SPOTFIRE® System, a polymerase chain reaction (PCR)-based in vitro diagnostic system for infectious disease testing. The BIOFIRE SPOTFIRE System Software executes the SPOTFIRE VG Panel test and interprets and reports the test results. The SPOTFIRE VG Panel was designed to be used in CLIA-waived environments.

A test is initiated by loading Hydration Solution into the hydration solution injection port of the SPOTFIRE VG Panel pouch and vaginal swab (VS) specimen, mixed with the provided Sample Buffer, into the other sample injection port of the SPOTFIRE VG Panel pouch and placing it in the SPOTFIRE System. The pouch contains all the reagents required for specimen testing and analysis in a freeze-dried format; the addition of Hydration Solution and Sample/Buffer Mix rehydrates the reagents. After the pouch is prepared, the SPOTFIRE System Software guides the user through the steps of placing the pouch into the instrument, scanning the pouch barcode, entering the sample identification, and initiating the run.

Nucleic acid extraction occurs within the SPOTFIRE VG Panel pouch using mechanical and chemical lysis followed by purification using standard magnetic bead technology. After extracting and purifying nucleic acids from the unprocessed sample, the SPOTFIRE System performs a nested multiplex PCR that is executed in two stages. During the first stage, the SPOTFIRE System performs a single, large volume, highly multiplexed PCR reaction. The products from first stage PCR are then diluted and combined with a fresh, primer-free master mix and a fluorescent double- stranded DNA binding dye (LC Green® *Plus*, BioFire Diagnostics). The solution is then distributed to each well of the array. Array wells contain sets of primers designed specifically to amplify sequences internal to the PCR products generated during the first stage PCR reaction. The 2nd stage PCR, or nested PCR, is performed in singleplex fashion in each well of the array. At the conclusion of the 2nd stage PCR, the array is interrogated by melt curve analysis for the detection of signature amplicons denoting the presence of specific targets. A digital camera placed in front of the 2nd stage PCR captures fluorescent images of the PCR reactions and software interprets the data.

The SPOTFIRE System Software automatically interprets the results of each DNA melt curve analysis and combines the data with the results of the internal pouch controls to provide a test result for each organism on the SPOTFIRE VG Panel .

SPOTFIRE Vaginal Swab Sample Collection Kit (SCK)

The SPOTFIRE Vaginal Swab SCK is intended for use in clinical settings to collect, preserve, and transport self-collected or clinician-collected vaginal swab specimens. The SPOTFIRE Vaginal Swab SCK consists of a swab inside of a rigid transport tube and a separate tube containing transport media (eNAT®). After collection of a vaginal swab specimen, the swab can be temporarily returned to the sterile transport tube it is provided in (for patients performing self-collection) or be placed directly into the eNAT® tube (for clinicians performing collection). The clinician (or other health care provider) places the swab into the eNAT® tube, breaks the swab off at the breakpoint, then recaps the tube. The remainder of the swab shaft and cap are then discarded.

Substantial Equivalence:

The SPOTFIRE VG Panel is substantially equivalent to the BD MAX Vaginal Panel , which was originally authorized via the De Novo pathway on October 28, 2016 (DEN160001), and determined to be a Class II device under regulation 21 CFR 866.3975. The BD MAX Vaginal Panel was subsequently cleared in K191957 and K201017.

A comparison between the SPOTFIRE VG Panel and the BD MAX Vaginal Panel is provided in Table 1.

Table 1: Similarities and differences between the SPOTFIRE VG Panel and the BD MAX Vaginal Panel

Element	BD MAX Vaginal Panel (K201017.)	SPOTFIRE VG Panel (this submission)
Intended Use	The BD MAX Vaginal Panel performed on the BD MAX System is an automated qualitative *in vitro* diagnostic test for the direct detection of DNA targets from bacteria associated with bacterial vaginosis (qualitative results reported based on detection and quantitation of targeted organism markers), *Candida* species associated with vulvovaginal candidiasis, and *Trichomonas vaginalis* from vaginal swabs in patients who are symptomatic for vaginitis/vaginosis. The test utilizes real-time polymerase chain reaction (PCR) for the amplification of specific DNA targets and utilizes fluorogenic target-specific hybridization probes to detect and differentiate DNA from: • Bacterial vaginosis markers (Individual markers not reported) o *Lactobacillus* spp. (*L. crispatus* and *L. jensenii)* o *Gardnerella vaginalis* o *Atopobium vaginae* o Bacterial Vaginosis Associated Bacteria-2 (BVAB-2) o *Megasphaera*-1 • *Candida* spp. (*C. albicans, C. tropicalis, C. parapsilosis, C. dubliniensis*) • *Candida glabrata* • *Candida krusei* • *Trichomonas vaginalis* The BD MAX Vaginal Panel is intended to aid in the diagnosis of vaginal infections in women with a clinical presentation consistent with bacterial vaginosis, vulvovaginal candidiasis and trichomoniasis.	The BIOFIRE SPOTFIRE Vaginitis (VG) Panel is an automated multiplexed polymerase chain reaction (PCR) test intended for use with BIOFIRE SPOTFIRE System for the simultaneous qualitative detection and identification of nucleic acids from multiple pathogens associated with bacterial vaginosis (BV), vulvovaginal candidiasis (VVC), and trichomoniasis. The BIOFIRE SPOTFIRE Vaginitis (VG) Panel is intended for use on clinician-collected or self-collected (in a clinical setting) vaginal swabs iobtained from individuals with symptoms of vaginitis/vaginosis. The following analytes are identified and differentiated using the BIOFIRE SPOTFIRE Vaginitis (VG) Panel: **Bacteria** Bacterial Vaginosis (BV) Analytes (not reported individually) *Fannyhessea (Atopobium) vaginaea* *Bacterial Vaginosis-Associated Bacterium 2 (BVAB2)* *Lactobacillus crispatus* *Lactobacillus gasseri/paragasseri* *Lactobacillus jensenii/Mulieris* *Sneathia vaginalis (amnii)/ sanguinegens* **Yeast** *Candida albicans* *Candida glabrata* *Candida krusei* *Candida parapsilosis* *Candida tropicalis* *Candida dubliniensis* **Parasite** *Trichomonas vaginalis* The BIOFIRE SPOTFIRE Vaginitis (VG) Panel is indicated as an aid in the diagnosis of specific agents of vaginitis/vaginosis and results should be used in conjunction with other clinical and laboratory findings. The BIOFIRE SPOTFIRE Vaginitis (VG) Panel is not intended to monitor treatment for vaginitis/vaginosis.
Specimen Types	Clinician and patient-collected vaginal swabs	Same

Organisms detected	Organisms associated with bacterial vaginosis (detected organisms not reported individually) • *Atopobium* spp. (*Atopobium vaginae*, *Atopobium* novel species CCUG 55226) • Bacterial Vaginosis-Associated Bacterium 2 (BVAB2) • *Megasphaera*-1 *Candida* spp. (*C. albicans, C. tropicalis, C. parapsilosis, C. dubliniensis*) *Candida glabrata/Candida krusei* *Trichomonas vaginalis*	**Bacteria** Bacterial Vaginosis (BV) Analytes (not reported) *Fannyhessea (Atopobium) vaginaea* *Bacterial Vaginosis-Associated Bacterium 2 (BVAB2)* *Lactobacillus crispatus* *Lactobacillus gasseri/paragasseri* *Lactobacillus jensenii/Mulieris* *Sneathia vaginalis (amnii)/ sanguinegens* **Yeast** *Candida albicans* *Candida glabrata* *Candida krusei* *Candida parapsilosis* *Candida tropicalis* *Candida dubliniensis* **Parasite** *Trichomonas vaginalis*
Analytes	DNA	Same
Technological Principles	Real Time PCR	Same
Specimen Collection	BD Molecular Swab Collection Kit	BIOFIRE SPOTFIRE Vaginal Swab Collection Kit
Instrumentation	BD MAX Systems	BIOFIRE SPOTFIRE System
Reagent Storage	Room Temperature	Same
Test Interpretation	Automated test interpretation and reporting.	Same

Clinical Performance Characteristics

Prospective Clinical Evaluation

The clinical performance (encompassing both accuracy and ease of use) of the SPOTFIRE® VG Panel was established during a prospective multi-center study that was further supplemented with contrived specimens. Five geographically distinct study sites in the US (comprising nine total enrollment locations), representative of the intended use setting participated in these studies from October 2024 to November 2025. Four VS specimens were collected from consented subjects and placed in each of the corresponding media specific to the SPOTFIRE® VG Panel and the three FDA-cleared nucleic acid amplification tests (NAATs) used as comparators. The clinician-collected (CC) VS specimen collection order was randomized for each subject for all four VS specimens. For the self-collected (SC) VS specimen collection, the SPOTFIRE® VG Panel VS specimen was always collected by the subject first, while the three other VS specimens were then collected by the clinician in a randomized order. All SPOTFIRE® VG Panel testing was performed according to the manufacturer's instructions by operators with training and educational backgrounds representative of those in the Clinical Laboratory Improvement Amendments of 1988 (CLIA)-waived or near-patient testing setting. No hands-on training was provided to the SPOTFIRE® VG Panel test operators; rather, training was limited to written materials (i.e., quick reference guides) that were intended to be included with the SPOTFIRE® System.

A total of 1535 VS specimens were enrolled from consented subjects who were at least 14 years of age for the prospective clinical study; 1432 of these VS specimens were from subjects showing symptoms of vaginitis (symptomatic enrollment) and 103 of these VS specimens were from subjects not showing symptoms of vaginitis (asymptomatic enrollment). Twenty (20) of the VS specimens from symptomatic subjects were excluded and three of the VS specimens from asymptomatic subjects were excluded. The most common reason for specimen exclusion was that a valid SPOTFIRE® VG Panel test was not obtained due to being unable to test the specimen in the designated timeframe. The final dataset consisted of 1512 VS specimens (1412 VS specimens from symptomatic subjects and 100 VS specimens from asymptomatic subjects).

Table 2 provides a summary of demographic information for the specimens included in the study.

Table 2: Demographic Summary for the SPOTFIRE® VG Panel Prospective Clinical Evaluation

Category		Symptomatic Subjects		Asymptomatic Subjects	
		CC VS	SC VS	CC VS	SC VS
Age	14-17 years	1 (0.1%)	6 (0.8%)	2 (4.1%)	0 (0%)
	18-29 years	339 (48.4%)	335 (47.1%)	23 (46.9%)	7 (13.7%)
	30-39 years	233 (33.3%)	222 (31.2%)	9 (18.4%)	16 (31.4%)
	40-49 years	94 (13.4%)	119 (16.7%)	9 (18.4%)	11 (21.6%)
	50+ years	33 (4.7%)	30 (4.2%)	6 (12.2%)	17 (33.3%)
Race/Ethnicity	Black or African American	366 (52.3%)	469 (65.9%)	31 (63.3%)	44 (86.3%)
	White (not Hispanic/ Latino)	90 (12.9%)	112 (15.7%)	13 (26.5%)	5 (9.8%)
	Hispanic/Latino	209 (29.9%)	97 (13.6%)	1 (2.0%)	0 (0%)
	Asian	8 (1.1%)	5 (0.7%)	0 (0%)	1 (2.0%)
	American Indian or Alaska Native	0 (0%)	1 (0.1%)	0 (0%)	0 (0%)
	Native Hawaiian or Other Pacific Islander	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	Other/Mixed/Unknown	27 (3.9%)	28 (3.9%)	4 (8.2%)	1 (2.0%)
Overall		**700**	**712**	**49**	**51**

For Bacterial Vaginosis (BV) and *Trichomonas vaginalis*, the performance of the SPOTFIRE® VG Panel was evaluated by comparing the test results to a composite infection status (CIS) and patient infected status (PIS) using three FDA-cleared nucleic acid amplification tests (NAATs). The comparator method for *Candida albicans*, *Candida dubliniensis*, *Candida parapsilosis*, and *Candida tropicalis* was an FDA-cleared multiplexed vaginitis pathogen panel followed by an analytically validated PCR assay confirmed by bidirectional sequencing (these analytes are not distinguished to the species level by the FDA-cleared panel). The comparator method for *Candida glabrata* and *Candida krusei* was the same FDA-cleared multiplexed vaginitis pathogen panel which provides species level identification for these two *Candida* species.

The performance for the prospective study is summarized in Table 3 and Table 4 for CC VS and SC VS specimens from symptomatic subjects, respectively. Sensitivity or positive percent agreement (PPA) for each analyte was calculated as 100% × (TP / (TP + FN)). True positive (TP) indicates that both the SPOTFIRE® VG Panel and the comparator method had a positive result for the specific analyte, and false negative (FN) indicates that the SPOTFIRE® VG Panel was negative while the comparator result was positive. Specificity or negative percent agreement (NPA) was calculated as 100% × (TN / TN + FP)). True negative (TN) indicates that both the SPOTFIRE® VG Panel and the comparator method had negative results, and FP indicates that the SPOTFIRE® VG Panel was positive while the comparator method was negative. The binomial proportion 95% confidence interval (95%CI) was calculated. Investigations of discrepant results are summarized in the footnotes.

Table 3: SPOTFIRE® VG Panel Clinical Performance Summary for CC VS Specimens[a]

SPOTFIRE® VG Panel Analyte	Sensitivity/PPA			Specificity/NPA		
	TP/(TP + FN)	%	95%CI	TN/(TN + FP)	%	95%CI
Bacteria						
Bacterial Vaginosis (BV)[b]	358/397	90.2	86.9-92.7%	295/303	97.4	94.9-98.7%
Yeast						
Candida albicans[c]	189/199	95.0	91.0-97.2%	481/499	96.4	94.4-97.7%
Candida dubliniensis[d]	0/1	0	-	696/697	99.9	99.2-100%
Candida glabrata[e]	15/16	93.8	71.7-98.9%	679/682	99.6	98.7-99.9%
Candida krusei	0/0	-	-	698/698	100	99.5-100%
Candida parapsilosis	0/0	-	-	698/698	100	99.5-100%
Candida tropicalis	1/1	100	-	697/697	100	99.5-100%
Parasites						
Trichomonas vaginalis[f]	49/49	100	92.7-100%	646/651	99.2	98.2-99.7%

[a] The performance measures of sensitivity and specificity only refer to instances where a CIS/PIS was used as the reference method (i.e., Bacterial Vaginosis (BV) and *Trichomonas vaginalis*).

[b] At least one negative Bacterial Vaginosis (BV) result was observed in 34/39 FN specimens using an independent molecular method or Amsel. In one additional FN specimen, a retest of the SPOTFIRE® VG Panel was BV positive. At least one positive Bacterial Vaginosis (BV) result was observed in all eight FP specimens using an independent molecular method or Amsel.

[c] *Candida albicans* was observed in 8/10 FN specimens: six using a SPOTFIRE® VG Panel retest and two using an independent molecular method. *Candida* spp. was observed in one additional FN specimen using a wet mount. *Candida albicans* was observed in 14/18 FP specimens using an independent molecular method. *Candida* spp. was observed in an additional two FP specimens using an independent molecular method.

[d] *Candida dubliniensis* was observed in the single FN specimen using a SPOTFIRE® VG Panel retest. *Candida dubliniensis* was observed in the single FP specimen using an independent molecular method.

[e] *Candida glabrata* was observed in the single FN specimen using an independent molecular method. *Candida glabrata* was observed in all three FP specimen using an independent molecular method.

[f] *Trichomonas vaginalis* was observed in all five FP specimens using an independent molecular method.

Table 4: SPOTFIRE® VG Panel Clinical Performance Summary for SC VS Specimens[a]

SPOTFIRE® VG Panel Analyte	Sensitivity/PPA			Specificity/NPA		
	TP/(TP + FN)	%	95%CI	TN/(TN + FP)	%	95%CI
Bacteria						
Bacterial Vaginosis (BV)[b]	380/413	92.0	89.0-94.3%	286/299	95.7	92.7-97.4%
Yeast						
Candida albicans[c]	177/193	91.7	87.0-94.8%	486/514	94.6	92.2-96.2%
Candida dubliniensis[d]	2/3	66.7	20.8-93.9%	704/704	100	99.5-100%
Candida glabrata[e]	17/18	94.4	74.2-99.0%	683/690	99.0	97.9-99.5%
Candida krusei[f]	0/0	-	-	707/708	99.9	99.2-100%
Candida parapsilosis[g]	1/2	50.0	-	697/705	98.9	97.8-99.4%
Candida tropicalis[h]	1/2	50.0	-	702/705	99.6	98.8-99.9%
Parasites						
Trichomonas vaginalis[i]	58/59	98.3	91.0-99.7%	646/653	98.9	97.8-99.5%

[a] The performance measures of sensitivity and specificity only refer to instances where a CIS/PIS was used as the reference method (i.e., Bacterial Vaginosis (BV) and *Trichomonas vaginalis*).

[b] At least one negative BV result was observed in 23/33 FN specimens using an independent molecular method or Amsel. In five additional FN specimens, a retest of the SPOTFIRE® VG Panel was BV positive. At least one positive BV result was observed in 11/13 FP specimens using an independent molecular method.

[c] *Candida albicans* was observed in 8/16 FN specimens: five using a SPOTFIRE® VG Panel retest and three using an independent molecular method. *Candida* spp. was observed in an additional seven FN specimens using an independent molecular method or wet mount. *Candida albicans* was observed in all 28 FP specimens using an independent molecular method.

[d] *Candida dubliniensis* was observed in the single FN specimen using a SPOTFIRE® VG Panel retest.

[e] *Candida glabrata* was observed in the single FN specimen using an independent molecular method. *Candida glabrata* was observed in 5/7 FP specimens using an independent molecular method. *Candida* spp. was observed in one additional FP specimens using a wet mount.

[f] *Candida krusei* was observed in the single FP specimen using an independent molecular method.

[g] *Candida parapsilosis* was observed in 7/8 FP specimens using an independent molecular method.

[h] *Candida* spp. was observed in the single FN specimen using an independent molecular method. *Candida tropicalis* was observed in all three FP specimens using an independent molecular method.

[i] *Trichomonas vaginalis* was observed in all seven FP specimens using an independent molecular method.

The performance for Bacterial Vaginosis (BV) specifically is further stratified by age range, race/ethnicity, and clinical condition in Table 5 and Table 6 for CC VS and SC VS specimens from symptomatic subjects, respectively.

Table 5: SPOTFIRE® VG Panel Bacterial Vaginosis (BV) Performance Stratified by Age, Race/Ethnicity, and Clinical Condition for CC VS Specimens

SPOTFIRE® VG Panel Bacterial Vaginosis (BV) Performance	Sensitivity			Specificity		
	TP/(TP + FN)	%	95%CI	TN/(TN + FP)	%	95%CI
Overall	*358/397*	*90.2*	*86.9-92.7%*	*295/303*	*97.4*	*94.9-98.7%*
Age						
14-17 years	0/0	-	-	1/1	100	-
18-29 years	175/194	90.2	85.2-93.6%	141/145	97.2	93.1-98.9%

SPOTFIRE® VG Panel Bacterial Vaginosis (BV) Performance	Sensitivity			Specificity		
	TP/(TP + FN)	%	95%CI	TN/(TN + FP)	%	95%CI
30-39 years	123/135	91.1	85.1-94.8%	96/98	98.0	92.9-99.4%
40-49 years	49/55	89.1	78.2-94.9%	38/39	97.4	86.8-99.5%
50+ years	11/13	84.6	57.8-95.7%	19/20	95.0	76.4-99.1%
Race/Ethnicity						
Black or African American	211/231	91.3	87.0-94.3%	129/135	95.6	90.6-97.9%
White (not Hispanic/Latino)	33/42	78.6	64.1-88.3%	48/48	100	92.6-100%
Hispanic/Latino	97/106	91.5	84.6-95.5%	103/103	100	96.4-100%
Asian	3/3	100	43.9-100%	4/5	80.0	37.6-96.4%
American Indian or Alaska Native	0/0	-	-	0/0	-	-
Native Hawaiian or Other Pacific Islander	0/0	-	-	0/0	-	-
Other/Mixed/Unknown	14/15	93.3	70.2-98.8%	11/12	91.7	64.6-98.5%
Clinical Condition[a]						
Pregnant	9/9	100	70.1-100%	6/6	100	61.0-100%
Menstruated within 24 hours	23/27	85.2	67.5-94.1%	25/25	100	86.7-100%
Premenarche	0/0	-	-	0/0	-	-
Perimenopause	5/7	71.4	35.9-91.8%	11/11	100	74.1-100%
Postmenopause	18/20	90.0	69.9-97.2%	14/15	93.3	70.2-98.8%
Using antibiotics	9/10	90.0	59.6-98.2%	9/9	100	70.1-100%
Using antifungals	8/9	88.9	56.5-98.0%	7/7	100	64.6-100%
Using estrogen therapy	1/1	100	-	2/2	100	34.2-100%
Using masculinizing GAHT[b]	2/2	100	34.2-100%	3/3	100	43.9-100%
Using hormone blockers	1/2	50.0	-	0/0	-	-
Recurrent symptoms	191/213	89.7	84.9-93.1%	141/145	97.2	93.1-98.9%
Recent intercourse	39/41	95.1	83.9-98.7%	26/28	92.9	77.4-98.0%

[a] Clinical Conditions are not mutually exclusive and may not add up to 100% of specimens included in performance calculations.

[b] GAHT = gender affirming hormone therapy

Table 6: SPOTFIRE® VG Panel Bacterial Vaginosis (BV) Performance Stratified by Age, Race/Ethnicity, and Clinical Condition for SC VS Specimens

SPOTFIRE® VG Panel Bacterial Vaginosis (BV) Performance	Sensitivity			Specificity		
	TP/(TP + FN)	%	95%CI	TN/(TN + FP)	%	95%CI
Overall	*380/413*	*92.0*	*89.0-94.3%*	*286/299*	*95.7*	*92.7-97.4%*
Age						
14-17 years	0/0	-	-	6/6	100	61.0-100%
18-29 years	172/191	90.1	85.0-93.5%	141/144	97.9	94.1-99.3%
30-39 years	120/133	90.2	84.0-94.2%	83/89	93.3	86.1-96.9%
40-49 years	77/78	98.7	93.1-99.8%	39/41	95.1	83.9-98.7%
50+ years	11/11	100	74.1-100%	17/19	89.5	68.6-97.1%
Race/Ethnicity						
Black or African American	288/311	92.6	89.1-95.0%	153/158	96.8	92.8-98.6%
White (not Hispanic/Latino)	41/44	93.2	81.8-97.7%	63/68	92.6	83.9-96.8%
Hispanic/Latino	38/45	84.4	71.2-92.3%	50/52	96.2	87.0-98.9%
Asian	1/1	100	-	4/4	100	51.0-100%
American Indian or Alaska Native	1/1	100	-	0/0	-	-
Native Hawaiian or Other Pacific Islander	0/0	-	-	0/0	-	-
Other/Mixed/Unknown	11/11	100	74.1-100%	16/17	94.1	73.0-99.0%
SPOTFIRE® VG Panel Bacterial Vaginosis (BV) Performance	**Sensitivity**			**Specificity**		
	TP/(TP + FN)	**%**	**95%CI**	**TN/(TN + FP)**	**%**	**95%CI**
Clinical Condition[a]						
Pregnant	1/1	100	-	1/1	100	-

Menstruated within 24 hours	24/25	96.0	80.5-99.3%	14/15	93.3	70.2-98.8%
Premenarche	0/0	-	-	0/0	-	-
Perimenopause	14/15	93.3	70.2-98.8%	5/6	83.3	43.6-97.0%
Postmenopause	15/15	100	79.6-100%	15/16	93.8	71.7-98.9%
Using antibiotics	11/16	68.8	44.4-85.8%	15/15	100	79.6-100%
Using antifungals	4/4	100	51.0-100%	9/9	100	70.1-100%
Using estrogen therapy	0/0	-	-	0/0	-	-
Using masculinizing GAHT[b]	2/2	100	34.2-100%	1/1	100	-
Using hormone blockers	1/1	100	-	0/0	-	-
Recurrent symptoms	216/235	91.9	87.7-94.8%	162/171	94.7	90.3-97.2%
Recent intercourse	17/22	77.3	56.6-89.9%	16/17	94.1	73.0-99.0%

[a] Clinical Conditions are not mutually exclusive and may not add up to 100% of specimens included in performance calculations.

[b] GAHT = gender affirming hormone therapy

The overall success rate for initial specimen tests was 97.9% (1482/1514). Fourteen (14) tests (14/1514; 0.9%) did not complete on the initial test attempt, resulting in an instrument success rate of 99.1% (1500/1514) for initial specimen tests. Of the 1500 tests that successfully produced a completed run on the initial test, 1482 had valid internal process controls. This represents a 98.8% (1482/1500) success rate for internal process controls in completed runs in the initial specimen tests.

While 1482 specimens were successful on the initial test, successful retesting of 31 specimens and the additional exclusion of one specimen unrelated to the system performance brings the total number of valid specimens analyzed in this study to 1512 (1412 VS specimens from symptomatic subjects and 100 VS specimens from asymptomatic subjects).

1.1. Contrived Testing

Several analytes were not observed in VS specimens in sufficient numbers to demonstrate the SPOTFIRE® VG Panel performance in the prospective study. Therefore, contrived specimens (n=164; at least 50 for each analyte) were created from unique, analyte-negative VS specimens in eNAT buffer. Specimens were spiked with a variety of isolates/strains for each organism at concentrations that spanned the detection range of each assay. At least half (50.0%) of the contrived positive specimens had analyte concentrations at 2× the limit of detection (LoD). Contrived specimens were randomized and coded along with 12 negative (unspiked) specimens such that the analyte status of each specimen was unknown to the users performing the testing. The coded contrived specimens were distributed to prospective clinical study sites for testing.

Performance results of the SPOTFIRE® VG Panel for contrived VS specimens are shown in Table 7.

Table 7: SPOTFIRE® VG Panel Contrived VS Specimen Performance Summary

Analyte	PPA			NPA		
	TP/(TP + FN)	%	95%CI	TN/(TN + FP)	%	95%CI
Yeast						
Candida dubliniensis	50/50	100.0%	92.9-100%	114/114	100.0%	96.7-100%
Candida glabrata	52/52	100.0%	93.1-100%	110/110	100.0%	96.6-100%
Candida krusei	50/50	100.0%	92.9-100%	114/114	100.0%	96.7-100%
Candida parapsilosis	50/50	100.0%	92.9-100%	113/113	100.0%	96.7-100%
Candida tropicalis	50/50	100.0%	92.9-100%	113/113	100.0%	96.7-100%

2. Analytical Performance Characteristics

2.1. Limit of Detection

The limit of detection (LoD) for the SPOTFIRE® VG Panel was established for various bacteria (composed of analytes for the Bacterial Vaginosis (BV) Algorithm), yeast, and parasitic analytes found in vaginal swab specimens. Contrived samples containing known concentrations for the yeast and parasitic organism were prepared in natural vaginal swab specimens (nVS), while the bacterial analytes, due to their naturally occurring presence in nVS, were prepared in artificial vaginal swab specimens (artificial vaginal matrix in eNAT® buffer; aVS). The LoD concentrations (shown in Table 8) were confirmed by detection in at least 95% (19/20) of replicates tested.

Table 8: Limit of Detection (LoD) for the SPOTFIRE® VG Panel

Analyte	Source ID	LoD Concentration
Bacteria[a]		
Fannyhessea (Atopobium) vaginae	ATCC® BAA-55™	9.0E+03 copies/mL (1.2E+03 cells/mL)
Lactobacillus crispatus	ATCC® 33820™	5.3E+04 copies/mL (2.9E+04 cells/mL)
Lactobacillus jensenii/mulieris	*Lactobacillus jensenii* ATCC® 25258™	6.0E+03 copies/mL (1.4E+03 cells/mL)
Lactobacillus gasseri/paragasseri	*Lactobacillus paragasseri* ATCC® 9857™	6.0E+03 copies/mL (1.1E+03 cells/mL)
Bacterial Vaginosis-Associated Bacterium 2 (BVAB2) (*Escherichia coli recombinant*)	SynBio BVAB2	2.7E+03 copies/mL (1.5E+02 CFU/mL)
Sneathia sanguinegens/vaginalis (amnii)	*Sneathia sanguinegens* CCUG 41628	6.0E+02 copies/mL (1.1E+01 cells/mL)
	Sneathia vaginalis CCUG 52889A	3.0E+02 copies/mL (6.6E+00 cells/mL)
Yeast		
Analyte	**Source ID**	**LoD Concentration**
Candida albicans	ATCC® MYA-2876™	2.0E+03 copies/mL (3.7E+03 cells/mL)
Candida dubliniensis	ATCC® MYA-646™	2.0E+03 copies/mL (2.7E+02 CFU/mL)
Candida glabrata	ATCC® 48435™	1.0E+02 copies/mL (7.6E+01 CFU/mL)
Candida krusei	ATCC® 34135™	2.0E+03 copies/mL (9.3E+03 CFU/mL)
Candida parapsilosis	ATCC® 22019™	9.0E+02 copies/mL (6.8E+02 CFU/mL)
Candida tropicalis	ATCC® 90874™	2.7E+03 copies/mL (4.3E+03 CFU/mL)
Parasites		
Trichomonas vaginalis	ATCC® 30001™	1.5E-01 copies/mL (8.6E-01 cells/mL)

[a] Assays contribute to the BV Interpretation

Note: LoD concentrations in copies/mL in Table 8 above are based on extraction of nucleic acids from isolate cultures followed by digital droplet PCR (ddPCR). The accuracy of concentrations may be affected by extraction efficiency, assay conditions, inhibitors, and/or sequence variance. The quantification has not been compared to a reference material or other quantification methods.

Note: For assessment of the BV algorithm in other analytical performance studies (with exception of analytical reactivity/inclusivity), it was not appropriate to base BV analyte concentrations on LoD values but rather on concentrations which are near target decision points within the BV algorithm. The lowest concentration of BV analyte where expected results are observed ≥95% of the time for each decision point within the algorithm will hereafter be referred to as BV algorithm cutoff concentrations.

2.2. Analytical Reactivity (Inclusivity)

The analytical reactivity (inclusivity) of the SPOTFIRE® VG Panel assays was assessed by testing at least 5 isolates, with the exception of only testing two isolates for BVAB2 due to limited strain availability, at 3× LoD. The panel was inclusive of all isolates tested except for the negativity for the *Candida glabrata* petite mutant. This result was expected as petite isolates have loss of mitochondrial DNA.

Table 9: Summary of Reactivity to *Fannyhessea (Atopobium) vaginae* isolates

Source ID	Strain	Result
CCUG 74734B	N/A	*Fannyhessea vaginae* Positive
CCUG 39382	N/A	
CCUG 44156	N/A	
CCUG 55227	N/A	
CCUG 74191	N/A	

Table 10: Summary of Reactivity BVAB2 isolates

Source ID	Strain	Result
ATCC® TSD-274™	UPII 610-J	BVAB2 Positive
ATCC® TSD-275™	KA00274	

Table 11: Summary of Reactivity to *Candida albicans* isolates

Source ID	Strain	Result
ATCC® 90028™	NCCLS 11	*Candida albicans* Positive
ATCC® 11651™	171D	
ATCC® 22972™	CBS 1912	
ATCC® 14053™	NIH 3172	
ATCC® 10231™	3147	

Table 12: Summary of Reactivity to *Candida dubliniensis* isolates

Source ID	Strain	Result
ATCC® MYA-179™	CD410	*Candida dubliniensis* Positive
ATCC® MYA-578™	H12	
ATCC® 44508™	75/043	
ATCC® MYA-180™	CD411	
ATCC® MYA-577™	H1	

Table 13: Summary of Reactivity to *Candida glabrata* isolates

Source ID	Strain	Result
ATCC® 64677™	LRA 85.10.75	*Candida glabrata* Positive
ATCC® 32554™	26247-1	
ATCC® 15126™	Mutant TMAGR-23	
ATCC® 2001™	CBS 138	
ATCC® 15545™	NRRL YB-4025	
Clinical Isolate[a]	N/A	*Candida glabrata* Negative

[a] Isolate characterized as 'petite mutant' with loss of mitochondrial function/mitochondrial DNA.

Note: The assay for detection of *C. glabrata* amplifies a gene within the mitochondrial genome and 'petite' strains that have lost mitochondrial DNA will not be detected.

Table 14: Summary of Reactivity to *Candida krusei* isolates

Source ID	Strain	Result
ATCC® 28870™	CBS 2052	*Candida krusei* Positive
ATCC® 6258™	NRRL Y-7179	
ATCC® 90878™	B74	
ATCC® 14243™	N/A	
ATCC® 201748™	89-08-008	

Table 15: Summary of Reactivity to *Candida parapsilosis* isolates

Source ID	Strain	Result
CDC ATLANTA USA AR-BANK#0335	N/A	*Candida parapsilosis* Positive
CDC ATLANTA USA AR-BANK#0336	N/A	
ATCC® 96138™	MCO433	
ATCC® 34136™	ST-89	
CDC ATLANTA USA AR-BANK#0344	N/A	

Table 16: Summary of Reactivity to *Candida tropicalis* isolates

Source ID	Strain	Result
ATCC® 201380™	API® 90 01 105	*Candida tropicalis* Positive
ATCC® MYA-2734™	508-12.1	
ATCC® 66029™	AmMS 227	
ATCC® 1369™	NRRL Y-11860	
ATCC® 750™	NRRL Y-12699	

Table 17: Summary of Reactivity to *Lactobacillus crispatus* isolates

Source ID	Strain	Result
CCUG 42897	N/A	*Lactobacillus crispatus* Positive
ATCC® 55221™	N/A	
ATCC® 55844™	N/A	
ATCC® 53545™	N/A	
ATCC® 33197™	VPI 7635	

Table 18: Summary of Reactivity to *Lactobacillus gasseri*/*paragasseri* isolates

Source ID	Strain	Result
ATCC® BAA-2841™	IDCC 3101	*Lactobacillus gasseri*/*paragasseri* Positive
ATCC® 4962™	3	
DSMZ 107525	D99	
ATCC® 33323™	DSM 20243	
ATCC® 29601™	NCIB 8820	
DSMZ 20077	F164	

Table 19: Summary of Reactivity to *Lactobacillus jensenii*/*mulieris* isolates

Source ID	Strain	Result
CCUG 49306	N/A	*Lactobacillus jensenii*/*mulieris* Positive
CCUG 38672	N/A	
CCUG 44492	N/A	
CCUG 63907	N/A	
ZeptoMetrix 0804260	Z264	
DSMZ 108704	c10Ua161M	

Table 20: Summary of Reactivity to *Sneathia sanguinegens*/*vaginalis (amnii)* isolates

Source ID	Strain	Result
CCUG 60204	N/A	*Sneathia sanguinegens*/*vaginalis (amnii)* Positive
CCUG 53806	N/A	
CCUG 64613	N/A	
CCUG 64370	N/A	
BEI NR-50515	Sn35	
CCUG 38322	N/A	
CCUG 42621	N/A	
CCUG 45289	N/A	
CCUG 52978	N/A	
CCUG 54983	N/A	

Table 21: Summary of Reactivity to *Trichomonas vaginalis* isolates

Source ID	Strain	Result
ATCC® 30184™	123414	*Trichomonas vaginalis* Positive
ATCC® 30187™	165307-1	
ATCC® 30238™	JH 32A #4	
ATCC® 30240™	JH 37A #2	
ATCC® 30245™	TVC	
ATCC® 50139™	RU 357	
ATCC® 50141™	RU 382	
ATCC® 50167™	B7RC2	
ATCC® 50143™	CDC 085	
ATCC® PRA-91™	JRS-TV-120	
ATCC® PRA-95™	JRS-TV-VB102	

2.3. Analytical Specificity (Cross-Reactivity and Exclusivity)

The potential for non-specific amplification and detection (cross-reactivity) by the SPOTFIRE® VG Panel assays was evaluated by in silico analysis of available sequences and, when available, by empirically testing on- and off-panel organisms at high concentrations. The organisms evaluated included relevant bacterial, fungal, viral, and parasitic organisms that may be phenotypically related to on-panel organisms, or pathogenic/commensal organisms that may be present in a vaginal swab specimen. Analysis of on-panel analytes was performed to evaluate the potential for intra-panel cross-reactivity (i.e., detection of an analyte by an assay other than the target-specific assay for that analyte). Each organism was tested in aVS in triplicate at the highest concentration possible (≥1.0E+06 units/mL for bacteria and fungi and ≥1.0E+05 units/mL for viruses and parasites).

Based on a combination of the in silico and empirical testing data, a summary of the potential cross-reactivities of the SPOTFIRE® VG Panel is provided in Table 22. The on-panel and off-panel organisms tested are listed in Table 23 and Table 24, respectively. Erroneous results due to cross-reactivity with organisms that were not evaluated or due to cross-reactivity with emerging or novel sequences are also possible.

Table 22: Predicted and Observed Cross-Reactivity for the SPOTFIRE® VG Panel

SPOTFIRE® VG Panel Analyte Result	Cross-Reactive Organism	Description
Lactobacillus gasseri/paragasseri	*Lactobacillus jensenii*	The listed *Lactobacillus* species may be amplified as *Lactobacillus gasseri* at high concentrations[a]. BV algorithm reporting will not be impacted.
Candida krusei	*Candida inconspicua* *Pichia fermentans* *Pichia norvegensis* *Pichia membranifaciens*[b]	The listed *Candida* species and *Pichia* species may be detected as *Candida krusei*
Candida tropicalis	*Candida kefyr*[c] *Candida maltosa* *Candida buenavistaensis*[d] *Candida neerlandica*[d] *Candida sanvaensis*[d]	The listed *Candida* species may be detected as *Candida tropicalis* at high concentrations[a]
Candida glabrata	*Candida nivariensis* *Candida bracarensis*[d]	The listed *Candida* species may be detected as *Candida glabrata* at high concentrations[a]

[a] Cross-reactivity observed at concentrations ≥1.0E+07 cells/mL.

[b] Cross-reactivity resulting in false positive *Candida krusei* results were not observed with ATCC® 2254™. However, cross-reactivity is supported by in silico predictions.

[c] Cross-reactivity resulting in false positive *Candida tropicalis* results have not been observed in empirical testing. However, non-specific amplification products with Tm values close to the assay specific Tm range have been observed and the potential for false positive *Candida tropicalis* results exist.

[d] Analytes were not tested empirically but cross reactivity is supported by in silico predictions.

Table 23: Summary of Results for On-Panel Organisms Tested During Evaluation of Analytical Specificity of the SPOTFIRE® VG Panel

Organism	Source ID	Observed Cross-Reactivity[a]
Bacteria		
Fannyhessea (Atopobium) vaginae	ATCC® BAA-55™	None
Bacterial Vaginosis-Associated Bacteria 2 (BVAB2)	SynBio BVAB2	None
Lactobacillus crispatus	ATCC® 33820™	None
Lactobacillus paragasseri	ATCC® 9857™	None
Lactobacillus jensenii[b]	ATCC® 25258™	*Lactobacillus gasseri*
Sneathia sanguinegens	CCUG 41628	None
Sneathia vaginalis (amnii)	CCUG 52889A	None
Yeast		
Candida albicans	ATCC® MYA-2876™	None
Candida dubliniensis	ATCC® MYA-646™	None
Candida glabrata	ATCC® 48435™	None
Candida krusei	ATCC® 34135™	None
Candida parapsilosis	ATCC® 22019™	None
	ATCC® 90875™	None
Candida tropicalis	ATCC® 90874™	None
Parasites		
Trichomonas vaginalis	ATCC® 30001™	None
	ATCC® 30238™	None

[a] Observed cross-reactivity results determined by target assay results

[b] Additional testing at lower concentrations was conducted. Cross-reactivity was observed at concentrations ≥1.0E+07 cells/mL.

Table 24: Summary of Results for Off-Panel Organisms Tested During Evaluation of Analytical Specificity of the SPOTFIRE® VG Panel

Organism	Source ID	Observed Cross-Reactivity[a]
Bacteria		
Acinetobacter baumannii	ATCC® 9955™	None
Acinetobacter calcoaceticus	ATCC® 23055™	None
Acinetobacter lwoffii	ATCC® 15309™	None
Actinomyces israelii	ATCC® 12102™	None
Actinomyces naeslundii	ATCC® 12104™	None
Actinomyces pyogenes	ATCC® 49698™	None
Aerococcus viridans	ATCC® 11563™	None
Alcaligenes faecalis	ATCC® 8750™	None
Amylolactobacillus amylophilus	ATCC® 49845™	None
Anaerococcus tetradius	ATCC® 35098™	None
Atopobium minutum	ATCC® 33267™	None
Bacillus atrophaeus	ATCC® 49760™	None
Bacteroides caccae	ATCC® 43185™	None
Bacteroides fragilis	ATCC® 43937™	None
Bacteroides stercoris	ATCC® 43183™	None
Bifidobacterium adolescentis	ATCC® 15703™	None
Bifidobacterium breve	BEI Resources HM-412	None
Bifidobacterium longum	ATCC® 15697™	None
Bordetella pertussis	ATCC® 10380™	None
Brevibacterium linens	ATCC® 9172™	None
Burkholderia cepacia	ATCC® 51671™	None
Campylobacter jejuni	ATCC® 700819™	None
Campylobacter ureolyticus	ATCC® 33387™	None
Chlamydia trachomatis	ATCC® VR-347™	None

Citrobacter freundii	ATCC® 8090™	None
Clostridium difficile	ATCC® 43598™	None
Clostridium perfringens	ATCC® 9081™	None
Corynebacterium genitalium	CCUG 38989T	None
Corynebacterium xerosis	ATCC® 373™	None
Cutibacterium acnes	ATCC® 6919™	None
Dialister invisus	ATCC® 51894™	None
Dialister micraerophilus	CCUG 59490	None
Dialister pneumosintes	ATCC® 33048™	None
Dialister propionicifaciens	CCUG 49291T	None
Dialister succinatiphilus	CCUG 76561T	None
Eggerthella lenta	ATCC® 43055™	None
Eikenella corrodens	ATCC® BAA-1152™	None
Enterobacter aerogenes	ATCC® 51697™	None
Enterobacter cloacae	ATCC® 13047™	None
Enterococcus faecalis	ATCC® 51299™	None
Enterococcus faecium	ATCC® 700221™	None
Erysipelothrix rhusiopathiae	ATCC® 19414™	None
Escherichia coli	ATCC® 43888™	None
Finegoldia magna	ATCC® 53516™	None
Fusobacterium nucleatum	ATCC® 25586™	None
Gardnerella vaginalis	ATCC® 14018™	None
Gemella haemolysans	ATCC® 10379™	None
Haemophilus ducreyi	ATCC® 51620™	None
Kingella denitrificans	ATCC® 33394™	None
Klebsiella oxytoca	ATCC® 13182™	None
Klebsiella pneumoniae	ATCC® BAA-1144™	None
Kocuria rhizophila	ATCC® 9341™	None
Lactobacillus acetotolerans	ATCC® 43578™	None
Lactobacillus acidophilus	ATCC® 314™	None
Lactobacillus delbrueckii	ATCC® 11842™	None
Lactobacillus helveticus	ATCC® 15009™	None
Lactobacillus iners	ATCC® 55195™	None
Lactobacillus johnsonii	Clinical Isolate	None
Lactobacillus vaginalis	ATCC® 49540™	None
Lancefieldella parvula	ATCC® 33793™	None
Lancefieldella rimae	ATCC® 49626™	None
Legionella pneumophila	ATCC® 33152™	None
Leptotrichia buccalis	ATCC® 14201™	None
Leptotrichia hongkongensis	DSMZ 23441	None
Levilactobacillus brevis	ATCC® 14869™	None
Ligilactobacillus animalis	ATCC® 35046™	None
Listeria monocytogenes	ATCC® 13932™	None
Mageeibacillus indolicus	CCUG 59143	None
Megasphaera elsdenii	ATCC® 25940™	None
Megasphaera hutchinsoni	ATCC® TSD-206™	None
Megasphaera lornae	DSMZ 111201	None
Megasphaera vaginalis	DSMZ 111203	None
Mobiluncus curtisii	ATCC® 35242™	None
Mobiluncus mulieris	ATCC® 43064™	None
Moraxella catarrhalis	ATCC® 43627™	None
Morganella morganii	ATCC® 25830™	None
Mycobacterium smegmatis	ATCC® 700084™	None

Organism	Source ID	Observed Cross-Reactivity[a]
Mycoplasmoides genitalium	ATCC® 33530™	None
Mycoplasma hominis	ATCC® 23114™	None
Neisseria gonorrhoeae	Clinical Isolate	None
Neisseria meningitidis	ATCC® 43744™	None
Olsenella uli	CCUG 31166	None
Pantoea agglomerans	ATCC® 27155™	None
Peptostreptococcus anaerobius	ATCC® 27337™	None
Peptoniphilus asaccharolyticus	ATCC® 14963™	None
Plesiomonas shigelloides	ATCC® 51572™	None
Porphyromonas asaccharolytica	ATCC® 25260™	None
Prevotella bivia	ATCC® 29303™	None
Prevotella disiens	CCUG 9558T	None
Prevotella histicola	BEI HM471	None
Prevotella melaninogenica	ATCC® 25845™	None
Prevotella oralis	ATCC® 33322™	None
Proteus mirabilis	ATCC® 35659™	None
Proteus vulgaris	ATCC® 29905™	None
Providencia stuartii	CDC Atlanta USA AR-BANK#0026	None
Pseudomonas aeruginosa	ATCC® 10145™	None
Salmonella enterica	NCTC 5800	None
Salmonella typhimurium	Calgary University SGSC 2181	None
Serratia marcescens	ATCC® 13880™	None
Shigella flexneri	ATCC® 700930™	None
Staphylococcus aureus	ATCC® BAA-1700™	None
Staphylococcus epidermidis	ATCC® 29887™	None
Streptococcus agalactiae	ATCC® 13813™	None
Streptococcus mitis	ATCC® 15914™	None
Streptococcus mutans	ATCC® 25175™	None
Streptococcus pneumoniae	ATCC® 49619™	None
Streptococcus pyogenes	ATCC® 12348™	None
Streptococcus salivarius	ATCC® 13419™	None
Streptococcus sanguinis	ATCC® 10556™	None
Streptococcus thermophilus	ATCC® 19258™	None
Treponema pallidum	UWS (Nichols)	None
Ureaplasma parvum	ATCC® 33697™	None
Ureaplasma urealyticum	ATCC® 27618™	None
Veillonella atypica	ATCC® 17744™	None
Veillonella parvula	ATCC® 10790™	None
Vibrio parahaemolyticus	ATCC® 17803™	None
Yersinia enterocolitica	ATCC® 9610™	None
Yeast/Fungi		
Candida auris	CDC AR-BANK#0390	None
Candida famata	ATCC® 4144™	None
Candida guilliermondii	ATCC® 38290™	None
Candida haemulonii	CDC AR-BANK#0393	None
Candida inconspicua[b]	ATCC® 16783™	*Candida krusei*
Candida intermedia	CDC Atlanta USA AR-BANK#0589	None
Candida kefyr[c] (also known as *Kluyveromyces marxianus*)	ATCC® 16045™	None
Candida lusitaniae	ATCC® 34449™	None
Candida maltosa[d]	ATCC® 28140™	*Candida tropicalis*

Organism	Source ID	Observed Cross-Reactivity[a]
Candida metapsilosis	ATCC® 96143™	None
Candida nivariensis[e]	CCUG 56432	*Candida glabrata*
Candida norvegica	ATCC® 96301™	None
Candida orthopsilosis	ATCC® 96139™	None
Candida utilis	ATCC® 22023™	None
Diutina catenulata	ATCC® 10565™	None
Diutina rugosa	ATCC® 10571™	None
Cryptococcus neoformans	ATCC® MYA-4567™	None
Eremothecium gossypii	ATCC® 10895™	None
Kodamaea ohmeri	CDC Atlanta USA AR-BANK#0396	None
Pichia fermentans[f]	ATCC® 22695™	*Candida krusei*
Pichia kluyveri	ATCC® 90421™	None
Pichia membranifaciens	ATCC® 2254™	None
Pichia norvegensis[g]	ATCC® 22977™	*Candida krusei*
Pichia occidentalis	ATCC® 22686™	None
Saccharomyces cerevisiae	ATCC® 18824™	None
Parasites		
Pentatrichomonas hominis	ATCC® 30000™	None
Trichomonas gallinae	ATCC® 30002™	None
Trichomonas tenax	ATCC® 30207™	None
Viruses		
Hepatitis B virus	Zeptometrix 0810031C	None
Herpes simplex virus, type I	Zeptometrix 0810005CF	None
Herpes simplex virus, type II	Zeptometrix 0810006CF	None
Human herpesvirus 3	ATCC® VR-1832™	None
Human immunodeficiency virus	Zeptometrix NATHIV1-LIN	None
Human papilloma virus 16	ATCC® 45113D™	None

a Observed cross-reactivity results determined by target assay results.

b Additional testing at lower concentrations was conducted. Cross-reactivity was observed at concentrations >1E+01 cell/ml

c Cross-reactivity resulting in false positive *Candida tropicalis* results have not been observed in empirical testing. However, non-specific amplification products with Tm values close to the assay specific Tm range have been observed and the potential for false positive *Candida tropicalis* results exist.

d Additional testing at lower concentrations was conducted. Cross-reactivity was observed at concentrations >1.0E+06 cells/mL

e Additional testing at lower concentrations was conducted. Cross-reactivity was observed at concentrations >1.0E+07 cells/mL

f Additional testing at lower concentrations was conducted. Cross-reactivity was observed at concentrations >9.4E+06 cells/mL

g Additional testing at lower concentrations was conducted. Cross-reactivity was observed at concentrations >8.9E+01 cells/mL

2.4. Near-LoD/Reproducibility Evaluation

A multi-variable study was performed to evaluate the precision (reproducibility) of the SPOTFIRE® VG Panel. Testing included trained operators (at internal bioMérieux site) and intended user operators (at three distinct external clinical sites).

Contrived samples contained combinations of the SPOTFIRE® VG Panel analytes prepared in aVS at or near (1× to 3×) the LoD for the yeast and parasite, while the BV analytes (bacteria) were prepared at various concentrations to give a Bacterial Vaginosis (BV) Positive or Bacterial Vaginosis (BV) Negative interpretation result. The mixes tested at internal and external sites (Mix 1-4) were prepared to include different combinations of representative analytes (various on-panel *Candida* species, *Trichomonas vaginalis,* and BV-analytes*)* detected by the SPOTFIRE® VG Panel. Positive sample mixes were prepared to include each analyte at either a low positive (1× LoD or BV algorithm cutoff concentration) or moderate positive (3× LoD or BV algorithm cutoff concentration) concentration. For the BV interpretation, four additional sample mixes (Mix 5-8) were tested internally and prepared with different combinations of representative BV analytes to produce

a Bacterial Vaginosis (BV) Positive or Bacterial Vaginosis (BV) Negative interpretation result based on the BV algorithm.

For testing performed at clinical sites, samples were tested over five testing events (non-consecutive days) by two operators during their normal workday routine. Each site was equipped with two modules on one SPOTFIRE® System. Testing at all three sites was performed with a single reagent lot. For each testing event, each operator ran two replicate pouches for a total of 20 replicates per site and 60 total replicates across all three sites. For testing performed at bioMérieux, samples were tested over five consecutive workdays, on three different SPOTFIRE® Systems, by two operators per System, using three different reagent kit lots. Each day of testing, the two operators each tested three replicates per System for a total of 30 replicates per System and 90 total replicates across all Systems.

A summary of results (percent (%) agreement with the expected result) for each analyte (by site and system) is provided in Table 25. For all non-BV analytes, agreement with the expected Detected interpretation results (all systems/sites) was ≥97.8%. The reproducibility of negative (Not Detected) results was evaluated using a combination of results from a sample negative for all analytes and results from the samples that were not spiked with the relevant analyte. The agreement with the expected negative (Not Detected) results was ≥99.7% for all non-BV analytes.

Similarly, the reproducibility of negative assay results of the BV analytes was evaluated using a combination of results from a sample negative for all analytes and results from the samples that were not spiked with the relevant analyte. The agreement with the expected negative assay results for BV analytes was ≥98.0%. Agreement with the expected positive assay results (all systems/sites) is not shown for individual BV analytes as these analytes were spiked above LoD in the mixes and evaluated on the BV algorithm cutoff concentrations. All had 100% positive assay results as expected in the respective mixes.

Table 25: Summary of Non-BV Reproducibility Results for the SPOTFIRE® Vaginitis Panel

Analyte	Test Concentration	SPOTFIRE® System testing								All Systems/ Sites [95% CI]
		bioMérieux				Clinical Sites				
		System A	System B	System C	Total	Site 1	Site 2	Site 3	Total	
Candida albicans	**6.0E+03 copies/mL** (3× LoD)	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)	20/20 (100%)	20/20 (100%)	19/20 (95.0%)	59/60 (98.3%)	149/150 (99.3%) [96.3-99.9%]
	2.0E+03 copies/mL (1× LoD)[a]	24/30 (80.0%)	25/30 (83.3%)	26/30 (86.7%)	75/90 (83.3%)	14/20 (70.0%)	15/20 (75.0%)	19/20 (95.0%)	48/60 (80.0%)	123/150 (82.0%) [75.1-87.3%]
	2.0E+03 copies/mL (1× LoD)[a]	28/30 (93.3%)	30/30 (100%)	30/30 (100%)	88/90 (97.8%)					88/90 (97.8%) [92.3-99.4%]
	Negative	179/179[b] (100%)	180/180 (100%)	180/180 (100%)	539/539 (100%)	40/40 (100%)	40/40 (100%)	40/40 (100%)	120/120 (100%)	659/659 (100%) [99.4-100%]
Candida dubliniensis	**6.0E+03 copies/mL** (3× LoD)	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)	20/20 (100%)	20/20 (100%)	20/20 (100%)	60/60 (100%)	150/150 (100%) [97.5-100%]
	2.0E+03 copies/mL (1× LoD)	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)	20/20 (100%)	20/20 (100%)	20/20 (100%)	60/60 (100%)	150/150 (100%) [97.5-100%]
	Negative	209/209[b] (100%)	210/210 (100%)	210/210 (100%)	629/629 (100%)	40/40 (100%)	40/40 (100%)	40/40 (100%)	120/120 (100%)	749/749 (100%) [99.5-100%]
Candida glabrata	**3.0E+02 copies/mL** (3× LoD)	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)	20/20 (100%)	20/20 (100%)	20/20 (100%)	60/60 (100%)	150/150 (100%) [96.3-99.9%]
	1.0E+02 copies/mL (1× LoD)	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)	20/20 (100%)	20/20 (100%)	20/20 (100%)	60/60 (100%)	150/150 (100%) [96.3-99.9%]
	Negative	209/209[b] (100%)	210/210 (100%)	210/210 (100%)	629/629 (100%)	40/40 (100%)	40/40 (100%)	40/40 (100%)	120/120 (100%)	749/749 (100%) [99.5-100%]
Candida krusei	**6.0E+03 copies/mL** (3× LoD)	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)	20/20 (100%)	20/20 (100%)	20/20 (100%)	60/60 (100%)	150/150 (100%) [97.5-100%]
	2.0E+03 copies/mL (1× LoD)	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)	19/20 (95.0%)	20/20 (100%)	20/20 (100%)	59/60 (98.3%)	149/150 (99.3%) [96.3-99.9%]
	Negative	209/209[b] (100%)	210/210 (100%)	210/210 (100%)	629/629 (100%)	40/40 (100%)	40/40 (100%)	40/40 (100%)	120/120 (100%)	749/749 (100%) [99.5-100%]
Candida parapsilosis	**2.7E+03 copies/mL** (3× LoD)	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)	20/20 (100%)	20/20 (100%)	20/20 (100%)	60/60 (100%)	150/150 (100%) [97.5-100%]
	9.0E+02 copies/mL (1× LoD)	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)	20/20 (100%)	19/20 (95.0%)	19/20 (95.0%)	58/60 (96.7%)	148/150 (98.7%) [95.3-99.6%]
	Negative	208/209[b] (99.5%)	210/210 (100%)	210/210 (100%)	628/629 (99.8%)	40/40 (100%)	40/40 (100%)	39/40 (97.5%)	119/120 (99.2%)	747/749 (99.7%) [98.8-99.9%]
Trichomonas vaginalis	**4.5E-01 copies/mL** (3× LoD)	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)	20/20 (100%)	20/20 (100%)	20/20 (100%)	60/60 (100%)	150/150 (100%) [97.5-100%]
	1.5E-01 copies/mL (1× LoD)	30/30 (100%)	29/30 (96.7%)	30/30 (100%)	89/90 (98.9%)	20/20 (100%)	20/20 (100%)	20/20 (100%)	60/60 (100%)	149/150 (99.3%) [96.3-99.9%]
	Negative	208/209[b] (99.5%)	210/210 (100%)	210/210 (100%)	628/629 (99.8%)	40/40 (100%)	40/40 (100%)	39/40 (97.5%)	119/120 (99.2%)	747/749 (99.7%) [99.0-99.9%]

Analyte	Test Concentration	SPOTFIRE® System testing								All Systems/ Sites [95% CI]
		bioMérieux				Clinical Sites				
		System A	System B	System C	Total	Site 1	Site 2	Site 3	Total	
Total Detected Agreement (%) by System/Site		**382/390** (98.0%)	**384/390** (98.5%)	**386/390** (99.0%)	**1152/1170** (98.5%)	**233/240** (97.1%)	**234/240** (97.5%)	**237/240** (98.8%)	**704/720** (97.7%)	**1856/1890** (98.2%) [97.5-98.7%]
Overall Detected Agreement (%) [95% CI]										

[a] *Candida albicans* was re-tested with refrigerated samples in accordance with recommended sample storage conditions. All other testing was performed on previously frozen samples to accommodate shipping to clinical sites

[b] Repeat for invalid result for one replicate in Mix 1 (negative mix) was not performed

The reproducibility of negative assay results of the BV analytes was evaluated using a combination of results from a negative sample (no analyte) and results for un-spiked analytes in the other samples Table 26.

Table 26: Summary of Negative BV Analyte Assay Results for the SPOTFIRE® Vaginitis Panel

Analyte	Test Concentration	SPOTFIRE® System testing								All Systems/ Sites [95% CI]
		bioMérieux				Clinical Sites				
		System A	System B	System C	Total	Site 1	Site 2	Site 3	Total	
Total Negative for Bacterial Vaginosis Analytes[a]										
Fannyhessea (Atopobium) vaginae	**Negative**	29/29[b] (100%)	30/30 (100%)	30/30 (100%)	89/89 (100%)	20/20 (100%)	20/20 (100%)	20/20 (100%)	60/60 (100%)	149/149 (100%) [97.5-100%]
BVAB2	**Negative**	179/179 (100%)	180/180 (100%)	180/180 (100%)	539/539 (100%)	80/80 (100%)	80/80 (100%)	80/80 (100%)	240/240 (100%)	779/779 (100%) [99.5-100%]
Lactobacillus crispatus	**Negative**	239/239[b] (100%)	240/240 (100%)	240/240 (100%)	719/719 (100%)	60/60 (100%)	60/60 (100%)	60/60 (100%)	180/180 (100%)	899/899 (100%) [99.6-100%]
Lactobacillus jensenii	**Negative**	239/239[b] (100%)	240/240 (100%)	240/240 (100%)	719/719 (100%)	60/60 (100%)	60/60 (100%)	60/60 (100%)	180/180 (100%)	899/899 (100%) [99.6-100%]
Lactobacillus paragasseri	**Negative**	209/209[b] (100%)	210/210 (100%)	210/210 (100%)	629/629 (100%)	58/60[c] (96.7%)	49/60[c] (81.7%)	54/60[c] (90.0%)	161/180[c] (89.4%)	790/809[c] (98.0%) [96.4-98.5%]
Sneathia vaginalis (amnii)	**Negative**	239/239[b] (100%)	240/240 (100%)	240/240 (100%)	719/719 (100%)	80/80 (100%)	80/80 (100%)	80/80 (100%)	240/240 (100%)	959/959 (100%) [99.6-100%]

[a] Data for BV analytes spiked at various concentrations are not shown since these analytes were tested at cutoff concentrations and not LoD. All analytes had 100% detections for all instances.

[b] Repeat for invalid result for one replicate in Mix 1 (negative mix) was not performed.

[c] All false positive assay results were observed in samples with high concentrations of *Lactobacillus jensenii* which is known to cross-react with the assay for *Lactobacillus paragasseri*. These individual assay false positive results did not compromise the expected BV interpretation result based on the BV algorithm.

Reproducibility was evaluated based on the percent agreement with the expected Bacterial Vaginosis (BV) Negative or Bacterial Vaginosis (BV) Positive interpretation for the BV analytes. Table 27 presents the overall reproducibility of each BV mix calculated as a percent (%) agreement with the expected results for that mix with a 95% confidence interval.

Table 27: Reproducibility of the Bacterial Vaginosis (BV) Algorithm

Mix	Analyte	Expected Result	SPOTFIRE® System testing								All Systems/ Sites [95% CI]
			bioMérieux				Clinical Sites				
			System A	System B	System C	Total	Site 1	Site 2	Site 3	Total	
1	**Negative**	(BV) Negative	29/29[a] (100%)	30/30 (100%)	30/30 (100%)	89/89 (100%)	20/20 (100%)	20/20 (100%)	20/20 (100%)	60/60 (100%)	149/149 (100%) [97.5-100%]
2	***Fannyhessea (Atopobium) vaginae Lactobacillus jensenii***	Bacterial Vaginosis (BV) Negative	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)	20/20 (100%)	20/20 (100%)	20/20 (100%)	60/60 (100%)	150/150 (100%) [97.5-100%]

Mix	Analyte	Expected Result	SPOTFIRE® System testing								All Systems/ Sites [95% CI]
			bioMérieux				Clinical Sites				
			System A	System B	System C	Total	Site 1	Site 2	Site 3	Total	
3	***Fannyhessea (Atopobium) vaginae Lactobacillus paragasseri***	Bacterial Vaginosis (BV) Low Positive	29/30 (96.7%)	27/30 (90.0%)	25/30 (83.3%)	81/90 (90.0%)	18/20 (90.0%)	19/20 (95.0%)	19/20 (95.0%)	56/60 (93.3%)	137/150 (91.3%) [85.7-94.9%]
4	***Fannyhessea (Atopobium) vaginae Lactobacillus paragasseri***	Bacterial Vaginosis (BV) Moderate Positive	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)	20/20 (100%)	20/20 (100%)	20/20 (100%)	60/60 (100%)	150/150 (100%) [97.5-100%]
			System A	**System B**	**System C**	**Total [95% CI]**					
5	***Fannyhessea (Atopobium) vaginae* BVAB2**	Bacterial Vaginosis (BV) High Negative	2/30 (6.7%)	6/30 (20.0%)	7/30 (23.3%)	15/90 (16.7%) [10.4-25.7 %]					
6	***Fannyhessea (Atopobium) vaginae* BVAB2**	Bacterial Vaginosis (BV) Low Positive	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%) [95.9-100 %]					
7	***Fannyhessea (Atopobium) vaginae Sneathia vaginalis (amnii) Lactobacillus paragasseri***	Bacterial Vaginosis (BV) Low Positive	30/30 (100%)	27/30 (90.0%)	29/30 (96.7%)	86/90 (95.6%) [89.1-98.3 %]					
8	***Fannyhessea (Atopobium) vaginae* BVAB2**	Bacterial Vaginosis (BV) Moderate Positive	29/30 (96.7%)	30/30 (100%)	29/30 (96.7%)	88/90 (97.8%) [92.3-99.4 %]					

[a] Repeat for invalid result for one replicate was not performed.

The standard deviation (SD) and coefficient of variation (CV) between-sites/system, between-pouch lots, between-days, between-operators, between-runs, and within-runs were calculated using the assay crossing point (Cp) values and were differentiated by mixes that were performed both internally and externally (Table 28), or internally only (Table 29).

Table 28: Summary of Precision Study Data for Mixes Tested Internally and Externally

Assay Target	BV Category/Fold-LoD	n	Between-Sites/System		Between-Operator		Between-Day		Between-Lot		Between-Run		Within-Run (Residual)		Total	
			SD	CV	SD	CV	SD	CV	SD	CV	SD	CV	SD	CV	SD	CV
Fannyhessea (Atopobium) vaginae	BV negative	150	0.2	3.5%	0.0	0.4%	0.1	1.4%	0.2	4.0%	0.5	9.3%	0.2	3.6%	1.2	22.2%
	BV low positive	150	0.1	0.8%	0.0	0.0%	0.0	0.0%	0.1	0.9%	0.4	4.9%	0.1	1.2%	0.7	7.9%
	BV moderate positive	150	0.2	2.6%	0.1	2.0%	0.0	0.0%	0.3	4.2%	0.4	5.8%	0.2	3.2%	1.2	17.7%
Candida albicans	1× LoD	211	0.4	1.5%	0.8	3.2%	0.2	0.7%	0.2	0.8%	1.1	4.5%	0.9	3.9%	3.5	14.6%
	3× LoD	149	0.3	1.5%	0.0	0.0%	0.1	0.3%	0.2	0.8%	0.8	3.6%	0.4	2.1%	1.8	8.2%
Candida dubliniensis	1× LoD	150	0.4	1.8%	0.2	1.1%	0.0	0.0%	0.0	0.0%	1.1	5.6%	0.4	1.8%	2.1	10.3%
	3× LoD	150	0.0	0.0%	0.3	1.8%	0.0	0.0%	0.1	0.4%	0.6	3.2%	0.2	1.1%	1.2	6.5%
Candida glabrata	1× LoD	150	0.0	0.0%	0.3	1.5%	0.3	1.6%	0.2	0.9%	1.9	9.2%	0.4	1.8%	3.1	15.1%
	3× LoD	150	0.0	0.0%	0.0	0.0%	0.0	0.0%	0.1	0.8%	1.2	6.5%	0.2	1.2%	1.6	8.5%

Assay Target	BV Category/ Fold-LoD	n	Between-Sites/System		Between-Operator		Between-Day		Between-Lot		Between-Run		Within-Run (Residual)		Total	
			SD	CV	SD	CV	SD	CV	SD	CV	SD	CV	SD	CV	SD	CV
Candida krusei	1× LoD	149	0.0	0.0%	0.0	0.0%	0.1	0.4%	0.0	0.0%	1.3	6.2%	0.5	2.4%	1.9	9.0%
	3× LoD	150	0.0	0.0%	0.4	2.0%	0.0	0.0%	0.2	0.8%	0.6	3.3%	0.3	1.4%	1.5	7.5%
Candida parapsilosis	1× LoD	148	0.0	0.0%	0.2	1.2%	0.2	0.8%	0.2	0.8%	0.9	4.4%	0.4	1.8%	1.8	8.9%
	3× LoD	150	0.1	0.7%	0.1	0.4%	0.2	1.0%	0.1	0.7%	0.7	3.9%	0.2	1.0%	1.3	7.7%
Lactobacillus crispatus	BV moderate positive	150	0.1	1.0%	0.1	1.3%	0.0	0.0%	0.2	2.3%	0.4	3.6%	0.1	1.0%	1.0	9.3%
Lactobacillus paragasseri	BV low positive	150	0.1	1.4%	0.0	0.0%	0.0	0.0%	0.2	2.2%	0.6	7.4%	0.1	1.3%	0.9	12.3%
Lactobacillus jensenii	BV negative	150	0.0	0.0%	0.0	0.0%	0.0	0.0%	0.1	1.1%	0.4	7.0%	0.0	0.1%	0.4	8.2%
Trichomonas vaginalis	1× LoD	149	0.0	0.0%	0.0	0.0%	0.0	0.0%	0.0	0.0%	0.8	3.9%	0.1	0.7%	0.9	4.5%
	3× LoD	150	0.1	0.6%	0.1	0.7%	0.0	0.0%	0.2	0.8%	0.4	2.2%	0.1	0.6%	0.9	4.9%

Table 29: Summary of Precision Study Data for Mixes Tested Internally

Assay Target	BV Category	n	Between-System		Between-Operator		Between-Day		Between-Lot		Between-Run		Within-Run (Residual)		Total	
			SD	CV	SD	CV	SD	CV	SD	CV	SD	CV	SD	CV	SD	CV
Fannyhessea (Atopobium) vaginae	BV negative	90	0.1	0.6%	0.0	0.0%	0.0	0.0%	0.0	0.0%	0.6	5.2%	0.1	0.8%	0.7	6.6%
	BV low positive	90	0.0	0.0%	0.1	0.7%	0.1	0.8%	0.1	0.9%	0.3	3.0%	0.1	0.7%	0.7	6.0%
	BV low positive	90	0.0	0.0%	0.0	0.0%	0.0	0.0%	0.2	3.4%	0.4	5.3%	0.2	2.6%	0.8	11.2%
	BV moderate positive	89	0.0	0.0%	0.1	0.5%	0.0	0.3%	0.2	1.6%	0.3	2.7%	0.1	0.8%	0.7	5.9%
BVAB2	BV negative	90	0.1	0.8%	0.0	0.0%	0.0	0.3%	0.2	1.6%	0.5	4.3%	0.0	0.3%	0.9	7.3%
	BV low positive	90	0.0	0.0%	0.1	0.9%	0.1	0.9%	0.2	1.7%	0.3	2.4%	0.0	0.4%	0.7	6.3%
	BV moderate positive	90	0.0	0.0%	0.1	1.5%	0.0	0.0%	0.3	2.9%	0.5	5.5%	0.0	0.5%	1.0	10.4%
Sneathia sanguinegens/vaginalis	BV low positive	90	0.1	0.5%	0.0	0.3%	0.0	0.0%	0.1	0.9%	0.3	1.7%	0.1	0.4%	0.6	3.9%

2.5. Interference

Potentially interfering substances/microorganisms that could be present in vaginal swab specimens were evaluated for their effect on the SPOTFIRE® VG Panel performance. A total of 70 potential interferants (7 endogenous, 43 exogenous, and 20 microorganisms) were tested. Samples with and without representative analytes were prepared in aVS and spiked with potential interferents at levels expected to represent a high level that may be found in natural clinical specimens. Of the 70 potential interferants tested, only the five listed in Table 30 interfered with panel performance. Interference was not observed with any of the 20 microorganisms tested or with the other 45 potentially interfering substances evaluated (Table 31). While interference was not observed with the tested probiotic suppository, any substances containing *Lactobacillus* species may increase the chance of false negative BV results.

Table 30: Summary of Substances with Observed Interference on the SPOTFIRE® VG Panel

Substance/Organism	Substance Type	Max Concentration Tested	Concentration of No Interference
Mucin	Endogenous	5% w/v	1.00% w/v

Substance/Organism	Substance Type	Max Concentration Tested	Concentration of No Interference
Baby powder	Exogenous	2% w/v	0.50% w/v
Vaginal deodorant powder	Exogenous	5% w/v	0.02% w/v
Boric acid vaginal suppository	Exogenous	5% w/v	1.25% w/v
Talcum powder	Exogenous	5% w/v	5% w/v[a]

[a] Initially, 1/3 of samples reported a negative result. Three additional replicates were tested, all of which produced the expected results for all analytes. While no interference was observed at this concentration, it is expected at levels higher than 5% w/v.

Table 31: Summary of Substance without Observed Interference on the SPOTFIRE® VG Panel

Substance/Organism	Substance Type	Concentration Tested
Estradiol	Endogenous	2.8E-05 µmol/L
Progesterone	Endogenous	2.9E+00 µmol/L
Albumin	Endogenous	60 mg/mL
Leukocytes	Endogenous	1.0E+06 cells/mL
Semen	Endogenous	5% w/v
Whole Blood	Endogenous	5% v/v
Vaginal lubricant (silicone based)	Exogenous	5% w/v
Vaginal lubricant (water based, with carbomers)	Exogenous	5% w/v
Vaginal moisturizer	Exogenous	5% w/v
Aloe Vera lubricant	Exogenous	5% w/v
Coconut oil	Exogenous	5% w/v
Aquasonic clear	Exogenous	5% w/v
Petroleum jelly	Exogenous	5% w/v
Hemorrhoidal cream	Exogenous	5% w/v
Vaginal douche (with vinegar)	Exogenous	5% v/v
Vaginal douche (Povidone-iodine 10% solution)	Exogenous	5% v/v
Deodorant suppositories	Exogenous	5% w/v
Whole body deodorant	Exogenous	5% w/v
Vaginal ointment (tioconazole 6.5%)	Exogenous	5% w/v
Vaginal cream (clotrimazole 2%)	Exogenous	5% w/v
Vaginal cream (miconazole nitrate 4%)	Exogenous	5% w/v
Vaginal anti-itch cream (benzocaine 20%, resorcinol 3%)	Exogenous	5% w/v
Anti-itch cream (hydrocortisone acetate 1%)	Exogenous	5% w/v
VagiBIOM vaginal probiotic suppository[a]	Exogenous	5% w/v
Vaginal contraceptive gel (nonyoxynol-9 4%)	Exogenous	5% w/v
Vaginal contraceptive film (nonyoxynol-9 28%)	Exogenous	5% w/v
Acyclovir	Exogenous	2.9E+02 µmol/L
Acetaminophen	Exogenous	1.0E+03 µmol/L
Acetylsalisylic acid	Exogenous	1.7E+02µmol/L
Ibuprofen	Exogenous	1.1E+03µmol/L
Naproxen	Exogenous	1.6E+03 µmol/L
Sodium salicylate[b]	Exogenous	6.6E+03 µmol/L
Phenazopyridine hydrochloride	Exogenous	9.1E-01µmol/L
Metronidazole	Exogenous	7.2E+02 µmol/L
Amoxicillin trihydrate	Exogenous	1.5E+02µmol/L
Azithromycin	Exogenous	1.5E+01 µmol/L
Ceftriaxone sodium salt hemiheptahydrate	Exogenous	1.5E+03 µmol/L
Doxycycline	Exogenous	4.1E+01 µmol/L
Erythromycin	Exogenous	1.9E+02µmol/L
Sulfamethoxazole	Exogenous	5.9E+03 µmol/L
Tetracycline HCl	Exogenous	5.4E+01µmol/L

Substance/Organism	Substance Type	Concentration Tested
Trimethoprim	Exogenous	1.5E+02 µmol/L
Spironolactone	Exogenous	1.3E+00 µmol/L
Leuprorelin acetate	Exogenous	2.5E-01 µmol/L
Histrelin acetate	Exogenous	2.5E-03 µmol/L
Atopobium vaginae	On Panel	1.8E+07 copies/mL
Candida albicans	On Panel	1.0E+06 copies/mL
Candida dubliniensis	On Panel	2.1E+06 copies/mL
Candida glabrata	On Panel	1.0E+06 copies/mL
Candida krusei	On Panel	1.0E+06 copies/mL
Candida parapsilosis	On Panel	1.0E+06 copies/mL
Candida tropicalis	On Panel	1.0E+06 copies/mL
Lactobacillus crispatus	On Panel	1.0E+07 copies/mL
Lactobacillus jensenii	On Panel	1.0E+07 copies/mL
Trichomonas vaginalis	On Panel	9.3E+04 copies/mL
Chlamydia trachomatis	Off Panel	1.0E+06 copies/mL
Corynebacterium xerosis	Off Panel	1.0E+08 copies/mL
Enterococcus faecalis	Off Panel	1.0E+08 copies/mL
Gardnerella vaginalis	Off Panel	3.2E+05 copies/mL
Lactobacillus acidophilus	Off Panel	1.0E+07 copies/mL
Lactobacillus brevis	Off Panel	1.0E+07 copies/mL
Mobiluncus curtsii	Off Panel	1.0E+06 copies/mL
Mycoplasma genitalium	Off Panel	1.5E+05 copies/mL
Proteus mirabilis	Off Panel	1.0E+08 copies/mL
Streptococcus agalactiae	Off Panel	1.0E+08 copies/mL

[a] Interference was not observed, however any substances containing *Lactobacillus* species may increase the chance of false negative BV results.

[b] Initially, 1/3 of samples reported negative results; therefore, three additional replicates were tested, all of which produced the expected results for all analytes.

To assess competitive interference, multiple mixes containing analytes near LoD or BV algorithm cutoff concentrations as well as multiple high concentration analytes (≥1.0E+6 copies/mL or 1.0E+04 copies/mL for *T. vaginalis*) were tested to evaluate potential inhibitory effects on low positive analytes and near BV algorithm cutoff concentrations due the presence of other on-panel analytes at high concentrations.

All expected results were observed across all panel analyte combinations tested with the SPOTFIRE® VG Panel. The study demonstrated that no competitive inhibitory effects were detected among the panel analytes.

Conclusion:

The results of the analytical and clinical performance studies summarized above demonstrated that the BIOFIRE SPOTFIRE Vaginitis (VG) Panel is substantially equivalent to the predicate device.