# 510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
# DECISION SUMMARY

## I Background Information:

### A 510(k) Number

K262136

### B Applicant

BIOFIRE Diagnostics, LLC

### C Proprietary and Established Names

BIOFIRE SPOTFIRE Vaginitis (VG) Panel

### D Regulatory Information

| Product Code(s) | Classification | Regulation Section | Panel |
|---|---|---|---|
| PQA | Class II | 21 CFR 866.3975 - Device That Detects Nucleic Acid Sequences From Microorganisms Associated With Vaginitis And Bacterial Vaginosis | MI - Microbiology |

## II Submission/Device Overview:

### A Purpose for Submission:

To obtain a substantial equivalence determination for BIOFIRE SPOTFIRE Vaginitis (VG) Panel performed on the BIOFIRE SPOTFIRE System.

### B Measurand:

The BIOFIRE SPOTFIRE Vaginitis (VG) Panel for use with BIOFIRE SPOTFIRE System is for the simultaneous qualitative detection and identification of nucleic acids from multiple pathogens associated with bacterial vaginosis (not differentiated): *Fannyhessea (Atopobium) vaginae*, Bacterial Vaginosis-Associated Bacterium 2 (BVAB2), *Lactobacillus crispatus, Lactobacillus gasseri/paragasseri, Lactobacillus jensenii/mulieris, Sneathia vaginalis (amnii)/sanguinegens*; vulvovaginal candidiasis: *Candida albican, Candida glabrata, Candida*

*krusei, Candida parapsilosis, Candida tropicalis, Candida dubliniensis*; and trichomoniasis: *Trichomonas vaginalis*.

**C Type of Test:**

Multiplex nucleic acid assay for use with the BIOFIRE SPOTFIRE System for the qualitative detection of pathogens in patients with symptoms of vaginitis/vaginosis.

## III Intended Use/Indications for Use:

**A Intended Use(s):**
See Indications for Use below.

**B Indication(s) for 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:

<table>
<tr><th>Bacteria</th><th>Yeast</th><th>Parasites</th></tr>
<tr><td>Bacterial Vaginosis (BV)</td><td rowspan="3">Candida albicans<br>Candida glabrata<br>Candida krusei<br>Candida parapsilosis<br>Candida tropicalis<br>Candida dubliniensis</td><td rowspan="3">Trichomonas vaginalis</td></tr>
<tr><td>Analytes (not reported individually)</td></tr>
<tr><td>Fannyhessea (Atopobium) vaginae<br>Bacterial Vaginosis-Associated Bacterium 2 (BVAB2)<br>Lactobacillus crispatus<br>Lactobacillus gasseri/paragasseri<br>Lactobacillus jensenii/mulieris<br>Sneathia vaginalis (amnii)/sanguinegens</td></tr>
</table>

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.

**C Special Conditions for Use Statement(s):**
Rx – For Prescription Use Only

**D Special Instrument Requirements:**
BIOFIRE SPOTFIRE System

## IV Device/System Characteristics:

### A Device Description:

The BIOFIRE SPOTFIRE Vaginitis (VG) Panel (referred to as SPOTFIRE VG Panel in subsequent text) is a multiplexed nucleic acid-amplification-based test that simultaneously identifies eight different targets: bacterial vaginosis (encompasses multiple bacterial targets), six yeasts, and one parasite from clinician collected and self-collected vaginal swab specimens collected using BIOFIRE SPOTFIRE Vaginal Swab Collection Kit from individuals with signs and symptoms of vaginitis. The SPOTFIRE VG Panel is intended to be used with the BIOFIRE SPOTFIRE System. The SPOTFIRE System Software includes step-by-step on-screen instructions that guide the user through the process of starting a run on the instrument.

The BIOFIRE SPOTFIRE System (referred to as SPOTFIRE System in subsequent text) automates nucleic acid extraction and nested multiplex PCR in unitized, closed pouches. The resulting PCR products are evaluated using assay-specific DNA melting analysis. The SPOTFIRE System Software automatically interprets the results of each assay 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, without user intervention.

The SPOTFIRE System is comprised of between one and four modules that are connected to a single SPOTFIRE Control Station equipped with the SPOTFIRE System Software. The first module is placed on top of the Control Station and additional modules may be stacked on top as required. Each module can be accessed at random to perform a test, independent of the other modules attached to the same Control Station. The SPOTFIRE VG Panel Software is required to perform the testing with the SPOTFIRE VG Panel.

The SPOTFIRE VG Panel Reagent Kit includes the following components:

- SPOTFIRE VG Panel Pouches containing freeze-dried reagents packed in vacuum-sealed packaging
- Sample Preparation Reagent Kit (SPRK)
  - Individually packaged fixed volume transfer pipette for addition of the test sample to the Sample Injection Vial
  - Sample Buffer ampoule containing Sample Buffer for addition to the Sample Injection Vial
  - Sample Injection Vial (coded red) for mixing of the test sample and Sample Buffer
  - Hydration Injection Vial (coded blue) containing Hydration Solution for pouch rehydration

BIOFIRE SPOTFIRE Vaginal Swab Collection Kit is used to collect vaginal swabs from patients for testing and is supplied separately. Kit includes individually packed consumables to collect 50 samples:

- Flexible tip size Nylon flocked swab with 80mm breakpoint
- eNAT-Molecular Transport Media

### B Principle of Operation:

The SPOTFIRE VG Panel pouch is a closed system disposable that stores all the necessary reagents for sample preparation, PCR, and detection to isolate, amplify, and detect nucleic acid

from multiple pathogens from a single vaginal specimen. After sample collection, the user injects hydration solution and sample combined with Sample Buffer into the pouch. This process also rehydrates a cell-based DNA Process Control in the sample well of the pouch. The user then places the pouch into the SPOTFIRE System and starts a run. The entire run process takes about 20 minutes. All other operations are automated.

During a run, the System:

- Lyses the sample by agitation (bead beating) in addition to chemical lysis mediated by the Sample Buffer.
- Extracts and purifies all nucleic acids from the sample using magnetic bead technology.
- Performs nested multiplex PCR by:
    - First performing a single, large volume, multiplexed first stage PCR reaction (PCR1).
    - Then performing multiple simultaneous second-stage PCR reactions (PCR2) in the array to amplify sequences within the PCR1 products. Each analyte or control target in PCR2 is represented by amplification reactions in three wells. PCR array includes an assay control, the PCR2 Control, failure of which indicates failure in the second stage PCR and invalidates the run.
- When PCR2 is complete, the instrument performs a high-resolution DNA melting analysis (Tm) on the PCR products and records the change in fluorescence signal generated in each of the three wells. The software then performs several analyses and assigns a final assay result. For an assay to be called positive, at least two of the three wells must have a PCR product with a Tm that is within the specified range for that analyte. Assays that do not meet these criteria are reported as negative.

Positive and negative results for the assay controls (DNA Process Control and PCR2 Control) are compared to their expected values and assigned a "Pass" or "Fail" result. If either the DNA Process Control or PCR2 Control result is Fail, then the results for all the tests on the panel are not displayed and the specimen must be retested with a new pouch. For all targets detected by the SPOTFIRE VG Panel except for BV, the target is reported as "Detected" if a single corresponding assay is positive (at least two of the three assay wells on the array have similar positive melt peaks with Tm values that are within the assay-specific Tm range). The test result for BV depends on the results from more than one assay.

The SPOTFIRE VG Panel uses six assays in the algorithm for the detection of BV. These six assays target organisms associated with BV: *Fannyhessea (Atopobium) vaginae*, Bacterial Vaginosis-Associated Bacterium (BVAB2), *Lactobacillus crispatus, Lactobacillus gasseri/paragasseri, Lactobacillus jensenii/mulieris, and Sneathia sanguinegens/vaginalis amnii*. The software includes a BV algorithm that evaluates each of these six assays against pre-defined limits, and the results are combined to determine a positive or negative call. This panel does not individually report any of these six assays. If the BV algorithm is positive the result will be "BV Positive". If the BV algorithm is negative the result will be "BV Negative".

## C Instrument Description Information:

1. Instrument Name:

   BIOFIRE SPOTFIRE System

2. Specimen Identification:

   Specimen identification can be entered manually or by scanning a barcode.

3. Specimen Sampling and Handling:

   Use of the SPOTFIRE VG Panel requires a vaginal swab to be collected using the BIOFIRE SPOTFIRE Vaginal Swab Collection Kit and placed in the eNAT molecular transport medium. The SPOTFIRE System Software includes step-by-step on-screen instructions that guide the user through the process of starting a run on the instrument.

4. Calibration:

   The BIOFIRE SPOTFIRE System is factory calibrated. User calibration is not required.

5. Quality Control:

   *Internal Controls*
   Two process controls are included in each SPOTFIRE VG Panel pouch:

   1) *DNA Process Control*
      The DNA Process Control assay targets a DNA sequence from the yeast *Schizosaccharomyces pombe*. The yeast is present in the pouch in a freeze-dried form and becomes rehydrated when sample is loaded. The control material is carried through all stages of the test process, including lysis, nucleic acid purification, PCR1, PCR2 and DNA melting. Positive control results indicate that all steps carried out in the SPOTFIRE VG Panel pouch were successful.

   2) *PCR2 Control*
      The PCR2 control comprises a DNA target sequence along with the corresponding primers that is dried into specific wells used for the second stage amplification (PCR2). A positive result indicates that PCR2 was successful.

   A positive result is required for both DNA Process Control and PCR2 Control for the results for an individual pouch to be considered valid. If either control fails, the sample should be retested using a new pouch.

   *External Controls*
   BIOFIRE recommends testing external SPOTFIRE VG Positive and Negative Controls that are commercially available from Maine Molecular Quality Controls, Inc. when training a new operator, or when a new shipment of SPOTFIRE panels or a new control station is received. The Instructions for Use of the SPOTFIRE VG Panel indicate that external

Negative Control should be tested at least monthly for each testing location to monitor environmental contamination.

## V Substantial Equivalence Information:

### A Predicate Device Name(s):
BD MAX Vaginal Panel

### B Predicate 510(k) Number(s):
K201017

### C Comparison with Predicate(s):

| **Device & Predicate Device(s):** | K262136 | K201017 |
|---|---|---|
| Device Trade Name | BIOFIRE SPOTFIRE Vaginitis (VG) Panel | BD MAX Vaginal Panel |
| **General Device Characteristic Similarities** | | |
| Intended Use/Indications For 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 | 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 |

| | | |
|---|---|---|
| | vaginitis/vaginosis.<br><br>The following analytes are identified and differentiated using the BIOFIRE SPOTFIRE Vaginitis (VG) Panel:<br><br>Bacteria<br>Bacterial Vaginosis (BV)<br>Analytes (not reported individually)<br>*Fannyhessea (Atopobium) vaginaea*<br>Bacterial Vaginosis-Associated<br>Bacterium 2 (BVAB2)<br>*Lactobacillus crispatus*<br>*Lactobacillus gasseri/paragasseri*<br>*Lactobacillus jensenii/Mulieris*<br>*Sneathia vaginalis (amnii)/ sanguinegens*<br><br>Yeast<br>*Candida albicans*<br>*Candida glabrata*<br>*Candida krusei*<br>*Candida parapsilosis*<br>*Candida tropicalis*<br>*Candida dubliniensis*<br><br>Parasite<br>*Trichomonas vaginalis*<br><br>The BIOFIRE SPOTFIRE Vaginitis (VG) Panel is indicated as an aid in the diagnosis of specific agents of | DNA targets and utilizes fluorogenic target-specific hybridization probes to detect and differentiate DNA from:<br>• Bacterial vaginosis markers (Individual markers not reported)<br>o *Lactobacillus spp. (L. crispatus* and *L. jensenii*)<br>o *Gardnerella vaginalis*<br>o *Atopobium vaginae*<br>o Bacterial Vaginosis Associated Bacteria-2 (BVAB-2)<br>o Megasphaera-1<br>• Candida spp. *(C. albicans, C. tropicalis, C. parapsilosis, C. dubliniensis*)<br>• *Candida glabrata*<br>• *Candida krusei*<br>• *Trichomonas vaginalis*<br><br>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. |

| | | |
|---|---|---|
| | 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 self-collected vaginal swabs | Same |
| Organism detected | <u>Bacteria</u><br>(Associated with BV; Analytes not reported separately)<br>*Fannyhessea (Atopobium) vaginaea*<br>Bacterial Vaginosis-Associated Bacterium 2 (BVAB2)<br>*Lactobacillus crispatus*<br>*Lactobacillus gasseri/paragasseri*<br>*Lactobacillus jensenii/Mulieris*<br>*Sneathia vaginalis (amnii)/ sanguinegens*<br><br><u>Yeast</u><br>*Candida albicans*<br>*Candida glabrata*<br>*Candida krusei*<br>*Candida parapsilosis*<br>*Candida tropicalis*<br>*Candida dubliniensis*<br><br><u>Parasite</u><br>*Trichomonas vaginalis* | Same except some of the analytes:<br>• Includes *Megasphaera-1*<br>• Does not include *Lactobacillus gasseri/paragasseri* |
| Disease Syndrome | Vaginitis/vaginosis | Same |
| Analyte | DNA | Same |
| Test Interpretation | Automatic test interpretation and | Same |

| | | |
|---|---|---|
| | reporting | |
| Technological Principle | Real time PCR | Same |
| **General Device Characteristic Differences** | | |
| Instrument | BIOFIRE SPOTFIRE System | BD MAX System |

## VI Standards/Guidance Documents Referenced:

21 CFR 866.3975: Device that detects nucleic acid sequences from microorganisms associated with vaginitis and bacterial vaginosis.

## VII Performance Characteristics (if/when applicable):

### A Analytical Performance:

1. Precision/Reproducibility:

A multi-variable study was performed to evaluate the precision and reproducibility of the SPOTFIRE VG Panel. Testing sites included three external clinical sites and one internal site. The sample 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. An equivalence study demonstrated comparable performance of SPOTFIRE VG Panel between single or multi-spiked samples. Contrived positive sample mixes were prepared to include each analyte at either a low positive (1x LoD or BV algorithm cutoff concentration) or moderate positive (3x LoD or BV algorithm cutoff concentration) concentration in artificial vaginal swab matrix in eNAT media (aVS). 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 BV Positive or BV Negative interpretation result based on the BV algorithm.

*External Sites*
For testing performed at external sites, samples were tested over five non-consecutive days by two operators during their normal workday routine per site. Each site was equipped with two modules on one SPOTFIRE System. Testing at all three sites was performed with a single reagent lot. Each testing day, each operator ran two replicates for a total of 20 replicates per site and 60 total replicates across all three sites (2 operators x 2 replicates x 3 sites x 5 days = 60 replicates).

*Internal Site*
For testing performed at internal site, samples were tested over five consecutive workdays, on three different SPOTFIRE Systems (A, B and C), by two operators per System, using three different reagent kit lots (one per System per day). Each day of testing, the two operators tested three replicates per System for a total of 30 replicates per System and 90 total replicates across all systems (2 operators x 3 replicates x 3 Systems x 5 days = 90 days).

A summary of results (percent (%) agreement with the expected result) for each analyte stratified by site and system is provided below. 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 (Table 1).

**Table 1: Summary of Non-BV Reproducibility Results for the SPOTFIRE VG Panel**

| Analyte | Test Concentration | Agreement (%) Stratified by Site and SPOTFIRE System | | | | | | | | All Systems/ Sites [95% CI] |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Internal | | | | External | | | | |
| | | System A | System B | System C | Total | Site 1 | Site 2 | Site 3 | Total | |
| ***Candida albicans*** | 6.0E+03 copies/mL (3x 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 (1x 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 (1x 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 (1x 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 (3x 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 (3x 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 (1x 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 | 30/30 | 30/30 | 30/30 | 90/90 | 20/20 | 19/20 | 19/20 | 58/60 | 148/150 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | copies/mL (1x LoD) | (100%) | (100%) | (100%) | (100%) | (100%) | (95.0%) | (95.0%) | (96.7%) | (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 (3x 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 (1x 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%] |
| **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 the sites.

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

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% (Table 2). 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. All had 100% positive assay results as expected in the respective mixes.

**Table 2: Summary of Negative BV Analyte Assay Results for the SPOTFIRE VG Panel**

| Analyte | Test Concentration | Agreement (%) Stratified by Site and SPOTFIRE System | | | | | | | | All Systems/ Sites [95% CI] |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Internal | | | | External | | | | |
| | | 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 BV Negative or BV Positive interpretation for the BV analytes. Table 3 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 3: Reproducibility of the BV Algorithm**

| Mix | Analyte | Expected Result | Agreement (%) Stratified by Site and SPOTFIRE System | | | | | | | | All Systems/ Sites [95% CI] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Internal | | | | External | | | | |
| | | | 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*** | 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%] |
| 3 | ***Fannyhessea (Atopobium) vaginae* *Lactobacillus paragasseri*** | 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*** | 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** | 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** | 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*** | BV Low Positive | 30/30 (100%) | 27/30 (90.0%) | 29/30 (96.7%) | 86/90 (95.6%) [89-98.3 %] | | | | | |
| 8 | ***Fannyhessea (Atopobium) vaginae* BVAB2** | 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 4), or internally only (Table 5).

**Table 4: 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 | | 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*** | 1x 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% |
| | 3x 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*** | 1x 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% |
| | 3x 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*** | 1x 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% |
| | 3x 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% |
| ***Candida krusei*** | 1x 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% |
| | 3x 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*** | 1x 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% |
| | 3x 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*** | 1x 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% |
| | 3x 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 5: 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 | | 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. Linearity:

   Not applicable.

3. Analytical Specificity/Interference:

**Cross-reactivity Study**

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 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 laboratory testing data, the potential cross-reactivities of the SPOTFIRE VG Panel is noted in the device labeling, as summarized in Table 6.

**Table 6: 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*<br>*Pichia fermentans*<br>*Pichia norvegensis*<br>*Pichia membranifaciens*[b] | The listed *Candida* species and *Pichia* species may be detected as *Candida krusei* |
| *Candida tropicalis* | *Candida kefyr*[c]<br>*Candida maltosa*<br>*Candida buenavistaensis*[d]<br>*Candida neerlandica*[d]<br>*Candida sanvaensis*[d] | The listed *Candida* species may be detected as *Candida tropicalis* at high concentrations[a] |

| | | |
|---|---|---|
| *Candida glabrata* | *Candida nivariensis*<br>*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.

The on-panel and off-panel organisms empirically tested to evaluate the analytical specificity of SPOTFIRE VG Panel are listed in Table 7 and 8, respectively.

**Table 7: 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 | 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 individual assay results
[b] Additional testing at lower concentrations was conducted. Cross-reactivity was observed at concentrations ≥1.0E+07 cells/mL.

**Table 8: 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 |
| *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* |

| | | |
|---|---|---|
| *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 individual 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 >1E+06 cell/ml.

[e] Additional testing at lower concentrations was conducted. Cross-reactivity was observed at concentrations >1E+07 cell/ml.

[f] Additional testing at lower concentrations was conducted. Cross-reactivity was observed at concentrations >9.4E+07 cell/ml.

[g] Additional testing at lower concentrations was conducted. Cross-reactivity was observed at concentrations >8.9E+01 cell/ml.

## Interference Study

A study was conducted to evaluate the potential for assay interference due to the presence of endogenous and exogenous substances that may be present in the vaginal swab specimens. In

addition, testing was also performed with representative on-panel microorganisms at very high concentrations to evaluate the potential for competitive interference between analytes. Microbial interference was also tested with off-panel microorganisms. A total of 70 potential interferants (7 endogenous, 43 exogenous, and 20 microorganisms) were tested. Samples with and without representative SPOTFIRE VG Panel 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. Testing of each potentially interfering substance/microorganism was performed in at least three replicates.

Of the 70 potential interferants tested, five listed in Table 9 interfered with the SPOTFIRE Vaginitis Panel performance.

**Table 9: 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 |
| 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 samples was reported as negative. 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.

No interference was observed with any of the 20 microorganisms tested or with the other 45 potentially interfering substances evaluated (Table 10).

**Table 10: Summary of Substance without Observed Interference on the SPOTFIRE VG Panel**

| Substance/Organism | Type | Concentration Tested |
|---|---|---|
| **Endogenous and Exogenous Substance Interference** | | |
| 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 |
| 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 |
| **Competitive Interference (On-panel microorganisms)** | | |
| *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 |
| **Microbial Interference** | | |
| *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 samples was reported as negative. Three additional replicates were tested, all of which produced the expected results for all analytes.

To further assess competitive interference between on-panel microorganisms, multiple mixes containing analytes near LoD or BV algorithm cutoff concentrations alongside additional analytes at high concentrations (≥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 on-panel analyte combinations tested with the SPOTFIRE VG Panel. The study demonstrated that no competitive inhibitory effects were detected among the on-panel analytes.

4. Detection Limit and Assay Reportable Range:

**Analytical Sensitivity**

The limit of detection (LoD) for each of the bacteria, yeast and parasitic analytes on the SPOTFIRE VG Panel was established. Contrived samples containing known concentrations for the yeast and parasitic organism were prepared in natural vaginal swab matrix (nVS), while the bacterial analytes, due to their naturally occurring presence in nVS, were prepared in artificial vaginal swab matrix in eNAT media (aVS). An initial titration of each viral or bacterial stock was performed to estimate the LoD, which was then confirmed by testing 20 replicates at the estimated LoD concentration and 20 replicates at 3-fold above and 10-fold lower dilution. The LoD was confirmed if at least 95% (≥19/20) replicates at or above the estimated LoD and less than 95% (<19/20) replicates at a lower concentration were detected. The confirmed LoD concentrations for the analytes on the SPOTFIRE VG Panel are listed in the table below.

**Table 11: LoD for the SPOTFIRE VG Panel**

| Analyte | Source ID | LoD Concentration[b] |
|---|---|---|
| **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* | 6.0E+03 copies/mL |

| | | |
|---|---|---|
| | ATCC 25258 | (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** | | |
| *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 based on the BV algorithm.
[b] LoD concentrations in copies/mL in the table 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.

The SPOTFIRE VG Panel uses six assays (*Fannyhessea (Atopobium) vaginae*, Bacterial Vaginosis-Associated Bacterium (BVAB2), *Lactobacillus crispatus, Lactobacillus gasseri/paragasseri, Lactobacillus jensenii/mulieris*, and *Sneathia sanguinegens/vaginalis amnii*) in the BV algorithm for the detection of BV. The BV algorithm evaluates each of these six assays against pre-defined limits, and the results are combined to determine a final positive or negative BV call. For assessment of the BV algorithm in analytical performance studies, concentrations which are near target decision points within the BV algorithm were used. The lowest concentration of BV analytes where expected BV positive results are observed ≥95% of the time for each decision point within the algorithm was determined and will hereafter be referred to as BV algorithm cutoff concentrations. This concentration was used in preparing samples for the analytical performance studies except for inclusivity study.

**Inclusivity (Analytical Reactivity)**
The inclusivity of the SPOTFIRE VG Panel assays was evaluated by testing at least five isolates, with the exception of only testing two isolates for BVAB2 due to limited strain availability. Contrived samples containing individual panel analytes were prepared at approximately 3x LoD in aVS and tested in triplicate.

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 the targeted mitochondrial DNA. 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 12: 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 13: Summary of Reactivity BVAB2 Isolates**

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

**Table 14: 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 15: 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 16: 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 the targeted mitochondrial function/mitochondrial DNA.

**Table 17: 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 18: 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 19: 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 20: 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 21: 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 22: 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 23: 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 24: 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 | |

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

**Specimen Stability**

A study was conducted to evaluate stability of vaginal swab specimens collected in the eNAT media prior to testing with SPOTFIRE VG Panel. The study evaluated stability at 3-8 days for ambient (25°C) and refrigerated (4°C), 3 days at elevated (30°C) temperature, and 30 days in frozen (-20°C) condition. Two multi-analyte contrived sample mixes- Mix 1 (*Candida* sp. and *Trichomonas vaginalis* at 3x LoD) and Mix 2 (BV analytes at 3x BV algorithm cutoff concentration) - were evaluated in the study either immediately (baseline) or after storage under different conditions. Each mix was tested 10 times at each time point after storage under the applicable condition.

All the analytes were shown to be stable under the conditions tested. The results of this study support the claimed specimen storage conditions included in the SPOTFIRE VG Panel labelling: 7 days at 4-25°C prior to testing.

6. Assay Cut-Off:

DNA melt curve analysis is used to determine the presence or absence of specific amplification products corresponding to each analyte within the SPOTFIRE VG Panel. If the melt profile indicates the presence of a PCR product, then the analysis software calculates the melting temperature (Tm) of the curve and compares it against the expected Tm range for the assay. If the software determines that the Tm of the curve is within the assay-specific Tm range, the melt curve is called positive. If the software determines that the Tm of the curve is not in the appropriate Tm range, the melt curve is called negative. Once positive melt curves have been identified, the software evaluates the two replicates for each assay to determine the assay result. For an assay to be called positive, at least two of the three associated melt curves must be called positive, and the Tm for at least two of the three positive melt curves must be similar. Assays that do not meet these criteria are called negative. Where applicable, a Crossing Point value cutoff (Cp; PCR cycle at which the fluorescence in the amplification reaction rises above the background fluorescence) was also implemented for select assays to further reduce the risk of false positive results. The melt range parameters and Cp cutoffs were validated in the subsequent analytical and clinical studies. For the BV interpretation, results from multiple BV-associated assays are collectively evaluated against pre-defined limits by a BV algorithm to determine an overall BV positive or negative result.

7. Carry-over/Cross-contamination Study:

The SPOTFIRE VG Panel utilizes the same workflow and cleaning procedures as previously cleared BIOFIRE SPOTFIRE Respiratory/Sore Throat (R/ST) Panel (K232954). The carry-over/cross-contamination studies were conducted previously and may be referenced in the published Decision Summary for K232954.

**B Comparison Studies:**

1. Method Comparison with Predicate Device:

Not applicable.

2. Matrix Comparison:

Because the BV analytes detected by SPOTFIRE VG Panel are present in normal vaginal flora, it was necessary to use artificial vaginal swab (aVS) matrix for preparation of samples for some analytical studies. This study assessed the performance equivalence of SPOTFIRE VG Panel between aVS and natural clinical vaginal swab (nVS) matrix samples.

Contrived organism mix containing a subset of representative analytes (*Candida* species and *Trichomonas vaginalis*) was prepared in aVS or in nVS for the testing. The samples were serially diluted (in either aVS or nVS) to concentrations at 5x, 2x, and 0.2x LoD. Ten replicates were tested for each sample above (5x) and below (0.2x) the LoD, while 40 replicates were tested for near-LoD (2x) samples. A summary of the results is presented in the table below.

**Table 25: Matrix Equivalency Study Results in aVS and nVS Matrices**

| Analyte | Analyte Concentration | Detected Results | |
|---|---|---|---|
| | | aVS | nVS |
| *Candida albicans* | 5x | 10/10 (100%) | 10/10 (100%) |
| | 2x | 39/40 (97.5%) | 39/40 (97.5%) |
| | 0.2x | 4/10 (40%) | 6/10 (60%) |
| *Candida krusei* | 5x | 10/10 (100%) | 10/10 (100%) |
| | 2x | 40/40 (100%) | 40/40 (100%) |
| | 0.2x | 5/10 (50%) | 4/10 (40%) |
| *Trichomonas vaginalis* | 5x | 10/10 (100%) | 10/10 (100%) |
| | 2x | 40/40 (100%) | 40/40 (100%) |
| | 0.2x | 0/10 (0%) | 1/10 (10%) |

Expected results were generated for all the samples tested in both the matrices. SPOTFIRE VG Panel performed equivalently for samples prepared in aVS and nVS. Study results support the use of the artificial vaginal swab matrix as an alternative to natural clinical vaginal swab matrix in selected analytical studies.

## C Clinical Studies:

1. Clinical Sensitivity:

The clinical performance of the SPOTFIRE VG Panel was established during a prospective multi-center study. 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. Demographic and clinical data was collected from the subjects at the time of enrollment. The included subjects were of various ethnicities and ages 14 years and above. Four vaginal swab (VS) specimens were collected from each subject: one self-collected (SC) or clinician-collected (CC) swab for testing with SPOTFIRE VG Panel and three clinician-collected swabs for comparator testing. The CC VS specimen collection order was randomized for each subject for all four VS specimens. The SPOTFIRE VG Panel SC VS specimen was always collected by the subject first, while the three other VS specimens for comparator testing were then collected by the clinician in a randomized order.

For 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) respectively, using three FDA-cleared nucleic acid amplification tests (NAATs). The CIS/PIS was considered positive or negative if a minimum of two FDA-cleared NAATs were concordant. Specimens without two valid concordant FDA-cleared test results were excluded from the analysis. 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.

A total of 1535 VS specimens were initially enrolled from consented subjects 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 and three of the VS specimens from asymptomatic subjects were excluded due to inability to meet the inclusion criteria or getting a valid SPOTFIRE VG Panel or comparator test result. The final dataset consisted of 1512 VS specimens (1412 VS specimens from symptomatic subjects and 100 VS specimens from asymptomatic subjects) evaluable for at least one analyte. In addition, some specimens were excluded from the analysis of performance for one or more specific analytes due to improper storage of sample aliquots or due to failure to obtain results from the comparator method and inability to perform retesting due volume constraints.

The performance for the prospective study is summarized in Table 26 and Table 27 for CC VS and SC VS specimens from symptomatic subjects, respectively.

**Table 26: SPOTFIRE VG Panel Clinical Performance for CC VS Specimens**

| SPOTFIRE VG Panel Analyte | Sensitivity/PPA[a] | | | Specificity/NPA[a] | | |
|---|---|---|---|---|---|---|
| | TP/ (TP + FN) | % | 95%CI | TN/ (TN + FP) | % | 95%CI |
| **Bacteria** | | | | | | |
| 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% |

PPA: Positive Percent Agreement; NPA: Negative Percent Agreement: 95% CI: 95% score confidence interval; TP: True Positive; FP: False Positive; False Negative; True Negative (all as determined with respect to the comparator)

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

[b] At least one negative 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 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 specimens using an independent molecular method.

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

**Table 27: SPOTFIRE VG Panel Clinical Performance for SC VS Specimens**

| SPOTFIRE VG Panel Analyte | Sensitivity/PPA[a] | | | Specificity/NPA[a] | | |
|---|---|---|---|---|---|---|
| | TP/ | % | 95%CI | TN/ | % | 95%CI |

| | (TP + FN) | | | (TN + FP) | | |
|---|---|---|---|---|---|---|
| **Bacteria** | | | | | | |
| 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% |

PPA: Positive Percent Agreement; NPA: Negative Percent Agreement: 95% CI: 95% score confidence interval; TP: True Positive; FP: False Positive; False Negative; True Negative (all as determined with respect to the comparator)

[a] The performance measures of sensitivity and specificity only refer to instances were a CIS/PIS was used as the reference method (i.e., 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 BV was further stratified by subject characteristics in Table 28 and Table 29 for CC VS and SC VS specimens from symptomatic subjects, respectively.

**Table 28: SPOTFIRE VG Panel BV Performance Stratified by Subject Characteristics for CC VS Specimens**

| SPOTFIRE VG Panel 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% |
| 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% |

PPA: Positive Percent Agreement; NPA: Negative Percent Agreement: 95% CI: 95% score confidence interval; TP: True Positive; FP: False Positive; False Negative; True Negative (all as determined with respect to the comparator)

[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 29: SPOTFIRE VG Panel BV Performance Stratified by Subject Characteristics for SC VS Specimens**

| SPOTFIRE VG Panel 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% |
| **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% |

PPA: Positive Percent Agreement; NPA: Negative Percent Agreement: 95% CI: 95% score confidence interval; TP: True Positive; FP: False Positive; False Negative; True Negative (all as determined with respect to the comparator)

[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).

**Contrived Specimens for *Candida***

Several analytes were not observed in the VS specimens in sufficient numbers to demonstrate 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 medium. 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 2x LoD. Contrived specimens were randomized and coded along with 12 negative (unspiked) VS specimens such that the analyte status of each specimen was unknown to the operators 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 the table below.

**Table 30: SPOTFIRE VG Panel Performance in Contrived VS Specimens**

| 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% |

PPA: Positive Percent Agreement; NPA: Negative Percent Agreement: 95% CI: 95% score confidence interval; TP: True Positive; FP: False Positive; False Negative; True Negative (all as determined with respect to the comparator)

**Coinfection Detection by SPOTFIRE VG Panel**

Observed coinfections (as determined by the SPOTFIRE VG Panel) in symptomatic subjects during the prospective clinical evaluation are presented in Table 31. The SPOTFIRE VG Panel reported positive result due to at least one analyte in 71.1% CC VS specimens (498/700) and 74.9% SC VS specimens (533/712) in symptomatic subjects during the prospective clinical evaluation. Polymicrobial detections of up to three analytes were observed in CC VS specimens and up to four analytes were observed in SC VS specimens.

**Table 31: Coinfections Reported for the SPOTFIRE VG Panel in the Prospective Clinical Study**

| SPOTFIRE VG Panel Positive | Prospective CC VS Specimens | | Prospective SC VS Specimens | |
|---|---|---|---|---|
| | Number | % of Total (% of Positives) | Number | % of Total (% of Positives) |
| **At least one analyte result** | 498 | 71.1% (100%) | 533 | 74.9% (100%) |
| **One analyte result** | 358 | 51.1% (71.9%) | 384 | 53.9% (72.0%) |
| **Two analyte results** | 131 | 18.7% (26.3%) | 131 | 18.4% (24.6%) |
| **Three analyte results** | 9 | 1.3% (1.8%) | 15 | 2.1% (2.8%) |
| **Four analyte results** | 0 | - | 3 | 0.4% (0.6%) |

The SPOTFIRE VG Panel reported a total of 140 CC VS specimens (20.0% of all specimens, 140/700; 28.1% of positive specimens, 140/498) and a total of 149 SC VS specimens (20.9% of all specimens, 149/712; 28.0% of positive specimens, 149/533) with multiple analyte detections. The resulting co-detection combinations as reported by the SPOTFIRE VG Panel

are presented in Table 32 and Table 33 for CC VS and SC VS specimens, respectively. These tables also indicate the number of specimens with false positive (FP) results (based on comparator test results) for each co-detection combination, as well as the specific analyte(s) that were discrepant.

**Table 32: Coinfections Reported by the SPOTFIRE VG Panel in the CC VS Specimens in the Prospective Study**

| Co-Detection Combinations | | | Total Specimens with Co-Detections | Number of Specimens with False Positive Co-Detections | SPOTFIRE VG Panel False Positive Analyte(s)[a] |
|---|---|---|---|---|---|
| **Analyte 1** | **Analyte 2** | **Analyte 3** | | | |
| BV | *Candida albicans* | *Candida glabrata* | 1 | 1 | *Candida glabrata* (1) |
| BV | *Candida albicans* | *Candida tropicalis* | 1 | 0 | - |
| BV | *Candida albicans* | *Trichomonas vaginalis* | 5 | 1 | *Candida albicans* (1) |
| BV | *Candida glabrata* | *Trichomonas vaginalis* | 2 | 1 | *Candida glabrata* (1) |
| BV | *Candida albicans* | | 91 | 7 | BV (3)<br>*Candida albicans* (6) |
| BV | *Candida glabrata* | | 3 | 0 | - |
| BV | *Trichomonas vaginalis* | | 27 | 2 | *Trichomonas vaginalis* (2) |
| *Candida albicans* | *Candida glabrata* | | 4 | 2 | *Candida albicans* (1)<br>*Candida glabrata* (1) |
| *Candida albicans* | *Trichomonas vaginalis* | | 5 | 1 | *Trichomonas vaginalis* (1) |
| *Candida glabrata* | *Trichomonas vaginalis* | | 1 | 1 | *Trichomonas vaginalis* (1) |
| **Total Co-Detections** | | | **140** | **16** | **18/289[b,c]** |
| **Total Double Co-Detections** | | | **131** | **13** | **15/262[c]** |
| **Total Triple Co-Detections** | | | **9** | **3** | **3/27[c]** |

[a] Based on comparator test results.
[b] Of the 18 discrepant analytes (out of 289 total analytes reported by the SPOTFIRE VG Panel), 16 (88.9%) were confirmed as being present in the specimen during discrepancy investigation.
[c] Number of false positive/total

**Table 33: Coinfections Reported by the SPOTFIRE VG Panel in the SC VS Specimens in the Prospective Study**

| Distinct Co-Detection Combinations | | | | Total Specimens with Co-Detections | Number of Specimens with False Positive Co-Detections | False Positive Analyte(s)[a] |
|---|---|---|---|---|---|---|
| **Analyte 1** | **Analyte 2** | **Analyte 3** | **Analyte 4** | | | |
| BV | *Candida albicans* | *Candida glabrata* | *Candida tropicalis* | 1 | 0 | - |
| BV | *Candida albicans* | *Candida glabrata* | *Trichomonas vaginalis* | 1 | 1 | *Candida glabrata* (1) |

| | | | | | | |
|---|---|---|---|---|---|---|
| BV | *Candida dubliniensis* | *Candida glabrata* | *Trichomonas vaginalis* | 1 | 1 | *Trichomonas vaginalis* (1) |
| BV | *Candida albicans* | *Candida glabrata* | | 5 | 2 | *Candida albicans* (1)<br>*Candida glabrata* (1) |
| BV | *Candida albicans* | *Candida tropicalis* | | 1 | 1 | *Candida albicans* (1)<br>*Candida tropicalis*(1) |
| BV | *Candida albicans* | *Trichomonas vaginalis* | | 6 | 1 | *Candida albicans* (1) |
| BV | *Candida glabrata* | *Trichomonas vaginalis* | | 2 | 0 | - |
| BV | *Candida parapsilosis* | *Trichomonas vaginalis* | | 1 | 1 | *Candida parapsilosis* (1) |
| BV | *Candida albicans* | | | 79 | 16 | BV (2)<br>*Candida albicans* (14) |
| BV | *Candida dubliniensis* | | | 1 | 0 | - |
| BV | *Candida glabrata* | | | 2 | 1 | *Candida glabrata* (1) |
| BV | *Candida parapsilosis* | | | 3 | 3 | BV (1)<br>*Candida parapsilosis* (2) |
| BV | *Trichomonas vaginalis* | | | 39 | 4 | BV (1)<br>*Trichomonas vaginalis* (3) |
| *Candida albicans* | *Candida glabrata* | | | 2 | 1 | *Candida glabrata* (1) |
| *Candida albicans* | *Trichomonas vaginalis* | | | 3 | 1 | *Candida albicans* (1) |
| *Candida glabrata* | *Candida parapsilosis* | | | 1 | 1 | *Candida glabrata* (1)<br>*Candida parapsilosis* (1) |
| *Candida glabrata* | *Trichomonas vaginalis* | | | 1 | 0 | - |
| **Total Co-Detections** | | | | **149** | **34** | **36/319[b,c]** |
| **Total Double Co-Detections** | | | | **131** | **27** | **28/262[c]** |
| **Total Triple Co-Detections** | | | | **15** | **5** | **6/45[c]** |
| **Total Quadruple Co-Detections** | | | | **3** | **2** | **2/12[c]** |

[a] Based on comparator test results.
[b] Of the 36 discrepant analytes (out of 319 total analytes reported by the SPOTFIRE VG Panel), 33 (91.7%) were confirmed as being present in the specimen during discrepancy investigation.
[c] Number of false positive/total

**Evaluation of SPOTFIRE VG Panel in Asymptomatic Population**

The intended use of the SPOTFIRE VG Panel does not include an asymptomatic patient population. However, positivity rates as determined by the SPOTFIRE VG Panel were calculated from 49 CC VS specimens and 51 SC VS specimens collected from asymptomatic subjects during the prospective clinical study and are shown in the table below.

**Table 34: Positivity Rates for Specimens Collected from Asymptomatic Subjects During the SPOTFIRE VG Panel Prospective Clinical Evaluation**

| SPOTFIRE VG Panel Result | *Overall (N=100)* | 14-17 years (N=2) | 18-29 years (N=30) | 30-39 years (N=25) | 40-49 years (N=20) | 50+ years (N=23) |
|---|---|---|---|---|---|---|

| | | | | | | |
|---|---|---|---|---|---|---|
| **Bacteria** | | | | | | |
| BV | 47 (47.0%) | 1 (50.0%) | 12 (40.0%) | 13 (52.0%) | 11 (55.0%) | 10 (43.5%) |
| **Yeast** | | | | | | |
| *Candida albicans* | 16 (16.0%) | 1 (50.0%) | 6 (20.0%) | 3 (12.0%) | 4 (20.0%) | 2 (8.7%) |
| *Candida dubliniensis* | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) |
| *Candida glabrata* | 5 (5.0%) | 0 (0%) | 0 (0%) | 2 (8.0%) | 1 (5.0%) | 2 (8.7%) |
| *Candida krusei* | 1 (1.0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 1 (4.3%) |
| *Candida parapsilosis* | 4 (4.0%) | 0 (0%) | 0 (0%) | 0 (0%) | 1 (5.0%) | 3 (13.0%) |
| *Candida tropicalis* | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) |
| **Parasites** | | | | | | |
| *Trichomonas vaginalis* | 7 (7.0%) | 0 (0%) | 1 (3.3%) | 2 (8.0%) | 2 (10.0%) | 2 (8.7%) |

2. Clinical Specificity:

   See Clinical Sensitivity section above

3. Clinical Cut-Off

   Not applicable.

4. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

   Not applicable.

## D Expected Values/Reference Range:

In the prospective clinical evaluation of the panel, 700 CC VS specimens and 712 SC VS specimens were collected from symptomatic individuals and tested at five geographically diverse study sites across the United States (comprising nine enrollment locations) over approximately 14 months (October 2024 to November 2025). Expected value summaries (as determined by the SPOTFIRE VG Panel) are stratified by specimen enrollment location in Table 35 and Table 36 for CC VS specimens and SC VS specimens, respectively.

**Table 35: Expected Value as determined by SPOTFIRE VG Panel by Enrollment Location for CC VS Specimens Collected from Symptomatic Subjects During the Prospective Clinical Evaluation (October 2024 to November 2025)**

| SPOTFIRE VG Panel Result / #/SA[a] (EV[b]) | Bacteria | Yeast | | | | | | Parasites |
|---|---|---|---|---|---|---|---|---|
| | **Bacterial Vaginosis (BV)** | ***Candida albicans*** | ***Candida dubliniensis*** | ***Candida glabrata*** | ***Candida krusei*** | ***Candida parapsilosis*** | ***Candida tropicalis*** | ***Trichomonas vaginalis*** |
| ***Overall*** | ***366/700 (52.3%)*** | ***207/698 (29.7%)*** | ***1/698 (0.1%)*** | ***18/698 (2.6%)*** | ***0/698 (0%)*** | ***0/698 (0%)*** | ***1/698 (0.1%)*** | ***54/700 (7.7%)*** |
| Site 1A | 62/97 (63.9%) | 21/97 (21.6%) | 0/97 (0%) | 2/97 (2.1%) | 0/97 (0%) | 0/97 (0%) | 0/97 (0%) | 19/97 (19.6%) |
| Site 1B | 20/31 (64.5%) | 3/31 (9.7%) | 0/31 (0%) | 2/31 (6.5%) | 0/31 (0%) | 0/31 (0%) | 0/31 (0%) | 3/31 (9.7%) |
| Site 2A | 53/100 (53.0%) | 30/100 (30.0%) | 0/100 (0%) | 1/100 (1.0%) | 0/100 (0%) | 0/100 (0%) | 1/100 (1.0%) | 9/100 (9.0%) |

| SPOTFIRE VG Panel Result / #/SA[a] (EV[b]) | Bacteria | Yeast | | | | | | Parasites |
|---|---|---|---|---|---|---|---|---|
| | **Bacterial Vaginosis (BV)** | ***Candida albicans*** | ***Candida dubliniensis*** | ***Candida glabrata*** | ***Candida krusei*** | ***Candida parapsilosis*** | ***Candida tropicalis*** | ***Trichomonas vaginalis*** |
| Site 3A | 100/200 (50.0%) | 59/199 (29.6%) | 1/199 (0.5%) | 4/199 (2.0%) | 0/199 (0%) | 0/199 (0%) | 0/199 (0%) | 6/200 (3.0%) |
| Site 3B | 57/100 (57.0%) | 37/99 (37.4%) | 0/99 (0%) | 4/99 (4.0%) | 0/99 (0%) | 0/99 (0%) | 0/99 (0%) | 9/100 (9.0%) |
| Site 3C | 41/97 (42.3%) | 29/97 (29.9%) | 0/97 (0%) | 4/97 (4.1%) | 0/97 (0%) | 0/97 (0%) | 0/97 (0%) | 6/97 (6.2%) |
| Site 3D | 19/50 (38.0%) | 23/50 (46.0%) | 0/50 (0%) | 1/50 (2.0%) | 0/50 (0%) | 0/50 (0%) | 0/50 (0%) | 2/50 (4.0%) |
| Site 4A | 0/0 (-) | 0/0 (-) | 0/0 (-) | 0/0 (-) | 0/0 (-) | 0/0 (-) | 0/0 (-) | 0/0 (-) |
| Site 5A | 14/25 (56.0%) | 5/25 (20.0%) | 0/25 (0%) | 0/25 (0%) | 0/25 (0%) | 0/25 (0%) | 0/25 (0%) | 0/25 (0%) |

[a] #/SA = number of SPOTFIRE VG Panel detections (or positives for Bacterial Vaginosis (BV)) divided by the number of specimens analyzed (i.e., with valid comparator results) for that analyte

[b] EV = Expected Value

**Table 36: Expected Value as determined by SPOTFIRE VG Panel by Enrollment Location for SC VS Specimens Collected from Symptomatic Subjects During the Prospective Clinical Evaluation (October 2024 to November 2025)**

| SPOTFIRE VG Panel Result / #/SA[a] (EV[b]) | Bacteria | Yeast | | | | | | Parasites |
|---|---|---|---|---|---|---|---|---|
| | **Bacterial Vaginosis (BV)** | ***Candida albicans*** | ***Candida dubliniensis*** | ***Candida glabrata*** | ***Candida krusei*** | ***Candida parapsilosis*** | ***Candida tropicalis*** | ***Trichomonas vaginalis*** |
| ***Overall*** | ***393/712 (55.2%)*** | ***205/707 (29.0%)*** | ***2/707 (0.3%)*** | ***24/708 (3.4%)*** | ***1/708 (0.1%)*** | ***9/707 (1.3%)*** | ***4/707 (0.6%)*** | ***65/712 (9.1%)*** |
| Site 1A | 118/166 (71.1%) | 50/165 (30.3%) | 1/165 (0.6%) | 8/166 (4.8%) | 0/166 (0%) | 1/165 (0.6%) | 0/165 (0%) | 28/166 (16.9%) |
| Site 1B | 47/75 (62.7%) | 17/75 (22.7%) | 1/75 (1.3%) | 6/75 (8.0%) | 0/75 (0%) | 3/75 (4.0%) | 2/75 (2.7%) | 12/75 (16.0%) |
| Site 2A | 103/182 (56.6%) | 42/178 (23.6%) | 0/178 (0%) | 3/178 (1.7%) | 0/178 (0%) | 1/178 (0.6%) | 1/178 (0.6%) | 19/182 (10.4%) |
| Site 3A | 3/8 (37.5%) | 3/8 (37.5%) | 0/8 (0%) | 0/8 (0%) | 0/8 (0%) | 0/8 (0%) | 0/8 (0%) | 1/8 (12.5%) |
| Site 3B | 34/87 (39.1%) | 20/87 (23.0%) | 0/87 (0%) | 2/87 (2.3%) | 0/87 (0%) | 2/87 (2.3%) | 0/87 (0%) | 1/87 (1.1%) |
| Site 3C | 13/37 (35.1%) | 6/37 (16.2%) | 0/37 (0%) | 2/37 (5.4%) | 0/37 (0%) | 0/37 (0%) | 0/37 (0%) | 0/37 (0%) |
| Site 3D | 52/106 (49.1%) | 48/106 (45.3%) | 0/106 (0%) | 2/106 (1.9%) | 0/106 (0%) | 1/106 (0.9%) | 0/106 (0%) | 2/106 (1.9%) |
| Site 4A | 23/51 (45.1%) | 19/51 (37.3%) | 0/51 (0%) | 1/51 (2.0%) | 1/51 (2.0%) | 1/51 (2.0%) | 1/51 (2.0%) | 2/51 (3.9%) |
| Site 5A | 0/0 (-) | 0/0 (-) | 0/0 (-) | 0/0 (-) | 0/0 (-) | 0/0 (-) | 0/0 (-) | 0/0 (-) |

[a] #/SA = number of SPOTFIRE VG Panel detections divided by the number of specimens analyzed (i.e., with valid comparator results) for that analyte

[b] EV = Expected Value

## E Other Supportive Instrument Performance Characteristics Data:

Software, electrical safety and electromagnetic compatibility (EMC) testing and cybersecurity documentation were reviewed and found to be acceptable.

## VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

## IX Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.