510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT

I Background Information:

A 510(k) Number

K260021

B Applicant

Roche Molecular Systems, Inc.

C Proprietary and Established Names

cobas BV/CV

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 cobas BV/CV

B Measurand:

The assay detects the nucleic acids of the following organisms:

- Organisms associated with BV (detected organisms not reported individually):
  - *Gardnerella vaginalis*
  - *Atopobium vaginae*
  - *Lactobacillus* species (*L. crispatus*, *L. gasseri*, *L. jensenii*)
- Candida spp. (*C. albicans*, *C. dubliniensis*, *C. glabrata*, *C. krusei*, *C. tropicalis*, and *C. parapsilosis*; species not differentiated)

C Type of Test:

cobas BV/CV for use on the cobas 6800/8800 systems, is a real-time polymerase chain reaction (PCR) test for the direct detection of bacteria (*Gardnerella vaginalis*, *Lactobacillus crispatus*, *Lactobacillus gasseri*, *Lactobacillus jensenii* and *Atopobium vaginae*) associated with bacterial vaginosis (BV) and yeast (*C. albicans*, *C. dubliniensis*, *C. glabrata*, *C. krusei*, *C. tropicalis*, and *C. parapsilosis*; species not differentiated) associated with *Candida* vaginitis (CV) in vaginal specimens obtained from symptomatic individuals.

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

cobas BV/CV for use on the cobas 6800/8800 systems is an automated, qualitative in vitro nucleic acid diagnostic test that utilizes real-time polymerase chain reaction (PCR) for the direct detection of bacteria (*Gardnerella vaginalis*, *Lactobacillus* spp., and *Atopobium vaginae*) associated with bacterial vaginosis (BV) and yeast (*Candida* spp. listed below) associated with *Candida* vaginitis (CV) from self-collected vaginal swab specimens and clinician-collected vaginal swab specimens, all collected in cobas PCR Media. The assay amplifies specific DNA targets to detect organisms associated with BV (i.e., *G. vaginalis*, *L. crispatus*, *L. gasseri*, *L. jensenii*, and *A. vaginae*) and specific organisms associated with CV (i.e., *C. albicans*, *C. dubliniensis*, *C. glabrata*, *C. krusei*, *C. tropicalis*, and *C. parapsilosis*) but does not differentiate which BV and/or CV organism(s) is present. This test is intended as an aid in the diagnosis of BV and/or CV in symptomatic individuals with a clinical presentation consistent with vaginitis, vaginosis, or both.

C Special Conditions for Use Statement(s)Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

cobas BV/CV is performed on cobas 6800/8800 systems

IV Device/System Characteristics:

A Device Description:

cobas BV/CV for use on the cobas 6800/8800 systems is a qualitative in vitro nucleic acid diagnostic test that utilizes real-time PCR for the direct detection of bacteria (*Gardnerella vaginalis*, *L. crispatus*, *L. gasseri*, *L. jensenii*, and *Atopobium vaginae*) associated with BV and yeast (*C. albicans*, *C. dubliniensis*, *C. glabrata*, *C. krusei*, *C. tropicalis*, and *C. parapsilosis*)

associated with CV from vaginal swab specimens collected in cobas PCR Media. cobas BV/CV includes the reagents for the detection of DNA from BV organisms and *Candida* species. The test does not distinguish which BV and/or CV organism(s) is present but can give an overall qualitative result indicating the presence or absence of organism(s) associated with BV and/or CV. A DNA Internal Control (DNA-IC) is also included in cobas BV/CV. DNA-IC is added to each vaginal specimens at the sample processing step and is used to monitor the entire sample preparation and PCR amplification process.

Vaginal swab specimens for testing with cobas BV/CV may be self- or clinician-collected in cobas PCR Media using either the cobas PCR Media Uni Swab Sample Kit or cobas PCR Media Dual Swab Sample Kit, both of which are sold separately. In addition, cobas BV/CV utilizes external positive and negative controls which are also sold separately.

The cobas 6800/8800 systems automate and integrate sample preparation, nucleic acid extraction and amplification, and detection of the target sequences in vaginal specimens using real-time PCR assays. The systems consist of the sample supply module, the transfer module, the processing module, and the analytic module. Automated data management is performed by cobas 6800/8800 systems which assign test results for all tests as positive, negative, or invalid.

B Principle of Operation:

Nucleic acids from patient samples and DNA-IC, added into the specimens to monitor for inhibition of nucleic acid isolation and amplification, are simultaneously extracted. In summary, microbial nucleic acid is released by addition of proteinase and lysis reagent to the sample. The released nucleic acids bind to the silica surface of added magnetic glass particles. Unbound substances and impurities, such as denatured protein, cellular debris and potential PCR inhibitors, are removed with subsequent wash steps and purified nucleic acids are eluted from the magnetic glass particles with elution buffer at elevated temperature. External controls (positive and negative) are processed in the same way.

Selective amplification of target nucleic acid from the sample is achieved by the use of target-specific forward and reverse primers for the bacteria and yeast associated with BV and CV which are selected from highly conserved regions within the respective target organism (CV: *C. albicans, C. dubliniensis, C. glabrata, C. krusei, C. tropicalis, and C. parapsilosis)*, BV: *Gardnerella vaginalis, Atopobium vaginae*, and *Lactobacillus* species including *L. crispatus*, *L. gasseri*, *L. jensenii*). The bacteria and yeast associated with BV and CV, respectively, are detected by multiple sets of primers and probes. Selective amplification of DNA-IC is achieved by the use of sequence-specific forward and reverse primers which have no homology with any target regions for the bacteria and yeast associated with BV and CV. A thermostable DNA polymerase enzyme is used for PCR amplification. The target and DNA-IC sequences are amplified simultaneously utilizing a universal PCR amplification profile with predefined temperature steps and number of cycles.

cobas BV/CV master mix contains multiple detection probes specific for the CV associated target sequences, multiple detection probes specific for the BV associated target sequences and one for the DNA-IC. The probes are labeled with target specific fluorescent reporter dyes allowing simultaneous detection of CV associated targets, BV associated targets and DNA-IC in

five different channels. When not bound to the target sequence, the fluorescent signal of the intact probes is suppressed by a quencher dye. During the PCR amplification step, hybridization of the probes to the specific single-stranded DNA template results in cleavage of the probe by the 5' to 3' exonuclease activity of the DNA polymerase resulting in separation of the reporter and quencher dyes and the generation of a fluorescent signal. With each PCR cycle, increasing amounts of cleaved probes are generated and the cumulative signal of the reporter dye increases concomitantly. Real-time detection and discrimination of PCR products are accomplished by measuring the fluorescence of the released reporter dyes for the BV and CV associated targets and DNA-IC, respectively.

The results are interpreted automatically by cobas 6800/8800 systems which assign test results for all tests as positive, negative, or invalid. cobas BV/CV uses a BV algorithm which integrates real-time PCR signals from bacterial markers associated with BV (*Gardnerella vaginalis* and *Atopobium vaginae*) and normal vaginal flora (*Lactobacillus* species including *L. crispatus*, *L. gasseri*, *L. jensenii*) to calculate BV probability to determine the final BV result. A probability greater than or equal to 0.5 results in a BV positive result. The CV algorithm is a single target detection of *Candida* spp. (*C. albicans, C. dubliniensis, C. glabrata, C. krusei, C. tropicalis,* and *C. parapsilosis*) associated with CV. Results can be reviewed directly on the system screen, exported, or printed as a report.

C Instrument Description Information:

1. Instrument Name:

   cobas 6800/8800 systems

2. Specimen Identification:

   cobas 6800/8800 supports sample identification by barcodes. Loaded samples are automatically moved for barcode scanning and processing.

3. Specimen Sampling and Handling:

   All swab specimens containing a single swab in the cobas PCR Media tube can be directly loaded on the cobas 6800/8800 systems after uncapping. If desired, the swab may be removed before the specimen tube is loaded onto the instrument. Specimen processing is fully automated.

4. Calibration:

   No calibration is required by the user.

5. Quality Control:

   Please see Section VII.A.6.

V Substantial Equivalence Information:

A Predicate Device Name(s):

BD MAX Vaginal Panel

B Predicate 510(k) Number(s):

DEN160001

C Comparison with Predicate(s):

Device & Predicate Device(s):	K260021	DEN160001
Device Trade Name	Cobas BV/CV	BD MAX Vaginal Panel
General Device Characteristic Similarities		
Intended Use/Indications For Use	cobas BV/CV for use on the cobas 6800/8800 systems is an automated, qualitative in vitro nucleic acid diagnostic test that utilizes real-time polymerase chain reaction (PCR) for the direct detection of bacteria (*Gardnerella vaginalis*, *Lactobacillus* spp., and *Atopobium vaginae*) associated with bacterial vaginosis (BV) and yeast (*Candida* spp. listed below) associated with *Candida* vaginitis (CV) from self-collected vaginal swab specimens and clinician-collected vaginal swab specimens, all collected in cobas PCR Media. The assay amplifies specific DNA targets to	The BD MAX Vaginal Panel performed on the BD MAX System is an automated qualitative *in vitro* diagnostic test for the direct detection of DNA targets from bacteria associated with bacterial vaginosis (qualitative results reported based on detection and quantitation of targeted organism markers), *Candida* species associated with vulvovaginal candidiasis, and *Trichomonas vaginalis* from vaginal swabs in patients who are symptomatic for vaginitis/vaginosis. The test utilizes real-time polymerase chain reaction (PCR) for the amplification of specific DNA targets and utilizes

	detect organisms associated with BV (i.e., *G. vaginalis*, *L. crispatus*, *L. gasseri*, *L. jensenii*, and *A. vaginae*) and specific organisms associated with CV (i.e., *C. albicans*, *C. dubliniensis*, *C. glabrata*, *C. krusei*, *C. tropicalis*, and *C. parapsilosis*) but does not differentiate which BV and/or CV organism(s) is present. This test is intended as an aid in the diagnosis of BV and/or CV in symptomatic individuals with a clinical presentation consistent with vaginitis, vaginosis, or both.	fluorogenic target-specific hybridization probes to detect and differentiate DNA from: • Bacterial vaginosis markers (Individual markers not reported) • *Lactobacillus* spp. (*L. crispatus* and *L. jensenii*) • *Gardnerella vaginalis* • *Atopobium vaginae* • Bacterial Vaginosis Associated Bacteria-2 (BVAB-2) • *Megasphaera-1* • *Candida* spp. (*C. albicans*, *C. tropicalis*, • *C. parapsilosis*, *C. dubliniensis*) • *Candida glabrata* *Candida krusei* • *Trichomonas vaginalis* The BD MAX Vaginal Panel is intended to aid in the diagnosis of vaginal infections in women with a clinical presentation consistent with bacterial vaginosis, vulvovaginal candidiasis and trichomoniasis.
Specimen Type	Clinician and self-collected vaginal swabs	Same
Intended Population	Symptomatic	Same
Condition for Use	For prescription use	Same

Sample Preparation Procedure	Automated	Same
Assay Technology	Real-time PCR	Same
Result Analysis	Automated Software	Same
Assay Results	Qualitative	Same
General Device Characteristic Differences		
Target Organisms	BV (Bacteria): *G. vaginalis*, *Lactobacillus* spp. (*L. crispatus*, *L. gasseri*, *L. jensenii*), *A. vaginae* CV (yeast): *C. albicans, C. dubliniensis, C. glabrata, C. krusei, C. tropicalis, C. parapsilosis*	BV (Bacteria): *Lactobacillus* spp. (*L. crispatus* and *L. jensenii*), *G. vaginalis*, *A. vaginae*, BVAB-2, Megasphaera-1 CV (yeast): *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. dubliniensis*, *C. glabrata*, *C. krusei* Other: *Trichomonas vaginalis*

VI Standards/Guidance Documents Reference

- CLSI EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline - Third Edition.
- CLSI EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline - Second Edition
- CLSI EP07 3rd Edition, Interference Testing in Clinical Chemistry
- ISO 14971 Third Edition 2019-12, Medical devices - Application of risk management to medical devices
- ISO 15223-1Fourth Edition 2021-07, Medical devices - Symbols to be used with information to be supplied by the manufacturer - Part 1: General requirements
- IEC 62366-1 Edition 1.1 2020-06, Medical devices - Part 1: Application of usability engineering to medical devices

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

Precision

Within-laboratory precision was examined with a panel consisting of three concentration levels - high negative (0.3x LoD), low positive (1x LoD) and moderate positive (3x LoD) - for each CV strain (*Candida albicans*, *Candida glabrata* and *Candida krusei*) or co-

formulated BV target compositions. Samples were prepared by spiking simulated vaginal matrix stabilized in cobas PCR Media with the BV or CV target organisms. For BV, three different sample compositions (A, B, and C) were prepared to represent the variety of target organism combinations found in vaginal specimens with bacterial vaginosis:

- Composition A contained all three microorganisms: *L. crispatus*, *G. vaginalis*, and *A. vaginae*
- Composition B contained two microorganisms: *G. vaginalis* and *A. vaginae*
- Composition C contained the same three microorganisms as Composition A (*L. crispatus*, *G. vaginalis*, and *A. vaginae*), but at different concentration ratios

The panel also included simulated vaginal matrix without analytes as a negative panel member. The panel members were tested using three lots of cobas BV/CV reagents, three instruments and three operators over six testing days with a total of 36 runs. Per testing day, two runs per instrument were performed with three replicates of each panel member tested per run. Results from the study are shown in Table 1. All negative panel members tested negative.

Table 1: Summary of Within-Laboratory Precision for cobas BV/CV on cobas 6800/8800

Target	Target Level	Number of Positive/Valid Replicates	% Positivity	95% CI
Negative	-	0/108	0%	0-3.36%
BV[1]	High Negative	302/324	93.2%	89.9-95.7%
BV[1]	Low Positive	324/324	100%	98.87-100%
BV[1]	Moderate Positive	324/324	100%	98.87-100%
CV[2]	High Negative	289/324	89.2%	85.3-92.4%
CV[2]	Low Positive	324/324	100%	98.87-100%
CV[2]	Moderate Positive	324/324	100%	98.87-100%

[1]Performance for BV includes combined results from replicates of three panel members containing different BV target organism compositions (A, B and C).

[2]Performance for CV includes combined results from replicates of three panel members containing *Candida albicans*, *Candida glabrata* and *Candida krusei*.

Note: CI = Confidence Interval

Analysis of standard deviation (SD) and percent coefficient of variation (%CV) of the BV probability and CV Ct values from valid tests performed on positive panel members are presented in **Error! Reference source not found.** and **Error! Reference source not found.**.

Table 2: Overall Mean, Standard Deviations and Coefficients of Variation (%) for Probability - BV Positive Panels

BV Panel Member	% Positivity	Mean Probability[1]	Instrument-to-Instrument		Lot-to-Lot		Day-to-Day		Run-to-Run		Within Run		Total	
			SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV
High Negative/ Composition A	100	0.63	0.03	4.21	0.01	2.17	0.02	2.60	0.00	0.69	0.04	6.16	0.05	8.22
High Negative/ Composition B	80.56	0.57	0.02	3.38	0.02	3.44	0.00	0.00	0.03	4.95	0.03	5.21	0.04	6.23

High Negative/ Composition C	99.07	0.72	0.02	2.90	0.00	0.00	0.03	3.83	0.00	0.00	0.15	21.43	0.16	21.96[2]
Low Positive/ Composition A	100	0.77	0.01	1.48	0.02	2.92	0.01	1.60	0.00	0.00	0.03	4.09	0.04	5.48
Low Positive/ Composition B	100	0.76	0.01	1.40	0.00	0.00	0.01	1.51	0.00	0.00	0.05	6.93	0.05	7.20
Low Positive/ Composition C	100	0.90	0.00	0.44	0.00	0.39	0.00	0.31	0.00	0.00	0.02	2.30	0.02	2.70
Moderate Positive/ Composition A	100	0.96	0.00	0.30	0.00	0.48	0.00	0.29	0.00	0.00	0.01	0.82	0.01	1.04
Moderate Positive/ Composition B	100	0.97	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.01	0.52	0.01	0.52
Moderate Positive/ Composition C	100	0.99	0.00	0.05	0.00	0.10	0.00	0.16	0.00	0.05	0.00	0.31	0.00	0.38

[1]Probability is calculated using the Ct values of the three BV targets *(G. vaginalis, A. vaginae, L. crispatus)*. Samples with a probability score greater than 0.5 are called BV positive.

[2]The elevated variance is due to the intermittent detection of *L. crispatus* at the specific concentration used in this panel member, which lies at the assay's limit of detection.

Note: SD = Standard Deviation; CV = Coefficient of Variation

Table 3: Overall Mean, Standard Deviations and Coefficients of Variation (%) for Probability - CV Positive Panels

CV Panel Member	% Positivity	Mean Ct	Instrument-to-Instrument		Lot-to-Lot		Day-to-Day		Run-to-Run		Within Run		Total	
			SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV
C. albicans/ High Negative	79.63	35.13	0.00	0.00	0.17	0.48	0.11	0.30	0.00	0.00	0.63	1.80	0.66	1.89
C. glabrata/ High Negative	99.07	34.06	0.00	0.00	0.03	0.07	0.10	0.30	0.00	0.00	0.78	2.30	0.79	2.32
C. krusei/ High Negative	88.89	34.78	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.65	1.86	0.65	1.86
C. albicans/ Low Positive	100	32.67	0.00	0.00	0.11	0.34	0.08	0.25	0.19	0.58	0.50	1.53	0.55	1.69
C. glabrata/ Low Positive	100	31.43	0.00	0.00	0.12	0.39	0.00	0.00	0.00	0.00	0.62	1.99	0.64	2.02
C. krusei/ Low Positive	100	33.21	0.00	0.00	0.06	0.18	0.00	0.00	0.01	0.04	0.48	1.44	0.48	1.45
C. albicans/ Moderate Positive	100	30.97	0.00	0.00	0.00	0.00	0.13	0.43	0.22	0.70	0.31	1.01	0.40	1.30
C. glabrata/ Moderate Positive	100	29.41	0.18	0.62	0.00	0.00	0.20	0.67	0.23	0.79	0.78	2.67	0.86	2.93
C. krusei/ Moderate Positive	100	31.96	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.51	1.61	0.51	1.61

Note: Ct = Cycle threshold; SD = Standard Deviation; CV = Coefficient of Variation

Reproducibility

A reproducibility study was performed across different sites, reagent lots, operators and days, for cobas BV/CV. Testing was performed at three sites including two external sites and one internal site. The reproducibility panel consisted of 13 panel members formulated by spiking simulated vaginal matrix stabilized in cobas PCR Media with BV and CV target organisms. The panel included one negative panel member, three low-positive (1x LoD), three moderate-positive (3x LoD), and three high-negative (0.3x LoD) members that were representative of BV (Composition A, B and C as described in precision above). The reproducibility panel also

included one low-positive, one moderate-positive, and one high-negative *Candida albicans* as representative of CV.

The study tested 3744 samples, using three lots of cobas BV/CV reagents over six days. Nine operators participated in the study across three sites. Of the 3744 tests, there were 3729 (99.6%) valid and 15 (0.4%) invalid test results. Table 4 below shows the percent agreement of cobas BV/CV test results with the expected result, along with the corresponding 95% confidence intervals.

Table 4: Percentage of Agreement of cobas BV/CV Test with the Expected Result, Presented by Pathogen and Level of Infection on cobas 6800/8800

Target	Target Level	Valid Replicates Tested (N)	Tests with Expected Results (n)	Percent Agreement (n/N) x 100%	95% CI[3]
BV	Negative	288	288	100	98.7-100%
BV[1]	High Negative	856	618	72.2	69.1-75.1%
BV[1]	Low Positive	862	862	100	99.6-100%
BV[1]	Moderate Positive	861	861	100	99.6-100%
CV	Negative	288	286[2]	99.3	97.5-99.8%
CV	High Negative	288	40	13.9	10.4-18. %
CV	Low Positive	286	286	100	98.7-100%
CV	Moderate Positive	288	288	100	98.7-100%

[1]Performance for BV includes combined results from replicates of three panel members containing different BV target organism compositions (A, B and C).
[2]Two samples were unexpectedly positive with high Ct-values suggesting contamination at the testing site.
[3]Confidence Intervals are calculated using Clopper-Pearson's exact method.
Note: CI = Confidence Interval

Analysis of SD and % CV of the BV probability and CV Ct values from valid tests performed on positive panel members are presented in Table 5 and 6.

Table 5: Standard Deviations and Coefficients of Variation of Probability Values across Testing Site, Reagent Lot, Days, and Runs Presented by Positive Panel Member on cobas 6800/8800 - BV

BV Probability[1]			Overall			Between - Site		Between - Lot		Between - Day		Between - Run		Within - Run	
BV Panel Member	(n/N)[2]	Positivity Rate%	Mean	SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV
High Negative/ Composition A	228/286	79.72	0.59	0.05	8.69	0.02	3.15	0.02	3.07	0.01	2.55	0.01	1.86	0.04	6.79
High Negative/ Composition B	131/286	45.8	0.57	0.06	10.0	0.00	0.00	0.01	2.27	0.01	2.10	0.00	0.00	0.05	9.51
High Negative/ Composition C	259/284	91.2	0.84	0.20	24.3[3]	0.00	0.00	0.01	1.17	0.00	0.00	0.00	0.00	0.20	24.3
Low Positive/ Composition A	288/288	100	0.80	0.05	6.25	0.03	3.83	0.01	1.25	0.02	2.87	0.01	1.69	0.03	3.43
Low Positive/ Composition B	288/288	100	0.79	0.06	7.11	0.02	2.66	0.00	0.00	0.02	2.03	0.02	2.28	0.05	5.85
Low Positive/ Composition C	286/286	100	0.87	0.07	8.15	0.03	3.02	0.02	1.74	0.02	1.93	0.00	0.00	0.06	7.11
Moderate Positive/ Composition A	287/287	100	0.96	0.01	1.22	0.01	0.57	0.00	0.46	0.01	0.57	0.00	0.40	0.01	0.69

BV Probability[1]			Overall			Between - Site		Between - Lot		Between - Day		Between - Run		Within - Run	
BV Panel Member	(n/N)[2]	Positivity Rate%	Mean	SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV
Moderate Positive/ Composition B	287/287	100	0.98	0.00	0.48	0.00	0.10	0.00	0.05	0.00	0.11	0.00	0.11	0.00	0.44
Moderate Positive/ Composition C	287/287	100	0.99	0.00	0.24	0.00	0.15	0.00	0.03	0.00	0.08	0.00	0.04	0.00	0.16

[1]Probability is calculated using the Ct values of the three BV targets *(G. vaginalis, A. vaginae, L. crispatus)*. Samples with a probability score greater than 0.5 are called BV positive

[2]n is the number of positive tests, which contribute probability values to the analysis. N is the total number of valid tests for the panel member.

[3]The elevated variance is due to the intermittent detection of *L. crispatus* at the specific concentration used in this panel member, which lies at the assay's limit of detection.

Note: CV = Coefficient of Variation; SD = Standard Deviation.

Table 1: Standard Deviations and Coefficients of Variation of Cycle Threshold (Ct) Values across Testing Site, Reagent Lot, Days, and Runs Presented by Positive Panel Member on cobas 6800/8800 - CV

Cycle Threshold (Ct)			Overall			Between - Site		Between - Lot		Between - Day		Between - Run		Within - Run	
CV Panel Member	(n/N)[1]	Positivity Rate%	Mean	SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV
High Negative	40/288	13.89	35.48	0.44	1.25	0.13	0.37	0.00	0.00	0.22	0.62	0.34	0.95	0.13	0.38
Low Positive	286/286	100	34.40	0.48	1.38	0.23	0.68	0.00	0.00	0.08	0.23	0.16	0.45	0.38	1.09
Moderate Positive	288/288	100	32.79	0.44	1.35	0.30	0.90	0.00	0.00	0.14	0.41	0.08	0.24	0.29	0.88

[1]n is the number of positive tests, which contribute Ct values to the analysis. N is the total number of valid tests for the panel member.

Note: CV = Coefficient of Variation; SD = Standard Deviation.

Tables 7 to 9 show the variance component analysis for individual BV organisms on the Ct values (*Gardnerella vaginalis*, *Lactobacillus crispatus*, and *Atopobium vaginae*) for all BV panel members.

Table 7: Standard Deviations and Coefficients of Variation of Ct Values across Testing Site, Reagent Lot, Days, and Runs Presented by Positive Panel Member on cobas 6800/8800 – BV (*Gardnerella vaginalis* target only)

Cycle Threshold (Ct)			Overall			Between - Site		Between - Lot		Between - Day		Between - Run		Within - Run	
BV Panel Member	(n/N)[1]	Positivity Rate %	Mean	SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV
High Negative/ Composition A	286/286	100	21.07	0.23	1.07	0.07	0.32	0.06	0.30	0.06	0.29	0.13	0.61	0.15	0.70
High Negative/ Composition B	286/286	100	27.73	0.22	0.79	0.07	0.24	0.06	0.23	0.06	0.20	0.12	0.42	0.15	0.54
High Negative/ Composition C	284/284	100	23.23	0.21	0.90	0.00	0.00	0.06	0.25	0.11	0.45	0.12	0.52	0.12	0.52
Low Positive/ Composition A	288/288	100	20.35	0.23	1.12	0.07	0.34	0.06	0.29	0.11	0.54	0.14	0.70	0.11	0.52
Low Positive/ Composition B	288/288	100	26.45	0.19	0.73	0.00	0.00	0.06	0.24	0.05	0.20	0.14	0.52	0.11	0.40
Low positive/ Composition C	286/286	100	22.23	0.20	0.89	0.00	0.00	0.03	0.13	0.09	0.42	0.12	0.56	0.12	0.53

Cycle Threshold (Ct)			Overall			Between - Site		Between - Lot		Between - Day		Between - Run		Within - Run	
BV Panel Member	(n/N)[1]	Positivity Rate %	Mean	SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV
Moderate Positive/ Composition A	287/287	100	18.54	0.22	1.19	0.05	0.24	0.06	0.32	0.09	0.47	0.13	0.69	0.14	0.74
Moderate Positive/ Composition B	287/287	100	24.83	0.17	0.69	0.03	0.13	0.02	0.07	0.07	0.26	0.11	0.44	0.11	0.44
Moderate Positive/ Composition C	287/287	100	19.71	0.18	0.91	0.03	0.17	0.04	0.19	0.07	0.35	0.09	0.47	0.13	0.65

[1]n is the number of positive tests, which contribute Ct values to the analysis. N is the total number of valid tests for the panel member.
Note: CV = Coefficient of Variation; SD = Standard Deviation.

Table 8: Standard Deviations and Coefficients of Variation of Ct Values across Testing Site, Reagent Lot, Days, and Runs Presented by Positive Panel Member on cobas 6800/8800 – BV (*Lactobacillus crispatus* target only)

Cycle Threshold (Ct)			Overall			Between - Site		Between - Lot		Between - Day		Between - Run		Within - Run	
BV Panel Member	(n/N)[1]	Positivity Rate %	Mean	SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV
High Negative/ Composition A	286/286	100	27.85	0.21	0.74	0.08	0.28	0.00	0.00	0.07	0.26	0.11	0.40	0.14	0.49
High Negative/ Composition B[2]	0/286	0.00	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
High Negative/ Composition C	122/284	42.96	35.56	0.30	0.83	0.05	0.15	0.00	0.00	0.05	0.13	0.00	0.00	0.29	0.81
Low Positive/ Composition A	288/288	100	27.09	0.20	0.73	0.08	0.31	0.00	0.00	0.10	0.36	0.11	0.40	0.10	0.37
Low Positive/ Composition B[2]	0/288	0.00	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Low Positive/ Composition C	242/286	84.62	35.22	0.47	1.34	0.13	0.36	0.01	0.04	0.14	0.41	0.00	0.00	0.43	1.22
Moderate Positive/ Composition A	287/287	100	25.26	0.21	0.83	0.10	0.41	0.00	0.00	0.10	0.39	0.12	0.46	0.10	0.41
Moderate Positive/ Composition B[2]	0/287	0.00	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Moderate Positive/ Composition C	287/287	100	33.11	0.39	1.17	0.23	0.70	0.00	0.00	0.13	0.39	0.01	0.02	0.28	0.84

[1]n is the number of positive tests, which contribute Ct values to the analysis. N is the total number of valid tests for the panel member.
[2]Composition B does not contain *Lactobacillus crispatus* and can therefore not be evaluated for these panel members.
Note: CV = Coefficient of Variation; N = Number; SD = Standard Deviation; N/A = Not Applicable.

Table 9: Standard Deviations and Coefficients of Variation of Ct Values across Testing Site, Reagent Lot, Days, and Runs Presented by Positive Panel Member on cobas 6800/8800 – BV (*Atopobium vaginae* target only)

Cycle Threshold (Ct)			Overall			Between - Site		Between - Lot		Between - Day		Between - Run		Within - Run	
BV Panel Member	(n/N)[1]	Positivity Rate%	Mean	SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV
High Negative/ Composition A	286/286	100	26.93	0.19	0.71	0.06	0.21	0.00	0.00	0.00	0.00	0.11	0.43	0.14	0.52
High Negative/ Composition B	286/286	100	32.81	0.31	0.95	0.06	0.18	0.00	0.00	0.00	0.00	0.13	0.39	0.28	0.85
High Negative/ Composition C	284/284	100	29.16	0.23	0.80	0.07	0.25	0.00	0.00	0.11	0.38	0.09	0.30	0.17	0.59

Cycle Threshold (Ct)			Overall			Between - Site		Between - Lot		Between - Day		Between - Run		Within - Run	
BV Panel Member	(n/N)[1]	Positivity Rate%	Mean	SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV
Low Positive/ Composition A	288/288	100	26.25	0.18	0.69	0.03	0.11	0.00	0.00	0.07	0.26	0.12	0.45	0.12	0.44
Low Positive/ Composition B	288/288	100	31.54	0.25	0.80	0.01	0.03	0.05	0.14	0.04	0.13	0.15	0.48	0.19	0.61
Low positive/ Composition C	286/286	100	28.04	0.22	0.79	0.06	0.20	0.00	0.00	0.06	0.21	0.15	0.53	0.14	0.50
Moderate Positive/ Composition A	287/287	100	24.45	0.18	0.73	0.04	0.16	0.02	0.09	0.05	0.22	0.11	0.44	0.13	0.51
Moderate Positive/ Composition B	287/287	100	30.16	0.22	0.74	0.06	0.21	0.03	0.09	0.08	0.26	0.10	0.34	0.17	0.57
Moderate Positive/ Composition C	287/287	100	25.34	0.19	0.74	0.05	0.20	0.00	0.00	0.08	0.33	0.09	0.36	0.13	0.52

[1]n is the number of positive tests, which contribute Ct values to the analysis. N is the total number of valid tests for the panel member.
Note: CV = Coefficient of Variation; SD = Standard Deviation.

2. Linearity:

Not applicable. Device reports qualitative results.

3. Analytical Specificity/Interference:

Cross-Reactivity and Microbial Interference Study
cobas BV/CV was evaluated for potential cross-reactivity and microbial interference with samples containing phylogenetically related species and other organisms likely to be present in vaginal specimens. A panel of 109 bacteria, fungi, viruses, parasites and plasmids were tested with cobas BV/CV in the study. Unless otherwise noted, the organisms listed in Table 10 were spiked at concentrations of approximately 1E+06 units/mL for bacteria, fungi, parasites and plasmid and approximately 1E+05 units/mL for viruses into simulated vaginal matrix stabilized in cobas PCR Media. Testing was performed with each potential interfering organism in three replicates in absence and presence of BV (*Atopobium vaginae, Gardnerella vaginalis, Lactobacillus crispatus)* and CV (*Candida albicans)* targets spiked at approximately 3x LoD.

Candida orthopsilosis, Lactobacillus acidophilus, Lactobacillus helveticus and *Lactobacillus johnsonii* were detected by cobas BV/CV as predicted based on the *Lactobacillus* sp. and *Candida* sp. target oligo design. The other microorganisms tested did not interfere with the cobas BV/CV. A limitation is included in the device labeling regarding the detection of these microorganisms by cobas BV/CV.

Table 10: Microorganism Tested for Cross-Reactivity and Microbial Interference

Microorganism	Concentration Tested	Microorganism	Concentration Tested
Acinetobacter baumannii	1.00E+06 CFU/mL	*Kodamaea ohmeri*	6.00E+05 CFU/mL
Acinetobacter calcoaceticus	1.00E+06 CFU/mL	*Lactobacillus acidophilus*[1]	1.00E+06 CFU/mL
Actinomyces israelii	1.00E+06 CFU/mL	*Lactobacillus helveticus*[1]	1.00E+06 CFU/mL
Aerococcus viridans	1.00E+06 CFU/mL	*Lactobacillus iners*	1.00E+06 CFU/mL

Microorganism	Concentration Tested	Microorganism	Concentration Tested
Alcaligenes faecalis	1.00E+06 CFU/mL	*Lactobacillus johnsonii*[1]	1.00E+06 CFU/mL
Anaerococcus tetradius	1.00E+06 CFU/mL	*Legionella pneumophila*	1.00E+06 CFU/mL
Atopobium minutum	1.00E+06 CFU/mL	*Mageeibacillus indolicus*	1.00E+06 CFU/mL
Atopobium parvulum	1.00E+06 CFU/mL	*Megasphaera elsdenii*	1.00E+06 CFU/mL
Atopobium rimae	1.00E+06 CFU/mL	*Mobiluncus curtisii*	1.00E+06 CFU/mL
Bacillus subtilis	1.00E+06 CFU/mL	*Mobiluncus mulieris*	1.00E+06 CFU/mL
Bacteroides caccae	1.00E+06 CFU/mL	*Moraxella catarrhalis*	1.00E+06 CFU/mL
Bacteroides fragilis	1.00E+06 CFU/mL	*Morganella morganii*	1.00E+06 CFU/mL
Bacteroides stercoris	1.00E+06 CFU/mL	*Mycobacterium smegmatis*	1.00E+06 CFU/mL
Bacteroides ureolyticus/ Campylobacter ureolyticus	1.00E+06 CFU/mL	*Mycoplasma genitalium*	1.00E+06 CFU/mL
Bifidobacterium adolescentis	1.00E+06 CFU/mL	*Mycoplasma hominis*	1.00E+06 CFU/mL
Bifidobacterium breve	1.00E+06 CFU/mL	*Neisseria gonorrhoeae*	1.00E+06 CFU/mL
Bifidobacterium longum	1.00E+06 CFU/mL	*Olsenella uli*	1.00E+06 CFU/mL
Brevibacterium linens	1.00E+06 CFU/mL	*Pantoea agglomerans*	1.00E+06 CFU/mL
Burkholderia cepacia	1.00E+06 CFU/mL	*Pentatrichomonas hominis*	1.00E+06 CFU/mL
BVAB1 plasmid	1.00E+06 copies/mL	*Peptoniphilus asaccharolyticus*	1.00E+06 CFU/mL
Campylobacter jejuni	1.00E+06 CFU/mL	*Peptostreptococcus anaerobius*	1.00E+06 CFU/mL
Candida catenulata/Diutina catenulata	1.00E+06 CFU/mL	*Pichia fermentans*	5.60E+05 CFU/mL
Candida famata	1.00E+06 CFU/mL	*Pichia norvegensis*	1.00E+06 CFU/mL
Candida haemulonii	1.00E+06 CFU/mL	*Pichia occidentalis*	1.00E+06 CFU/mL
Candida inconspicua	1.00E+06 CFU/mL	*Plesiomonas shigelloides*	1.00E+06 CFU/mL
Candida intermedia	1.00E+06 CFU/mL	*Porphyromonas asaccharolytica*	1.00E+06 CFU/mL
Candida kefyr	1.00E+06 CFU/mL	*Prevotella bivia*	1.00E+06 CFU/mL
Candida lusitaniae	1.00E+06 CFU/mL	*Prevotella melaninogenica*	1.00E+06 CFU/mL
Candida norvegica	1.00E+06 CFU/mL	*Prevotella oralis/Hoylesella oralis*	1.00E+06 CFU/mL
Candida orthopsilosis[1]	1.00E+06 CFU/mL	*Propionibacterium acnes*	1.00E+06 CFU/mL
Candida rugosa/Diotima rugosa	1.00E+06 CFU/mL	*Proteus mirabilis*	1.00E+06 CFU/mL
Candida utilis	1.00E+06 CFU/mL	*Providencia stuartii*	1.00E+06 CFU/mL
Chlamydia trachomatis	1.00E+06 CFU/mL	*Pseudomonas aeruginosa*	1.00E+06 CFU/mL
Citrobacter freundii	1.00E+06 CFU/mL	*Saccharomyces cerevisiae*	1.00E+06 CFU/mL
Clostridium perfringens	1.00E+06 CFU/mL	*Salmonella typhimurium*	1.00E+06 CFU/mL
Corynebacterium genitalium	1.00E+06 CFU/mL	*Serratia marcescens*	1.00E+06 CFU/mL
Dialister micraerophilus	1.00E+06 CFU/mL	*Shigella flexneri*	1.00E+06 CFU/mL

Microorganism	Concentration Tested	Microorganism	Concentration Tested
Eikenella corrodens	1.00E+06 CFU/mL	*Sneathia sanguinegens*	1.00E+06 CFU/mL
Enterobacter aerogenes	1.00E+06 CFU/mL	*Sneathia vaginalis*	1.00E+06 CFU/mL
Enterococcus faecalis	1.00E+06 CFU/mL	*Staphylococcus aureus*	1.00E+06 CFU/mL
Enterococcus faecium	1.00E+06 CFU/mL	*Staphylococcus epidermidis*	1.00E+06 CFU/mL
Erysipelothrix rhusiopathiae	1.00E+06 CFU/mL	*Streptococcus agalactiae*	1.00E+06 CFU/mL
Escherichia coli	1.00E+06 CFU/mL	*Streptococcus mitis*	1.00E+06 CFU/mL
Finegoldia magna	1.00E+06 CFU/mL	*Streptococcus mutans*	1.00E+06 CFU/mL
Fusobacterium nucleatum	1.00E+06 CFU/mL	*Streptococcus salivarius*	1.00E+06 CFU/mL
Gemella haemolysans	1.00E+06 CFU/mL	*Treponema pallidum*	1.00E+06 CFU/mL
Hepatitis B virus	1.00E+05 IU/mL	*Trichomonas tenax*	1.00E+06 CFU/mL
Hepatitis C virus	1.00E+05 IU/mL	*Trichomonas vaginalis*	2.30E+05 CFU/mL
Herpes simplex virus I/HSV type 1	1.00E+05 IU/mL	*Trueperella pyogenes/ Actinomyces pyogenes/ Corynebacterium pyogenes*	1.00E+06 CFU/mL
Herpes simplex virus II/ Human herpesvirus 2	1.00E+05 IU/mL	Varicella-zoster virus	1.00E+05 IU/mL
HIV-1	1.00E+05 IU/mL	*Veillonella atypica*	1.00E+06 CFU/mL
Human papilloma virus	1.00E+05 IU/mL	*Veillonella parvula*	1.00E+06 CFU/mL
Kingella denitrificans	1.00E+06 CFU/mL	*Vibrio parahaemolyticus*	1.00E+06 CFU/mL
Klebsiella pneumoniae	1.00E+06 CFU/mL	*Yersinia enterocolitica*	1.00E+06 CFU/mL
Kocuria rhizophila	1.00E+06 CFU/mL	-	-

[1]*Candida orthopsilosis*, *Lactobacillus acidophilus*, *Lactobacillus helveticus* and *Lactobacillus johnsonii* were detected by the test due to oligo design. The detection of these organisms by cobas BV/CV does not compromise the final BV or CV result.

Interfering Substances Study

Interference testing was conducted to assess the effects of substances that may be present in vaginal swab specimens on cobas BV/CV performance. These included exogenous substances such as over-the-counter and prescription products (Table 11), as well as endogenous substances (Table 12). Samples were prepared in simulated vaginal matrix stabilized in cobas PCR Media and spiked with potential interferents at levels expected from normal patient usage, in absence and presence of BV (*Atopobium vaginae, Gardnerella vaginalis, Lactobacillus crispatus)* and CV (*Candida albicans)* targets spiked at approximately 3x LoD.

Exogenous Substances Interference Study

Table 11 below lists the substances tested for interference in the study. Of the products tested, six substances including Crinone 8% Vaginal Gel, Gynaedron, Metronidazole Vaginal Gel, NUVESSA, RepHresh Odor Eliminating Gel and Vagisil showed interference and produced invalid or false negative results at levels that may be present in vaginal swab specimens. These products contain carbomer(s) or polycarbophil which have been shown to generate invalid and false results. Utrogestan interfered with cobas BV/CV and generated

false positive results for CV. A limitation is included in the device labeling listing all substances that demonstrated interference with cobas BV/CV.

Table 11: Exogenous Substances Tested for Interference

Exogenous Product/Substance	Ingredient	Concentration of Product/Substance Tested [mg/mL]	Concentration of Active Ingredient Tested [mg/mL]
Acyclovir Cream	Acyclovir	33.64	1.68
AFI LactoSpore	Bacillus coagulans	123.13	1.16E+09 CFU/mL
Arilin[2]	Metronidazole	63.60	26.07
Clindamycin Phosphate Vaginal Cream	Clindamycin Phosphate	41.54	0.83
7 Day Vaginal Cream	Clotrimazole	37.82	0.38
Crinone 8% Vaginal Gel[1]	Progesterone	33.72	2.70
Daktarin	Miconazole Nitrate	38.56	0.77
Estradiol Vaginal Cream	Estradiol	46.64	0.01
Globe Hemorrhoidal Cream	Glycerol	47.22	6.80
	Pramoxine HCl		0.47
	Phenylephrine HCl		0.12
	Petrolatum		7.08
Gynaedron[1]	Dexpanthenol	36.01	0.72
	Lactic acid		0.29
	Carbomer		N/A[3]
Gynofit	Glycerin, Hydroxyethyl Cellulose	48.24	N/A[3]
Imiquimod Cream	Imiquimod	39.72	1.99
K-Y Ultragel	Propylene Glycol	75.01	N/A[3]
Lactacyd	Glycerin, Propylene Glycol	57.07	N/A[3]
Monistat	Hydrocortisone	36.39	0.36
Metronidazole Vaginal Gel[1]	Metronidazole	41.93	0.31
NutraBlast Boric Acid Vaginal Supp.	Boric Acid	138.99	139.53
Norforms	Benzethonium Chloride	490.66	N/A[3]
NUVESSA[1]	Metronidazole	61.14	0.79
Premarin	Conjugated Estrogens	33.76	0.02
Progesterone	N/A	1.57	1.57[4]

Exogenous Product/Substance	Ingredient	Concentration of Product/Substance Tested [mg/mL]	Concentration of Active Ingredient Tested [mg/mL]
RepHresh Odor Eliminating Gel[1]	Glycerin; Sodium Hydroxide; Carbomer	51.76	N/A[3]
Summer's Eve Ultimate Odor Protection Spray	Isobutane, Isopropyl M Myristate	28.14	N/A[3]
Tioconazole 1 Ointment	Tioconazole	56.06	3.65
Utrogestan[1]	Progesterone	170.92	46.51
VCF Vaginal Contraceptive Gel	Nonoxynol-9	71.18	2.85
Vagisan	Povidone-iodine	55.92	5.90
Vagisil[1]	Benzocaine	40.12	8.02
	Resorcinol		1.20
YeastGard Advanced	*Candida albicans, Candida parapsilosis, Pulsatilla, Bacillus coagulans*	495.46	N/A[3]

[1]Products that showed interference at levels that may be present in vaginal swab specimens.
[2]Product containing metronidazole which did not show interference. All other metronidazole-containing products tested in the study showed interference.
[3]Concentrations of the ingredients not available from the manufacturer.
[4]Progesterone was tested as a pure active ingredient dissolved in DMSO along with DMSO solvent alone. Both yielded 100% expected results, demonstrating no interference from progesterone at the tested concentration.

Endogenous Substances Interference Study

None of the tested substances (whole blood, mucin, leukocyte and seminal fluid) showed interference with cobas BV/CV performance at the tested concentrations. The levels of endogenous substances tolerated by the assay are shown in Table 12.

Table 12: Endogenous Substances Tested for Interference

Substance	Tested Concentration
Whole Blood	44.8 mg/mL
Leukocytes	36.5 mg/mL
Mucin	0.2 - 0.5%
Seminal fluid	42.3 mg/mL

Competitive Interference

To assess competitive inhibition between BV and CV targets detected by cobas BV/CV, low and moderate positive concentrations of one target were mixed with very high positive concentrations of the other target. Low and moderate positive target concentrations were defined as approximately 1x and 3x LoD, respectively. High concentrations were defined at approximately 1E+06 CFU/mL for all the target analytes. The study samples were prepared in simulated vaginal matrix stabilized in cobas PCR Media.

Testing results indicated that when BV targets were present at a high concentration, CV was detected at low and moderate levels. Results also indicated that when CV was present at a high concentration, BV was detected at low and moderate levels. The specificity of CV was not affected by the presence of high concentrations of BV and the specificity of BV was not affected by the presence of high concentrations of CV. The study demonstrated that competitive inhibitory effects were not observed between cobas BV/CV analytes for the relevant co-infections evaluated in the study.

4. Detection Limit and Assay Reportable Range:

A study was conducted to determine the limit of detection (LoD) for a representative strain of each targeted organism (*Atopobium vaginae*, *Gardnerella vaginalis*, three *Lactobacillus* species and six *Candida* species) detected by cobas BV/CV. Serial dilutions of targeted strains were inoculated into simulated vaginal matrix stabilized in cobas PCR Media for *Atopobium vaginae*, *Gardnerella vaginalis*, three *Lactobacillus* species and in clinical vaginal swab matrix for six *Candida* species. Panels of five to six concentration levels were tested over three lots of cobas BV/CV test reagents to determine the LoD for each target. The study also included simulated vaginal matrix without analytes as negative control. Table 13 lists the LoD for strains evaluated in the study.

Table 13: Limit of Detection for Individual cobas BV/CV Targets

Target	Strain / Isolate	LoD (CFU/mL)	95% LoD PROBIT CFU/mL (95% CI)	Mean Ct at LoD
Lactobacillus crispatus	*Lactobacillus crispatus 33820 (ATCC)*	1.50E+00	1.64E+00 (1.38E+00 – 2.09E+00)	34.9
Lactobacillus gasseri	*Lactobacillus gasseri* HM-644 (BEI Resources)	1.60E+02	1.14E+02 (9.12E+01 – 1.54E+02)	34.24
Lactobacillus jensenii	*Lactobacillus jensenii* HM-374 (BEI Resources)	8.70E+01	8.51E+01 (6.73E+01 – 1.25E+02)	34.6
Gardnerella vaginalis	*Gardnerella vaginalis* 72422 (CCUG)	5.00E+02	3.44E+02 (2.93E+02 – 4.32E+02)	32.14
Atopobium vaginae	*Atopobium vaginae* 55227 (CCUG)	1.50E+02	1.35E+02 (1.08E+02 – 1.95E+02)	34.83
Candida parapsilosis	*Candida parapsilosis* 22019 (ATCC)	1.60E+02	1.41E+02 (1.10E+02 – 2.00E+02)	33.24
Candida tropicalis	*Candida tropicalis* 750 (ATCC)	6.67E+01	5.66E+01 (4.58E+01 – 7.61E+01)	33.79
Candida dubliniensis	*Candida dubliniensis* MYA-646 (ATCC)	1.20E+03	8.19E+02 (6.59E+02 – 1.12E+03)	33.29

Target	Strain / Isolate	LoD (CFU/mL)	95% LoD PROBIT CFU/mL (95% CI)	Mean Ct at LoD
Candida krusei	*Candida krusei* 14243 (ATCC)	1.50E+02	9.39E+01 (7.68E+01 – 1.23E+02)	33.82
Candida albicans	*Candida albicans* 14053 (ATCC)	7.00E+02	6.77E+02 (5.34E+02 – 9.30E+02)	33.36
Candida glabrata	*Candida glabrata* MYA-275 (ATCC)	7.00E+02	3.52E+02 (2.75E+02 – 5.01E+02)	31.86

Table 14 provides a summary of LoD for BV target combinations used in various non-clinical performance studies. These combinations served as representative compositions of bacterial targets (*Atopobium vaginae*, *Gardnerella vaginalis*, three *Lactobacillus* species) to reflect the BV LoD because the BV algorithm integrates real-time PCR signals from multiple bacterial targets to calculate the final BV result. The LoD for BV is defined as the lowest concentrations of BV target organism combinations that generate a BV positive test result for ≥95% of sample replicates. LoD for two BV combinations was established by testing serial dilutions of each combination prepared in simulated vaginal matrix stabilized in cobas PCR Media.

Table 14: LoD for BV Target Combinations for cobas BV/CV

Strain/Isolate	LoD (CFU/mL)
Atopobium vaginae 55227 (CCUG) *Gardnerella vaginalis* 72422 (CCUG)	*Atopobium vaginae* = 2.01E+03 *Gardnerella vaginalis* = 2.15E+04
Atopobium vaginae 55227 (CCUG), *Gardnerella vaginalis* 72422 (CCUG) *Lactobacillus crispatus 33820 (ATCC)*	*Atopobium vaginae* = 7.41E+04, *Gardnerella vaginalis* = 1.52E+06 *Lactobacillus crispatus* = 2.98E+01

5. Analytical Inclusivity:

The inclusivity of cobas BV/CV was confirmed by testing minimum of five strains of each BV target *(Lactobacillus crispatus*, *Lactobacillus gasseri*, *Lactobacillus jensenii*, *Gardnerella vaginalis*, and *Atopobium vaginae)* and five strains of each CV target (*Candida parapsilosis, Candida tropicalis, Candida dubliniensis, Candida krusei, Candida albicans, and Candida glabrata*). All BV strains were detected at approximately 3x LoD compared to the respective LoD strain. Four of the five strains of *Candida tropicalis* were detected at 4-8x LoD. All other CV strains were detected at approximately 3x LoD compared to the respective LoD strain. A limitation regarding detection of *Candida tropicalis* strains at higher concentrations was included in the device labeling.

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

Controls
A DNA Internal Control (DNA-IC) is introduced into each specimen during sample processing and monitors specimens for substances that may interfere with nucleic acid

isolation and PCR amplification. DNA-IC contains a non-BV/CV related DNA construct containing primer and probe specific sequence regions.

External quality controls, cobas BV/CV Control Kit (positive control) and cobas Buffer Negative Control Kit (negative control) are provided separately. These controls are processed through sample preparation and PCR procedures that are identical to those used for the vaginal specimens. Validation of results is performed automatically by the cobas 6800/8800 software based on the control performance. If one of the controls is invalid, repeat testing of all controls and all associated samples is required. Positive and/or negative controls may be tested in accordance with local, state, and/or federal regulations or accreditation requirements and internal quality control policy.

Specimen Stability
A study was conducted to evaluate stability of vaginal swab specimens collected in cobas PCR Media prior to testing with cobas BV/CV. The study evaluated stability at two temperatures (2-8°C and 30°C) at different time intervals (0, 66 and 99 days) of storage. Negative clinical vaginal swab samples, pre-screened and determined to be negative for BV and CV, were pooled to create five negative sample pools. Positive samples were created by spiking BV (*Atopobium vaginae*, *Gardnerella vaginalis*, *Lactobacillus* spp.) and CV (*Candida albicans)* analytes to the negative sample pools at approximately 3x LoD. Three replicates of each sample pool (negative and positive) were evaluated for a total of 15 replicates per time point and per temperature condition.

For negative samples, all replicates stored at 2-8°C and 30°C at all time points generated the expected negative results for all cobas BV/CV targets. For positive samples, all replicates stored at 2 - 8°C and 30°C at all time points generated the expected positive results for all cobas BV/CV targets. Additional evaluation showed no significant shift in mean Ct values for cobas BV/CV analytes over the storage duration.

The study results support the claimed specimen storage conditions included in cobas BV/CV labeling: storage at 2-30°C for up to 90 days.

Opened Kit and On-board Stability of cobas BV/CV
Reagents loaded onto the cobas 6800/8800 systems are stored at appropriate temperatures and their expiration is monitored and enforced by the system. A study was conducted to determine the opened kit and on-board stability (outside on-board refrigerator, as applicable) of cobas BV/CV reagents on the cobas 6800/8800 systems. Testing was done at several timepoints for up to 181 days at 2-8°C for open kit stability and for up to 41 hours at 37°C for on-board stability. The results of the study support the claimed storage conditions included in cobas BV/CV labeling: open kit stability of 180 days from first usage at 2-8°C and on-board stability of 40 hours at 37°C.

7. Assay Cut-Off:

cobas BV/CV cut-offs include defined Ct value cutoffs for each organism target (*Gardnerella vaginalis*, *Lactobacillus* spp., *Candida* spp., *Atopobium vaginae*) as well as the internal control (DNA-IC) determined in pre-clinical studies. Data from cobas BV/CV feasibility

studies were compiled and analyzed to identify the Ct cutoff for each of the target channels. All single target channel cut-offs were subsequently verified during technical performance verification studies demonstrating that all or nearly all environmental contamination events fell above the established cut-offs. Bacterial vaginosis results are determined using a validated algorithm that integrates Ct values from three BV-related targets (*Gardnerella vaginalis, Lactobacillus* spp., and *Atopobium vaginae*) plus the internal control to indicate a positive or negative BV final result.

8. Carry-Over/Cross-Contamination Study:

A carry-over/cross-contamination study was conducted to assess the potential for specimen and/or amplicon carryover contamination from high positive to negative samples when tested with cobas BV/CV on the cobas 6800/8800 systems. The study included alternating high positive and negative samples tested over multiple runs. Negative samples consisted of simulated vaginal matrix stabilized in cobas PCR Media without the analytes. High positive samples in the study were co-formulated with *Gardnerella vaginalis* and *Candida albicans* in simulated vaginal matrix stabilized in cobas PCR Media to a concentration that generated Ct values of approximately 14 and 24 cycles, respectively, representing the Ct value lower than that observed in 95% of positive results in the intended use population

Positive and negative samples were processed in a checkerboard configuration in five runs on cobas 8800 System for a total of 470 samples (46 high positive samples and 48 negative samples per run). Study results included 240 negative samples correctly reported as negative and 230 positive samples correctly reported as positive for each of the tested cobas BV/CV analyte, demonstrating no cross-contamination when tested with cobas BV/CV on the cobas 6800/8800 Systems.

B Comparison Studies:

1. Method Comparison with Predicate Device:

Not applicable

2. Matrix Comparison:

Because the BV analytes detected by cobas BV/CV are present in normal vaginal flora, it was necessary to use a simulated vaginal matrix for preparation of samples for some analytical studies. This study assessed the performance equivalence of cobas BV/CV between simulated vaginal matrix stabilized in cobas PCR Media and clinical vaginal swab matrix samples. Pooled CV-negative clinical vaginal swab matrix and negative simulated vaginal matrix were individually spiked with *Candida* strains (*Candida albicans* or *Candida glabrata*) at the low positive (1-2x LoD), below LoD (<1x LoD) and moderate positive (3-5x LoD) concentrations. The samples evaluated in the study along with their corresponding expected results are shown below in Table 15.

Table 15: Matrix Equivalency Study and Expected Results

Target Level	Number of Replicates per CV Strain	Expected Result (% Positive)
Moderate Positive (3-5x LoD)	11	100%
Low Positive (1-2x LoD)	42	≥ 95%
Below LoD (<1x LoD)	21	10 – 90%
Negative	11	0%

Expected results were generated for all the samples tested in both the matrices. cobas BV/CV performed equivalently for samples prepared in simulated vaginal matrix and pooled clinical vaginal swab matrix for negative samples as well as positive samples prepared at <1x, 1-2x and 3-5x LoD levels for both the representative organism targets (*C. albicans* and *C. glabrata*). Study results support the use of the simulated 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 cobas BV/CV was established in a prospective clinical study performed at eight geographically diverse sites in the U.S. and one site in Europe. 716 total subjects were screened for inclusion in this study, with 711 subjects included in the final data set. Demographic and clinical data was collected from the subjects at enrollment. Among the 711 subjects included, 552 were symptomatic and 159 were asymptomatic subjects. Symptomatic patients had a clinical presentation consistent with vaginitis, vaginosis, or both.

Each symptomatic subject had multiple vaginal swabs collected.

- One self-collected vaginal swab for cobas BV/CV testing
- Three clinician-collected vaginal swabs (randomized in collection sequence) for BV and CV comparator testing
- One clinician-collected vaginal swab for cobas BV/CV testing

Each asymptomatic subject had one clinician-collected vaginal swab for cobas BV/CV testing only. The testing with cobas BV/CV was performed at four geographically diverse laboratory testing sites.

cobas BV/CV performance in symptomatic subjects was compared to BV and CV status determined by comparator algorithms based on a combination of three FDA-cleared nucleic acid amplification tests (NAATs). A positive status was determined when at least two of the comparator NAATs were positive. A negative status was determined when at least two of the comparator NAATs were negative. When the results from the comparator algorithm were inconclusive, the result was excluded from the final performance calculations.

Of the 711 subjects enrolled in the study, 17 symptomatic subjects and 5 asymptomatic subjects were non-evaluable due to invalid or missing results due to protocol deviations on

cobas BV/CV and/or comparator tests. The remaining 689 subjects (535 symptomatic and 154 asymptomatic) were evaluable for either BV and/or CV. The evaluable subjects included women of various ethnicities and ages 18 years and above.

BV Performance

After excluding specimens without the results from comparator method and/or cobas BV/CV, 530 clinician-collected and 528 self-collected vaginal swab specimens from symptomatic subjects were included in BV performance calculations.

Table 16 shows the clinical performance of cobas BV/CV for detecting BV in symptomatic population, stratified by clinician- and self-collected vaginal swab samples. Of the 530 evaluable clinician-collected specimens, 213 (40.2%; 213/530) were detected on cobas BV/CV with concordant comparator method results. Of the 528 evaluable self-collected specimens, 214 (40.5%; 214/528) were detected on cobas BV/CV with concordant comparator method results.

Table 16: Summary of cobas BV/CV Performance in Symptomatic Subjects for BV by Collection Method

Collection Method	Total N	SENS	95% Score CI	SPEC	95% Score CI	PPV	95% Score CI	NPV	95% Score CI
CC	530	95.9% (213/222)	92.5-97.9%	93.8% (289/308)	90.6-96.0%	91.8% (213/232)	87.6-94.7%	97% (289/298)	94.4-98.4%
SC	528	96.8% (214/221)	93.6-98.5%	92.8% (285/307)	89.4-95.2%	90.7% (214/236)	86.3-93.8%	97.6% (285/292)	95.1-98.8%

Note: N is the total number of evaluable subjects; CC = Clinician-Collected; SC = Self-Collected.
CI = Confidence Interval, NPV = Negative Predictive Value, PPV = Positive Predictive Value, SENS = Sensitivity, SPEC = Specificity
Note: The predictive values shown above reflect performance specific to the clinical study population and may not be applicable to all individuals in the intended use population.

There were no clinically significant differences when performance of the device was evaluated stratified by demographic characteristics such as ethnicity, race and age. Table 17 and Table 18 show BV performance of cobas BV/CV in symptomatic subjects with respect to the subjects' clinical characteristics from clinician-collected and self-collected vaginal swabs, respectively.

Table 17: Summary of cobas BV/CV Performance for BV in Symptomatic Subjects Stratified by Subject Clinical Characteristics – Clinician-Collected Vaginal Swab Specimens

Factor	N	TP/(TP+FN)	Sensitivity (%)	95% CI	TN/(TN+FP)	Specificity (%)	95% CI
Overall	530	213/222	95.9%	92.5-97.9%	289/308	93.8%	90.6-96%
Hormone Therapy - Yes	61	21/22	95.5%	78.2-99.2%	34/39	87.2%	73.3-94.4%
Hormone Therapy - No	469	192/200	96%	92.3-98%	255/269	94.8%	91.5-96.9%
Menses - Yes	20	16/17	94.1%	73-99%	2/3	66.7%	20.8-93.9%
Menses - No	510	197/205	96.1%	92.5-98%	287/305	94.1%	90.9-96.2%

Factor	N	TP/(TP+FN)	Sensitivity (%)	95% CI	TN/(TN+FP)	Specificity (%)	95% CI
Pregnancy - No	507	200/206	97.1%	93.8-98.7%	282/301	93.7%	90.4-95.9%
Pregnancy - Yes	19	10/13	76.9%	49.7-91.8%	6/6	100%	61-100%
Pregnancy - Unknown	4	3/3	100%	43.9-100%	1/1	100%	20.7-100%

Note: N = Total number of paired samples; CI = Confidence Interval.
TP = Number of samples where both the cobas BV/CV and the comparator are positive.
FP = Number of samples where the cobas BV/CV is positive and the comparator is negative.
FN = Number of samples where the cobas BV/CV is negative and the comparator is positive.
TN = Number of samples where both the cobas BV/CV and the comparator is negative.

Table 18: Summary of cobas BV/CV Performance for BV in Symptomatic Subjects Stratified by Subject Clinical Characteristics – Self-Collected Vaginal Swab Specimens

Factor	N	TP/(TP+FN)	Sensitivity (%)	95% CI	TN/(TN+FP)	Specificity (%)	95% CI
Overall	528	214/221	96.8%	93.6-98.5%	285/307	92.8%	89.4-95.2%
Hormone Therapy - Yes	61	21/22	95.5%	78.2-99.2%	34/39	87.2%	73.3-94.4%
Hormone Therapy - No	467	193/199	97%	93.6-98.6%	251/268	93.7%	90.1-96%
Menses - Yes	20	16/17	94.1%	73-99%	2/3	66.7%	20.8-93.9%
Menses - No	508	198/204	97.1%	93.7-98.6%	283/304	93.1%	89.7-95.4%
Pregnancy - No	505	200/205	97.6%	94.4-99%	278/300	92.7%	89.1-95.1%
Pregnancy - Yes	19	11/13	84.6%	57.8-95.7%	6/6	100%	61-100%
Pregnancy - Unknown	4	3/3	100%	43.9-100%	1/1	100%	20.7-100%

Note: N = Total number of paired samples; CI = Confidence Interval.
TP = Number of samples where both the cobas BV/CV and the comparator is positive.
FP = Number of samples where the cobas BV/CV is positive and the comparator is negative.
FN = Number of samples where the cobas BV/CV is negative and the comparator is positive.
TN = Number of samples where both the cobas BV/CV and the comparator is negative.

CV Performance

After excluding specimens without the results from comparator and/or cobas BV/CV, 529 clinician-collected and 526 self-collected vaginal swab specimens from symptomatic subjects were included in CV performance calculations.

Table 19 shows the clinical performance of cobas BV/CV for detecting CV in symptomatic population, stratified by clinician- and self-collected vaginal swab samples. Of the 529 evaluable clinician-collected specimens, 119 (22.5%; 119/529) were detected on cobas BV/CV with concordant comparator method results. Of the 526 evaluable self-collected specimens, 120 (22.8%; 120/526) were detected on cobas BV/CV with concordant comparator method results.

Table 19: Summary of cobas BV/CV performance for CV in Symptomatic Subjects by Collection Method

Collection Method	Total N	PPA	95% Score CI	NPA	95% Score CI

CC	529	93% (119/128)	87.2-96.3%	97.8% (392/401)	95.8-98.8%
SC	526	94.5% (120/127)	89.1-97.3%	93% (371/399)	90-95.1%

Note: N is the total number of evaluable subjects; CC = Clinician-Collected; SC = self-Collected.
CI = Confidence Interval, PPA= Positive Percent Agreement, NPA= Negative Percent Agreement

Table 20 and Table 21 show performance of cobas BV/CV in symptomatic subjects with respect to the subjects' clinical characteristics from clinician-collected and self-collected vaginal swabs, respectively for CV.

Table 20: Summary of cobas BV/CV Performance for CV in Symptomatic Subjects Stratified by Subject Characteristics – Clinician-Collected Vaginal Swab Specimens

Factor	N	TP/(TP+FN)	PPA (%)	PPA 95% CI	TN/(TN+FP)	NPA (%)	NPA 95% CI
Overall	529	119/128	93%	87.2-96.3%	392/401	97.8%	95.8-98.8%
Hormone Therapy - Yes	61	20/20	100%	83.9-100%	40/41	97.6%	87.4-99.6%
Hormone Therapy - No	468	99/108	91.7%	84.9-95.6%	352/360	97.8%	95.7-98.9%
Menses - Yes	20	2/2	100%	34.2-100%	18/18	100%	82.4-100%
Menses - No	509	117/126	92.9%	87-96.2%	374/383	97.7%	95.6-98.8%
Pregnancy - No	507	114/123	92.7%	86.7-96.1%	375/384	97.7%	95.6-98.8%
Pregnancy - Yes	18	5/5	100%	56.6-100%	13/13	100%	77.2-100%
Pregnancy - Unknown	4	0/0	NC	NC	4/4	100%	51-100%

N = Total number of paired samples; PPA = Positive Percent Agreement; NPA = Negative Percent Agreement.
TP = Number of samples where both the cobas BV/CV and the comparator is positive.
FP = Number of samples where the cobas BV/CV is positive and the comparator is negative.
FN = Number of samples where the cobas BV/CV is negative and the comparator is positive.
TN = Number of samples where both the cobas BV/CV and the comparator is negative.
NC = Not calculable

Table 21: Summary of cobas BV/CV Performance for CV in Symptomatic Subjects Stratified by Subject Characteristics – Self-Collected Vaginal Swab Specimens

Factor	N	TP/(TP+FN)	PPA (%)	PPA 95% CI	TN/(TN+FP)	NPA (%)	NPA 95% CI
Overall	526	120/127	94.5%	89.1-97.3%	371/399	93%	90-95.1%
Hormone Therapy - Yes	61	19/20	95%	76.4-99.1%	40/41	97.6%	87.4-99.6%
Hormone Therapy - No	465	101/107	94.4%	88.3-97.4%	331/358	92.5%	89.2-94.8%
Menses - Yes	20	2/2	100%	34.2-100%	17/18	94.4%	74.2-99.0%
Menses - No	506	118/125	94.4%	88.9-97.3%	354/381	92.9%	89.9-95.1%
Pregnancy - No	504	115/122	94.3%	88.6-97.2%	354/382	92.7%	89.6-94.9%
Pregnancy - Yes	18	5/5	100%	56.6-100%	13/13	100%	77.2-100%
Pregnancy - Unknown	4	0/0	NC	NC	4/4	100%	51-100%

N = Total number of paired samples; PPA = Positive Percent Agreement; NPA = Negative Percent Agreement.
TP = Number of samples where both the cobas BV/CV and the comparator is positive.
FP = Number of samples where the cobas BV/CV is positive and the comparator is negative.
FN = Number of samples where the cobas BV/CV is negative and the comparator is positive.
TN = Number of samples where both the cobas BV/CV and the comparator is negative.
NC = Not calculable

Co-infection Detection Rate by cobas BV/CV
Table 22 provides the BV and CV co-infection rates by collection method in symptomatic population observed in the prospective clinical study using cobas BV/CV. The data presented includes evaluable subjects with valid results for both BV and CV targets.

Table 22: cobas BV/CV Co-infection Detection Rates by Collection Method

Co-Infection	Clinician-collected	Self-collected
BV and CV	9.0% (47/525)	10.7% (56/523)

Evaluation of cobas BV/CV Performance in Asymptomatic Population
The BV and CV organisms that are detected by cobas BV/CV are also considered part of the normal vaginal microbiome. Although cobas BV/CV is not intended for testing specimens from asymptomatic women, the performance of the assay was evaluated in asymptomatic population with demographic characteristics similar to the intended use population. Of the 159 asymptomatic subjects enrolled, 154 subjects were evaluable. The positivity rate of BV and CV in the asymptomatic population is presented in Table 23 below.

Table 23: cobas BV/CV Positivity Rates for BV and CV in the Asymptomatic Population

Analyte	Total N	cobas BV/CV Positive Result	Positivity Rate (%)	95% CI
BV	154	50	32.5%	25.6-40.2%
CV	154	19	12.3%	8-18.5%

2. Clinical Specificity:

   See Clinical Sensitivity section above

3. Clinical Cut-Off

   See Assay Cut-off Section VII A.7 above

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

D Expected Values/Reference Range:

In cobas BV/CV prospective clinical trial, reportable results from specimens in the symptomatic evaluable population were obtained from nine geographically diverse sites. The number and percentage of positive cases per target, as determined by cobas BV/CV, are presented in Table 24 below.

Table 24: cobas BV/CV Positivity Rates by Collection Method

Collection Type	BV	CV
Clinician-collected	43.8% (232/530)	24.2% (128/529)
Self-collected	44.7% (236/528)	28.1% (148/526)

E Other Supportive Instrument Performance Characteristics Data:

Software, electrical safety and electromagnetic compatibility (EMC) testing and cybersecurity documentation was 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.