cobas® BV/CV

For use on the cobas® 5800/6800/8800 systems

For in vitro diagnostic use

cobas® BV/CV	P/N: 09988815190
cobas® BV/CV Control Kit	P/N: 09988807190
cobas® Buffer Negative Control Kit	P/N: 09051953190

Table of contents

Intended use

cobas® BV/CV for use on the **cobas**® 5800/6800/8800 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.) associated with candida vaginitis (CV) from clinician-instructed 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.

Summary and explanation of the test

Background

Vaginitis is characterized by symptoms that can include abnormal discharge, abnormal odor, itching, irritation, and/or burning.[1,2] Most women and other persons with vaginas will have at least one episode of vaginitis during their lifetime.[3] The three most common causes of infectious vaginitis are bacterial vaginosis (BV), candida vaginitis (CV; also known as vulvovaginal candidiasis), and *Trichomonas vaginalis*, with prevalences of 40%-50%, 20%-25%, and 15%-20%, respectively.

Bacterial vaginosis

BV is a condition that results from a dysbiosis of the vaginal microbiome. A healthy vaginal tract consists of a microbiome that is predominantly colonized by *Lactobacillus* spp. This Gram-positive organism provides protection against infections by maintaining a low pH due to the production of lactic acid. When the healthy vaginal microbiome is disrupted, overgrowth of anaerobic bacteria can occur and result in the syndrome of vaginitis.[4] The development of BV has been associated with complications, such as adverse pregnancy outcomes (preterm delivery and low birthweight babies), as well as an increased risk of pelvic inflammatory diseases and post-abortal sepsis.[1,5] It has also been reported that patients with BV are at an increased risk of acquiring sexually transmitted infections with pathogens, such as human immunodeficiency virus, *Neisseria gonorrhoeae, Chlamydia trachomatis, T. vaginalis, Mycoplasma genitalium*, human papillomavirus, and herpes simplex virus; they are also at increased risk for complications after gynecologic surgery, complications of pregnancy, and recurrence of BV.[6]

Candida vaginitis

Vaginal yeast colonization with Candida spp. can occur in up to 30% of healthy asymptomatic individuals.[7] It is not entirely understood what factors specifically cause the development of CV, but it is believed to be a combination of factors inherent to the patient (e.g., genetic predisposition, host factors), factors external to the patient (behavioral and microbial), and previous CV episodes. CV is predominantly caused by *Candida albicans* in up to 90% of cases[3], while *C. glabrata* is the second leading cause of CV (~8% of cases).[8-10]

Diagnosis

The diagnosis of vaginitis, particularly for bacterial vaginosis (BV) and candida vaginitis (CV), has traditionally relied on clinical signs and symptoms, as well as non-molecular tests. For BV, the Amsel criteria—developed in 1983—requires meeting at least three of the following: presence of homogenous vaginal discharge, vaginal pH >4.5, whiff test (fishy odor after adding 10% potassium hydroxide), and presence of clue cells on wet mount microscopy. [11]

The Amsel criteria's sensitivity ranges from 37-70%, with high specificity (94-99%), meaning diagnosis might be missed.[6] Another method used is the Nugent score analysis—a Gram stain assessing various bacteria morphotypes in the vaginal discharge—is considered the gold standard, with a reported sensitivity of 65% and specificity of 97%.[12] However, interpretation is very subjective relying on the microscopist expertise. Additional data on clinician practices showed that 37-42% of clinicians do not perform microscopy and >90% do not perform pH measurements, which are both part of the Amsel criteria. [13]

A CV diagnosis can also be done from visual examination of the vaginal discharge material identifying yeast hyphae via microscopy. [3]Vaginal cultures for *Candida* can be performed, however, this is not a timely method and may not be performed routinely.[6]

Overall, traditional methods for diagnosing BV and CV have limitations regarding subjectivity and sensitivity. Newer nucleic acid amplification tests (NAATs) can provide improved detection of organisms associated with BV and detection of *Candida* species associated with CV.

Explanation of the test

cobas® BV/CV for use on the **cobas**® 5800 system, **cobas**® 6800 system, and **cobas**® 8800 system is an automated, qualitative *in vitro* real-time PCR diagnostic test that detects bacteria and yeast associated with BV and CV, respectively in patient specimens.

Appropriate specimens are as follows:

- Collected in **cobas**® PCR Media (Roche Molecular Systems, Inc.):
    - Self-collected vaginal swab specimens (collected in a clinical setting)
    - Clinician-collected vaginal swab specimens

The test does not distinguish among the pathogens but can give an overall qualitative result indicating the presence or absence of pathogen(s) associated with BV and/or CV. The DNA Internal Control, used to monitor the entire sample preparation and PCR amplification process, is introduced into each specimen during sample processing. In addition, the test utilizes external controls (a positive control and a negative control).

Principles of the procedure

cobas® BV/CV is based on fully automated sample preparation (nucleic acid extraction and purification) followed by PCR amplification and detection. The **cobas**® 5800 system is designed as one integrated instrument. The **cobas**® 6800/8800 systems consist of the sample supply module, the transfer module, the processing module, and the analytic module. Automated data management is performed by the **cobas**® 5800 system or **cobas**® 6800/8800 systems software which assigns test results for all tests as positive, negative or invalid. Results can be reviewed directly on the system screen, exported, or printed as a report.

Nucleic acid from patient samples and added internal control DNA (DNA-IC) molecules are simultaneously extracted. In summary, nucleic acid is released by addition of proteinase and lysis reagent to the sample. The released nucleic acid binds to the silica surface of the 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 acid is 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: *Candida* species, BV: *Gardnerella vaginalis, Atopobium vaginae*, and *Lactobacillus* species). 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 are selected to 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. The master mix includes deoxyuridine triphosphate (dUTP), instead of deoxythimidine triphosphate (dTTP), which is incorporated into the newly synthesized DNA (amplicon). Any contaminating amplicon from previous PCR runs is eliminated by the AmpErase enzyme, which is included in the PCR master mix, during the first thermal cycling step. However, newly formed amplicons are not eliminated since the AmpErase enzyme is inactivated once exposed to temperatures above 55°C.

The **cobas**® BV/CV master mix contains multiple probes specific for the CV associated target sequences, multiple 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 target 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 is accomplished by measuring the fluorescence of the released reporter dyes for the BV and CV associated targets and DNA-IC, respectively.

Limitations

- A negative result does not preclude a possible infection with another pathogen(s) that can cause a similar clinical presentation.
- *Candida* species and bacterial compositions associated with CV and BV can be present as part of normal vaginal flora and results should be considered in conjunction with available clinical information.
- Additional testing may be needed to identify *C. glabrata* and *C. krusei* in patients with a CV detected result due to the increased likelihood of resistance to azole antifungal treatments.
- Reliable results are dependent on adequate specimen collection, transport, storage, and processing. Failure to observe proper procedures in any one of these steps can lead to incorrect results. See **Specimen collection, transport, and storage** for instructions. For detailed information, refer to the respective collection kit instructions for use.

Reagents and materials

cobas® BV/CV reagents and controls

The materials provided for **cobas**® BV/CV can be found in Table 1. Materials required, but not provided can be found in Table 2 to Table 4 and Table 9 to Table 11.

All unopened reagents and controls shall be stored as recommended in Table 1 to Table 4.

Table 1 cobas® BV/CV

(BV/CV)

Store at 2-8°C
192 test cassette (P/N 09988815190)

Kit components	Reagent ingredients	Quantity per kit 192 tests
Proteinase Solution (PASE)	Tris buffer, < 0.05% EDTA, Calcium chloride, Calcium acetate, 8% Proteinase*, glycerol EUH210: Safety data sheets available on request. EUH208: Contains subtilisin. May produce an allergic reaction.	22.3 mL
DNA Internal Control (**DNA-QS**)	Tris buffer, < 0.05% EDTA, < 0.001% non-BV/CV related DNA construct containing primer and probe specific sequence regions, < 0.1% Sodium azide	21.2 mL
Elution Buffer (EB)	Tris buffer, 0.2% Methyl-4 hydroxibenzoate	21.2 mL
Master Mix Reagent 1 (MMX-R1)	Manganese acetate, Potassium hydroxide, < 0.1% Sodium azide	7.5 mL
BV/CV Master Mix Reagent 2 (BV/CV MMX-R2)	Tricine buffer, Potassium acetate, EDTA, Glycerol, < 18% Dimethyl sulfoxide, < 0.12% dATP, dCTP, dGTP, dUTPs, < 0.1% Tween 20, < 0.1% Sodium azide, < 0.1% Z05 DNA polymerase, < 0.1% AmpErase (uracil-N glycosylase) enzyme (microbial), < 0.01% Internal Control forward and reverse primers, < 0.01% Upstream and downstream *Gardnerella vaginalis, Lactobacillus spp., Atopobium vaginae,* and *Candida spp.* primers, < 0.01% Fluorescent-labeled oligonucleotide probes specific for *Gardnerella vaginalis, Lactobacillus spp., Atopobium vaginae, Candida spp.*, and the DNA Internal Control, < 0.01% Oligonucleotide aptamer	9.7 mL

*Hazardous substance

Table 2 cobas® BV/CV Control Kit

(BV/CV CTL)

Store at 2–8°C

(P/N 09988807190)

Kit components	Reagent ingredients	Quantity per kit
BV/CV Control (BV/CV (+) C)	Tris buffer, < 0.05% Sodium azide, < 0.005% EDTA, < 0.003% Poly rA, <0.01% Non-infectious plasmid DNA (microbial) containing, <0.01% Non-infectious plasmid DNA (microbial) containing *Gardnerella vaginalis, Lactobacillus*, *Atopobium vaginae,* and *Candida*	16 mL (16 x 1mL)

Table 3 cobas® Buffer Negative Control Kit

(BUF (–) C)

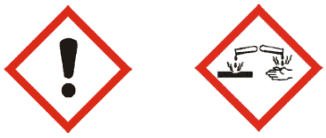
Store at 2–8°C

(P/N 09051953190)

Kit components	Reagent ingredients	Quantity per kit
cobas® Buffer Negative Control (BUF (–) C)	Tris buffer, < 0.1% sodium azide, EDTA, < 0.002% Poly rA RNA (synthetic)	16 mL (16 x 1mL)

cobas® omni reagents for sample preparation

Table 4 cobas® omni reagents for sample preparation

Reagents	Reagent ingredients	Quantity per kit	Safety symbol and warning*
cobas® omni MGP Reagent (MGP) Store at 2–8°C (P/N 06997546190)	Magnetic glass particles, Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide	480 tests	Not applicable
cobas® omni Specimen Diluent (SPEC DIL) Store at 2–8°C (P/N 06997511190)	Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide	4 x 875 mL	Not applicable
cobas® omni Lysis Reagent (LYS) Store at 2–8°C (P/N 06997538190)	42.56% (w/w) guanidine thiocyanate**, 5% (w/v) polydocanol**, 2% (w/v) dithiothreitol**, dihydro sodium citrate	4 x 875 mL	 **DANGER** H302: Harmful if swallowed. H314: Causes severe skin burns and eye damage. H412: Harmful to aquatic life with long lasting effects. EUH032: Contact with acids liberates very toxic gas. EUH071: Corrosive to the respiratory tract. P273: Avoid release to the environment. P280: Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection. P301 + P330 + P331: IF SWALLOWED: Rinse mouth. Do NOT induce vomiting. P303 + P361 + P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water. P304 + P340 + P310: IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/ doctor. P305 + P351 + P338 + P310: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/ doctor. 593-84-0 Guanidinium thiocyanate 9002-92-0 Poly(oxy-1,2-ethanediyl), α-dodecyl-ω-hydroxy- 3483-12-3 (R*,R*)-1,4-dimercaptobutane-2,3-diol
cobas® omni Wash Reagent (WASH) Store at 15–30°C (P/N 06997503190)	Sodium citrate dihydrate, 0.1% methyl-4 hydroxybenzoate	4.2 L	Not applicable

* Product safety labeling primarily follows EU GHS guidance

**Hazardous substance

Reagent storage requirements

Reagents shall be stored as specified in Table 5.

When reagents are not loaded on the **cobas**® 5800 system or **cobas**® 6800/8800 systems, store them at the corresponding temperature specified in Table 5.

Table 5 Reagent storage (when reagent is not on the system)

Reagent	Storage temperature
cobas® BV/CV	2–8°C
cobas® BV/CV Control Kit	2–8°C
cobas® Buffer Negative Control Kit	2–8°C
cobas® **omni** Lysis Reagent	2–8°C
cobas® **omni** MGP Reagent	2–8°C
cobas® **omni** Specimen Diluent	2–8°C
cobas® **omni** Wash Reagent	15–30°C

Reagent handling requirements for cobas® 5800 system or cobas® 6800/8800 systems

Reagents loaded onto the cobas® 5800 system or cobas® 6800/8800 systems are stored at appropriate temperatures and their expiration is monitored and enforced by the system. The system allows reagents to be used only if all of the reagent handling conditions shown in Table 6, Table 7, and Table 8 are met. The system automatically prevents use of expired reagents. Remaining open-kit stability and number of kit use information for assay specific reagents is accessible through the system user interface.

Table 6 Reagent expiry conditions monitored and enforced by the **cobas**® 5800 system

Reagent	Open-kit stability	Number of Kit uses	On-board stability
cobas® BV/CV	180 days from first usage	80	36 days from loading
cobas® BV/CV Control Kit	Singe vial use	16	36 days from loading
cobas® Buffer Negative Control Kit	Singe vial use	16	36 days from loading

Table 7 Reagent expiry conditions monitored and enforced by the **cobas**® 6800/8800 systems

Reagent	Open-kit stability	Number of Kit uses	On-board stability (outside on board refrigerator, as applicable)
cobas® BV/CV	180 days from first usage	40	40 hours
cobas® BV/CV Control Kit	Singe vial use	16	10 hours
cobas® Buffer Negative Control Kit	Singe vial use	16	10 hours

Table 8 shows the open-kit stability of the **cobas**® **omni** reagents. Prior to each run, the system verifies the open-kit stability and ensures sufficient fill volume. Therefore, these reagents have no number of kit uses or on-board stability assigned.

Table 8 **cobas**® **omni** reagent expiry condition enforced by the **cobas**® 5800/6800/8800 systems

Reagent	Open-kit stability
cobas® **omni** MGP Reagent	30 days from first usage
cobas® **omni** Lysis Reagent	30 days from loading
cobas® **omni** Specimen Diluent	30 days from loading
cobas® **omni** Wash Reagent	30 days from loading

Additional materials required for cobas® 5800/6800/8800 system

Table 9 Material for use on the **cobas**® 5800/6800/8800 systems

Material	P/N
cobas® **omni** Lysis Reagent	06997538190
cobas® **omni** MGP Reagent	06997546190
cobas® **omni** Specimen Diluent	06997511190
cobas® **omni** Wash Reagent	06997503190

Table 10 Consumables for use on the **cobas**® 5800 system*

Material
cobas® **omni** Processing Plate 24
cobas® **omni** Amplification Plate 24
cobas® **omni** Liquid Waste Plate 24
Tip CORE TIPS with Filter, 1ml
Tip CORE TIPS with Filter, 300 µl
cobas® **omni** Liquid Waste Container
Solid Waste Bag or Solid Waste Bag With Insert
16-position tube S-carrier complete
5-position Rack Carrier

* For Part Numbers please refer to the **cobas**® 5800 system User Assistance

Table 11 Materials and consumables for use on the **cobas**® 6800/8800 systems*

Material
cobas® **omni** Processing Plate
cobas® **omni** Amplification Plate
cobas® **omni** Pipette Tips
cobas® **omni** Liquid Waste Container
Solid Waste Bag and Solid Waste Container or Solid Waste Bag with Insert and Kit Drawer
STD-Rack. re-run R001-R025 PINK

* For Part Numbers please refer to the **cobas**® 6800/8800 system User Assistance

Instrumentation and software required

The **cobas**® 5800 software, the **cobas**® 6800/8800 systems software and **cobas**® BV/CV analysis package (ASAP) for **cobas**® 5800/6800/8800 systems shall be installed on the instrument(s).

For **cobas**® 5800 and the **cobas**® 6800/8800 systems with software 2.0 or higher, the x800 Data Manager Software and PC (or server) will be provided by the system.

For the **cobas**® 6800/8800 systems with software version 1.4, the Instrument Gateway (IG) server will be provided with the system.

Table 12 Instrumentation

Equipment	P/N
cobas® 5800 system	08707464001
cobas® 6800 system	05524245001 and 09575154001
cobas® 8800 system	05412722001 and 09575146001
Sample Supply Module for **cobas**® 6800/8800 systems	06301037001 and 09936882001

Refer to the **cobas**® 5800 system or **cobas**® 6800/8800 systems User Assistance for additional information.

Additional materials required for sample collection for cobas® BV/CV

Table 13 Specimen collection kits used with **cobas**® BV/CV

Collection Kit	P/N
cobas® PCR Media Kit	06466281190
cobas® PCR Media Uni Swab Sample Kit	07958030190
cobas® PCR Media Dual Swab Sample Kit	07958021190

cobas® BV/CV accepts the primary tube used for swab specimens collected in **cobas**® PCR Media kits listed above. Refer to the **cobas**® 5800 system or the **cobas**® 6800/8800 systems User Assistance for additional information for primary and secondary sample tubes accepted on the instruments.

Note: Contact your local Roche representative for a detailed order list for sample racks, racks for clotted tips and rack trays accepted on the instruments.

Additional materials required for sample aliquoting and sample loading for cobas® BV/CV

Table 14 Additional material used with **cobas**® BV/CV for sample aliquoting and loading

Material	P/N
cobas® PCR Media Secondary Tube Kit	07958048190
cobas® PCR Media Tube Replacement Cap Kit	07958056190
cobas® PCR Media Disposable Tube Stand (Optional)	07958064190
MPA RACK 16 MM LIGHT GREEN 2001-2050[a,b,c]	03143449001
RD5 RACK – RD Standard rack 0001-0050 LR[a,b,c]	11902997001

[a] RD5 or MPA racks are required in combination with the 5-position Rack Carrier on the **cobas**® 5800 system.

[b] MPA 16mm rack or 16-position tube carrier are the preferred racks for use with samples collected in **cobas**® PCR Media tubes.

[c] MPA and RD5 racks identified here are example materials and part numbers. Please contact your local Roche representative for a detailed order list for sample racks and rack carriers accepted on the instruments.

Precautions and handling requirements

Warnings and precautions

As with any test procedure, good laboratory practice is essential to the proper performance of this assay. Due to the high sensitivity of this test, care should be taken to keep reagents and amplification mixtures free of contamination.

- For in vitro diagnostic use only.
- All patient samples should be handled as if infectious, using good laboratory procedures as outlined in Biosafety in Microbiological and Biomedical Laboratories and in the CLSI Document M29-A4.[1,2]
  Only personnel proficient in handling infectious materials and in the use of **cobas**® BV/CV and **cobas**® 5800 system or **cobas**® 6800/8800 systems should perform this procedure.
- All human-sourced materials should be considered potentially infectious and should be handled with universal precautions. If spillage occurs, immediately disinfect with a freshly prepared solution of 0.5% sodium hypochlorite or potassium hypochlorite in distilled or deionized water or follow appropriate site procedures.
- Do not freeze any samples.
- Use only supplied or specified required consumables to ensure established test performance.
- Safety Data Sheets (SDS) are available on request from your local Roche representative.
- Closely follow procedures and guidelines provided to ensure that the test is performed correctly. Any deviation from the procedures and guidelines may affect established test performance.
- False positive results may occur if carryover of samples is not adequately controlled during sample handling and processing.
- **cobas**® PCR Media (from primary specimen tube) contains guanidine hydrochloride. **Do not allow direct contact between guanidine hydrochloride and sodium hypochlorite (bleach) or other highly reactive reagents such as acids or bases. These mixtures can release a noxious gas.** If liquid containing guanidine hydrochloride is spilled, clean with suitable laboratory detergent and water. If the spilled liquid contains potentially infectious agents, **FIRST** clean the affected area with laboratory detergent and water, and then with at least 0.5% sodium hypochlorite or 0.5% potassium hypochlorite solution.
- Inform your local competent authority and manufacturer about any serious incidents which may occur when using this assay.

Reagent handling

- Handle all reagents, controls, and samples according to good laboratory practice in order to prevent carryover of samples, reagents, or controls.
- Before use, visually inspect each reagent cassette, diluent, lysis reagent, and wash reagent to ensure that there are no signs of leakage. If there is any evidence of leakage, do not use that material for testing.
- **cobas**® **omni** Lysis Reagent contains guanidine thiocyanate, a potentially hazardous chemical. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur.
- Do not allow **cobas**® **omni** Lysis Reagent, which contains guanidine thiocyanate, to contact sodium hypochlorite or potassium hypochlorite solution. This mixture can produce a highly toxic gas.
- Expended control kits contain pierced vials with residual reagent; special care should be taken during disposal to

avoid spills and contact.

- **cobas**® BV/CV kit, **cobas**® BV/CV Control kit, **cobas**® Buffer Negative Control kit, **cobas**® **omni** MGP Reagent, and **cobas**® **omni** Specimen Diluent contain sodium azide as a preservative. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur. If these reagents are spilled, dilute with water before wiping dry.
- Dispose of all materials that have come in contact with samples and reagents in accordance with country, state, and local regulations.

Good laboratory practice

- Do not pipette by mouth.
- Do not eat, drink, or smoke in designated work areas.
- Wear laboratory gloves, laboratory coats, and eye protection when handling samples and reagents. Avoid contaminating gloves when handling samples and controls. Gloves must be changed between handling samples and **cobas**® BV/CV kit, **cobas**® BV/CV Control kit, **cobas**® Buffer Negative Control kit**,** and **cobas**® **omni** reagents to prevent contamination.
- Wash hands thoroughly after handling samples and reagents, and after removing the gloves.
- Thoroughly clean and disinfect all laboratory work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite or potassium hypochlorite solution. Follow by wiping the surface with 70% ethanol.
- If spills occur on the **cobas**® 5800 instrument or **cobas**® 6800/8800 instruments, follow the instructions in the **cobas**® 5800 System or **cobas**® 6800/8800 Systems User Assistance to properly clean and decontaminate the surface of instrument(s).

Specimen collection, transport, and storage

Note: Handle all samples and controls as if they are capable of transmitting infectious agents.

Specimen collection

Vaginal swab specimens collected with the woven polyester swab in either the **cobas**® PCR Media Uni Swab Sample Kit or **cobas**® PCR Media Dual Swab Sample Kit may be used with **cobas**® BV/CV (see Table 13 for a list of all collection kits). Follow the instructions provided in the respective collection kit's IFU for collecting all swab specimens.

Note: Use only the woven polyester swab in either the **cobas**® PCR Media Dual Swab Sample Kit or the **cobas**® PCR Media Uni Swab Sample Kit to collect vaginal swab specimens. **cobas**® BV/CV has not been validated for use with other swab collection devices or media types. Using **cobas**® BV/CV with other swab collection devices (eg. flocked swab) or media types may lead to false negative, false positive, and/or invalid results.

Specimen transport

All specimen types listed in the **Specimen collection** section can be transported at 2-30°C. Transportation of specimens in **cobas**® PCR Media must comply with country, federal, state and local regulations for the transport of etiologic agents.

Specimen storage

Table 15 Summary of acceptable specimen storage conditions prior to testing with **cobas**® BV/CV

Specimen Type	2-8°C	15-30°C
Samples in **cobas**® PCR Media	90 days	90 days

Note: **cobas**® PCR Media specimens should not be frozen.

Vaginal swab specimens

- Use only the woven polyester swab in either the **cobas**® PCR Media Dual Swab Sample Kit or the **cobas**® PCR Media Uni Swab Sample Kit to collect vaginal swab specimens. **cobas**® BV/CV has not been validated for use with other swab collection devices or media types. Using **cobas**® BV/CV with other swab collection devices (eg. flocked swab) or media types may lead to false negative, false positive, and/or invalid results.
- To avoid cross contamination of processed specimens, additional caps for **cobas**® PCR Media tubes in an alternate color (see **Additional materials required for sample aliquoting and sample loading for cobas® BV/CV**) should be used to recap specimens after processing.
- All swab specimens containing a single swab in the **cobas**® PCR Media tube can be directly processed on the **cobas**® 5800 system or **cobas**® 6800/8800 systems. If desired, the swab may be removed before the specimen tube is loaded onto the instrument, however utmost care must be exercised to avoid cross contamination.
- A properly collected swab specimen should have a single swab with the shaft broken at the score line. Swab shafts which are broken above the score line will appear longer than normal and may also be bent over to fit into the **cobas**® PCR Media tube. This can create an obstruction to the pipetting system which may cause the loss of sample, test results and/or mechanical damage to the instrument. In the event that a swab specimen has an improperly broken shaft, remove the swab prior to sample processing on the **cobas**® 5800 system or **cobas**® 6800/8800 systems. Use caution when disposing of specimen swabs; avoid splashing or touching swabs to other

surfaces during disposal to prevent contamination.

- Incoming primary swab specimen tubes, with no swabs or with two swabs, have not been collected according to the instructions in their respective collection kit IFU and should not be tested.
- Occasionally, incoming swab specimens contain excessive mucus which may induce a pipetting error (e.g., clot or other obstruction) on the **cobas**® 5800 system or **cobas**® 6800/8800 systems. Prior to retesting of specimens that exhibited clots during initial processing, remove and discard the swab, then re-cap and vortex these specimens for 30 seconds to disperse the excess mucus.
- Swab specimens can be assayed twice on the **cobas**® 5800 system or **cobas**® 6800/8800 systems while the swab is in the collection tube. If additional testing is required, or if the first test fails due to specimen pipetting error (e.g., clot or other obstruction), the swab must be removed and the remaining fluid must have a minimum volume of 1.0 mL.

Instructions for use

Procedural notes

- Do not use **cobas**® BV/CV, **cobas**® BV/CV Control Kit, **cobas**® Buffer Negative Control Kit, or **cobas**® **omni** reagents after their expiry dates.
- Do not reuse consumables. They are for one-time use only.
- **cobas**® BV/CV can be run with a minimum required sample volume of 1.0 mL.

Running cobas® BV/CV on cobas® 5800/6800/8800 systems

- The operation of the instrument is described in detail in the **cobas**® 5800/6800/8800 systems User Assistance. Figure 1 and Figure 2 summarize the procedure.
- Refer to the **cobas**® 5800 system or **cobas**® 6800/8800 systems User Assistance for proper maintenance of instruments.
- Swab specimens should be uncapped and loaded directly onto racks for processing on the **cobas**® 5800 system or **cobas**® 6800/8800 systems.
- Specimens collected in **cobas**® PCR Media should be processed using the sample type selection in the user interface (UI) of the **cobas**® BV/CV as described in Table 16.
- Ensure that specimen barcode labels on sample tubes are visible through the openings on the side of sample carriers. Refer to the **cobas**® 5800 system or **cobas**® 6800/8800 systems User Assistance for proper barcode specifications and additional information on loading sample tubes.

Table 16 Sample type selection in the user interface of the **cobas**® 5800/6800/8800 systems

Specimen	Specimen	Collection kit type	Process as Sample Type
Female	Vaginal swab	**cobas**® PCR Media Uni or Dual Swab Sample Kit	Swab

Figure 1 **cobas**® BV/CV test procedure on **cobas**® 5800 system

1	Log onto the system

2	Loading specimens onto the system:
	• For each vaginal swab in **cobas**® PCR Media
	  ○ Uncap tube
	  ○ Transfer tube directly to rack
	• Load sample rack
	Confirm samples have been accepted into the system
	Order tests
	• Choose “swab” for ordering swab specimens collected in **cobas**® PCR Media
	Choose the test name

3	Refill reagents and consumables as prompted by the system • Load test specific reagent cassette • Load control mini racks • Load processing tips • Load elution tips • Load processing plates • Load amplification plates • Load liquid waste plates • Load MGP Reagent Refill Specimen Diluent Refill Lysis Reagent Refill Wash Reagent
4	Start the run by choosing the Start processing button on the user interface, all subsequent runs will start automatically if not manually postponed
5	Review and export results
6	Remove sample tubes. If needed, cap any sample tubes meeting the minimum volume requirements for future use. Clean up instrument • Unload empty test specific reagent cassette(s) • Unload empty control mini racks • Empty amplification plate drawer • Empty liquid waste • Empty solid waste

Figure 2 **cobas**® BV/CV test procedure on **cobas**® 6800/8800 systems

1	Log onto the system Press Start to Prepare the system Order tests • Choose “swab” for ordering swab specimens collected in **cobas**® PCR Media Choose the Test
2	Refill reagents and consumables as prompted by the system • Load test specific reagent cassette • Load control cassettes • Load pipette tips • Load processing plates • Load MGP Reagent • Load amplification plates • Refill Specimen Diluent • Refill Lysis Reagent • Refill Wash Reagent
3	Loading specimens onto the system • For each primary vaginal swab in **cobas**® PCR Media ○ Uncap tube ○ Transfer tube directly to rack • Load sample rack and clot tip racks into the sample supply module • Confirm samples have been accepted into the transfer module
4	Start run
5	Review and export results
6	Remove and cap any sample tubes meeting the minimum volume requirements if needed for future use. Clean up instrument • Unload empty control cassettes • Empty amplification plate drawer • Empty liquid waste • Empty solid waste

Results

The **cobas**® 5800 system and **cobas**® 6800/8800 systems automatically detect and discriminate bacteria and yeast associated with BV and CV simultaneously for samples and controls, displaying test validity, overall results, as well as individual target results.

Quality control and validity of results on the cobas® 5800 system and cobas® 6800/8800 systems with software version 2.0 or higher

- One **cobas**® Buffer Negative Control [(-) Ctrl] and one **cobas**® BV/CV Control [BV/CV (+) C] must be processed at least every 72 hours or with every new kit lot. Positive and/or negative controls can be scheduled more frequently based on laboratory procedures and/or local regulations.
- The results of the controls are shown in the "Controls" app of the software.
- In the software and/or report, check for flags to ensure the validity of the corresponding test results (refer to the x800 Data Manager User Assistance for a 'List of flag codes').
- The controls are valid if no flags appear for either control.
- Controls are marked with "Valid" in the column "Control result" if the respective targets of the control are reported valid. Controls are marked with 'Invalid' in the column "Control result" if the respective targets of the control are reported invalid.
- Controls marked with 'Invalid' show a flag in the "Flags" column. More information on why the control is reported invalid including flag information will be shown in the detail view.
  If one of the controls is invalid, repeat testing of all controls and all associated samples is required.

Validation of results is performed automatically by the instrument based on control results.

NOTE: The **cobas**® 5800 system and the **cobas**® 6800/8800 systems with software version 2.0 or higher will be delivered with the standard setting of running a set of controls (positive and negative) with every run but can be configured to a less frequent scheduling up to every 72 hours based on laboratory procedures and/or local regulations. Please contact your Roche service engineer and/or Roche customer technical support for more information.

Quality control and validity of results on the cobas® 6800/8800 systems with software version 1.4

- One **cobas**® Buffer Negative Control [BUF (-) C] and one **cobas**® BV/CV Control [BV/CV (+) C] are processed with each batch of a requested result type.
- In the software and/or report, check for flags and their associated results to ensure batch validity.
- All flags are described in the **cobas**® 6800/8800 systems User Assistance.
- The batch is valid if no flags appear for all controls. If the batch is invalid, repeat testing of the entire batch is required.

Validation of results is performed automatically by the instrument software based on control result.

Interpretation of results on the cobas® 5800 system and cobas® 6800/8800 systems with software version 2.0 or higher

The results of the samples are shown in the "Results" app of the software. Result display examples are shown in Figure 3.

Figure 3 Example of **cobas**® BV/CV results display for **cobas**® 5800 system and **cobas**® 6800/8800 systems with software version 2.0 or higher

Sample ID	Test	Control results	Flag*	Result
BV/CV_01	BV/CV	Valid		**BV Negative CV Negative**
BV 01_	BV	Valid		**BV Positive**
BV/CV_02	BV/CV	Valid		**BV Invalid CV Invalid**
CV 01	CV	Valid		**CV Negative**
BV/CV_03	BV/CV	Valid		**BV Positive CV positive**
BVCV_Tpos	BV/CV	Valid		**BV Positive CV Positive**
BVCV_Tneg	BV/CV	Valid		**BV Negative CV Negative**
BVCV_Tinv	BV/CV	Valid		**BV Invalid CV Invalid**
BVCV_BVpos_CVneg	BV/CV	Valid		**BV Positive CV Negative**
BVCV_BVneg_CVpos	BV/CV	Valid		**BV Negative CV Positive**
BVCV_BVpos_CVinv	BV/CV	Valid		**BV Positive CV Invalid**
BVCV_BVneg_CVinv	BV/CV	Valid		**BV Negative CV Invalid**
BVCV_BVinv_CVpos	BV/CV	Valid		**BV Invalid CV Positive**
BVCV_BVinv_CVneg	BV/CV	Valid		**BV Invalid CV Negative**
BVCV_Tpos_ICneg	BV/CV	Valid		**BV Positive CV Positive**
BVCV_Tpos_ICinv	BV/CV	Valid		**BV Invalid CV Invalid**
BVCV_Level detection	BV/CV	Invalid		**BV Invalid CV Invalid**
BV_Tpos	BV	Valid		**BV Positive**
BV_Tneg	BV	Valid		**BV Negative**
BV_Tinv	BV	Valid		**BV Invalid**
BV_Tpos_ICneg	BV	Valid		**BV Positive**
BV_Tpos_ICinv	BV	Valid		**BV Invalid**
BV_Level detection	BV	Invalid		**BV Invalid**
CV_Tpos	CV	Valid		**CV Positive**
CV_Tneg	CV	Valid		**CV Negative**
CV_Tinv	CV	Valid		**CV Invalid**
CV_Tpos_ICneg	CV	Valid		**CV Positive**
CV_Tpos_ICinv	CV	Valid		**CV Invalid**
CV_Level detection	CV	Invalid		**CV Invalid**

* The result overview shows a flag symbol in case of invalid results. Detailed flag descriptions are available in the result details.

Check each individual sample for flags in the software and/or report. The result interpretation should be as follows:

- Samples associated with valid controls are shown as 'Valid' in the "Control result" column.
- Samples associated with a failed control are shown as 'Invalid' in the "Control result" column.

- If the associated controls of a sample result are invalid, a specific flag will be added to the sample result as follows:
    - Q05D: Result validation failure because of an invalid positive control
    - Q06D: Result validation failure because of an invalid negative control
- The values in "Results" column for individual sample target result should be interpreted as shown in

- Figure 4, Figure 5, and Figure 6.
- If one or more sample targets are marked with "Invalid" the software shows a flag in the "Flags" column. More information on why the sample target(s) is reported invalid including flag information is shown in the detail view.
- Invalid results for one or more target combinations are possible with the BV/CV result request and are reported out specifically for each target. Refer to retesting instructions.
- Results of this test should only be interpreted in conjunction with information available from clinical evaluation of the patient and patient history.

Interpretation of results on the cobas® 6800/8800 systems with software version 1.4

Result display examples are shown in

Figure 4, Figure 5, and Figure 6, respectively.

Figure 4 Example of **cobas**® BV/CV results display for BV/CV result request for **cobas**® 6800/8800 systems with software version 1.4

Test	Sample ID	Valid	Flags	Sample type	Overall result	Target 1	Target 2
BV/CV	SB_BVCVNeg_01	NA		Swab	**NA**	BV Negative	CV Negative
BV/CV	SB_BVCVInv_01	NA	Y40T	Swab	**NA**	Invalid	Invalid
BV/CV	SB_BVCVNegPos_B1	NA		Swab	**NA**	BV Negative	CV Positive
BV/CV	SB_BVCVPos_B2	NA		Swab	**NA**	BV Positive	CV Positive
BV/CV	SB_BVCVPosNeg_A6	NA		Swab	**NA**	BV Positive	CV Negative
BV/CV	SB_BVCVPosInv_01	NA	C01H2	Swab	**NA**	BV Positive	Invalid
BV/CV	SB_BVCVInvPos_A2	NA	C01H1	Swab	**NA**	Invalid	CV Positive
BV/CV	BVCV_BVneg_CVinv	NA	C02H2	Swab	**NA**	BV Negative	Invalid
BV/CV	BVCV_BVinv_CVneg	NA	C02H1	Swab	**NA**	Invalid	CV Negative
BV/CV	C161420284090428828404	Yes		(-) Ctrl	**Valid**	Valid	Valid
BV/CV	C161420284093009580264	Yes		BV/CV (+) C	**Valid**	Valid	Valid

Figure 5 Example of **cobas**® BV results display for BV result request for **cobas**® 6800/8800 systems with software version 1.4

Test	Sample ID	Valid	Flags	Sample type	Overall result	Target 1
BV	SB_BVInv_01	NA	Y40T	Swab	**NA**	Invalid
BV	SB_BVPos_A5	NA		Swab	**NA**	BV Positive
BV	SB_BVNeg_01	NA		Swab	**NA**	BV Negative
BV	C161420284093009580263	Yes		(-) Ctrl	**Valid**	Valid
BV	C161420284090428828403	Yes		BV/CV (+) C	**Valid**	Valid

Figure 6 Example of **cobas**® CV results display for CV result request for **cobas**® 6800/8800 systems with software version 1.4

Test	Sample ID	Valid	Flags	Sample type	Overall result	Target 1
CV	SB_ CVNeg_A1	NA		Swab	**NA**	CV Negative
CV	SB_CVInv_01	NA	Y40T	Swab	**NA**	Invalid
CV	SB_CVPos_A2	NA		Swab	**NA**	CV Positive
CV	C16142028409300950734	Yes		BV/CV (+) C	**Valid**	Valid
CV	C161420284090428828402	Yes		(-) Ctrl	**Valid**	Valid

For a valid batch, check each individual sample for flags in the **cobas**® 6800/8800 systems with software version 1.4 and/or report. The result interpretation should be as follows:

- A valid batch may include both valid and invalid sample results.
- The "Valid" and "Overall Result" columns are not applicable (NA) to sample results for the **cobas**® BV/CV and are marked with "NA". Values reported in these columns are not applicable and **do not** impact the validity of results reported within individual Target Result columns.
- Reported target results for individual samples are valid unless indicated as "Invalid" within the individual target result column.
- Invalid results for one or more target combinations are possible with the BV/CV result request and are reported out specifically for each target. Refer to the retesting instructions for respective specimen type.
- Results of this test should only be interpreted in conjunction with information available from clinical evaluation of the patient and patient history.

Interpretation of results for cobas® 5800/6800/8800 systems

Results and their corresponding interpretation for detecting BV and CV (Table 17), BV only (Table 18) and CV only (Table 19) are shown in Table 17 to Table 19.

cobas® BV/CV uses algorithms to report qualitative results for BV and CV. The BV algorithm integrates data from multiple bacterial markers associated with Bacterial Vaginosis (BV) to calculate the final BV result. The CV algorithm is a single target detection (*Candida spp.*) associated with Candida vaginitis (CV).

Table 17 cobas® BV/CV results and interpretation for the BV/CV result request

Result		Interpretation
BV Positive	CV Positive	All requested results were valid. Sample is positive for BV and CV.
BV Positive	CV Negative	All requested results were valid. Sample is positive for BV. Sample is negative for CV.
BV Negative	CV Positive	All requested results were valid. Sample is negative for BV. Sample is positive for CV.
BV Negative	CV Negative	All requested results were valid. Sample is negative for BV or CV.
BV Positive	Invalid	Not all requested results were valid. Sample is positive for BV. BV result is valid. CV result is invalid. Original specimen should be re-tested to obtain valid CV results. If the result is still invalid, a new specimen should be obtained.

Result		Interpretation
Invalid	CV Positive	Not all requested results were valid. BV result is invalid. Original specimen should be re-tested to obtain valid BV results. If the result is still invalid, a new specimen should be obtained. Sample is positive for CV. CV result is valid.
BV Negative	Invalid	Not all requested results were valid. Sample is negative for BV. BV result is valid. CV result is invalid. Original specimen should be re-tested to obtain valid CV results. If the result is still invalid, a new specimen should be obtained.
Invalid	CV Negative	Not all requested results were valid. BV result is invalid. Original specimen should be re-tested to obtain valid BV results. If the result is still invalid, a new specimen should be obtained. Sample is negative for CV. CV result is valid.
Invalid	Invalid	Both BV and CV results are invalid. Original specimen should be re-tested to obtain valid BV and CV results. If the results are still invalid, a new specimen should be obtained.

Table 18 cobas® BV/CV results and interpretation for the BV result request

Result	Interpretation
BV Positive	The requested result was valid. Sample is positive for BV
BV Negative	The requested result was valid. Sample is negative for BV
Invalid	BV result is invalid. Original specimen should be re-tested to obtain valid BV results. If the result is still invalid, a new specimen should be obtained.

Table 19 cobas® BV/CV results and interpretation for the CV result request

Result	Interpretation
CV Positive	The requested result was valid. Sample is positive for CV
CV Negative	The requested result was valid. Sample is negative for CV
Invalid	CV result is invalid. Original specimen should be re-tested to obtain valid CV results. If the result is still invalid, a new specimen should be obtained.

Procedural limitations

- Use of this product must be limited to personnel trained in the techniques of PCR and the use of the **cobas**® 5800 system or **cobas**® 6800/8800 systems.
- **cobas**® BV/CV has been evaluated only for use in combination with the **cobas**® BV/CV Control Kit, **cobas**® Buffer Negative Control Kit, **cobas**® **omni** MGP Reagent, **cobas**® **omni** Lysis Reagent, **cobas**® **omni** Specimen Diluent, and **cobas**® **omni** Wash Reagent for use on the **cobas**® 5800/6800/8800 systems.
- Reliable results depend on proper sample collection, storage and handling procedures.
- Products containing carbomer(s), including vaginal lubricants, creams and gels may interfere with the test and should not be used during or prior to collecting vaginal swab specimens. See Interference results (Table 26) for further details.
- **cobas**® BV/CV has been validated for use with clinician-instructed self-collected vaginal swab specimens and

clinician-collected vaginal swab specimens. Assay performance has not been established with other collection media and/or specimen types. Use of other collection media and/or specimen types may lead to false positive, false negative or invalid results.

- **cobas**® BV/CV has not been evaluated in patients younger than 18 years of age.
- Detection of bacteria and yeast associated with BV and CV is dependent on the number of organisms present in the specimen and may be affected by specimen collection methods, patient factors (i.e., age, history of STI, presence of symptoms), stage of infection and/or infecting *Candida*, *Gardnerella vaginalis, Atopobium vaginae*, and *Lactobacillus* strains.
- The effects of other potential variables such as vaginal discharge, use of tampons, douching, etc. and specimen collection variables have not been evaluated.
- **cobas**® BV/CV has not been evaluated with patients who were currently being treated with antimicrobial agents active against BV or CV as well as patients with a history of hysterectomy.
- False negative or invalid results may occur due to polymerase inhibition. The Internal Control is included in **cobas**® BV/CV to help identify specimens which contain substances that may interfere with nucleic acid isolation and PCR amplification.
- The addition of AmpErase enzyme into the **cobas**® BV/CV Master Mix reagent enables selective amplification of target DNA; however, good laboratory practices and careful adherence to the procedures specified in this Instructions-For-Use document are necessary to avoid contamination of reagents.
- Though rare, mutations within the highly conserved regions of the genomic DNA of *Candida*, *Gardnerella vaginalis, Atopobium vaginae*, and *Lactobacillus* strains covered by **cobas**® BV/CV primers and/or probes may result in failure to detect the presence of the bacterium.
- Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to the next; users perform method correlation studies in their laboratory to qualify technology differences.
- This assay is intended for use in symptomatic individuals. BV and/or CV detected results in asymptomatic individuals may not be clinically significant.

Non-clinical performance evaluation

Assay performance equivalency between the cobas® 6800/8800 and cobas® 5800 systems

Assay performance equivalency between the **cobas**®6800/8800 and **cobas**® 5800 systems was demonstrated through performance studies. The data presented herein are applicable to the cobas® BV/CV test across all instruments.

Key performance characteristics

Limit of Detection (LoD)

The limit of detection of **cobas**® BV/CV was determined by analysis of serial dilutions of one *Atopobium vaginae*, one *Gardnerella vaginalis*, three *Lactobacillus* species and six Candida species. Panels of five to six concentration levels plus a blank were tested over three lots of **cobas**® BV/CV test reagents, multiple runs, days, operators, and instruments. The LoD for BV and CV targets are presented in Table 20.

Table 20 Limit of Detection for individual **cobas**® BV/CV targets

Target	Strain / Isolate	LoD by Hit Rate ≥95% (CFU/mL)	95% LoD PROBIT (CFU/mL)	Mean Ct at LoD by Hit Rate ≥ 95%
Lactobacillus crispatus	*Lactobacillus crispatus 33820 (ATCC)*	1.50E+00	1.64E+00 (95% CI: 1.38E+00 – 2.09E+00)	34.90
Lactobacillus gasseri	*Lactobacillus gasseri* HM-644 (BEI Resources)	1.60E+02	1.14E+02 (95% CI: 9.12E+01 – 1.54E+02)	34.24
Lactobacillus jensenii	*Lactobacillus jensenii* HM-374 (BEI Resources)	8.70E+01	8.51E+01 (95% CI: 6.73E+01 – 1.25E+02)	34.60
Gardnerella vaginalis	*Gardnerella vaginalis* 72422 (CCUG)	5.00E+02	3.44E+02 (95% CI: 2.93E+02 – 4.32E+02)	32.14
Atopobium vaginae	*Atopobium vaginae* 55227 (CCUG)	1.50E+02	1.35E+02 (95% CI: 1.08E+02 – 1.95E+02)	34.83
Candida parapsilosis	*Candida parapsilosis* 22019 (ATCC)	1.60E+02	1.41E+02 (95% CI: 1.10E+02 – 2.00E+02)	33.24
Candida tropicalis	*Candida tropicalis* 750 (ATCC)	6.67E+01	5.66E+01 (95% CI: 4.58E+01 – 7.61E+01)	33.79
Candida dubliniensis	*Candida dubliniensis* MYA-646 (ATCC)	1.20E+03	8.19E+02 (95% CI: 6.59E+02 – 1.12E+03)	33.29
Candida krusei	*Candida krusei* 14243 (ATCC)	1.50E+02	9.39E+01 (95% CI: 7.68E+01 – 1.23E+02)	33.82
Candida albicans	*Candida albicans* 14053 (ATCC)	7.00E+02	6.77E+02 (95% CI: 5.34E+02 – 9.30E+02)	33.36
Candida glabrata	*Candida glabrata* MYA-275 (ATCC)	7.00E+02	3.52E+02 (95% CI: 2.75E+02 – 5.01E+02)	31.86

Table 21 provides a summary of near clinical cutoff concentrations for BV target combinations used in various non-clinical performance studies.

Table 21 Near clinical cut-off concentration – BV targets

Target	Strain / Isolate	Near Cut-off Concentration (CFU/mL)
BV	*Atopobium vaginae* 55227 (CCUG) *Gardnerella vaginalis* 72422 (CCUG)	*A. vaginae* = 2.01E+03 *G. vaginalis* = 2.15E+04
	Atopobium vaginae 55227 (CCUG) *Gardnerella vaginalis* 72422 (CCUG) *Lactobacillus crispatus 33820 (ATCC)*	*A. vaginae* = 7.41E+04 *G. vaginalis* = 1.52E+06 *L. crispatus* = 2.98E+01

Inclusivity

The inclusivity of **cobas**® BV/CV was confirmed by testing 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*) strains. All BV strains were detected at approximately 3x LoD compared to the respective LoD strain and all CV strains at approximately 3-8x LoD compared to the respective LoD strain.

Precision (within laboratory)

In-house precision was examined using BV and CV cultures diluted into simulated clinical matrix stabilized in **cobas**® PCR Media. Sources of variability were examined with a panel consisting of three concentration levels per CV strain or co-formulated BV target plus blank, using three lots of **cobas**® BV/CV reagents and three instruments over a time course of 18 days and with a total of 36 runs. A description of the precision panels and the study performance positivity rates are shown in Table 22. All negative panel members tested negative throughout the study. Analysis of standard deviation and percent coefficient of variation of the BV probability and CV Ct values from valid tests performed on positive panel members are presented in Table 23 and Table 24.

Table 22 Summary of within laboratory precision

Target	Target Species Details	Level/ Concentration	Number of Positive / Valid Replicates	Hit Rate	95% two-sided CI (Clopper-Pearson)
BV	Condition A*	Below cutoff (PM 2)	108 / 108	100.00%	96.64 - 100.00%
		Near cutoff (PM 5)	108 / 108	100.00%	96.64 - 100.00%
		Above cutoff (PM 8)	108 / 108	100.00%	96.64 - 100.00%
	Condition B*	Below cutoff (PM 3)	87 / 108	80.56%	71.83 - 87.54%
		Near cutoff (PM 6)	108 / 108	100.00%	96.64 - 100.00%
		Above cutoff (PM 9)	108 / 108	100.00%	96.64 - 100.00%
	Condition C*	Below cutoff (PM 4)	107 / 108	99.07%	94.95 - 99.98%
		Near cutoff (PM 7)	108 / 108	100.00%	96.64 - 100.00%
		Above cutoff (PM 10)	108 / 108	100.00%	96.64 - 100.00%
CV	*C. albicans*	~ 0.3X LoD (PM 11)	86 / 108	79.63%	70.80 – 86.77%
		~ 1X LoD (PM 14)	108 / 108	100.00%	96.64 - 100.00%
		~ 3X LoD (PM 17)	108 / 108	100.00%	96.64 - 100.00%

Target	Target Species Details	Level/ Concentration	Number of Positive / Valid Replicates	Hit Rate	95% two-sided CI (Clopper-Pearson)
	C. glabrata	~ 0.3X LoD (PM 12)	107/ 108	99.07%	94.95 - 99.98%
		~ 1X LoD (PM 15)	108 / 108	100.00%	96.64 - 100.00%
		~ 3X LoD (PM 18)	108 / 108	100.00%	96.64 - 100.00%
	C. krusei	~ 0.3X LoD (PM 13)	96 / 108	88.89%	81.40 – 94.13%
		~ 1X LoD (PM 16)	108 / 108	100.00%	96.64 - 100.00%
		~ 3X LoD (PM 19)	108 / 108	100.00%	96.64 - 100.00%

*BV composition A and C consists of three microorganisms (*Lactobacillus crispatus*, *Gardnerella vaginalis*, and *Atopobium vaginae*), with different concentrations for each target comparing Condition A and Condition C. BV composition B consists of two microorganisms (*Gardnerella vaginalis* and *Atopobium vaginae*).

Table 23 Overall mean, standard deviations and coefficients of variation (%) for cycle threshold, BV positive panels

Composition*	Level	Hit Rate	BV Call Mean Probability	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%
A	**Below cutoff**	100.00%	62.92%	0.03	4.23	0.01	2.18	0.02	2.60	0.00	0.69	0.04	6.18	0.05	8.25
B	**Below cutoff**	100.00%	57.96%	0.02	3.33	0.02	3.39	0.00	0.00	0.03	4.87	0.03	5.12	0.04	6.12
C	**Below cutoff**	82.41%	72.44%	0.02	2.86	0.00	0.00	0.03	3.78	0.00	0.00	0.15	21.16	0.16	21.68
A	**Near cutoff**	99.07%	76.92%	0.01	1.48	0.02	2.92	0.01	1.59	0.00	0.00	0.03	4.09	0.04	5.47
B	**Near cutoff**	100.00%	76.24%	0.01	1.40	0.00	0.00	0.01	1.51	0.00	0.00	0.05	6.93	0.05	7.20
C	**Near cutoff**	99.54%	90.13%	0.00	0.44	0.00	0.38	0.00	0.31	0.00	0.00	0.02	2.29	0.02	2.68
A	**Above cutoff**	100.00%	95.69%	0.00	0.30	0.00	0.48	0.00	0.29	0.00	0.00	0.01	0.82	0.01	1.04
B	**Above cutoff**	100.00%	97.54%	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.01	0.52	0.01	0.52
C	**Above cutoff**	100.00%	98.69%	0.00	0.05	0.00	0.10	0.00	0.16	0.00	0.05	0.00	0.31	0.00	0.38

*BV composition A and C consists of three microorganisms (*Lactobacillus crispatus*, *Gardnerella vaginalis*, and *Atopobium vaginae*), with different concentrations for each target comparing Condition A and Condition C. BV composition B consists of two microorganisms (*Gardnerella vaginalis*, and *Atopobium vaginae*).

Table 24 Overall mean, standard deviations and coefficients of variation (%) for cycle threshold, CV positive panels

Target	Target Conc.	Hit Rate	Mean Ct	Instrument-to-Instrument / Operator-to-Operator		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	**~0.3X LoD**	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	**~0.3X LoD**	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	**~0.3X LoD**	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	**~1X LoD**	100.00%	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	**~1X LoD**	100.00%	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	**~1X LoD**	100.00%	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	**~3X LoD**	100.00%	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	**~3X LoD**	100.00%	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	**~3X LoD**	100.00%	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

Analytical specificity/cross reactivity

A panel of 105 bacteria, fungi, viruses, parasites and plasmids were tested with **cobas**® BV/CV to assess analytical specificity. The organisms listed in Table **25** were spiked at concentrations of approximately 1 x 10^6 units/mL for bacteria, fungi, parasites and plasmid and approximately 1 x 10^5 units/mL for viruses into simulated clinical matrix stabilized in **cobas**® PCR Media. Testing was performed with each potential interfering organism in absence and presence of BV and CV target (spiked at approximately 3x LoD). Tested microorganisms did not interfere with **cobas**® BV/CV with respect to sensitivity (100%) and specificity (100%).

Table 25 Microorganisms tested for analytical specificity/cross reactivity

Microorganism	Concentration Tested	Microorganism	Concentration Tested
Acinetobacter baumannii	1.00E+06 CFU/mL	*Kocuria rhizophila*	1.00E+06 CFU/mL
Acinetobacter calcoaceticus	1.00E+06 CFU/mL	*Kodamaea ohmeri*	6.00E+05 CFU/mL
Actinomyces israelii	1.00E+06 CFU/mL	*Lactobacillus iners*	1.00E+06 CFU/mL
Aerococcus viridans	1.00E+06 CFU/mL	*Legionella pneumophila*	1.00E+06 CFU/mL
Alcaligenes faecalis	1.00E+06 CFU/mL	*Mageeibacillus indolicus*	1.00E+06 CFU/mL
Anaerococcus tetradius	1.00E+06 CFU/mL	*Megasphaera elsdenii*	1.00E+06 CFU/mL
Atopobium minutum	1.00E+06 CFU/mL	*Mobiluncus curtisii*	1.00E+06 CFU/mL
Atopobium parvulum	1.00E+06 CFU/mL	*Mobiluncus mulieris*	1.00E+06 CFU/mL
Atopobium rimae	1.00E+06 CFU/mL	*Moraxella catarrhalis*	1.00E+06 CFU/mL
Bacillus subtilis	1.00E+06 CFU/mL	*Morganella morganii*	1.00E+06 CFU/mL
Bacteroides caccae	1.00E+06 CFU/mL	*Mycobacterium smegmatis*	1.00E+06 CFU/mL
Bacteroides fragilis	1.00E+06 CFU/mL	*Mycoplasma genitalium*	1.00E+06 CFU/mL
Bacteroides stercoris	1.00E+06 CFU/mL	*Mycoplasma hominis*	1.00E+06 CFU/mL
Bacteroides ureolyticus / Campylobacter ureolyticus	1.00E+06 CFU/mL	*Neisseria gonorrhoeae*	1.00E+06 CFU/mL
Bifidobacterium adolescentis	1.00E+06 CFU/mL	*Olsenella uli*	1.00E+06 CFU/mL
Bifidobacterium breve	1.00E+06 CFU/mL	*Pantoea agglomerans*	1.00E+06 CFU/mL
Bifidobacterium longum	1.00E+06 CFU/mL	*Pentatrichomonas hominis*	1.00E+06 CFU/mL
Brevibacterium linens	1.00E+06 CFU/mL	*Peptoniphilus asaccharolyticus*	1.00E+06 CFU/mL
Burkholderia cepacia	1.00E+06 CFU/mL	*Peptostreptococcus anaerobius*	1.00E+06 CFU/mL
BVAB1 plasmid	1.00E+06 cp/mL	*Pichia fermentans*	5.60E+05 CFU/mL
Campylobacter jejuni	1.00E+06 CFU/mL	*Pichia norvegensis*	1.00E+06 CFU/mL

Microorganism	Concentration Tested	Microorganism	Concentration Tested
Candida catenulata / Diutina catenulata	1.00E+06 CFU/mL	*Pichia occidentalis*	1.00E+06 CFU/mL
Candida famata	1.00E+06 CFU/mL	*Plesiomonas shigelloides*	1.00E+06 CFU/mL
Candida haemulonii	1.00E+06 CFU/mL	*Porphyromonas asaccharolytica*	1.00E+06 CFU/mL
Candida inconspicua	1.00E+06 CFU/mL	*Prevotella bivia*	1.00E+06 CFU/mL
Candida intermedia	1.00E+06 CFU/mL	*Prevotella melaninogenica*	1.00E+06 CFU/mL
Candida kefyr	1.00E+06 CFU/mL	*Prevotella oralis / Hoylesella oralis*	1.00E+06 CFU/mL
Candida lusitaniae	1.00E+06 CFU/mL	*Propionibacterium acnes*	1.00E+06 CFU/mL
Candida norvegica	1.00E+06 CFU/mL	*Proteus mirabilis*	1.00E+06 CFU/mL
Candida rugosa / Diotima rugosa	1.00E+06 CFU/mL	*Providencia stuartii*	1.00E+06 CFU/mL
Candida utilis	1.00E+06 CFU/mL	*Pseudomonas aeruginosa*	1.00E+06 CFU/mL
Chlamydia trachomatis	1.00E+06 CFU/mL	*Saccharomyces cerevisiae*	1.00E+06 CFU/mL
Citrobacter freundii	1.00E+06 CFU/mL	*Salmonella typhimurium*	1.00E+06 CFU/mL
Clostridium perfringens	1.00E+06 CFU/mL	*Serratia marcescens*	1.00E+06 CFU/mL
Corynebacterium genitalium	1.00E+06 CFU/mL	*Shigella flexneri*	1.00E+06 CFU/mL
Dialister micraerophilus	1.00E+06 CFU/mL	*Sneathia sanguinegens*	1.00E+06 CFU/mL
Eikenella corrodens	1.00E+06 CFU/mL	*Sneathia vaginalis*	1.00E+06 CFU/mL
Enterobacter aerogenes	1.00E+06 CFU/mL	*Staphylococcus aureus*	1.00E+06 CFU/mL
Enterococcus faecalis	1.00E+06 CFU/mL	*Staphylococcus epidermidis*	1.00E+06 CFU/mL
Enterococcus faecium	1.00E+06 CFU/mL	*Streptococcus agalactiae*	1.00E+06 CFU/mL
Erysipelothrix rhusiopathiae	1.00E+06 CFU/mL	*Streptococcus mitis*	1.00E+06 CFU/mL
Escherichia coli	1.00E+06 CFU/mL	*Streptococcus mutans*	1.00E+06 CFU/mL
Finegoldia magna	1.00E+06 CFU/mL	*Streptococcus salivarius*	1.00E+06 CFU/mL
Fusobacterium nucleatum	1.00E+06 CFU/mL	*Treponema pallidum*	1.00E+06 CFU/mL
Gemella haemolysans	1.00E+06 CFU/mL	*Trichomonas tenax*	1.00E+06 CFU/mL
Hepatitis B virus	1.00E+05 IU/mL	*Trichomonas vaginalis*	2.30E+05 CFU/mL
Hepatitis C virus	1.00E+05 IU/mL	*Trueperella pyogenes / Actinomyces pyogenes / Corynebacterium pyogenes*	1.00E+06 CFU/mL
Herpes simplex virus I / HSV type 1	1.00E+05 IU/mL	Varicella-zoster virus	1.00E+05 IU/mL
Herpes simplex virus II / Human herpesvirus 2	1.00E+05 IU/mL	*Veillonella atypica*	1.00E+06 CFU/mL
HIV-1	1.00E+05 IU/mL	*Veillonella parvula*	1.00E+06 CFU/mL
Human papilloma virus	1.00E+05 IU/mL	*Vibrio parahaemolyticus*	1.00E+06 CFU/mL
Kingella denitrificans	1.00E+06 CFU/mL	*Yersinia enterocolitica*	1.00E+06 CFU/mL
Klebsiella pneumoniae	1.00E+06 CFU/mL	-	-

NOTE: *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

The effect of over-the-counter and prescription products that may be present in vaginal swab specimens (Table 26) were evaluated with **cobas**® BV/CV. Testing was done using simulated clinical matrix stabilized in **cobas**® PCR media and spiked with potential interferents at levels expected from normal patient usage and in absence and presence of BV and CV target (spiked at approximately 3x LoD).

Of the products tested, Crinone® 8% Vaginal Gel, Gynaedron®, Metronidazole Vaginal Gel, NUVESSA®, RepHresh™ Odor Eliminating Gel and Vagisil produced false negative or invalid results. These products contain carbomer(s) or polycarbophil. Products containing carbomer(s) or polycarbophil(s) have been shown to generate false negative and invalid results. Utrogestan® interfered with the specificity for CV, but not the overall sensitivity. Table 26 lists the substances tested for interference and is not intended to be a comprehensive list of carbomer or polycarbophil containing products.

Table 26 List of substances tested for interference with **cobas**® BV/CV

Product Name		
Acyclovir Cream	Gynofit	RepHresh™ Odor Eliminating Gel*
AFI LactoSpore®	Imiquimod Cream	Summer's Eve Ultimate Odor Control Spray
Arilin®**	K-Y Ultragel	Tioconazole 1 Ointment
Cleocin®	Lactayd®	Utrogestan®*
Clotrimazole	Monistat	VCF Vaginal Contraceptive Gel
Crinone® 8% Vaginal Gel*	Metronidazole Vaginal Gel*	Vagisan
Daktarin®	NutraBlast Boric Acid Vaginal Supp.	Vagisil*
Estradiol Vaginal Cream	Norforms	YeastGard Advanced
Globe Hemorrhoidal Cream	NUVESSA®*	-
Gynaedron®*	Premarin®	-

* Crinone® 8% Vaginal Gel, Gynaedron®, Metronidazole Vaginal Gel, NUVESSA®, RepHresh™ Odor Eliminating Gel, Utrogestan® and Vagisil showed interference at levels that may be present in vaginal swab specimens.
**Product containing metronidazole which did not show interference

Endogenous substances which may be present in vaginal specimens were tested for interference. Testing was done using simulated clinical matrix stabilized in **cobas**® PCR media and spiked with potential interferents at elevated levels and in the absence and presence of BV and CV target (spiked at approximately 3x LoD).

None of the tested substances interfered with **cobas**® BV/CV performance by generating false-negative or false-positive results. The levels of endogenous substances tolerated by the assay are shown in Table 27.

Table 27 Summary of endogenous substance concentrations that do not show interference

Interferent	Concentration
Whole Blood	44.86 mg/mL*
Leukocytes	36.50 mg/mL*
Mucin	0.20% - 0.50%
Seminal fluid	42.32 mg/mL*

*Concentration is equivalent to a fully covered swab stabilized into 4.3 mL of **cobas**® PCR media

Competitive inhibition

To assess competitive inhibition between BV and CV targets, low and moderate concentrations of one target were mixed with very high concentrations of the opposite target. Low and moderate concentrations were defined as approximately 1x LoD and approximately 3x LoD, respectively, and high concentrations were defined at approximately 1E+06 CFU/mL.

Testing results indicated that when BV targets were present at a high concentration, CV was detected at both low and moderate levels. Results also indicated that when CV was present at a high concentration, BV was detected at both 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.

Whole system failure

The samples tested in the whole system failure study were simulated clinical matrix stabilized in **cobas**® PCR Media, co-formulated with BV and CV target to a concentration of approximately 3x LoD of the respective target. All tested replicates were valid and positive for both BV and CV, resulting in a whole system failure rate of 0%.

Cross contamination

Cross-contamination can cause false positive results. The sample to sample cross-contamination rate of **cobas**® BV/CV was determined to be 0.0% (0/240) when alternating very high positive and negative samples over multiple runs. Testing was done using samples prepared with simulated clinical matrix stabilized in **cobas**® PCR Media. High positive samples in the study were co-formulated with *Gardnerella vaginalis* and *Candida albicans* to generate a Ct-value for each target that would exceed 95% or more of signal observed in specimens of infected patients in the intended use population.

Clinical performance evaluation

The clinical performance of **cobas**® BV/CV was established in a multi-center, prospective clinical study by comparing the results to a Patient Infected Status (PIS) based on a combination of comparator reference tests for BV and based on culture for CV. The CV results from **cobas**® BV/CV were also compared to a Composite Reference Standard (CRS) established from the results of 3 CV NAATs.

716 total subjects were enrolled in this study, with 692 evaluable subjects included in the final data set. Among the 692 evaluable subjects, 538 were symptomatic and 154 were asymptomatic, which were from different sites in the US and Europe.

Each symptomatic subject had six vaginal swabs collected.

- One self-collected vaginal swab for **cobas**® BV/CV testing
- Four clinician-collected vaginal swabs (randomized in collection sequence) for NAAT1, NAAT2, NAAT3, and culture/MALDI-TOF testing
- One clinician-collected vaginal swab for **cobas**® BV/CV testing

Each asymptomatic subject had 1 clinician-collected vaginal swab for **cobas**® BV/CV testing only.

The BV PIS and CV CRS were determined as shown in Table 28 and the CV PIS was determined as shown in Table 29.

Table 28 Determination of BV PIS and CV CRS

-		NAAT2	NAAT3		
			Positive	**Negative**	**Invalid**
NAAT1	**Positive**	**Positive**	Infected	Infected	Infected
		Negative	Infected	Non-Infected	Indeterminate
		Invalid	Infected	Indeterminate	Indeterminate
	Negative	**Positive**	Infected	Non-Infected	Indeterminate
		Negative	Non-Infected	Non-Infected	Non-Infected
		Invalid	Indeterminate	Non-Infected	Indeterminate
	Invalid	**Positive**	Infected	Indeterminate	Indeterminate
		Negative	Indeterminate	Non-Infected	Indeterminate
		Invalid	Indeterminate	Indeterminate	Indeterminate

NAAT: nucleic acid amplification test.

Table 29 Determination of CV PIS

Culture	MALDI-TOF	CV PIS
Positive	Isolate determined to be one of the following *Candida* species: *C. albicans*, *C. dubliniensis*, *C. glabrata*, *C. krusei*, *C. tropicalis*, and *C. parapsilosis*	Positive
Positive	Isolate determined to be a *Candida* species other than *C. albicans*, *C. dubliniensis*, *C. glabrata*, *C. krusei*, *C. tropicalis*, and *C. parapsilosis*	Non-evaluable
Positive	Isolate determined to be a genus other than *Candida* spp.	Negative
Negative	Not applicable	Negative

CV: Candida vaginitis; MALDI-TOF: matrix-assisted laser desorption/ionization time-of-flight; PIS: patient infection status.

Symptomatic patients had a clinical presentation consistent with vaginitis, vaginosis, or both. Although not an inclusive list, symptoms may have included abnormal vaginal discharge, painful or frequent urination, vaginal itching or burning or irritation, painful or uncomfortable intercourse, and/or abnormal vaginal odor.

The clinical performance of **cobas**® BV/CV:

The PIS for BV was based on the results from the 3 comparator NAATs and are summarized below and presented in Table 30. Of the 552 subjects included in the study, 224 (40.6%) were infected for BV; 314 (56.9%) were Non-Infected; and 14 (2.5%) were indeterminate. Of the 224 subjects infected by BV, 181 (80.8%) were positive by all 3 NAATs. Of the 314 subjects that were Non-Infected, 249 (79.3%) were negative by all 3 NAATs.

- BV performance compared to the BV PIS ("two-out-of-three" rule with BV NAATs) as shown in Table 30:
    - For clinician – collected specimens (CC):
        - Clinical Sensitivity was 95.9% (213/222; 95% CI: 92.5%, 97.9%)
        - Clinical Specificity was 93.8% (289/308; 95% CI: 90.6%, 96.0%)
        - The PPV and NPV were 91.8% (213/232; 95% CI: 87.6%, 94.7%) and 97.0% (289/298; 95% CI: 94.4%, 98.4%), respectively
        - Prevalence was 43.8%
    - For self – collected specimens (SS):
        - Clinical Sensitivity was 96.8% (214/221; 95% CI: 93.6%, 98.5%)
        - Clinical Specificity was 92.8% (285/307; 95% CI: 89.4%, 95.2%)
        - The PPV and NPV were 90.7% (214/236; 95% CI: 86.3%, 93.8%) and 97.6% (285/292; 95% CI: 95.1%, 98.8%), respectively
        - Prevalence was 44.7%

For the CV PIS (based on culture/MALDI-TOF), there were 124 infected subjects, that were positive by culture and MALDI–TOF. The particular *Candida* species identified from CV Infected subjects are as follows: 67.7% (84/124) *Candida albicans*; 15.3% (19/124) *Candida glabrata*; 4.8% (6/124) *Candida parapsilosis*; 1.6% (2/124) *Candida krusei* and *Candida dubliniensis*; and 0.8% (1/124) *Candida tropicalis*. There were 361 CV Non-Infected subjects, of which 359 (99.4%) were negative on culture and 2 (0.6%) had growth on culture but the final yeast identifications were not *Candida* spp. (*Saccharomyces cerevisiae* and *Rhodotorula mucilaginosa*).

- CV performance compared to the CV PIS (culture/MALDI-TOF) as shown in Table 30:
    - For clinician – collected specimens (CC):
        - Clinical Sensitivity was 87.6% (106/121; 95% CI: 80.6%, 92.3%)
        - Clinical Specificity was 98.0% (349/356; 95% CI: 96.0%, 99.0%)
        - The PPV and NPV were 93.8% (106/113; 95% CI: 87.8%, 97.0%) and 95.9% (349/364; 95% CI: 93.3%, 97.5%), respectively
        - Prevalence was 23.7%
    - For self – collected specimens (SS):
        - Clinical Sensitivity was 89.3% (108/121; 95% CI: 82.5%, 93.6%)
        - Clinical Specificity was 92.4% (326/353; 95% CI: 89.1%, 94.7%)
        - The PPV and NPV were 80.0% (108/135; 95% CI 72.5%, 85.9%) and 96.2% (326/339; 95% CI: 93.6%, 97.7%), respectively
        - Prevalence was 28.5%

The composite reference standard (CRS) for CV was based on the results from 3 NAATs. Of the 552 subjects included in the study, 131 (27.3%) were Infected for CV; 411 (74.5%) were Non-Infected; and 10 (1.8%) were indeterminate. Of the 131 subjects infected by CV, 108 (82.4%) were positive by all 3 NAATs. Of the 411 subjects that were Non-Infected, 353 (85.9%) were negative by all 3 NAATs.

- CV performance compared to the CV CRS ("two-out-of-three" rule with CV NAATs) as shown in Table 30:
    - For clinician – collected specimens (CC):
        - Clinical Sensitivity was 93.0% (119/128; 95% CI: 87.2%, 96.3%)
        - Clinical Specificity was 97.8% (397/406; 95% CI: 95.8%, 98.8%)
        - The PPV and NPV were 93.0% (119/128; 95% CI: 87.2%, 96.3%) and 97.8% (397/406; 95% CI: 95.8%, 98.8%), respectively
        - Prevalence was 24.0%
    - For self – collected specimens (SS):
        - Clinical Sensitivity was 94.5% (120/127; 95% CI: 89.1%, 97.3%)
        - Clinical Specificity was 92.8% (375/404; 95% CI: 89.9%, 95.0%)
        - The PPV and NPV were reported as 80.5% (120/149; 95% CI: 73.4%, 86.1%) and 98.2% (375/382; 95% CI: 96.3%, 99.1%), respectively
        - Prevalence was 28.1%

Table 30 Summary of **cobas**® BV/CV Performance by collection method

-	Collection Method	Total N	SENS	95% Score CI	SPEC	95% Score CI	PREV (%)	PPV	95% Score CI	NPV	95% Score CI
BV (by PIS)	CC	530	95.9% (213/222)	(92.5%, 97.9%)	93.8% (289/308)	(90.6%, 96.0%)	43.8%	91.8% (213/232)	(87.6%, 94.7%)	97.0% (289/298)	(94.4%, 98.4%)
	SS	528	96.8% (214/221)	(93.6%, 98.5%)	92.8% (285/307)	(89.4%, 95.2%)	44.7%	90.7% (214/236)	(86.3%, 93.8%)	97.6% (285/292)	(95.1%, 98.8%)
CV (by PIS)	CC	477	87.6% (106/121)	(80.6%, 92.3%)	98.0% (349/356)	(96.0%, 99.0%)	23.7%	93.8% (106/113)	(87.8%, 97.0%)	95.9% (349/364)	(93.3%, 97.5%)
	SS	474	89.3% (108/121)	(82.5%, 93.6%)	92.4% (326/353)	(89.1%, 94.7%)	28.5%	80.0% (108/135)	(72.5%, 85.9%)	96.2% (326/339)	(93.6%, 97.7%)
CV (by CRS)	CC	534	93.0% (119/128)	(87.2%, 96.3%)	97.8% (397/406)	(95.8%, 98.8%)	24.0%	93.0% (119/128)	(87.2%, 96.3%)	97.8% (397/406)	(95.8%, 98.8%)
	SS	531	94.5% (120/127)	(89.1%, 97.3%)	92.8% (375/404)	(89.9%, 95.0%)	28.1%	80.5% (120/149)	(73.4%, 86.1%)	98.2% (375/382)	(96.3%, 99.1%)

Note: N is the total number of evaluable subjects; CC = Clinician-collected; SS = Self-collected, CI = confidence interval, NPV = negative predictive value, PPV = positive predictive value, PREV = prevalence, SENS = sensitivity, SPEC = specificity, PIS = Patient Infection Status, CRS = Composite Reference Standard

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.

Prevalence of BV and CV in Asymptomatic Individuals

The BV and CV organisms that are detected by **cobas**® BV/CV are also considered part of the normal vaginal microbiome. The development of BV and CV is a result of a microbiome imbalance, and the organism loads can increase in a diseased state compared to normal, healthy levels. The detection of these organisms by any highly sensitive and specific NAAT represents an inherent challenge. It is therefore important to understand how any diagnostic NAAT performs in an asymptomatic patient population. The diagnostic utility is highest for any BV and CV NAAT when testing individuals that are presenting with vaginitis/vaginosis symptoms.

There were 154 asymptomatic subjects enrolled and tested using **cobas**® BV/CV. The overall prevalence of BV and CV in the asymptomatic population was 32.5% and 12.3%.

Table 31 Prevalence of BV and CV in the Asymptomatic Population

Analyte	Total N	cobas® BV/CV Positive Result	Positivity Rate (%)	95% Score CI
BV	154	50	32.5%	(25.6, 40.2)
CV	154	19	12.3%	(8.0, 18.5)

Clinical performance equivalency between the cobas® 6800/8800 and cobas® 5800 systems

A clinical performance equivalency study was conducted to demonstrate equivalent performance between the **cobas**® 6800/8800 and **cobas**® 5800 system. All symptomatic prospective specimens for **cobas**® BV/CV were run on the **cobas**® 6800/8800 systems and then a subset of samples, chosen at random, were tested on the **cobas**® 5800 system. The results from each system were evaluated against the PIS/CRS, and the sensitivity and specificity was directly compared between the two systems. The results were equivalent between the instrument systems.

Additional information

Key assay features

Sample types	• Clinician-collected Vaginal swab collected in **cobas**® PCR Media • Self-collected Vaginal swab collected in **cobas**® PCR Media
Amount of sample required/processed	• ≥1000 µL required in sample tube for swab samples, instrument processes 400 µL
Test duration	• < 3.5 hours to first result

Symbols

The following symbols are used in labeling for Roche PCR diagnostic products.

Table 32 Symbols used in labeling for Roche PCR diagnostics products

Age/DOB Age or Date of Birth	Device not for near-patient testing	QS IU/PCR QS IU per PCR reaction, use the QS International Units (IU) per PCR reaction in calculation of the results.
SW Ancillary Software	Device not for self-testing	
Assigned Range [copies/mL] Assigned Range (copies/mL)	Distributor *(Note: The applicable country/region may be designated beneath the symbol)*	SN Serial number
Assigned Range [IU/mL] Assigned Range (IU/mL)	Do not re-use	Site Site
EC REP Authorized representative in the European Community	Female	Procedure Standard Standard Procedure
BARCODE Barcode Data Sheet	For IVD performance evaluation only	STERILE EO Sterilized using ethylene oxide
LOT Batch code	GTIN Global Trade Item Number	Store in dark
Biological risks	Importer	Temperature limit
REF Catalogue number	IVD In vitro diagnostic medical device	TDF Test Definition File
CE CE marking of conformity; this device is in conformity with the applicable requirements for CE marking of an in vitro diagnostic medical device	LLR Lower Limit of Assigned Range	This way up
	Male	Procedure UltraSensitive Ultrasensitive Procedure
Collect Date Collect date	Manufacturer	UDI Unique Device Identifier
Consult instructions for use	CONTROL − Negative control	ULR Upper Limit of Assigned Range
Σ Contains sufficient for <*n*> tests	NON STERILE Non-sterile	Urine Fill Line Urine Fill Line
CONTENT Content of kit	Patient Name	Rx only For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.
CONTROL Control	Patient number	Use-by date
Date of manufacture	Peel here	
Device for near-patient testing	CONTROL + Positive control	
Device for self-testing	QS copies / PCR QS copies per PCR reaction, use the QS copies per PCR reaction in calculation of the results.	

Technical support

For technical support (assistance) please reach out to your local affiliate:
https://www.roche.com/about/business/roche_worldwide.htm

Manufacturer and importer

Table 33 Manufacturer and importer

Roche Molecular Systems, Inc.
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Made in USA

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Sandhofer Strasse 116
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