Medical Policy

BlueCross BlueShield Kansas
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Title: Multitarget Polymerase Chain Reaction Testing for Diagnosis of Bacterial Vaginosis

Related Policies:	▪ *Identification of Microorganisms Using Nucleic Acid Probes*

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Populations	Interventions	Comparators	Outcomes
Individuals: • With signs or symptoms of bacterial vaginosis	Interventions of interest are: • Multitarget polymerase chain reaction testing	Comparators of interest are: • Clinical and microscopic evaluation, including scoring systems (e.g. Amsel criteria, Nugent score)	Relevant outcomes include: • Test validity • Symptoms • Change in disease status

DESCRIPTION

Bacterial vaginosis (BV) is a common medical condition resulting from an imbalance in the normal vaginal flora. Although the identification of *Gardnerella vaginalis* has traditionally been associated with BV, there is no single etiologic agent. Most cases are asymptomatic, and most symptomatic cases can be diagnosed using clinical and microscopic evaluation. Multitarget polymerase chain reaction (PCR) testing is proposed as an alternative to currently available laboratory tests to diagnose BV. This test may improve outcomes if it is a more accurate and reliable method to diagnose BV.

OBJECTIVE

The objective of this evidence review is to evaluate whether the technical performance, diagnostic accuracy, and clinical utility of multitarget polymerase chain reaction testing improve net health outcomes in patients with signs or symptoms of BV.

BACKGROUND

Bacterial Vaginosis

BV is a condition caused by an imbalance in the normal bacteria vaginal flora. It is common, especially in women of reproductive age. While there is no single known etiologic agent, there is a shift in vaginal flora that involves depletion of hydrogen peroxide-producing Lactobacillus species with a rise in vaginal pH and overgrowth of other bacteria, including *Gardnerella vaginalis*, *Mycoplasma hominis*, *Peptostreptococcus*, *Mobiluncus* species, and other anaerobic gram-negative rods

Vaginal culture is not an appropriate diagnostic method to identify BV because BV is not caused by the presence of a particular bacterial species.

Various commercial tests provide rapid and accurate pH evaluation and amine detection. For example, automated devices that measure the volatile gases produced from vaginal samples and a colorimetric pH test are commercially available.

Nucleic acid probes of DNA fragments are available to detect and quantify specific bacteria in vaginal fluid samples. Polymerase chain reaction (PCR) methods extract and amplify the DNA fragments using either universal or specific primers. The result can be qualitative (to assess whether a specific microorganism is present) or quantitative (to assess how many microorganisms are present). The technology can be used to measure multiple organisms (eg, those known to be associated with BV) at the same time and is commercially available as multitarget PCR testing.

Blue Cross Blue Shield of Kansas medical policy Identification of Microorganisms Using Nucleic Acid Testing addresses the use of direct or amplified nucleic acid probes with or without quantification to detect microorganisms of clinical significance, including single microorganisms associated with BV.

Multitarget PCR Tests

Five quantitative multiplex PCR assays are available: BD Max (Becton Dickinson), Aptima BV (Hologic), NuSwab VG (LabCorp), OneSwab BV Panel PCR with Lactobacillus Profiling by qPCR (Medical Diagnostic Laboratories), and SureSwab BV (Quest Diagnostics).

The SureSwab Total test involves obtaining vaginal swab specimens, extracting total DNA, and quantitating the 4 types of bacteria using PCR. Results are reported as log cells per milliliter for each organism and concentrations of all *Lactobacilli* species are reported together then classified into 1 of the following 3 categories: not supportive, equivocal, and supportive.

A classification of *not supportive* of BV diagnosis is based on:

- The presence of *Lactobacillus* species, *G. vaginalis* levels <6.0 log cells/mL, and absence of *Atopobium vaginae* and *Megasphaera* species; or
- The absence of *Lactobacillus* species, *G. vaginalis* levels <6.0 log cells/mL, and absence of *A. vaginae* and *Megasphaera* species; or
- The absence of all targeted organisms.

A classification of equivocal is based on:

- The presence of *Lactobacillus* species, plus *G. vaginalis* at least 6.0 log cells/mL, and/or presence of *A. vaginae* and/or *Megasphaera* species.

A classification of supportive of BV diagnosis is based on the absence of *Lactobacillus* species, and presence of *G. vaginalis* levels of at least 6.0 log cells/mL, and presence of *A. vaginae and*/or *Megasphaera* species.

The BD Max (Becton, Dickinson), tests for markers of BV and vaginitis. The test uses a similar process to that described for SureSwab. Vaginal swab specimens are collected, DNA is extracted, and real-time PCR is used to quantitate targeted organisms. Results of BV marker tests are not reported for individual organisms. Instead, qualitative BV results are reported as positive or negative for BV based on the relative quantity of the various organisms.

The Aptima BV Assay was cleared by the U.S. Food and Drug Administration with the BD Max as the predicate device. The Aptima assay is a nucleic acid amplification test (NAAT) for detection and quantitation of ribosomal RNA.

Medical Diagnostics Laboratory offers a Bacterial Vaginosis Panel. Markers are assessed using real-time PCR and *Lactobacillus* is profiled using quantitative PCR. GenPath Diagnostics also offers a bacterial vaginosis test.

The NuSwab Select BV test (Laboratory Corporation of America) uses semiquantitative PCR analysis of 3 predictive marker organisms of vaginal dysbiosis to generate a total score that is associated with the presence or absence of BV. In this test system, samples with a total score of 0 to 1 are considered negative for BV, samples with a score of 3 to 6 are positive for BV, and samples with a score of 2 are indeterminate for BV.

REGULATORY STATUS

Two assays are FDA cleared (BD Max and Aptima BV), and 3 (NuSwab VG, OneSwab BV Panel PCR with Lactobacillus Profiling by qPCR, and SureSwab BV) are laboratory-developed tests.

Several of the manufacturers of the BV tests also have extensions that include other causes of vaginitis such as *Trichomonas vaginalis* and *Candidiasis* species. For example, the BD Vaginal Panel was cleared in March 2023 with the BD Max as the predicate device. It is intended to aid in the diagnosis of vaginal infections in individuals with a clinical presentation consistent with bacterial vaginosis, vulvovaginal candidiasis and trichomoniasis.[1,]

Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests must meet the general regulatory standards of the Clinical Laboratory Improvement Act (CLIA). Laboratories that offer laboratory-developed tests must be licensed by the CLIA for high-complexity testing.

POLICY

Multitarget polymerase chain reaction testing for the diagnosis of bacterial vaginosis is considered **experimental / investigational**.

Please refer to the member's contract benefits in effect at the time of service to determine coverage or non-coverage of these services as it applies to an individual member.

RATIONALE

The evidence review has been updated regularly with searches of the PubMed database. The most recent literature review was performed through November 12 , 2024.

Evidence reviews assess whether a medical test is clinically useful. A useful test provides information to make a clinical management decision that improves the net health outcome. That is, the balance of benefits and harms is better when the test is used to manage the condition than when another test or no test is used to manage the condition.

The first step in assessing a medical test is to formulate the clinical context and purpose of the test. The test must be technically reliable, clinically valid, and clinically useful for that purpose. Evidence reviews assess the evidence on whether a test is clinically valid and clinically useful. Technical reliability is outside the scope of these reviews, and credible information on technical reliability is available from other sources.

Promotion of greater diversity and inclusion in clinical research of historically marginalized groups (e.g., People of Color [African-American, Asian, Black, Latino and Native American]; LGBTQIA (Lesbian, Gay, Bisexual, Transgender, Queer, Intersex, Asexual); Women; and People with Disabilities [Physical and Invisible]) allows policy populations to be more reflective of and findings more applicable to our diverse members. While we also strive to use inclusive language related to these groups in our policies, use of gender-specific nouns (e.g., women, men, sisters, etc.) will continue when reflective of language used in publications describing study populations.

INDIVIDUALS WITH SIGNS OR SYMPTOMS OF BACTERIAL VAGINOSIS

Clinical Context and Test Purpose

The purpose of multitarget polymerase chain reaction (PCR) testing in patients who have signs or symptoms of bacterial vaginosis (BV) is as a replacement to current diagnostic strategies so that appropriate treatment is selected and patient outcomes are improved.

This review evaluates whether multimarker PCR testing improves health outcomes compared with standard diagnostic tests. These tests have been proposed as a replacement for standard diagnostic tests such as Amsel criteria and Nugent score.

The following PICO was used to select literature to inform this review.

Populations

The relevant population of interest is individuals with signs or symptoms of BV. BV is a condition caused by an imbalance in the normal bacteria vaginal flora. It is common, especially in women

of reproductive age. While there is no single known etiologic agent, there is a shift in vaginal flora that involves depletion of Lactobacillus species and overgrowth of other bacteria, including *Gardnerella vaginalis*, *Mycoplasma hominis*, *Peptostreptococcus*, *Mobiluncus* species, and other anaerobic gram-negative rods. Prevalence of the condition is high, and it is asymptomatic in most cases. According to data from a nationally representative sample of women surveyed from 2001 to 2004, the prevalence of BV among women ages 14 to 49 years in the U. S. was 29%.[2,] BV may be confused with nonbacterial causes of vaginitis, including *candidiasis* and *trichomoniasis*.

When symptomatic, BV is associated with characteristic signs and symptoms. The most common sign of BV is an abnormal grayish-white vaginal discharge, generally with an unpleasant, often "fishy" smell in association with mild itching or irritation.

BV resolves spontaneously in a high percentage of women, treatment for symptomatic BV is usually a course of oral antibiotics, either metronidazole or clindamycin. Antibiotic treatment results in a high rate of remission of symptoms, but recurrences are common within the first year after treatment.

Interventions

The intervention of interest is a multitarget PCR test for BV. Nucleic acid probes of DNA fragments are available to detect and quantify the bacteria in vaginal fluid samples. Bacterial DNA is extracted and amplified by PCR methods, using either universal or specific primers The result can be qualitative (to assess whether a specific microorganism is present) or quantitative (to assess how many microorganisms are present). The technology can be used to measure multiple organisms (eg, those known to be associated with BV) at the same time and is commercially available as multitarget PCR testing.

Comparators

The comparators of interest are standard diagnostic approaches such as clinical examination and microscopic examination of vaginal specimens.

Gram staining of vaginal discharge samples is the conventional microscopic method of BV diagnosis and requires preparation and analysis of the specimen in the laboratory setting. It remains the historical research criterion standard for diagnosing BV. Gram-stained samples are analyzed using the Nugent criteria or a modified version by Ison and Hay.

For the Nugent criteria, levels of 3 types of bacteria (*Lactobacillus, Gardnerella/Bacteroides*, and *Mobiluncus)* in vaginal discharge samples are estimated. Levels of *Lactobacillus* and *Gardnerella/Bacteroides* are rated on a scale from 0 to 4 based on the number of cells per field magnified at 100 times, and levels of *Mobiluncus* are rated on a scale from 0 to 2. A composite score is calculated by summing the 3 subscores, as listed in Table 1.

Table 1. Nugent Criteria

Criterion	Scoring Range
Not consistent with BV	Score of 0-3; or score of 4-6 with clue cells not present
Consistent with BV	Score of 4-6 with clue cells present; or score of at least 7

Some clinicians include a third, middle category in Nugent scoring, with a total score of 0 to 3 considered normal, 4 to 6 as intermediate/equivocal, and 7 to 10 as definite BV.
BV: bacterial vaginosis.

Table 2 summarizes the simplified Ison and Hay criteria.

Table 2. Ison and Hay Criteria

Criterion	Scoring Range
Grade 1 (normal)	*Lactobacillus* morphotypes predominate
Grade 2 (intermediate)	Flora are mixed with some *Lactobacillus* morphotypes and some *Gardnerella* or *Mobiluncus* morphotypes are present
Grade 3 (bacterial vaginosis)	*Gardnerella* and/or *Mobiluncus* morphotypes predominate; *lactobacilli* morphotypes are few or absent

In practice, the diagnosis of BV can be made based on the presence of at least 3 Amsel criteria (characteristic vaginal discharge, elevated pH, clue cells, fishy odor),[3,] which is simple and has a sensitivity of over 90% and specificity of 77% compared with Gram stain.[4,]

More specifically, vaginal discharge is characterized as homogeneous, thin, and whitish-gray; clue cells are squamous epithelial cells that normally have a sharply defined cell border but in BV, have bacteria adherent to their surfaces and appear to be "peppered" with bacteria; pH of vaginal fluid greater than 4.5; and a "fishy" odor of vaginal discharge before or after addition of potassium hydroxide 10%.

Both comparator diagnostic methods (i.e., clinical diagnosis using the Amsel criteria and laboratory diagnosis using Nugent or Ison and Hay criteria)[5,6,] have subjective components and, therefore, may be imprecise. Moreover, Gram stain examination is time-consuming, requires substantial training, and it is difficult to determine an appropriate clinical response for intermediate scores. The 2 methods of diagnosis can also be used in combination to increase diagnostic accuracy.

Outcomes

The primary outcomes of interest are test validity, symptom resolution, and cure rate (absence of symptoms and normal vaginal flora).

Study Selection Criteria

For the evaluation of the clinical validity of the tests, studies that met the following eligibility criteria were considered:

- Reported on the accuracy of the marketed version of the technology (including any algorithms used to calculate scores)
- Included a suitable reference standard (Amsel, Nugent, or Hay/Ison criteria)

- Patient/sample clinical characteristics were described
- Patient/sample selection criteria were described
- Included a validation cohort separate from the development cohort.

REVIEW OF EVIDENCE

Excluded Publications

A publication by Hilbert et al (2016), funded through Medical Diagnostics Laboratory and evaluating markers in that laboratory's BV Panel, and Gaspar et al (2019) were not selected because they did not include a validation cohort independent of the development cohort.[7,] Two studies were excluded because they did not include a suitable reference standard.[8,9,] Other publications were not included because they analyzed data previously reported in Gaydos et al (2017).[10,11,]

Clinically Valid

A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

There are no published studies on the diagnostic accuracy of the SureSwab test or the GenPath test, but information is available on the diagnostic accuracy of the BD Max test, the Aptima BV test, and the NuSwab offered by LabCorp.

The characteristics of the studies are shown in Table 3 and the results are shown in Table 4. The studies are briefly described following the tables.

Table 3. Characteristics of Clinical Validity Studies Assessing BV Tests

Study	Study Population	Design	Reference Standard	Threshold for Positive Index Test	Timing of Reference and Index Tests	Blinding of Assessors
BD Max						
Aguirre-Quiñonero (2019)[12,]	Women ≥ 14 years old with or without symptoms in Spain; median age, 39 years; 5% pregnant	Prospective, unclear whether consecutive, single-center	Combination of Hay's criteria, the presence of clue cells, and a predominant growth of G. vaginalis; independent scoring by 2 microbiologists	NR	Simultaneous	Yes
van den Munckhof (2019)[13,]	Women with symptoms of BV visiting a single outpatient clinic in the Netherlands between	Prospective, unclear whether consecutive, single-center	Microbiota analysis	≤47% relative abundance of Lactobacillus and mainly anaerobes	Simultaneous	Yes

Study	Study Population	Design	Reference Standard	Threshold for Positive Index Test	Timing of Reference and Index Tests	Blinding of Assessors
	January and July 2015 and additional asymptomatic women from the same clinic; mean age, 34 years; majority of 'European origin'					
FDA decision summary[14]; Gaydos (2017)[10]	Women with symptoms of BV or vaginitis; samples collected in 2015; 53% African American; 25% white; age range, 18-29 y	Prospective, consecutive, multicenter	Nugent score; indeterminate by Nugent diagnosed with Amsel criteria	Automatic reporting based on algorithmic analysis of molecular DNA detection of lactobacilli and bacteria associated with BV	Simultaneous	Yes
NuSwab						
Cartwright (2018)[15]	Women with symptoms of vaginitis or BV; samples collected in 2016-2017; 34% African American, 38% white, age range, 18-49 y	Prospective, multicenter	Nugent score; indeterminate by Nugent diagnosed with Amsel criteria	Score of 3-6 indicates presence of BV	Simultaneous	Yes
Cartwright (2012)[16]; validation cohort	Women evaluated at 3 clinics in Alabama in 2011; 87% African American, 13% (50/402) white	Prospective, selection criteria not described	Nugent score; indeterminate by Nugent diagnosed with Amsel criteria	Score of 3-6 indicates presence of BV	Simultaneous	Yes
Aptima BV						
Schwebke (2020)[17]	Women ≥ 14 years old with	Prospective, multicenter	Nugent consensus	Nugent score ≥ 7 indicates	Simultaneous	Yes

Study	Study Population	Design	Reference Standard	Threshold for Positive Index Test	Timing of Reference and Index Tests	Blinding of Assessors
	symptoms of vaginitis evaluated at 21 US sites between June and October 2018; 50.2% African American, 22% white; mean age, 35.3 years		score, indeterminate by Nugent diagnosed with modified Amsel criteria	presence of BV		
Richter (2019)[18,]	Women with symptoms of vaginitis evaluated at Cleveland Clinic between May and December 2018	Prospective, selection criteria not described, single-center	Nugent score; indeterminate by Nugent diagnosed with ≥2 Amsel criteria	Nugent score ≥ 7 indicates presence of BV	Simultaneous	Yes

BV: bacterial vaginosis; FDA: U.S. Food and Drug Administration; NR: not reported.

Table 4. Results of Clinical Validity Studies Assessing BV Tests

Study	Initial N	Final N	Excluded Samples	Prevalence of Condition, %	Clinical Validity (95% Confidence Interval), %			
					Sensitivity	Specificity	PPV	NPV
BD Max								
Aguirre-Quiñonero (2019)[12,]	1000	1000	13 results were reported to be invalidated; unclear how these were coded for analysis	19.3	89.8 (85.0 to 93.1)	96.5 (95.1 to 97.6)	86.9 (81.9 to 90.7)	97.3 (96.0 to 98.2)
van den Munckhof (2019)[13,]	80 women; designed for 2 visits per women	115 for either visit; 63 in visit 1	14 women did not attend visit 2; data from 31 visits excluded because of insufficient sample volume or indeterminate outcome by at least 1 of the methods	31				

Study	Initial N	Final N	Excluded Samples	Prevalence of Condition, %	Clinical Validity (95% Confidence Interval), %			
Amsel criteria, Visit 1					70.8 (50.8 to 85.1)	92.3 (79.7 to 97.4)	85.0 (64.0 to 94.8)	83.7 (70.0 to 91.9)
Nugent score, Visit 1					70.8 (50.8 to 85.1)	100 (91.0 to 100)	100 (81.6 to 100)	84.8 (71.8 to 92.4)
BD Max, Visit 1					66.7 (46.7 to 82.0)	97.4 (86.8 to 99.6)	94.1 (73.0 to 99.0)	82.6 (69.3 to 90.9)
FDA decision summary[14,]; Gaydos (2017)[10,]	1763	1559[a] 1582[b]	• Protocol issues: withdrawn (13), informed consent process incorrect (7), asymptomatic patient enrolled (2), and >1 specimen obtained for same patient (1) • TPI: reference standard results not compliant with protocol (130); index test not compliant with protocol (8); index test results not reported (71)	56	90.5 (88.3 to 92.2)[a] 90.7 (88.6 to 92.5)[b]	85.8 (83.0 to 88.3)[a] 84.5 (81.6 to 87.0)[b]	89.0 (NR)[a] 88.1 (NR)[b]	87.7 (NR)[a] 87.8 (NR)[b]
NuSwab								
Cartwright (2018)[15,]	1595	1484	Incomplete testing (16); test indeterminate (95)	34	96 (94 to 98)	90 (88 to 92)	83 (81 to 86)	98 (97 to 99)

Study	Initial N	Final N	Excluded Samples	Prevalence of Condition, %	Clinical Validity (95% Confidence Interval), %			
Cartwright (2012)[16]; validation cohort	227	213	Indeterminate (14)	49	99 (NR)	91 (NR)	NR	NR
Aptima BV								
Schwebke (2020)[17]	1519	1413[a] 1405[b]	Ineligibility (17); test not evaluable (58); test not available (26); indeterminate score could not be resolved (1)	49.5	95.0 (93.1 to 96.4)[a] 97.3 (95.8 to 98.2)[b]	89.6 (87.1 to 91.6)[a] 85.8 (83.1 to 88.2)[b]	95.6 (93.9 to 96.9)[a] 93.3 (91.4 to 94.9)[b]	95.9 (94.1 to 97.2)[a] 97.7 (96.3 to 98.7)[b]
Richter (2019)[18]	111	111	-	40.5	84.4 (70.9 to 92.6)	86.3 (75.9 to 92.9)	80.9 (67.2 to 89.8)	89.1 (78.8 to 94.9)

BV: bacterial vaginosis; FDA: U.S. Food and Drug Administration; NPV: negative predictive value; NR: not reported; PPV: positive predictive value; TPI: test performance issues.
[a] Clinician.
[b] Self.

BD Max Test

The U.S. Food and Drug Administration (FDA) decision summary and Gaydos et al (2017) for the BD Max test includes a description of a prospective clinical diagnostic accuracy study.[14,10] The study included 1763 women with symptoms of BV or vaginitis. Both clinician-collected and self-collected vaginal swabs were obtained and were analyzed independently. A total of 1559 (88%) clinician-detected and 1582 (90%) self-detected samples were available for analysis.

Aguirre-Quiñonero et al (2019) describes the results of the BD MAX in 1000 vaginal swabs from women ≥ 14 years old (median age, 33 years) presenting with or without symptoms from a single institution in Spain.[12] Consistent with the inclusion of asymptomatic women, the prevalence of BD was lower in this study at 19%.

van den Munckhof (2019) compared BD MAX to Amsel and Nugent with microbiota analysis as a reference standard in 60 symptomatic women and 20 women treated for other reasons from a single institution in the Netherlands.[13] Samples were collected at 2 visits approximately 4 weeks apart. It is unclear what treatments women received between the visits. The performance characteristics for samples collected at visit 1 are included in Table 4. The authors used microbiota analysis as the reference standard and therefore performance characteristics of BD MAX may not be comparable to other studies. The confidence intervals for the performance characteristics of Amsel and BD MAX were highly overlapping

NuSwab

Cartwright et al (2012) published data on a multitarget semiquantitative PCR test including 3 organisms: *Atopobium vaginae*, *Megasphaera* type 1, and *BVAB2*.[16,] The investigators used separate samples for the development and validation phases and compared the diagnostic accuracy of the multitarget panel with an accepted reference standard. The patient population consisted of 402 women presenting at a clinic for sexually transmitted infections (n=299) or a personal health clinic (n=103). Samples from 169 women were included in the development phase, of which 108 (64%) were positive for BV and 61 (36%) were negative for BV. In the validation phase, the multitarget PCR test was assessed using an additional 227 samples. Results were similar in Cartwright et al (2018), which reported on a multicenter study of 1579 women of whom 538 were positive and 1041 were negative for BV.[15,] In this publication, the authors proposed an α-diversity score generated from next-generation sequencing that could be used to resolve discordant PCR and Nugent/Amsel results.

Aptima BV

Schwebke et al (2020) compared the Aptima BV assay (Hologic, Inc.) to Nugent score as reference standard in 1,417 symptomatic women.[17,] Both clinician- and patient-collected swabs were assessed. Clinicians utilized modified Amsel criteria for the resolution of indeterminate Nugent scores. Performance characteristics for evaluable samples are included in Table 4.

Richter et al (2019) compared the accuracy of testing with Aptima BV, Hologic Analyte Specific Reagent, and the direct-probe BD Affirm test to Nugent score as the reference standard in 111 symptomatic women.[18,] Modified Amsel criteria were used for the resolution of indeterminate Nugent scores. Performance characteristics for the commercially-marketed nucleic acid amplification Aptima BV test are included in Table 4.

The purpose of limitations tables (see Tables 5 and 6) is to display notable limitations identified in each study. This information is synthesized as a summary of the body of evidence following each table and provides the conclusions on the sufficiency of the evidence supporting the position statement.

Table 5. Study Relevance Limitations

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of Follow-Up[e]
Aguirre-Quiñonero (2019)[12,]	4. Includes asymptomatic women		3. No comparison to clinical diagnosis by Amsel alone		
van den Munckhof (2019)[13,]	4. Includes asymptomatic women		2: Used microbiota analysis as the reference standard		
FDA decision summary[14,]; Gaydos (2017)[10,]			3. No comparison to clinical diagnosis by Amsel alone		

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of Follow-Up[e]
Cartwright (2018)[15,]			3. No comparison to clinical diagnosis by Amsel alone		
Cartwright (2012)[16,]	3,4. Unclear if women had symptoms of vaginosis		3. No comparison to clinical diagnosis by Amsel alone		
Schwebke (2020)[17,]			3. No comparison to clinical diagnosis by Amsel alone; modified Amsel criteria used		
Richter (2019)[18,]	3. Patient clinical characteristics not described.		3. No comparison to clinical diagnosis by Amsel alone, modified Amsel criteria used		

The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
FDA: U.S. Food and Drug Administration.
[a] Population key: 1. Intended use population unclear; 2. Clinical context is unclear; 3. Study population is unclear; 4. Study population not representative of intended use.
[b] Intervention key: 1. Classification thresholds not defined; 2. Version used unclear; 3. Not intervention of interest.
[c] Comparator key: 1. Classification thresholds not defined; 2. Not compared to credible reference standard; 3. Not compared to other tests in use for same purpose.
[d] Outcomes key: 1. Study does not directly assess a key health outcome; 2. Evidence chain or decision model not explicated; 3. Key clinical validity outcomes not reported (sensitivity, specificity, and predictive values); 4. Reclassification of diagnostic or risk categories not reported; 5. Adverse events of the test not described (excluding minor discomforts and inconvenience of venipuncture or noninvasive tests).
[e] Follow-Up key: 1. Follow-up duration not sufficient with respect to natural history of disease (true-positives, true-negatives, false-positives, false-negatives cannot be determined).

Table 6. Study Design and Conduct Limitations

Study	Selection[a]	Blinding[b]	Delivery of Test[c]	Selective Reporting[d]	Data Completeness[e]	Statistical[f]
Aguirre-Quiñonero (2019)[12,]	1. Unclear if selection was consecutive					
van den Munckhof (2019)[13,]					2. >20% of samples excluded	
FDA decision summary[14,]; Gaydos (2017)[10,]					2. >10% of samples excluded	

Study	Selection[a]	Blinding[b]	Delivery of Test[c]	Selective Reporting[d]	Data Completeness[e]	Statistical[f]
Cartwright (2018)[15,]						
Cartwright (2012)[16,]	1. Selection criteria not clear					1. CIs not reported for subgroup in validation cohort
Schwebke (2020)[17,]	1. Selection criteria not described				2. >8% of samples excluded	
Richter (2019)[18,]	1. Selection criteria not described					

The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
CI: confidence interval; FDA: U.S. Food and Drug Administration.
[a] Selection key: 1. Selection not described; 2. Selection not random or consecutive (i.e., convenience).
[b] Blinding key: 1. Not blinded to results of reference or other comparator tests.
[c] Test Delivery key: 1. Timing of delivery of index or reference test not described; 2. Timing of index and comparator tests not same; 3. Procedure for interpreting tests not described; 4. Expertise of evaluators not described.
[d] Selective Reporting key: 1. Not registered; 2. Evidence of selective reporting; 3. Evidence of selective publication.
[e] Data Completeness key: 1. Inadequate description of indeterminate and missing samples; 2. High number of samples excluded; 3. High loss to follow-up or missing data.
[f] Statistical key: 1. Confidence intervals and/or p values not reported; 2. Comparison with other tests not reported.

Other Tests

Several studies have reported on the validation of multitarget PCR tests not currently commercially available in the U.S.[19,20,21,22,]These tests will not be reviewed in full until such time they become available in the U.S.

Section Summary: Clinically Valid

Several studies have evaluated the diagnostic accuracy of multitarget PCR tests for BV, including 5 studies evaluating commercially available tests. The studies found sensitivities of 84% to 95% and specificities of 85% to 97%, compared with a reference standard combination of the Amsel criteria and Nugent or Hay score. Several studies generally included symptomatic women; 2 studies included symptomatic and asymptomatic women.

Clinically Useful

A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct Evidence

Direct evidence of clinical utility is provided by studies comparing health outcomes for patients managed with and without the test. Preferred evidence comes from randomized controlled trials. No published studies were identified that evaluated changes in health outcomes when a multitarget PCR test was used to diagnose BV compared with standard methods of diagnosis.

Chain of Evidence
Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

Diagnostic accuracy studies have found that multitarget PCR tests for BV have a sensitivity ranging from approximately 90% to 95% and specificity ranging from approximately 85% to 90% compared with a reference standard combining Amsel criteria and Nugent score. The studies have not reported the concurrent measurement of the diagnostic accuracy of Amsel criteria alone.

The multitarget PCR tests have also not demonstrated improvement in other health outcomes. The tests are not less invasive nor less burdensome for patients because they use the same type of specimen obtained during a pelvic exam that would be needed for microscopy. The multitarget PCRs test also does not provide a diagnosis with a faster turn-around than using Amsel criteria. Therefore, a chain of evidence to demonstrate an improvement in the net health outcome compared with Amsel criteria cannot be constructed.

Section Summary: Clinically Useful
A useful test provides information to make a clinical management decision that improves the net health outcome. To improve the net health outcome, the multitarget PCR tests should either improve diagnostic accuracy (sensitivity, specificity) or have similar diagnostic accuracy with improvements in other health outcomes such as patient burden or timeliness of diagnosis.

- If the multitarget PCR tests could demonstrate improved diagnostic accuracy, a chain of evidence could be created because improvements in diagnosis should lead to improvements in appropriate treatment and therefore an improvement in health outcomes.
- Nugent is the criterion standard for the diagnosis of BV in research studies of BV. The studies of multitarget PCR tests used Nugent criteria as the reference standard with the Amsel criteria used when Nugent were indeterminate.
- Given that the criterion standard is how true- and false-positives and -negatives are defined, multitarget PCR tests cannot show higher sensitivity or specificity than the Nugent criteria.
- To demonstrate improvement in diagnostic accuracy over the *criterion standard* would require direct evidence through reporting of health outcomes such as symptom resolution and recurrences.

In the absence of evidence of improved diagnostic accuracy, to demonstrate improvement in the net health outcome, multitarget PCR tests should have similar diagnostic accuracy with improvements in other health outcomes such as patient burden or timeliness of diagnosis.

- In the reported studies, sensitivities ranged from approximately 90% to 95% and specificities ranged from approximately 85% to 90% compared with the Nugent criterion standard.
- Guidelines have recommended that Amsel criteria can be used to diagnose BV in practice. Therefore, to understand the diagnostic accuracy of multitarget PCR tests compared with Amsel criteria, studies should have also concurrently compared Amsel criteria with the Nugent criterion standard. The sensitivity and specificity of Amsel criteria alone compared with the Nugent criterion were not reported.

- The multitarget PCR tests are no less invasive nor less burdensome for patients than Amsel criteria for diagnosis because they use the same type of specimen obtained during a pelvic exam that would be needed for microscopy.
- The multitarget PCRs test also does not provide a diagnosis with a faster turn-around than Amsel criteria.
- Multitarget PCR tests might provide benefits in the differential diagnosis of vaginitis. However, the other most common causes of vaginitis (vulvovaginal candidiasis and trichomoniasis) can also be diagnosed using the clinical information assessed when applying the Amsel criteria (signs/symptoms, vaginal pH, amine test, microscopy).

In summary, the present studies have not demonstrated improvements in diagnostic accuracy or improvements in health outcomes compared with Amsel criteria alone or compared with the Nugent criterion standard.

SUPPLEMENTAL INFORMATION

The purpose of the following information is to provide reference material. Inclusion does not imply endorsement or alignment with the evidence review conclusions.

Practice Guidelines and Position Statements

Guidelines or position statements will be considered for inclusion in 'Supplemental Information' if they were issued by, or jointly by, a US professional society, an international society with US representation, or National Institute for Health and Care Excellence (NICE). Priority will be given to guidelines that are informed by a systematic review, include strength of evidence ratings, and include a description of management of conflict of interest.

American College of Obstetricians and Gynecologists

Published in 2012 and reaffirmed in 2018, the American College of Obstetricians and Gynecologists (ACOG) has produced a Practice Bulletin on the prediction of preterm birth. The Bulletin stated that BV testing is not recommended as a screening strategy in asymptomatic pregnant women at increased risk of preterm birth.[23,]

Published in 2020, the ACOG has issued a Practice Bulletin on vaginitis in nonpregnant patients.[24,] The Bulletin made the following recommendations on the initial evaluation of patients with symptoms of vaginitis, citing CDC guidelines:

"A complete medical history, physical examination of the vulva and vagina, and clinical testing of vaginal discharge (i.e., pH testing, a potassium hydroxide "whiff test," and microscopy) are recommended for the initial evaluation of patients with vaginitis symptoms."

The Bulletin noted that single-swab multiplex PCR testing "may be a promising alternative to microscopy," but that its clinical utility is still under evaluation.

Centers for Disease Control and Prevention

In 2021, the Centers for Disease Control and Prevention updated its guidelines on sexually transmitted infections.[25,] Regarding the diagnosis of bacterial vaginosis (BV), the guidelines stated:

"BV can be diagnosed by....clinical criteria (i.e., Amsel's Diagnostic Criteria) or by determining the Nugent score from a vaginal Gram stain. Vaginal Gram stain, considered the reference standard laboratory method for diagnosing BV, is used to determine the relative concentration of lactobacilli …"

The guidelines state that multiplex PCR assays are available, but noted that traditional methods of BV diagnosis, including the Amsel criteria, Nugent score, and the Affirm VP III assay, remain useful for diagnosing symptomatic BV because of their lower cost and ability to provide a rapid diagnosis. The guidelines also stated that BV nucleic acid amplification tests should be used among symptomatic women only (eg, women with vaginal discharge, odor, or itch) because their accuracy is not well defined for asymptomatic women.

U.S. Preventive Services Task Force Recommendations
The USPSTF (2020) recommendations on screening for BV in pregnancy[26,] have stated that:

"The USPSTF recommends against screening for bacterial vaginosis in pregnant persons who are not at increased risk for preterm delivery." (Grade D recommendation)

"The USPSTF concludes that the current evidence is insufficient to assess the balance of benefits and harms of screening for bacterial vaginosis in pregnant persons who are at increased risk for preterm delivery." (I statement)

Ongoing and Unpublished Clinical Trials
A search of ClinicalTrials.gov in November 2024 did not identify any ongoing or unpublished trials that would likely influence this review.

CODING

The following codes for treatment and procedures applicable to this policy are included below for informational purposes. This may not be a comprehensive list of procedure codes applicable to this policy.

Inclusion or exclusion of a procedure, diagnosis or device code(s) does not constitute or imply member coverage or provider reimbursement. Please refer to the member's contract benefits in effect at the time of service to determine coverage or non-coverage of these services as it applies to an individual member.

The code(s) listed below are medically necessary ONLY if the procedure is performed according to the "Policy" section of this document.

CPT/HCPCS	
81513	Infectious disease, bacterial vaginosis, quantitative realtime amplification of RNA markers for Atopobium vaginae, Gardnerella vaginalis, and Lactobacillus species, utilizing vaginal-fluid specimens, algorithm reported as a positive or negative result for bacterial vaginosis
81514	Infectious disease, bacterial vaginosis and vaginitis, quantitative real-time amplification of DNA markers for Gardnerella vaginalis, Atopobium vaginae, Megasphaera type 1, Bacterial Vaginosis Associated Bacteria-2 (BVAB2), and Lactobacillus species (L. crispatus and L. jensenii), utilizing vaginal-fluid specimens, algorithm reported as a positive or negative for high likelihood of bacterial vaginosis, includes separate detection of Trichomonas vaginalis and/or Candida species (C. albicans, C. tropicalis, C. parapsilosis, C. dubliniensis), Candida glabrata, Candida krusei, when reported
81515	Infectious disease, bacterial vaginosis and vaginitis, realtime PCR amplification
0330U	Infectious agent detection by nucleic acid (DNA or RNA), vaginal pathogen panel, identification of 27 organisms, amplified probe technique, vaginal swab
0505U	Infectious disease (vaginal infection), identification of 32 pathogenic organisms, swab, real-time PCR, reported as positive or negative for each organism

REVISIONS	
12-12-2023	Policy added to the bcbsks.com web site.
10-01-2024	Updated Coding Section ▪ Added 0505U (eff. 10-01-2024)
01-01-2025	Updated Coding Section ▪ Added 81515 (eff. 01-01-2025) ▪ Removed deleted code 0352U (eff. 01-01-2025)
01-28-2025	Updated Description Section
	Updated Rationale Section
	Updated References Section

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