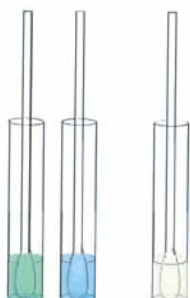


Interpretation of Results



POSITIVE NEGATIVE

A32, Rev 4

BVBLUE®

This is a CLIA Waived Test

For clinics in the US: A CLIA Certificate of Waiver is needed to perform testing in waived settings. Read all instructions carefully before use. If a laboratory modifies the following test instructions including Quality Control the test will be considered High Complexity and no longer considered Waived.

INTENDED USE:

The BVBLUE Test is an enzyme activity test for use in the detection of vaginal fluid specimens for sialidase activity, an enzyme produced by bacterial pathogens such as *Gardnerella vaginalis*, *Bacteroides spp.*, *Prevotella spp.*, and *Mobiluncus spp.*

The BVBLUE Test is indicated for use in women suspected of having Bacterial Vaginosis (BV) infection, e.g., women with vaginal discharge typical of BV and/or women with previous history of BV, as an aid in the diagnosis of BV infection. Test results should be considered in conjunction with other clinical and patient information (see Limitations of the Procedure).

For In Vitro Diagnostic Use Only: The BVBLUE Test is indicated for professional use only and may be used at the point of care and/or in physician's offices. It is not intended for home use.

SUMMARY AND EXPLANATION OF THE TEST:

Vaginosis is one of the most common reasons that women visit obstetricians or gynecologists.^{1,2} BV is the most common form of infectious vaginitis. The causative agents of the infection are bacterial pathogens such as *Gardnerella vaginalis*, *Bacteroides spp.*, *Prevotella spp.*, and *Mobiluncus spp.*

Complications associated with BV include sialpingitis, endometritis, post-hysterectomy infections, recurrent UTIs, and an increased risk of PID and HIV.^{3,4} BV represents a serious danger in women, due to its significant association with placental infection, premature rupture of membranes, and preterm birth.^{5,6}

Studies have shown elevated sialidase activities in women with BV and an increased risk for preterm birth and low birth weight infants in patients exhibiting elevated sialidase activity.^{7,8,9} The BVBLUE Test is designed to provide a clear, simple indication of elevated sialidase activity in patient vaginal fluid samples. The generation of a blue or green color indicates a positive test result; a yellow color indicates a negative test result.

PRINCIPLES OF BVBLUE:

The BVBLUE Test includes a chromogenic substrate of bacterial sialidase. In the test procedure, a vaginal fluid sample is placed in the BV Test Vessel. The sample then reacts with the chromogenic substrate. A Developer Solution is added after the reaction.

If the sample has a high level of sialidase, a blue or green color will be seen in the BV Test Vessel or on the head of the swab. If the sample has no sialidase, or has very low levels, a yellow color will be seen in the BV Test Vessel.

REAGENTS / MATERIALS:

- BX-4041 component (0.25 mg/est)
- potassium acetate (24.5 mg/est)
- sodium hydroxide (1.0 mg/est)

Materials Provided:

- Either 10 or 25 Test Vessels each containing 0.25 mg BX-4041 component in 0.5 mL of an aqueous potassium acetate buffer solution (49mg/mL, 0.5 M, pH 5.5-6.0) (40mg/mL, 1.0 M, pH > 11.0)
- 1 Directional Insert / Quick Reference Instruction

Materials Required But Not Provided:

- BVBLUE Control Kit
- Timer or watch
- Cotton Swabs

WARNINGS AND PRECAUTIONS:

- For *In Vitro* diagnostic use only.
- Do not use after the expiration date printed on the kit.
- Do not store the kit at temperatures above 26°C (79°F).
- Do not store the kit in strong light.
- Follow your laboratory safety guidelines in the collection, handling, storage and disposal of patient specimens and all items exposed to patient specimens.
- Used tests should never be re-used.
- This product is intended for vaginal fluid use only.

SPECIMEN COLLECTION AND STORAGE:

- Collect specimens with a cotton swab from the lower one-third of the vaginal wall. Collecting specimens from the cervix should be avoided because (a) it might increase risk to OB patients, and (b) cervical sialidase activity is usually higher than vaginal sialidase activity.
- Do not use specimens from patients who have (a) used a vaginal cream or ointment product, (b) douched, or (c) used spermicides, vaginal lubricants or feminine sprays within 72 hours of testing.

• Use the patient specimen as soon as possible after collection.

- If you do not perform the BVBLUE test immediately, store the swabs either at room temperature for up to 48 hours or refrigerated for up to 7 days. To transport patient specimens, place each swab in a clean, dry container such as a plastic or glass tube. Do not use any transport media.
- If you do not collect enough sample or collect from a patient undergoing antimicrobial therapy the test may give a false negative result.

STORAGE AND STABILITY:

Store the kit at controlled temperature, 2°–26°C (36°–79°F), out of direct sunlight. Store vessels inside the box. Kit contents are stable until the expiration date printed on the outer box.

INDICATIONS OF INSTABILITY:

- Signs of possible product instability include:
 - A blue color in a BV Test Vessel when one drop of Developer Solution is added to the BV Test Vessel in the absence of a patient specimen.
 - Positive control does not give expected results.
 - Negative control does not give expected results.

QUALITY CONTROL:

1. Internal Quality Control

The BVBLUE Test contains two types of internal quality control with each test run. For daily quality control, Gynpus Diagnostics recommends documenting these controls on each day of testing.

- **Type 1 Control:** Before adding a patient specimen, inspect the Test Vessel. It should contain a colorless liquid without precipitates (sediment).
- If the testing vessel contains a precipitate, the test is invalid. Do not use the Test Vessel.

- **Type 2 Control:** The BVBLUE Test has a two-color result format: blue/green is positive, yellow is negative. After running the test according to the instructions for use, the appearance of either a uniform yellow, blue, or green color in the testing vessel or a blue or green color on the swab assures proper mixing of the reagent and sample has occurred. If the test fails to provide either a blue, green, or a yellow color result the test is invalid. Do not report patient results if either the Type 1 Control or the Type 2 Control does not produce expected results.

2. External Quality Controls

External Controls (manufactured by Gynpus Diagnostics, LLC) are used to test that the reagents are working properly. Also use the Controls to test that you are able to correctly perform the test procedure.

- A Control Kit that contains a positive control and a negative control may be purchased separately, Catalog No. S751.
- Refer to the Control Kit Directional Insert for instructions on how to interpret the results of the control.
- If QC testing fails:
 - Check expiration dates of the test kit and controls
 - Ensure the instructions for testing were followed
- Repeat the test.

- If the controls still do not perform as expected contact Technical Support at 1-800-924-4195.

2a. For CLIA Waived Labs

You should follow the manufacturer's guidelines for QC testing. Gynpus Diagnostics recommends that external controls be run with each new lot, each new shipment and with each new untrained operator.

2b. For CLIA Non-Waived Labs

Quality Control requirements should be established in accordance with local, state and federal regulations or accreditation requirements. Minimally, Gynpus Diagnostics recommends that external controls be run with each new lot, each new shipment and with each new untrained operator.

LIMITATIONS OF THE PROCEDURE:

- Do not use samples from the cervix.
- Patients may have mixed infections. The BVBLUE test shows that sialidase enzyme is active in the sample. The BVBLUE test does not show if other organisms such as yeast and parasitic organisms are present in the sample.
- Test results should be considered in conjunction with other clinical and patient information.
- Test operators must follow all instructions to a) collect the sample, b) store the sample, and c) use the test procedure properly. If the instructions are not followed, the BVBLUE test may not give correct results.

EXPECTED VALUES:

The BVBLUE Test can show sialidase activity in vaginal fluid at levels of ≥ 7.8 U. There are two possible results: (a) positive or (b) negative. If the test fails to provide a blue, green, or yellow color result, the test is invalid.

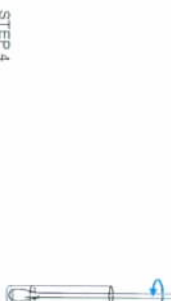
INSTRUCTIONS FOR USE:

- STEP 1**
Remove one BV Test Vessel and the Developer Solution Bottle from the kit prior to use. Remove the cap from the BV Test Vessel.

- STEP 2**
Collect a vaginal fluid sample with a swab. Contact the swab with the lower one-third of the vaginal wall. Collect as much fluid as possible.

NOTE: Do not use samples from patients who have used vaginal cream products within 72 hours before testing. Do not touch or collect fluid near the cervix.

- STEP 3**
Put the swab into the BV Test Vessel. Gently swirl the mixture.



- STEP 4**
Let the BV Test Vessel containing the swab stand for 10 minutes between 17 and 37°C.



- STEP 5**
Add one drop of Developer Solution to the BV Test Vessel containing the swab. Gently swirl the mixture. Read the results immediately.



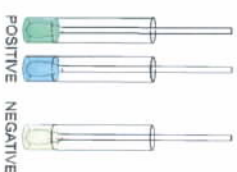
CAUTION: The Developer Solution is a dilute alkaline solution. This may cause skin and eye irritation. If the solution comes in contact with the skin or eyes, flush with large volumes of water.

INTERPRETATION OF TEST RESULTS:

- There are two possible results: (a) positive result or (b) negative result.
- a) Positive Result: A blue or green color in the BV Test Vessel or on the head of the swab
- b) Negative Result: A yellow color in the BV Test Vessel.

NOTE: You may need to remove the swab to read the test results.

A Positive Result shows a high level of sialidase activity. A Negative Result shows a normal level of sialidase activity.



1



2



3

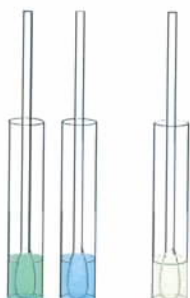
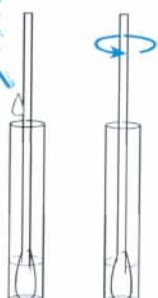


4



10 min.
17°C - 37°C
(62.6°F - 98.6°F)

5



POSITIVE NEGATIVE

TABLE 1. RESULTS OF A STUDY OF 118 WOMEN.

Patient Type	Mean Vaginal Fluid Sialidase Activity
Patients with BV	12.3 U (95% CI 8.1-16.6 U)
Healthy Controls	2.7 U (95% CI 2.3-3.2 U)
Patients with Candidiasis	3.7 U (95% CI 2.8-4.6 U)
Patients with Trichomoniasis	1.99 U (95% CI 0.6-3.4 U)

PERFORMANCE CHARACTERISTICS:

MINIMUM DETECTION LIMIT (MDL):

The BVBLUE Test was evaluated by different users using control samples to demonstrate the MDL of 0.3 µg (7.8 U). A total of 64 samples with sialidase levels above the MDL were evaluated. Complete agreement of results was obtained on every sample. A total of 233 samples with sialidase levels below the MDL were evaluated. Complete agreement of results was obtained on 99% of samples.

REPRODUCIBILITY STUDIES:

The BVBLUE Test was evaluated at three clinics by three different users (MLTs and RNs) for reproducibility within and between runs and clinics. Each site evaluated 5 coded control samples in triplicate on each of 3 days. A total of 45 samples were evaluated by each clinic. Three of the 5 samples were positive samples and 2 were negative samples. Complete agreement of results was obtained on every sample by each of the three sites, demonstrating the inter-operator, inter-site, intra-site, and inter-day reproducibility of the test.

INTERFERENCE STUDIES:

In all clinical studies, no evidence of interference was observed for menses (n=118), blood (n=620), semen (n=620), birth control methods (n=36) including birth control pills (Depo-Provera, Norplant, IUDs, condoms, or tubal ligation), or microorganisms (n=118) including *Staphylococcus*, *Streptococcus*, *E. coli*, *Candida albicans*, *Lactobacillus*, among others.

METHOD COMPARISON:

The BVBLUE Test was evaluated at five clinics by different users (MDs, MLTs, and RNs) in the US. A total of 620 women were evaluated. Patients treated with a vaginal cream or ointment product within 72 hours prior to testing were excluded.

Independent investigators evaluated the performance of the BVBLUE Test compared to Amsel Criteria in 620 women (TABLE 2). A clinical diagnosis of BV required the following three symptoms: vaginal fluid pH > 4.5, the presence of vaginal fluid amines, and the presence of clue cells (>20%). Of the 164 symptomatic women, 65% were diagnosed with BV. Of the 456 asymptomatic women, <1% were diagnosed with BV. The sensitivity and specificity of BVBLUE compared to Amsel Criteria was found to be 95.2% and 99.6% respectively.

Independent investigators evaluated the performance of BVBLUE compared to Gram's stain in 118 women (TABLES 3 and 5). A clinical diagnosis of BV required a Gram's stain score of 7-10⁺. Of the 27 symptomatic women, 78% were diagnosed with BV. Of the 91 asymptomatic women, 11% were diagnosed with BV. The sensitivity and specificity of BVBLUE compared to Gram's stain was found to be 90.3% and 96.6% respectively.

Independent investigators evaluated the performance of BVBLUE compared to Gram's stain in 220 women (TABLE 6). The sensitivity and specificity of BVBLUE compared to Gram's stain was found to be 92.8% and 98.0%, respectively.

TABLE 2. UNRECONCILED PERFORMANCE OF BVBLUE COMPARED TO AMSEL CRITERIA.

All patients tested (n = 620)	BVBLUE Positive
145 (23.4%)	145 (23.4%)
Results in patients with BV by Amsel Criteria (n = 108)	92 (85.2%)
Results in patients without BV by Amsel Criteria (n = 512)	53 (10.4%)

TABLE 3. UNRECONCILED PERFORMANCE OF BVBLUE COMPARED TO GRAM'S STAIN.

All patients tested (n = 118)	BVBLUE Positive
31 (26.3%)	31 (26.3%)
Results in patients with BV by Gram's Stain (n = 31)	28 (90.3%)
Results in patients without BV by Gram's Stain (n = 87)	3 (3.4%)

MIXED INFECTIONS:

The BVBLUE Test was investigated in 265 patients stratified by clinical diagnosis. The results of this study are presented in TABLE 4.

TABLE 4. RESULTS OF BVBLUE IN PATIENTS STRATIFIED BY CLINICAL DIAGNOSES

No. of Patients	Clinical Diagnosis			BVBLUE
	Amsel Criteria	Wet Mount	Tric	
0	+	+	+	0
1	+	+	+	0
2	+	+	+	2
3	+	+	+	0
4	+	+	+	0
5	+	+	+	0
6	+	+	+	0
7	+	+	+	0
8	+	+	+	0
9	+	+	+	0
10	+	+	+	0
11	+	+	+	0
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50	+	+	+	0
51	+	+	+	0
52	+	+	+	0
53	+	+	+	0
54	+	+	+	0
55	+	+	+	0
56	+	+	+	0
57	+	+	+	0
Total (255)	46	6	63	

TABLE 5. PERFORMANCE OF BVBLUE AND EIGHT OTHER CLINICAL METHODS COMPARED TO GRAM'S STAIN RESULTS IN 118 PATIENTS.

Test vs. Gram's Stain	Sensitivity (%)	Specificity (%)	Overall Accuracy (%)
BVBLUE	90.3	96.6	94.9
Amsel Criteria	58.1	95.4	83.6
Vaginal Fluid pH ⁺ (n = 117)	90.3	65.1	71.8
Vaginal Fluid Amines (n = 117)	67.7	93.0	86.3
Vaginal Fluid pH and Vaginal Fluid Amines ⁺ (n = 117)	67.7	94.2	88.4
Wet Prep (>20% clue cells) (n = 117)	71.0	89.5	84.6
Wet Prep (any clue cells) (n = 117)	77.4	84.9	82.9
Semiquantitative Identification of Morphotypes Associated with BV:	100	86.2	89.8
Clinically Significant Culture of Microorganisms Associated with BV/	55.6	85.5	78.4

⁺Vaginal fluid pH > 4.5 considered positive result. Vaginal fluid pH > 4.5 and presence of vaginal fluid amines considered positive result. All other combinations considered negative result. Includes identification and scoring of *Gardnerella vaginalis*, *Bacteroides* spp., *Pteroisilia* spp., and/or *Mobiluncus* spp. using Gram's stain. Score of 2-1 considered positive result for *Mobiluncus* spp. All other morphotypes required score of 2-3+. Includes typing and scoring of *Gardnerella vaginalis*, *Bacteroides* spp., and/or *Pteroisilia* spp. Score of 2-3+ for each microorganism considered positive result.

FIGURE 1. ASSOCIATION BETWEEN GRAM'S STAIN SCORE AND TEST RESULTS FROM BVBLUE AMSEL CRITERIA.

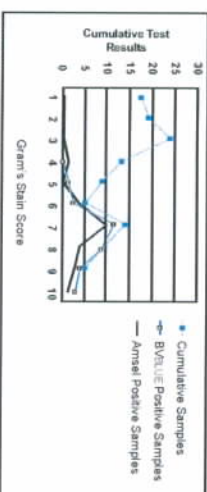


TABLE 6. PERFORMANCE OF BVBLUE COMPARED TO GRAM'S STAIN

Sample Type	Correct	Incorrect	Agreement (95% CI)
All patients tested (n=220) (P=0.0001)	212	8	96.4% (93.9-98.8%)
Results in patients with BV by Gram's Stain (n=69)	64	5	92.8% (86.6-98.9%)
Results in patients without BV by Gram's Stain (n=151)	148	3	98.0% (95.9-100%)

CLIA WAIVER PERFORMANCE:

The BVBLUE Test was evaluated by seventy-five non-trained operators at three non-clinical lab sites. Each operator at each site tested four samples from a randomly coded panel of strong negative (25), weak negative (25), weak positive (25), and strong positive samples (25). Three trained lab operators at one lab site ran all 300 samples. Agreement among non-trained operators and known sample distribution was as follows:

TABLE 7. CLIA WAIVER PERFORMANCE OF BVBLUE

Sample (sialidase activity)	Agreement
Strong Negative (0.15 U)	98.7%
Weak Negative (6.08 U)	100%
Weak Positive (9.15 U)	100%
Strong Positive (20.1 U)	100%

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ASSISTANCE:

For technical assistance call Gryphus Diagnostics Technical Support at 1-800-924-1195.

RE-ORDER:

BVB25 BVBLUE® (25 Tests)
S751 BVBLUE® Control Kit

Manufactured by Gryphus Diagnostics, LLC
BVBLUE® is a registered trademark of Gryphus Diagnostics, LLC.
US Patent nos 6,612,100, 6,667,161, 6,812,332
Australian patent nos 781,391, 768,886. Other patents pending.

KEY TO COMPONENT LABELLING

Use by YYYY-MM

Batch code

Catalog number

Contents sufficient for <n> tests

In vitro diagnostic medical device

Temperature limitation

Consult instructions for use

Authorized representative in the European Community

MANUFACTURED BY:

Gryphus Diagnostics
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www.gryphus.com