

Bacterial Vaginosis

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0. Public Release Data Set Information

This document details the Lab Protocol for NHANES 2001-2002 data.

A list of the released analytes follows:

Lab	Analyte	SAS Label	Description
I34_b	LBXBV	Bacterial Vaginosis	Bacterial Vaginosis
I34_b	LBXBVPH	PH of Bacterial Vaginosis Specimen	PH, Bacterial Vaginosis Specimen

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

Gram stain of vaginal secretions:

The NHANES participant self collects the appropriate cotton swab. The NHANES laboratory staff then tests the pH of the vaginal fluid on the cotton swab and makes a smear for gram staining by rolling the swab across the surface of the frosted edge slide. The slides are air dried and stored at room temperature for shipping.

Scan the slide using a low power objective to locate any clusters of epithelial cells. The flora in these areas should be noted. Switch to the oil immersion lens (x1000) and examine between 10 and 20 representative fields to observe cell morphology and Gram reaction. The BV score for Gram staining will be calculated by Nugent's method (1991). Briefly, the average number of lactobacilliary morphotypes per oil immersion field will be quantitated. These organisms are usually filamentous, gram positive rods of varying length that often form chains, but occasionally, they may stain gram negative. Also quantitate the average number of Gardnerella spp. and anaerobic gram negative rods. These may appear as small, gram variable pleomorphic coccobacilli. Finally, look for and quantitate the amount of Mobiluncus morphotypes present. They are often thin, wispy, eyelash-like faintly staining curved gram negative rods. Alternatively they may be much smaller "banana-like" forms with pointed ends. Occasionally, they may stain gram positive. These bacteria are often absent from gram stain smears of patients with other bacterial morphotypes.

2. SAFETY PRECAUTIONS

- a. The technologist will wear gloves, laboratory coat and safety glasses while carrying out the staining procedure.
- b. All Gram stain reagents will be properly handled and stored in accordance with the written hospital safety regulations.
- c. The alcohol/acetone decolorization solution is stored in a safety cabinet designed to hold flammable liquids.

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

- a. Each shipment of specimens received from the NHANES mobile unit contains a corresponding transmittal sheet and an ANSI data file (XXXXXXX.TXT) is emailed as an attachment. The data file, containing the specimen ID, collection date, and type of sample (i.e. slide) is checked against the information on the transmittal sheet and specimen label prior to the assay.
- b. After the data is calculated and the final values are approved by the reviewing supervisor for release, all results are entered onto the NHANES ANSI data file by using Excel.
- c. After the results are entered, back-up copies are made and stored in locked areas.
- d. The results are emailed, as an attachment, to NCHS.

4. SPECIMEN COLLECTION, STORAGE, AND HANDLING PROCEDURES; CRITERIA FOR SPECIMEN REJECTION

- a. The smears should be left to air dry before being shipped at room temperature to the contractor's laboratory for fixation, staining, reading and interpretation.
- b. The BV smear will be heat fixed at the receiving laboratory before being Gram stained.
- c. The ideal BV smear should upon visual inspection cover at least two-thirds of the surface area of a properly labeled glass slide and air-dried.
- d. Swabs containing vaginal fluid for BV smears must be immediately rolled onto a properly labeled glass slide at the time of collection, and allowed to air-dry properly.
- e. The slide should be properly labeled including the date of collection, using an indelible marker that will not wash off during the decolorization step of the Gram staining procedure.
- f. The BV smears should be packaged so as to protect the glass slides from breaking en route, and maintain

ambient temperature during shipping.

- g. Once the slides have arrived at the contractor's facility, they will be logged in, heat fixed and stained as described previously.

Criteria for rejection

- 1) Inadequately, unlabeled, or illegibly labeled slides.
- 2). Broken slides.
- 3) Slides lacking specimen inoculum.
- 4) Slides containing ≤ 2 epithelial cells per oil powered field; indicates sample collected from wrong site; cervical, not vaginal.
- 5). Slides containing only cellular debris.

5. PROCEDURES FOR MICROSCOPIC EXAMINATIONS; CRITERIA FOR REJECTION OF INADEQUATELY PREPARED SLIDES

- a. Slides that have been sprayed with a fixative, such as a pap smear cytology spray fixative, will not Gram stain properly.
- b. Slides containing epithelial cells only, and no bacteria are unusual and will be reported out with such a comment.

6. EQUIPMENT AND INSTRUMENTATION, MATERIALS, REAGENT PREPARATION, CALIBRATORS (STANDARDS), AND CONTROLS

a. Instrumentation

- 1) Microscope

b. Other Materials

- 1) Crystal violet
- 2) Iodine solution
- 3) Alcohol/acetone solution
- 4) Safranin

7. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

a. Gram Stain Procedure

- 1) Heat-fix the glass slide containing the specimen.
- 2) Flood the fixed slide with crystal violet.
- 3) Allow stain to remain on for 1 min.
- 4) Rinse slide gently under running tap water.
- 5) Flood slide with the iodine solution.
- 6) Allow the solution to remain for 1 min.
- 7) Rinse slide gently under running tap water.

- 8) Decolorize slide by letting the alcohol/acetone solution flow over the smear, while the slide is held at an angle. Stop applying decolorizer when the solution runs clear.
- 9) Rinse slide gently under running tap water.
- 10) Flood slide with safranin.
- 11) Allow stain to remain for 30 sec.
- 12) Rinse slide gently under running tap water.
- 13) Drain slide and air-dry it in an upright position.

b. Reading Stained Slides

- 1) Scan the slide using a low power objective to locate any clusters of epithelial cells. The flora in these areas should be noted.
- 2) Switch to the oil immersion lens (x1000) and examine between 10 and 20 representative fields to observe cell morphology and Gram reaction.
- 3) The BV score for Gram staining will be calculated by Nugent's method (1991).
- 4) The average number of lactobacilliary morphotypes per oil immersion field will be quantitated. These organisms are usually filamentous, gram positive rods of varying length that often form chains, but occasionally, they may stain gram negative.
- 5) Quantitate the average number of Gardnerella spp. and anaerobic gram negative rods. These may appear as small, gram variable pleomorphic coccobacilli.
- 6) Look for and quantitate the amount of Mobiluncus morphotypes present. They are often thin, wispy, eyelash-like faintly staining curved gram negative rods. Alternatively they may be much smaller "banana-like" forms with pointed ends. Occasionally, they may stain gram positive. These bacteria are often absent from gram stain smears of persons with other bacterial morphotypes.
- 7) The relative amounts of each of the three classes of observed morphotypes will be reported. Each morphotype will be quantitated from 0 to 4+ with regard to the numbers of organisms present per oil immersion field as described in Table 1.

Table 1. Calculating Individual Scores Based upon Morphotype.

MORPHOTYPE	NUMBERS OF ORGANISMS/OIL IMMERSION FIELD				
	NONE	<1	1-4	5-30	>30
Lactobacilli spp.	4	3	2	1	0
Gardnerella & anaerobic GNR	0	1	2	3	4
Mobiluncus spp.	0	1	1	2	2

The individual scores for each of the three morphotypes of bacteria; Lactobacilli spp., Gardnerella spp./anaerobic gram negative rods and Mobiluncus spp., respectively should be added together to obtain the total score. The final BV score and the result interpretation are described below:

c. Recording of Data

(1) Analytical Results

<u>BV Score</u>	<u>Interpretation</u>
1-3	"Normal vaginal flora"
4-6	"Intermediate"

7-10 "Indicative of Bacterial Vaginosis"

d. Calculations

The BV score for Gram staining will be calculated by Nugent's method (1991).

9. REPORTABLE RANGE OF RESULTS

<u>BV Score</u>	<u>Interpretation</u>
1-3	"Normal vaginal flora"
4-6	"Intermediate"
7-10	"Indicative of Bacterial Vaginosis"

10. QUALITY CONTROL (QC) PROCEDURES

- a. Gram stain reagents are checked weekly and also when a new lot of stain is to be put into use, as per the CLIA Quality Control regulatory notice (published July 18, 1997).
- b. Gram stain reagents are evaluated by staining the following recommended bacterial strains; ATCC 25923, Staphylococcus aureus and ATCC 25922, Escherichia coli. The Staphylococcus aureus should appear as deep violet, gram-positive cocci, while the Escherichia coli should appear as pink, gram-negative rods.
- c. Gram stain QC will be performed with every run of person samples and will include Staphylococcus aureus and E. coli. The results of this QC will be recorded.

11. REMEDIAL ACTION IF CALIBRATION OR QC SYSTEMS FAIL TO MEET ACCEPTABLE CRITERIA

None. There is no mechanism to recollect the specimen.

12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

Not applicable to this procedure.

13. REFERENCE RANGES (NORMAL VALUES)

A score of 1-3 is expressed as "normal vaginal flora".

14. CRITICAL CALL RESULTS ("PANIC VALUES")

Not applicable to this procedure.

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

The slides are stored in the slide boxes at ambient temperature.

16. ALTERNATIVE METHODS FOR PERFORMING TEST OR STORING SPECIMENS IF TEST SYSTEM FAILS

If the microscope is inoperable, the slides are stored at ambient temperature until it is repaired.

17. TEST RESULT REPORTING SYSTEM; PROTOCOL FOR REPORTING CRITICAL CALLS (IF APPLICABLE)

Not applicable for this procedure.

18. TRANSFER OR REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING

For the NHANES 2001-2002 the slides are stored at ambient temperature for six years after analysis and then discarded.

19. Summary Statistics and QC graphs

Qualitative assays are qualitative assays with a normal vaginal flora, intermediate, or indicative of bacterial vaginosis result.