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Documentation for Laboratory Results

Laboratory 34- Bacterial vaginosis (BV) and *Trichomonas vaginalis*

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(2) Documentation File Name- Laboratory 34- Bacterial vaginosis (BV) and *Trichomonas vaginalis*

(3) Survey Years Included in this File Release-2001-2002

(4) Component Description

Bacterial vaginosis (BV) and trichomoniasis are two of the most common vaginal conditions affecting women of childbearing age. In the United States, BV varies depending on the population studied, from 17% in some prenatal and family planning settings to 37% in STD clinics. The same is true for trichomoniasis, with between 3% and 48% of sexually active women diagnosed in various clinical settings. Recent studies have linked BV to adverse pregnancy and gynecologic outcomes, such as preterm labor and delivery, low birth weight, premature rupture of membranes, post-Cesarean endometritis, and post-abortal and post-hysterectomy infections. Also, trichomoniasis has been associated with preterm labor, preterm delivery, and low birth weight. More recently, both BV and trichomoniasis have been linked to an increased risk of HIV acquisition and transmission. However, no national surveillance system exists to measure the full burden of these two diseases, and no reliable national population estimate of BV or trichomoniasis exists. NHANES offers a unique opportunity to assess the prevalence of BV and Trichomonas vaginalis infections in the general population, to identify and confirm risk factors, and to monitor trends in prevalence as detection and treatment programs are established and expanded.

(5) Sample Description:

5.1 Eligible Sample

Female participants aged 14 to 49 years were tested.

(6) Description of the Laboratory Methodology

6.1 Bacterial vaginosis

After the slide was gram stained the slide was scanned under a microscope using low power objective to locate clusters of epithelial cells. The flora in these areas was noted. The oil immersion lens (x1000) was switched and between 10 and 20

representative fields was examined to observe cell morphology and gram reaction. The BV score was calculated by Nugent's method. Briefly, the average number of lactobacillary morphotypes per oil immersion field was quantitated. These organisms were usually filamentous, gram positive rods of varying length that often form chains, but occasionally, they stained gram negative. Also, the average number of Gardnerella spp. and anaerobic gram negative rods were quantitated. These may appear as small, gram variable pleomorphic coccobacilli. Finally, the amount of Mobiluncus morphotypes present was quantitated. 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 were often absent from gram stain smears of patients with other bacterial morphotypes. The relative amounts of each of the three classes of observed morphotypes was reported. Each morphotype was 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

	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

6.2 *Trichomonas vaginalis*

During PCR testing, Taq polymerase and DNA primers complimentary to a unique sequence of target DNA were used to greatly amplify that region if the target was present in the sample. This greatly enhanced the sensitivity of assays used to detect that specific sequence of DNA. After a series of successive cycles of amplification, the presence of double stranded DNA product was visualized on agarose gels stained with ethidium bromide. Specificity of the product was confirmed with hybridization to a labeled probe. Samples were tested for the presence of amplifiable DNA and absence of inhibitors by performing beta globin PCR. Beta globin is common to all mammalian cells and it is reasonable to expect that some human cells will be present in the sample. If the presence of beta globin couldn't be demonstrated, the validity of the sample wasn't determined.

***Trichomonas vaginalis*, a sexually transmitted human parasite, was detected by performing PCR with primers from a region of the 18S rRNA gene that produce a 312 base pair product. The specificity of the product was confirmed by hybridization to a digoxigenin-labeled probe according to the method described in Boehringer Mannhein's Genius System User's Guide for Filter Hybridization.**

(7) Laboratory Quality Control and Monitoring

The NHANES quality control and quality assurance protocols (QA/QC) meet the 1988 Clinical Laboratory Improvement Act mandates. Detailed quality control and quality assurance instructions are discussed in the NHANES Laboratory/Medical Technologists Procedures Manual (LPM). Read the LABDOC file for detailed QA/QC protocols.

(8) Data Processing and Editing

Vaginal swabs were processed, stored and shipped to Magee-Women's Hospital, Pittsburgh, Pennsylvania. Detailed specimen collection and processing instructions are discussed in the NHANES Laboratory/Medical Technologists Procedures Manual (LPM). Read the LABDOC file for detailed data processing and editing protocols. The analytical methods are described in the Description of the Laboratory Methodology section.

(9) Data Access:

All data are publicly available.

(10) Analytic Notes for Data Users:

10.1 The analysis of NHANES 2001-2002 laboratory data must be conducted with the key survey design and basic demographic variables. The NHANES 2001-2002 Household Questionnaire Data Files contain demographic data, health indicators, and other related information collected during household interviews. They also contain all survey design variables and sample weights for these age groups. The phlebotomy file includes auxiliary information such as the conditions precluding venipuncture. The household questionnaire and phlebotomy files may be linked to the laboratory data file using the unique survey participant identifier SEQN.

10.2 Final BV score and interpretative reporting

BV score	Intrepretation
0-3	"Normal vaginal flora"
4-6	"Intermediate"
7-10	"Indicative of Bacterial Vaginosis"

10.3 The results of the Trichomonas PCR test will be finalized as follows:

A sample will be considered positive if it yields a 102 base pair fragment after PCR amplification that is recognized by the Trichomonas specific DNA probe upon Southern blot hybridization.

A sample will be considered negative if it does not yield the 102 base pair fragment after PCR amplification, or when the PCR product's identity cannot be confirmed by Southern blot hybridization.
A sample will be considered uninterpretable if the specimen was found to be both Trichomonas and Beta globulin negative by PCR. This can be due to either the presence of inhibitors or the lack of DNA or both.