Ministry of Health

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Contents Table:

Acknowledgment

The diagnostic laboratories services at the Directorate General of Specialized Medical Care (DGSMC) at Ministry of Health (MOH) would like to thank and appreciate the great effort of the Microbiology documents development team. Participated and contributed personnel are:

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Acronyms:

BV	bacterial vaginosis
BA	Blood agar
CA	Chocolate agar
MAC	MacConkey
ATCC	American Type Culture Collection
H&S	Health and Safety
ID	Identification
IQC	Internal Quality Control
MDRO	Multidrug Resistant Organism
MRSA	Methicillin Resistant Staph. Aureus
SOP	Standard operating procedure
TAT	Turnaround time
WHO	World Health Organization

1. **Purpose**

This SOP describes laboratory examination of high vaginal swab for the presence of yeasts, group B streptococcus, Trichomonas vaginalis and bacterial vaginosis (BV).

2. **Scope**

This document is applicable for all medical laboratories under MOH and other collaborative governmental and non-governmental health institutions.

3. **Definitions**

3.1 Bacterial vaginosis: is a condition that happens when there is existence of certain bacteria in the vagina. This changes the normal balance of bacteria in the vagina.

4. **Procedure**

4.1.Clinical background:

Vaginal discharge is a common presentation in general practice. Infections of the lower genital tract (vulva, urethra, vagina and cervix) are generally caused by sexually transmitted pathogens such as *Neisseria gonorrhoea*, *Trichomonas vaginalis* ,*Chlamydia trachomatis* or by organisms which may be part of the normal vaginal flora such yeasts and those associated with bacterial vaginosis. The discharge commonly pools in the posterior vaginal fornix where the High vaginal swab (HVS) is collected. HVS investigation is indicated in females presenting with vaginal discharge. HVS microscopy and culture are useful tools to diagnose some causes of vaginal discharge such as Bacterial vaginosis (BV), *Trichomonas vaginalis* (TV) and Candidiasis (Vaginal thrush). HVS is also useful tool to screen pregnant ladies for transient colonization with Group B *streptococcus.*

Normal vaginal flora

vaginal flora normally contains a large variety of organisms including aerobic and anaerobic organisms such as Lactobacillus species, streptococci, enterococci and coagulase-negative staphylococci. Some organisms such anaerobic cocci, Gardnerellavaginalis and yeasts may also be present as part of the normal vaginal flora, but they have also been implicated as a cause of vaginal infections.

Vaginal candidosis

Cases with vaginal candidosis commonly present with pruritus, dysuria and a whitish discharge. It occurs in conditions associated with alterations in the vaginal environment allowing yeasts which are often present as commensal vaginal flora to over-proliferate.

Bacterial vaginosis (BV)

- BV is characterised by shift in vaginal flora and a decrease in numbers of lactobacilli which are replaced by a mixed group of organisms. This includes *Prevotella* species, *Gardnerella Vaginalis, Mobiluncus*species, *Peptostreptococcus* species, *Mycoplasma hominis.*
- Clue cells are epithelial cells to which Gram-variable rods are attached in large numbers, obscuring the cell border. They are reported as being highly specific (almost 100%) but not as sensitive as using other aspects of a Gram stain to detect BV. Gram staining (using the criteria of Nugent) of vaginal smears is the most sensitive method for the laboratory diagnosis of BV. It is not necessary to see clue cells to make a diagnosis of BV. One of the key features is the absence of typical lactobacilli and their replacement with Gram-variable or Gram-negative rods.
- In typical smears from patients with BV, clue cells are accompanied by a mixed flora consisting of very large numbers of small Gram-negative rods (predominantly *Prevotella* species) and Gram-variable rods and coccobacilli (predominantly *G. vaginalis*) in the absence of larger Gram-positive rods (*Lactobacillus* species). Curved Gram-variable rods (*Mobiluncus* species) may also be present.
- It is recommended to examine all vaginal swabs from women of child bearing age for the presence of BV by Gram film.

Trichomoniasis

- Trichomoniasis is caused by the flagellate protozoan, *Trichomonavaginalis,* is almost always sexual acquired. Symptoms include an increased vaginal discharge, pruritus and dysuria.
- Microscopy of a wet preparation is highly specific and easily performed, but it fails to detect 30-50% of TV.

Vulvovaginitis

- Vulvovaginitis is mainly seen in pre-pubertal females, but may affect women of any age. Common causative organisms include Lancefield group A streptococcus, *Staphylococcus aureus, C. Albicans, Haemophilus influenza and N. gonorrhoeae.*
- Other unusual organisms may cause vulvovaginitis include *Salmonella* and *Shigella* species. Threadworm infestation may predispose to vulvovaginitis.

4.2.Principle:

- Swab specimens are collected from the vagina to determine the presence of organisms associated with sexually transmitted or non-sexually transmitted infections.
- Appropriate specimens are often difficult to obtain because of frequent contamination with faecal flora during the collection of specimens.
- Certain important organisms are not amenable to detection by routine culture (e.g. Ureaplasmaurealyticum, Treponema pallidum, Mycoplasma hominis, and Chlamydia trachomatis).
- Cultures for gonorrhea should be obtained directly from the uterine cervix.
- Anaerobic cultures should not be performed except on abscess fluid aspirated by syringe and needle from a paravaginal abscess.
- High vaginal swab is also not an optimal specimen for the diagnosis of suspected cases of pelvic inflammatory diseases, IUCD infections, or postpartum infections.

4.3.Pre – analytical stage:

4.3.1. Sample:

- It is important to avoid contamination with faecal flora during collection of specimens.
- The swab is received in Amies transport medium with charcoal and processed as soon as possible.
- If processing is delayed of more than 2 hours, refrigeration is preferable to storage at ambient temperature. Delays of over 48hr are undesirable.
- Note: Cultures for Gonorrhea should be obtained directly from the uterine cervix and not from high vaginal swab.

4.3.2. Material:

Reagents	Consumables/Supplies	Equipment
Strept grouping kit Staphaurex kit Catalase Tube coagulase normal saline	Blood Agar (BA) Choclate Agar (CA) Sabouraud Agar (SDA) CO_2 Kit Slides Cover slip Loops Marker pen	Anaerobic Jar Microscope Incubator

4.3.3. Safety precaution:

- All specimens need to be treated as potentially infectious. Standard procedures for handling of biohazard material must be followed at all times. Universal Precautions must be practiced at all stages of these procedures.

4.3.4. Quality control:

- Laboratories should ensure that all commercial and in-house tests, swabs and media used such as: agars, have been checked for quality insurance and validated and shown to be fit for purpose.
- Laboratories should participate in external quality assessment (EQA) schemes and undertake relevant internal quality control procedures.
- Follow the quality control procedure for culture media, stains, reagents, biochemical, identification & susceptibility systems.

4.4.Analytical stage:

4.4.1. Wet preparation for the detection of TV

- After inoculation of all agar plates, prepare a wet prep by rotating and tapping the swab on a clean microscope slide with a drop of normal saline.
- Place a cover slip over the wet inoculum and examine with a low power objective (10X).

4.4.2. Microscopy for BV

- Perform Gram stain and apply Nugent's criteria
- Methods for staining procedures are included in separate SOP.

4.4.3. Culture and Investigation

- Inoculate each agar plate with swab as follow in table 1.
- For the isolation of individual colonies, spread inoculum with a sterile loop.

Table 1: Types of Culture media, conditions and organisms

Standard media	**Incubation**				
	Temp °C	**Atmos**	**Time**	**Cultures read**	**Target organism(s)**
Blood agar	35-37	5-10% CO_2	16-24hr	16-24hr	*S. aureus* Lancefield Groups A, C and G streptococci Other organisms may be significant e.g. Lancefield group B streptococci in pregnancy
Chocholate agar	35-37	5-10% CO_2	40-48hr	daily	*H. influenza*
Sabouraud agar	35-37	Air	40-48h	≥40h	Yeasts

4.4.4. Identification:

Minimum level of identification in the laboratory as follows in table 2.

Table 2: level of organism's identification

Organism	**Identification level**
β-haemolytic streptococci	Lancefield group level
Other streptococci and enterococci	genus level
Enterobacteriaceae	"coliforms" level
Haemophilus	species level
S. aureus	species level
Yeasts	"yeasts" level Note: if suspected treatment failure, identify Candida to species level

Organisms may be further identified if this is clinically or epidemiologically indicated.

4.4.5. Antimicrobial susceptibility testing

Refer to National Antimicrobial Susceptibility Guidelines.

4.5.Post – analytical stage:

4.5.1. MicroscopyReporting Procedure

4.5.1.1.Gram films

- Report on yeasts and WBCs if present and on the presence or absence of intracellular Gram-negative diplococci.
- Report on clue cells if present and whether microscopy is suggestive of BV according to the criteria of Nugent (number of organisms per high power, oil immersion field (hpf) at approximately x1000 magnification) as follows in table 3:

Table3: Nugent's criteria

Numbers of *Lactobacillus*morphotypes seen (large gram positive bacillus)	Score	Numbers of *Gardnerella*and *Prevotella*morphotypes seen (tiny Gram variable coccobacilli or rods)	Score	Numbers of *Mobiluncus*morphotypes seen (curved gram variable rods)	Score
>30/hpf	0	>30/hpf	4	>30/hpf	4
5-30/hpf	1	5-30/hpf	3	5-30/hpf	3
2-4/hpf	2	2-4/hpf	2	2-4/hpf	2
1/hpf	3	1/hpf	1	1/hpf	1
none	4	none	0	none	0

Code each morphotype separately according to numbers of organisms seen as indicated in the table above and add individual scores together. Interpret scores as follows in table4

Table 4: Interpretation of Nugent's criteria

Score	**Interpretation**
0-3	Normal
4-6	Intermediate: Suggestive of Bacterial Vaginosis Please correlate clinically and send repeat for confirmation.
≥7	Abnormal: Indicative of Bacterial Vaginosis

- A vaginal smear should be requested on any swab that is suggestive of BV or if examination for BV is specifically requested.

4.5.1.2.Wet preparations:

- Report on WBCs, yeasts and trichomonads seen.

Note:A negative microscopy result does not exclude the possibility of TV infection.

4.5.1.3.Culture

Report clinically significant organisms isolated or

Report other growth (e.g. normal Vaginal flora isolated) or

Report absence of growth.

Note: The absence of *N. gonorrhoeae* in vaginal swabs should not be reported as these are not the specimen of choice for the isolation of *N. gonorrhoeae*.

4.5.1.4. Antimicrobial susceptibility testing

Report susceptibilities as clinically indicated.

5. Responsibilities

5.1. Responsible staff:

- To ensure the adherence to critical result communication procedure
- To facilitate the alternative channels once needed

5.2. Quality manager /officer

- To follow up the implementation of the procedure
- To monitor regularly communication of critical results and raise non-conformance with corrective action once needed.

5.3. All lab staff:

- To adhere to the procedure.
- To document record and release results as recommended
- To report test failures or incident

6. Document History and Version Control

Version	Description	Review Date
1	Initial Release	May 2026

7. References

Title of book/ journal/ articles/ Website	Author	Year of publication	Page
UK Standards for Microbiology Investigations Investigation of Genital Tract and Associated Specimens	Public Health England	2017	
Genital Tract Culture Manual	University health network/Mount Sinai hospital, Department of Microbiology	2021	
Policy & Procedure Of High Vaginal Swab	Al Masarra hospital, Laboratory Department	2020	

8. **Annexes:**

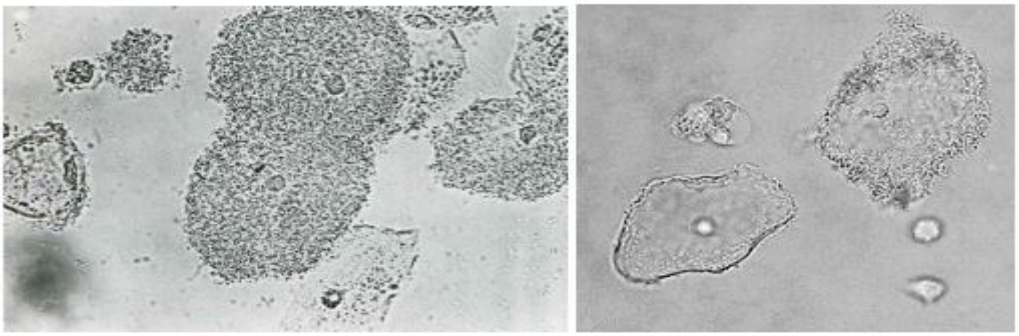
Nugents Criteria

Culture Media

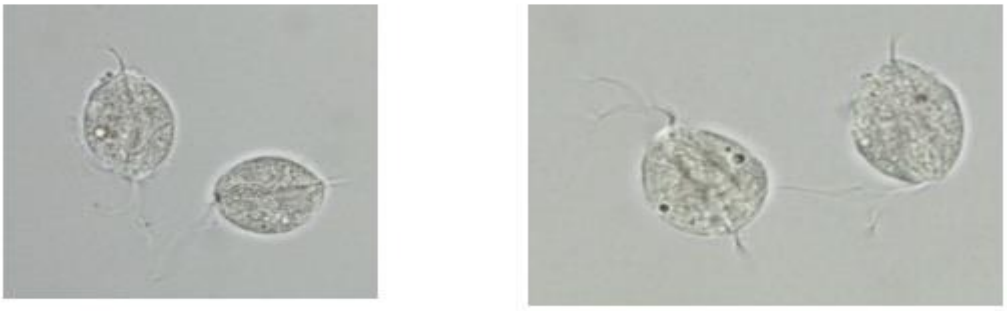
Additional information contained e.g. In a table, forms

Wet Mount using 0.9 Normal saline and examine under 10x objectives.

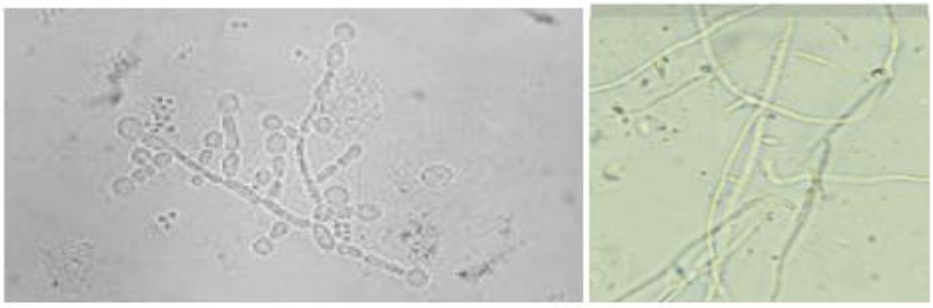
"Clue Cells" (*Gardnerella vaginalis* – Bacterial Vaginosis)



Trichomonads (*Trichomonas Vaginalis*)



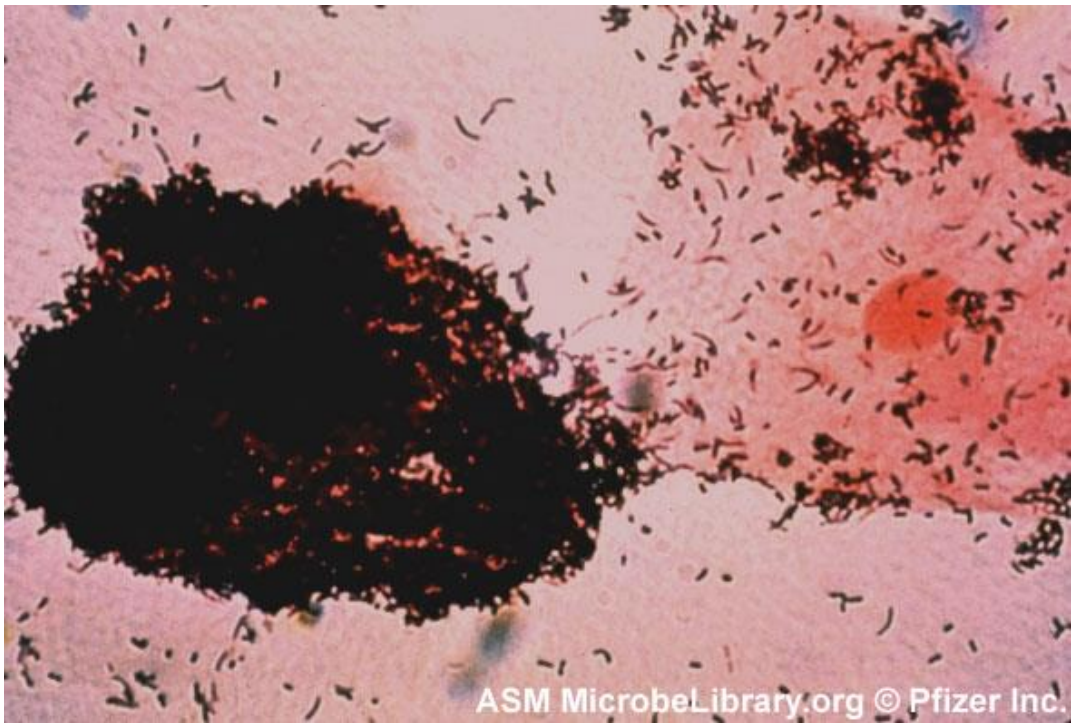
Yeast and/or Pseudohyphae (*Candida albicans*)



University of California, San Francisco – Department of Laboratory Medicine Zuckerberg San Francisco General Hospital and Trauma Center, 1001 Potrero Avenue, San Francisco, CA 94110

Clinical Laboratory – Barbara Haller, MD, PhD, Director Title:Saline and KOH Vaginal Wet Mounts, Document No.: 48667.258 (version 3.1).

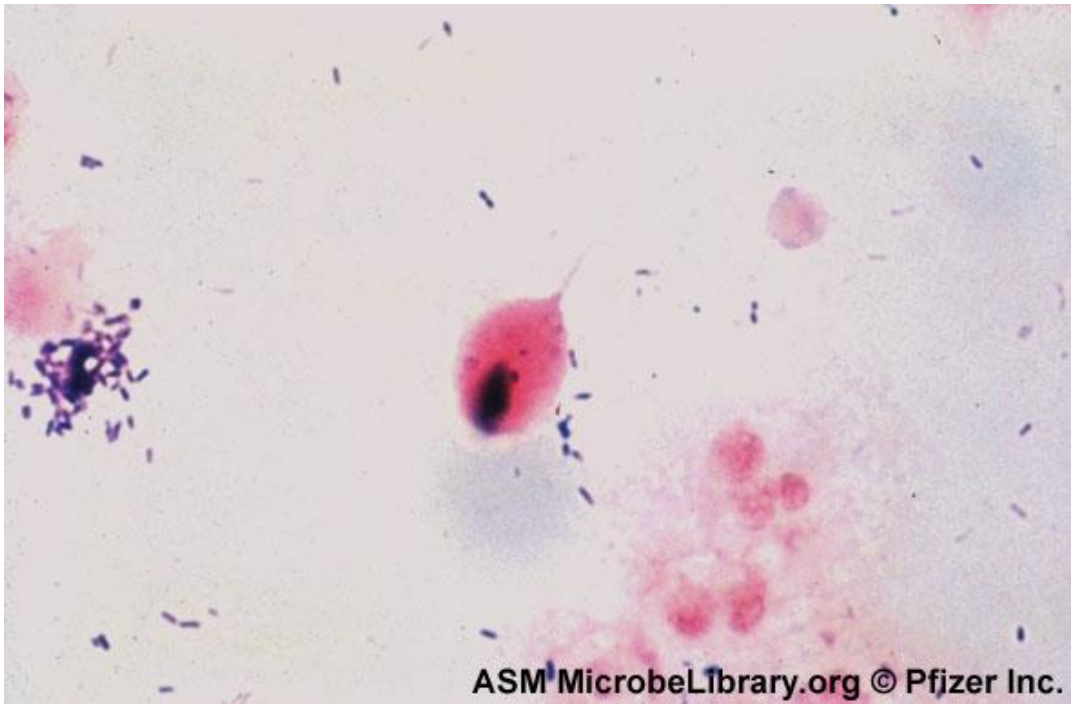
Grams stain of High Vaginal swab examined under 100x objectives with Oil imirsion.



Gardnerella vaginalis (Enlarged view) Bacterial vaginosis
This epithelial cell is a "clue cell" to which large numbers of Gardnerella vaginalis adhere. The presence of clue cells is an important criterion in diagnosing
bacterial vaginosis, apparently a synergistic infection involving G. vaginalis and anaerobic bacteria. Gardnerella vaginalis frequently stains gram-variable, as does Mobiluncus curtisii, an anaerobic bacterium often associated with bacterial vaginosis and visible here as gram-positive curved rods.

Adopted from: American Society For Microbiology

Grams stain of High Vaginal swab examined under 100x objectives with Oil imirsion.



Trichomonas vaginalis (Enlarged view)

This Gram-stained specimen shows a large oval organism with an axostyle, which is a supporting rod running through the body of a trichomonad and protruding posteriorly. Trichomonas vaginalis, a protozoan that causes vaginitis, is usually more easily detected on a wet mount than on a Gram stain.

Adopted from: American Society For Microbiology