#### Copy of version 1.0 (approved and current)

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Periodic Review Completed		Printed By	Lisa Lee		
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#### Approval and Periodic Review Signatures

Туре	Description	Date	Version	Performed By	Notes
Periodic review	Designated Review er	10/27/2022	1.0	Lisa G. Lee N	MT (ASCP) ATC
Approval	Lab Director	1/22/2021	1.0	Aml Girgis	Recorded when document added to MediaLab
Periodic review	Designated Review er	1/22/2021	1.0	Aml Girgis	Recorded when document addec to MediaLab

Approvals and periodic reviews that occured before this document was added to the MediaLab Document Control system may not be listed. V

#### Version History

Version	Status	Туре	Date Added	Date Effective	Date Retired
1.0	Approved and Current	First version in Document Control	12/22/2021	1/22/2021	Indefinite

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#### SCOPE OF USE:

Testing should be performed by licensed providers that meet designated educational and competency criteria approved by regulatory and accrediting agencies. Narrative results may be entered into the provider note as part of a provider performed examination. Diagnoses should be made in conjunction with other clinical information including patient history, symptoms and results from other laboratory and/or diagnostic tests.

#### PRINCIPLE:

The female genital tract has a complex "ecosystem" that must remain in balance. If this balance is upset by hormonal changes, antibiotics or sexually transmitted diseases, it will lead to symptoms that range from mild discomfort to life-threatening infections. Many of the most common problems can be identified with the use of simple tests at the point of care (Women's Health Clinic). Microscopic observation of unfixed "wet mounts" of clinical specimens, either stained or unstained, can be useful for the rapid detection of the presence of bacterial, fungal, and parasitic organisms. Presumptive identification can be made, based on morphology and motility. The presence or absence of white blood cells and "clue cells" may also be demonstrated, and a number of well-recognized pathologic conditions may be identified.

#### SPECIMEN:

Specimen types include swabs of the vaginal mucosa and vaginal pool secretions that must be tested without delay at site of collection during physical examination and collected by provider.

#### **Patient Preparation:**

Specimens for direct wet mount are usually collected during an internal examination of the female genital tract. Specimens from other sources such as skin scrapings, hair, and nail analyses are collected during an appropriate exam.

#### Type:

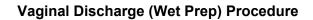
Specimen types include swabs of the vaginal mucosa and vaginal pool or scraping of suspected skin area.

#### Handling Conditions:

Swabs used to collect specimens should be placed into tubes containing approximately 0.5 mL sterile physiologic saline, then resealed. To preserve the motility of *Trichomonas*, specimens should not be refrigerated and should be examined as soon as possible following collection. All tubes must be properly labeled with the patient's name and ID number.

EQUIPMENT AND MATERIALS:
Equipment:
Microscope with 10X and 40X objectives
Materials:
1. Latex gloves
2. Sterile swab
3. 0.85% saline
4. Collection container
5. Glass slides w/coverslips
6. 10% KOH (potassium hydroxide)

7. Plastic pipettes



#### **Preparation:**

- 1. A slide can be prepared directly from the discharge, or
- 2. Swab can be placed in a small amount (<1.0ml) of non-bacteriostatic saline.

#### **Performance Parameters:**



KOH and pH strips must be checked for performance. There are no controls or proficiency tests for these analytes. However, proficiency testing is available and done for organism identification, presence or absence of organisms, and cell identification.

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## Storage Requirements:

Specimens must be fresh and kept at room temperature and read microscopically within 30 minutes of collection.

## CALIBRATION:

N/a

## **Standard Preparation:**

None

#### **Calibration Procedure:**

None

#### **QUALITY CONTROL:**

Commercial controls are not currently available for wet mount preparations. Multiple examiners and/or pathology confirmation can help determine accuracy of the test results. Check normal saline solution for olled cr clarity of solution and no growth or precipitation.

## **PROCEDURE - STEPWISE:**

- 1. Place 1 to 3 drops of normal saline onto a clean microscope slide.
- 2. Twirl swab onto slide.
- 3. Place a coverslip over sample and tamp down lightly.
- 4. Examine slide with a brightfield microscope.

#### ANALYSIS:

# 1. <u>Color:</u>

When the vaginal discharge has been visualized, select the description that best matches the color of the discharged:

CLE	EAR	
YEL	LLOW	4
GR	REY	0
WF	HITE	$c^{\circ}$
GR	REEN	6
CLEAR		100
		0
2. <u>Consist</u>	tency:	ALL.

When the vaginal discharge has been visualized, select the description that best matches the consistency of the discharge:

FLOCCULAR

CHEESY

FROTHY (w/bubbles)

HOMOGENOUS

## 3. <u>Blood:</u>

When the vaginal discharge has been visualized, observe the discharge for the presence of gross blood. The presence of blood is reported as: PRESENT or NONE.

## 4. WHIFF Test:

The WHIFF Test is actually a test for certain aromatic amines that are characteristic of bacterial vaginitis. Place a drop of the vaginal discharge on a slide. Add one drop of 10% KOH. The test is positive if there is the release of a "fishy" odor, otherwise the test is NEGATIVE.

#### 5. Microscopic Examination:

A swab of vaginal fluid can be touched to a glass slide or a drop of saline from the swab transport container to prepare a wet prep. A coverslip is placed over the specimen on the glass slide. The specimen is then examined microscopically using high power (40X) objective for the following:

#### A. Yeast:

Yeast will appear as a spherical object, often with budding daughter cells. Strands of pseudohypae may also be present.

#### B. Trichomonas:

These mobile organisms can be found by observing the whip-like movement of flagella. Trichomonads will be very difficult to visualize if dead. They will die if the specimen is not read within 1 hour, or if the specimen is allowed to dry.



## C. Clue Cells:

"Clue cells" are squamous epithelial cells that are studded with bacteria, often to the point that the cell margins cannot be seen. The bacteria appear as tiny refractile spots on the cell.

#### D. White Cells:

White blood cells measure 14 to 16  $\mu$ m and exhibit a granular cytoplasm. White blood cells (with characteristic multilobed nucleus) are usually present in vaginal specimens in rare to scanty numbers. If they appear to be 3+, abnormal flora may be suspected. Conditions usually associated with 3+ white blood ells include Trichomonas, vaginal Candidiasis, N. gonorrhea, herpes simplex, and severe atrophic vaginitis.

#### E. Squamous Cells:

Squamous cells measure 25 to 70  $\mu$ m and demonstrate a polygonal "flagstone" appearance. These cells are almost always present in vaginal fluid.

## F. Red Blood Cells:

Red blood cells (RBCs)appear as biconcave discs measuring 7 to 8 µm in diameter. They are normally smooth, but may be greatly distorted in vaginal and urine specimens. The cytoplasm is clear and does not contain a nucleus. Red blood cells may be confused with yeast. They will lyse with addition of KOH, which is helpful in distinguishing between RBCs and yeast. RBCs may be present in vaginal fluid as a result of current or recent menses, or due to Desquamative inflammatory process.

#### G. Parabasal Cells:

Parabasal cells measure 16 to 40  $\mu$ M in diameter and appear oval to round in shape. They have a nucleus to cytoplasm ratio of 1:1 to 1:2. Less mature epithelial cells may be found in increased numbers at the time of menstruation and postmenopause. Parabasal cells, if present with large numbers of WBCs and altered flora in vaginal fluid, are suggestive of Desquamative inflammatory vaginitis.



#### H. Basal Cells:

Basal cells measure 10 to 16  $\mu$ m and appear round. They have a nucleus to cytoplasm ration of 1:2. Similar in size to WBCs, they are distinguished by round rather than lobed nucleus. Basal cells are deep tissue cells, and their presence with large numbers of WBCs and altered flora in vaginal fluid strongly suggests Desquamative inflammatory vaginitis.

#### 6. Bacteria and Parasites:

#### A. Gardnerella vaginalis:

G. vaginalis appears as small, nonmotile, coccobacillus that attaches to the epithelial cell (gram negative/gram-variable, facultative anaerobe). It is one of the major bacterial species associated with bacterial vaginosis (clue cells). It produces a characteristic "amine odor" when KOH is added to the specimen.

#### B. Mobiluncus species:

M. spp. Appears as thin, curved, gram negative anaerobic bacilli, demonstrating corkscrew motility in wet preparations. An "amine odor" is produced with addition of KHO. Best demonstrated on gram stain. If the specimen is amine positive and clue cell negative, a gram stain may identify Mobilucus.

## C. Trichomonas vaginalis:

Typical T. vaginalis trophozoite measures approximately 7 x 15  $\mu$ m and is oval in shape. Size and shape may vary greatly. A key feature is a long protruding flagella which bends back along the outer edge of the undulating membrane creating a "jerky motility." The pointed tip may be instrumental in attachment and may also produce tissue damage. T. vaginalis may be found in vaginal specimens and urine specimens (especially those contaminated with vaginal secretions), as well as secretions from the urethra, prostate, and epididymis.



## D. Lactobacilli:

Lactobacilli are present as normal flora in the vaginal fluid of postpubescent females and produce lactic acid. It is this metabolic waste which helps maintain the pH in the acidic range of 3.8 to 4.2 in the normal vaginal environment. They are relatively large, nonmotile rods (gram positive). Hydrogen peroxideproducing strains are thought to be responsible for protection against pathogenic organisms. If lactobacilli are absent or rare relative to squamous cells, abnormal flora may be suspected.

## 8. <u>Yeast and Fungi:</u>

The two basic structures used to confirm the presence of yeast and fungi are:

1) Hyphae – long filaments which grow and form a mat (mycelium); and

2) **Blastospores** – buds formed during the reproductive process of yeasts. Multiple buds that do not detach can form chains known as pseudohypae.

Some fungi will produce both forms (although usually not both at the same time) n tissue depending upon environmental conditions – these are the dimorphic fungi; others will produce only one form or the other. Most pathogenic fungi are dimorphic.

Fungal hyphae, spores, and other fungal forms can be distorted in clinical specimens by the host's inflammatory response.

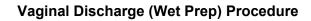


## DIFFERENTIAL DIAGNOSIS OF VULVOVAGINITIS:

DIAGNOSIS	HISTORY	PHYSICAL FEATURES	LAB FINDINGS
Bacterial vaginosis	Mildly odorous	Gray, mucoid, pasty	Studded with clue
	discharge	discharge, pH 5-6	cells
Candidiasis	Recurrent pruritic	Creamy, curdly	KOH prep: mycelia,
	discharge	discharge, pH 4-5	buds, culture as
			needed
Tichomonal vaginitis	Odorous leucorrhea,	Greenish yellow	Motile flagellate
	dysuria	discharge, pH 5-6.5,	protozoa
		friable hyperemic	5
		cervix	. %
Reactive vaginitis	Use of hygienic sprays	Foreign bodies,	None
	and douches, odorous discharge	erythema	
Atrophic vaginitis	Dyspareunia, burning	Sticky brown	None
		discharge, thin vaginal	
		tissue	
Normal cervical or	Minimal discharge	Clear mucoid	Few leukocytes and
vaginal discharge	$\sim$	discharge, pH 4.5,	epithelial cells
vaginitis	100	ectropion	

## CALCULATIONS:

N/A



## **REPORTING RESULTS:**

QUANTITATION, DIRECT EXAMS				
Rare	<than 1-2="" cells="" hpf<="" or="" organisms="" th=""></than>			
Few	>than 1-5 organism or cell/hpf			
Moderate	>5 to 30 organisms or cells/hpf			
Many	>than 30 organisms or cells/hpf			

\*Per Microbiology Standard Reporting\*

The "Vaginal Discharge Analysis" is a profile that consists of 8 individual tests that will each be documented uncontroll separately. They are:

COLOR

CONSISTENCY

BLOOD

WHIFF TEST

YEAST

**TRICHOMONAS** 

CLUE CELLS

**PMNs** 

Results will be entered manually in the 'Progress Notes' of the patient record. (There is no computer Laboratory report – only Progress Note documented by provider.)

## **PROCEDURE NOTES:**

Vaginal discharge fluid analysis may only be performed by healthcare professionals (providers) who have undergone a formal training program and are validated as competent. Competency is verified on an annual basis by a qualified peer healthcare provider (MD) or Ancillary Testing Coordinator. Competency verification will be required more frequently if:

- -There is a change in technology or equipment
- -Individual is observed to have poor testing technique
- -There is failure to comply with test interpretation/procedure guideline.
- -Proficiency testing failure(s)

Individuals must participate at regular intervals in a peer review program and documented.

Individuals performing the analyses must be tested for visual color discrimination (color change on pH indicators).

**Reference Ranges:** 



•	tion of Microscopi				
Finding	Abnormal Due	Abnormal	Abnormal	Abnormal	Reference
	to Bacterial	Due to	Due to	Due to	Values
	Vaginitis	Vaginal	Trichomonas	Desquamative	
	w/Gardnerella	Candidiasis	Vaginitis	Inflammatory	
				Vaginitis	
White					
Blood Cells	Rare	3+ to 4+	2+ to 4+	3+ to 4+	<u>&lt;</u> 2+
Lacto-					
Bacilli	Rare	Present	Present/	Reduced/	Predominant
			Absent	Absent	
"Clue Cells"					
	Occasional to	Absent	Absent/		Absent
	4+		Present		$\sim$
Other Cells		Large clumps		Occasional	Absent
		of epithelial		parabasal/	(except RBCs
		cells		Basal cells	during
				0	menses)
Other	Predominant	Budding	Trichomonas	2+ bacteria	Other
Organisms	Gardnerella	yeast			lactobacilli
	morphotypes	pseudohypae	.0		subgroups &
					occasional
					yeast

# **Procedures for Abnormal Results:**

A specimen may be sent to the clinical Laboratory, Microbiology department, for confirmation of preliminary results if needed.

**Reporting Format:** 

**Progress Note** 



## LIMITATIONS OF THE PROCEDURE:

- 1. Interpretation by individuals performing the test
- 2. Degree of color change
- 3. Accurate recognition of cellular elements

4. Many intravaginal medications will leave oil droplets which can make interpretation of direct wet mounts difficult. It is often useful in such situations to perform a gram stain. If there are any unusual circumstances, a gram stain can be a very helpful aid.

5. Primary Care Service Line Manager is responsible for supervising and ensuring personnel performing tests are in accordance with ancillary testing and hospital policies.

6. Only authorized staff will perform testing.

7. Annual competency will be assessed on at least two of the following: proficiency testing results, direct observation, review of QC and test records, or written assessment.

8. Inadequate smear/slide/specimen quality.

## **REFERENCES:**

- 1. "Women's Primary Care Guide", Department of Veterans Affairs, 1<sup>st</sup> edition, 2001.
- 2. Rakel, R.E. (1993), *Essentials of Family Practice*, W.B. Saunders: Philadelphia, p. 297.