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Valley Laboratories

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Owner:	*Lindsey Westerbeck: Dir, Lab*
Policy Area:	*Lab - Urinalysis*
References:	
Applicability:	*Valley Laboratories*

Performing a Wet Mount Examination

PURPOSE

This procedure provides instructions for performing a microscopic direct examination using phase contrast for identification of clue cells, yeast, and Trichomonas in a saline suspension of vaginal secretions, along with identification of yeast and Trichomonas in a variety of other specimens.

POLICY

- Wet mounts are to be performed STAT and read within 1 hour of collection.
  - If sample is received >1 hour of collection, wet mount can be performed but the following ETCs must be appended to the result if Trichomonas is not present: **UTR1-UTR2.** This translates to: *"Unable to rule out Trichomonas. Time since collection is more than 1 hr."*
- Order Code: **WET** (*Wet Mount Smear*)

EQUIPMENT, REAGENTS AND SUPPLIES

- Phase contrast microscope
- Centrifuge (for fluid specimens only)
- Glass microscope slides and coverslips
- Plastic transfer pipettes

SPECIMEN REQUIREMENTS

- Vaginal (*including cervical*) specimens, urethral discharges, prostatic secretions and sediments of fresh urine may be examined for the presence of WBCs, yeast and Trichomonas. Clue cell presence is only reported for vaginal specimens.
- Flocked collection swabs are the preferred swab. Rayon swabs are also acceptable.
- Dry or cotton swabs, flocked swabs received in Eswab containers or Universal Viral Transport (UVT) will be rejected.
- Swabs must be received in a properly labeled sterile container with at least 0.5 ml of sterile saline.
- Specimens must be transported to the lab at room temp within 1 hr of collection to ensure motility of Trichomonas can be detected.

SPECIMEN PREPARATION

Prepare specimen based on specimen received:

If	Then
Collection swab in 0.5-1.0 ml of saline	• **Vortex** tube for 3-5 seconds • Place 1-2 drops of liquid onto labeled glass slide (Do not use swab to roll onto slide) • Apply cover slip
Collection swab in >1.0 ml of saline or Body Fluid >1.0 ml	• **Vortex** tube for 3-5 seconds (if swab sample) • Centrifuge specimen for 5 minutes • Remove supernatant to bring sample to at least 1.0 ml • Resuspend sediment and place 1-2 drops of liquid onto labeled glass slide • Apply cover slip
Body Fluid <1.0 ml	• Mix sample well • Place 1-2 drops of liquid onto labeled glass slide • Apply cover slip

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PROCEDURE

Follow the steps to perform a wet mount examination:

Step	Action
1.	Using phase contrast microscopy, examine the *entire slide* on low power (10X) for the presence of motile Trichomonas and pseudohyphae. Using low power is also helpful to evaluate the distribution of cellular material on the slide.
2.	Re-examine 20-40 fields on high power (40X) to evaluate the presence or absence of clue cells, weakly motile Trichomonas, WBCs and yeast (budding/non-budding, and pseudohyphae).
3.	Record findings on *Form A: Wet Mount Report*, using the following grading criteria: **Amount Seen** / **Report** >10 per High Power Field / Many 5 to 10 per High Power Field / Moderate 1 to 4 per High Power Field / Few 1 to 4 per entire slide / Rare 0 per entire slide / None Seen Also include the following information on the form: • Patient Name/MRN/Accession number • Source • Tech Code • Date/Time testing performed
4.	Discard specimen in the appropriate biohazard waste container.

REPORTING RESULTS

Step	Action
1.	In Sunquest Microbiology Result Entry function, enter the patient accession number to be resulted.
2.	Confirm source of sample matches what is reflected in Sunquest. If necessary, modify source description under "Misc. Updates" tab - SDES result field.
3.	Using designated result keys <>, enter Wet Mount results under the Direct Exam tab. Press <F8> to display the keyboard on screen. Enter all findings that are present before entering findings that are absent using the approved corresponding keys below: • The presence or absence of WBCs, Yeast or Trichomonas must always be reported. • For Vaginal specimens only: also report the presence or absence of clue cells. • Do not report the presence or absence of any other cell type/findings. ◦ **NOTE:** If a finding (not normally reported) may be of clinical significance, refer to pathologist and site specific procedures for further guidance. **If** / **Then enter** WBCs, Yeast, Trichomonas or Clue Cells are seen / • Quantity followed by finding description: • Rare <5> • Few <6> • Moderate <7> • Many <8> • WBCs <W> • Trichomonas <T> • Yeast <Y> • Clue Cells <U> • Repeat for each finding seen *Example:*

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		• *Many <8> Yeast <Y>* • *Few <6> Trichomonas <T>*
	No WBCs are seen	<S> key
	No Yeast or Trichomonas are seen	<B> key
	No Trichomonas, clue cells, or yeast are seen (**Vaginal** specimens)	<N> key
	No Trichomonas or clue cells are seen (**Vaginal** specimens)	<V> key
	No Trichomonas are seen	<F> key
	No Yeast are seen	<H> key
	No Clue Cells are seen	<G> key
4.	Enter each observation on a separate line, reporting each finding present before reporting what is not seen. Press Tab to move from one line to another.	
5.	Final the report by selecting the period key <.>.	
6.	Before selecting Save, ensure that findings for WBCs, Trichomonas and yeast, along with clue cells (vaginal specimen), have been reported. If a correction needs to be made, press "." in the result field to remove final status and make corrections. Then repeat Step 5 to finalize result.	
7.	Select "Save" to finish resulting of Wet Mount.	
8.	Verify correct manual entry of results.	
9.	File *Form A: Wet Mount Report* in the designated area of the laboratory.	

REFERENCE RANGE

- No WBCs, yeast or Trichomonas
- No Clue Cells (vaginal specimens)

PROCEDURE NOTES

- **Trichomonas vaginalis**: Pear shaped organisms that are larger than WBCs, slightly refractile with a granular appearance and have long flagella. They move with a rapid, jerky, rotating and non-directional leaf-like motion. Their numbers vary in clinical specimens, so it is essential to scan the entire slide. Trichomonas has limited survival outside the host and examining the specimen immediately is critical. As viability decreases they become spherical and non-motile. Identification should be made upon visualization of flagella movement.
- **Yeast/Pseudohyphae:** Yeast will appear refractile and can be seen as a single cell with or without a bud and is about the same size as an RBC. Hyphae elements appear as thick walled tube like structures that may be single or branching. Do not confuse hyphae with natural fibers from a swab. Candida albicans is the most frequently encountered Candida sp. Associated with vaginal infections but the organism does occur in low numbers as part of the normal vaginal flora.
- **Clue Cells:** Epithelial cells of vaginal origin covered with bacteria. The bacterium coating the cells gives the cytoplasm a characteristic refractile, stippled, or granular appearance with shaggy or bearded cell borders. Most of the cell surface should be covered by bacteria for it to be identified as a clue cell. Epithelial cells may have occasional irregular kerato-hyaline granules in the cytoplasm that should not be mistaken for adherent bacteria. Bacterial vaginosis (BV) is the most common type of vaginal infection and is associated with the presence of clue cells. A key microscopic feature of BV is the lack of inflammatory cells and the absence of or reduction in normal flora.

REFERENCES

- Bailey and Scott's *Diagnostic Microbiology*, 12th Edition, Mosby Elsevier, 2007.
- Braude, Abraham L. *Medical Microbiology and Infectious Diseases, Volume II.* WB Saunders Company.
- Centers for Disease Control and Prevention, *2015 Sexually Transmitted Diseases Treatment Guidelines - Diseases Characterized by Vaginal Discharge*, 6/2015.
- Egan, ME. Lipsky, MS. *Diagnosis of Vaginitis.* Amer Fam Physician 2000; 62: 1995.

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- Garcia, LS. Buickner, DA. *Diagnostic Medical Parasitology*, 2nd Edition, American Society for Microbiology, Washington, D.C., 1993.
- Henry, JB. *Clinical Diagnosis and Management by Laboratory Methods*, WB Saunders Company, Philadelphia, 2001.
- Kern, M. *Medical Mycology*, FA Davis Company, Philadelphia, 1990.
- W Geisler, S Yu, M Venglarik, and J Schwebke, *Vaginal leukocyte counts in women with bacterial vaginosis: relation to vaginal and cervical infections*, Sexually Transmitted Infections, BMJ, 2004 Oct; 80(5): 401–405.

All revision dates:

Attachments:

Form A: Wet Mount Report

Approval Signatures

Step Description	Approver	Date
Lab Medical Directors	Kristen Vandewalker: MD	pending
Lab Medical Directors	Andrea Ong: MD	pending
Lab Medical Directors	Hannah Wong: MD	pending
Lab Medical Directors	Rowberry Ron: MD	pending
Lab Medical Directors	Jamie Cassity: MD	pending
Lab Medical Directors	Mary Keohane: MD	pending
Lab Medical Directors	Marian Butcher: MD	pending
	Lindsey Westerbeck: Dir, Lab	10/2/2019

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