

Wet Mount

Purpose

This procedure provides instructions to follow in processing, performing, and resulting wet mount analysis of vaginal secretions for aid in the diagnosis of vaginal infections by yeast, *Trichomonas*, or bacteria (*Gardnerella vaginalis*).

Principle/ Introduction

The identification of *Trichomonas vaginalis*, *Monilia* (*Candida*) and clue cells is usually based on the examination of wet preparation of vaginal and urethral discharges and prostatic secretions.

Trichomonas vaginalis is a parasitic pathogen of the urogenital tract in men and women causing, Trichomoniasis, one of the most common nonviral sexually transmitted diseases. The organism is 5-18 ul in diameter, has four anterior flagella, and an undulating membrane that extends half the length of the body. In a wet mount, the trophozoite has a characteristic jerky motility, and the motion of the flagella and undulating membrane may be observed.

Yeast may appear as single budding cells or as clumps and chains of elongated hypha-like buds (pseudohyphae). On microscopic examination of the membranous material in wet preparation, the observation of a tangled mass of segmented mycelium or budding yeast like cells is indicative of *Candida* (*Monilia*) *albicans*.

Clue cells are vaginal squamous epithelial cells coated with the bacteria, *Gardnerella vaginalis*. Normal vaginal squamous epithelial cells have distinct margins and lack granularity, whereas clue cells show coccobacilliary organisms attached in clusters on the cell surface making the border indistinct or stippled.

Trichomonas vaginalis, yeast, and clue cell infections are primarily diagnosed from direct examination, after addition of saline as the diluting media (wet mount), thru observance of live motile flagellates, yeast or pseudohyphae, and clue cells.

Scope

This procedure is intended for Clinical Laboratory Scientists (CLS).

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Wet Mount, Continued

Policy

- Specimen should be delivered to the Laboratory as soon as possible after collection, keeping the tube upright to avoid spillage.
- Wet mount should be examined as soon as possible.
- Specimen must be examined within two hours of collection to report the presence of *Trichomonas*.
- Motility is best seen at lower temperatures of 15-25°C.

Safety

All specimens, reagents and controls should be handled as though capable of transmitting infectious diseases. Wear appropriate personal protective equipment when running patient samples or performing related activities.

Specimen collection & handling

- Specimen should be collected by the provider, either by the use of swab or by means of the spoon-shaped depression of an unlubricated vaginal speculum.
- Cervix, urethral-mucosa scrapings, or penile, urethral, or vaginal discharges specimens are acceptable. Submit specimens in at least 1ml of sterile saline.
- It is very important to transport the specimen to the testing lab as soon as possible because the *Trichomonas* organisms will lose their motility after 2 hours.

Specimen transport & storage

- Ensure cap of sterile tube is screwed tightly to avoid leakage during transport.
- Do not refrigerate.

Specimen rejection

- The following specimens will be rejected:
- Specimens not labeled according to laboratory protocol (unlabeled, mislabeled, or mismatched specimens).
 - Specimens that have leaked from container and/or are grossly contaminated.
 - Specimens greater than 24 hours old, improperly stored, or transported specimens (refrigerated specimens).
 - Dry swab received.

Reagent/Media

Saline (0.85-0.90% Sodium Chloride – NaCl)

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Wet Mount, Continued

Materials and supplies

The following is the list of materials and supplies required.

- Glass slides
- Coverslips
- Disposable pipettes

Equipment

Microscope

Quality Assurance

Commercial controls are not available. Quality assurance for this procedure is maintained thru available activities:

	Activity
1	Microscope preventive maintenance
2	Proficiency testing participation
3	Annual staff competency assessment
4	Reference material photographs
5	Saline inspection *Open a fresh preparation if organism is observed.

Performing the Examination Procedure

Follow the steps below to perform Wet Mount.

Step	Action
1	Assess the specimen to ensure that it is suitable for testing by being properly labeled and appears properly preserved. Note time of collection if prolonged.
2	Prepare a saline suspension of vaginal or urethral scrapings. <ul style="list-style-type: none"> • If a dry swab is received, reject the sample. • If more moisture is needed, add a few drops of saline but not exceeding 1 mL.
3	Gently mix the specimen while capped.

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Wet Mount, Continued

Performing the Examination Procedure,
 continued

Step	Action												
4	Prepare the specimen for placement onto the glass slide. <ul style="list-style-type: none"> Press the swab against tube wall to squeeze out as much material as possible. Mix saline-specimen suspension using a disposable plastic pipette. 												
5	If visible, pick out white flecks for testing. <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 50%;">If ...</th> <th style="width: 50%;">Then ...</th> </tr> </thead> <tbody> <tr> <td>Specimen contains swab</td> <td>May use swab to transfer material onto slide by rolling swab onto slide.</td> </tr> <tr> <td>Specimen does not contain swab</td> <td>Use a pipette to transfer 1-2 dops of resuspended specimen onto slide.</td> </tr> </tbody> </table>	If ...	Then ...	Specimen contains swab	May use swab to transfer material onto slide by rolling swab onto slide.	Specimen does not contain swab	Use a pipette to transfer 1-2 dops of resuspended specimen onto slide.						
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6	Cover specimen material with a clean glass cover slip.												
Microscopic Examination of Elements													
7	Thoroughly scan to examine the specimen under low power (10x) objective (LPO).												
8	Switch to high power (40x) objective (HPO) for higher magnification of suspicious elements.												
9	<ul style="list-style-type: none"> Semi-quantitate the number of white blood cells (WBC) seen by examining ten fields under HPO. Report as None seen/ Rare/ Few/ Moderate or Many using quantification chart below. 												
10	For the detection of yeast: <ul style="list-style-type: none"> Examine for budding yeast that may appear as single budding cells or as clumps and chains of elongated hyphae-like buds (pseudohyphae). Report as None/ Rare/ Few/ Moderate, or Many using the quantification chart below. <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 50%;">Reporting quantification for WBC & yeast</th> <th style="width: 50%;">Observed number of elements</th> </tr> </thead> <tbody> <tr> <td>None seen</td> <td>0 cells or organisms seen</td> </tr> <tr> <td>Rare</td> <td>1-5 cells or organisms seen on entire preparation</td> </tr> <tr> <td>Few</td> <td>1-5 cells or organisms per HPF (40X)</td> </tr> <tr> <td>Moderate</td> <td>6-30 cells or organisms per HPF (40X)</td> </tr> <tr> <td>Many</td> <td>Greater than 30 cells or organisms per HPF (40X)</td> </tr> </tbody> </table>	Reporting quantification for WBC & yeast	Observed number of elements	None seen	0 cells or organisms seen	Rare	1-5 cells or organisms seen on entire preparation	Few	1-5 cells or organisms per HPF (40X)	Moderate	6-30 cells or organisms per HPF (40X)	Many	Greater than 30 cells or organisms per HPF (40X)
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Wet Mount, Continued

Performing the Examination Procedure, continued

Step	Action
11	For the detection of Trichomonas: <ul style="list-style-type: none"> Under high power field, look for the motile anterior flagella. Only motile forms are reported as Present. Report as None seen or Present.
12	For the detection of clue cells: <ul style="list-style-type: none"> Observe under high power field for an abundance of cells with many small bacteria superimposed on them. These should be observed evenly distributed on the epithelial cells. Report as None seen or Present.

Reporting Procedure

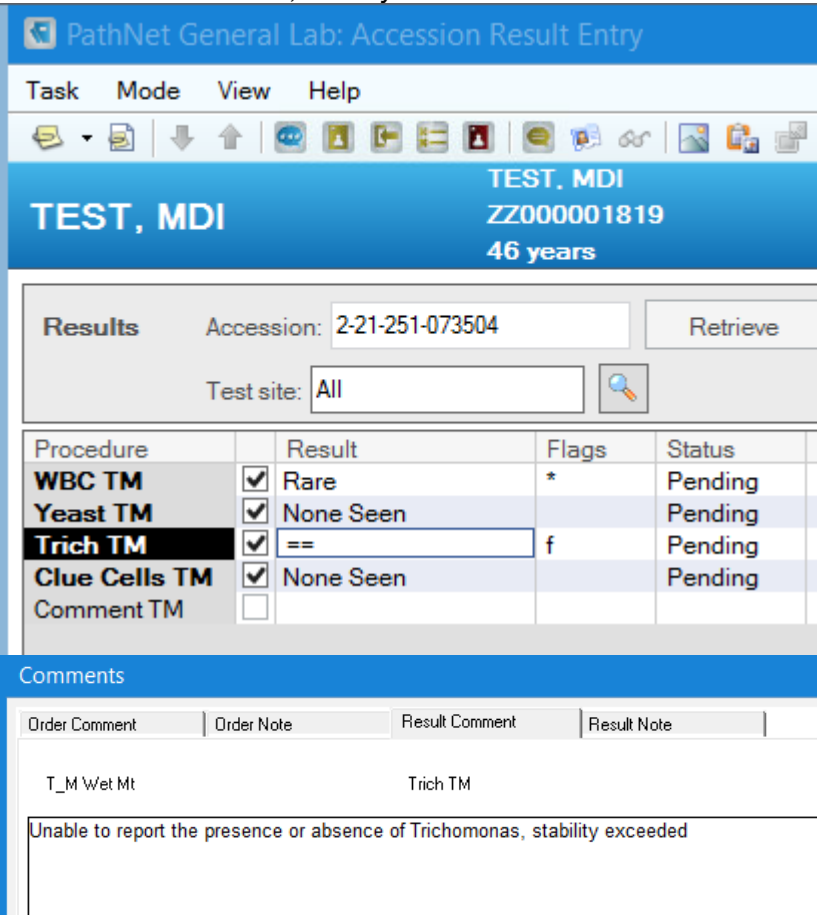
Follow the steps below to report the observed elements of Wet Mount.

Step	Action																		
1	In Cerner, go to Accession Result Entry (ARE).																		
2	Scan Cerner barcode of the specimen and click Retrieve.																		
3	Use the dropdown arrows in the result field for each element to select one of the predetermined ranges that reflect the microscopic observation as result for each.																		
4	The following results will appear: <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th>Test Ordered</th> <th>Test</th> <th>Results</th> </tr> </thead> <tbody> <tr> <td>T_M WET MT</td> <td>WBC TM</td> <td>None seen Rare Few Moderate Many</td> </tr> <tr> <td></td> <td>Yeast TM</td> <td>None seen Rare Few Moderate Many</td> </tr> <tr> <td></td> <td>Trich TM</td> <td>None seen Present</td> </tr> <tr> <td></td> <td>Clue Cells TM</td> <td>None seen Present</td> </tr> <tr> <td></td> <td>Comment TM</td> <td></td> </tr> </tbody> </table>	Test Ordered	Test	Results	T_M WET MT	WBC TM	None seen Rare Few Moderate Many		Yeast TM	None seen Rare Few Moderate Many		Trich TM	None seen Present		Clue Cells TM	None seen Present		Comment TM	
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5	Click Perform.																		

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Wet Mount, Continued

Reporting Procedure, continued

Step	Action
6	Review entered results. Click Verify to finalize results. All pending results should have been resultated out.
For specimen received ≥ 2 hours from time of collection:	
10	<p>Do not report the presence of Trichomonas unless a motile Trichomonas is observed. Instead, right click and choose free text under the result entry for Trichomonas. Enter (double equal sign) “= =” to acknowledge of not reporting result. Under the Result Comment, enter <i>“Unable to report the presence or absence of Trichomonas, stability exceeded.”</i></p> 
11	Review entered results. Click Verify to finalize results.

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Wet Mount, Continued

**Reference
Range**

Specimens from negative patients should have zero WBCs, and no yeast, *Trichomonas*, or clue cells.

The following are flagged as Abnormal in Cerner:

WBC TM	Rare, Few, Moderate, Many
Yeast TM	Rare, Few, Moderate, Many
Trichomonas TM	Present
Clue Cells TM	Present

**Non-Controlled
Documents**

The following non-controlled documents support this procedure.

- Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. Fed Register. 2003(Jan 24): [42CFR493.1256(h)].
- Provider-Performed Microscopy Procedures (PPMP) booklet, Centers for Disease Control and Prevention (CDC), Division of Laboratory Systems, July 2019 edition.
- CLSI. Physician and Nonphysician Provider-Performed Microscopy Testing; Approved Guideline – Second Edition, CLSI document POCT10-A2, Vol. 31 No. 24. Wayne, PA: Clinical and Laboratory Standards Institute; 2011.

Author(s)

SCPMG Microbiology Working Group
