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|  Wet Prep for *Trichomonas vaginalis*, Clue Cells and Yeast |
| **Purpose** | This procedure provides instruction for performing Wet Preps. |
| **Principal and Clinical Significance** | Vaginal drainage, penile drainage, and urine may be directly examined for the presence of clue cells, *Trichomonas vaginalis*, and yeast using saline wet mounts.The presence of clue cells and few or no PMNs indicate bacterial vaginosis. Clue cells are epithelial cells, which have obscured borders covered with sheets of small bacteria adhering to their surface, giving a stippled appearance.*Trichomonas vaginalis* infections are diagnosed primarily by detecting live motile flagellates from direct saline wet mounts. Microscopic slides made from patient specimens can be examined under low and high power for the presence of actively moving organisms.Yeast cells and pseudohyphae may be observed directly in a saline wet mount; however, mucous and other cellular material may need to be dissolved to reveal the presence of yeast. This is accomplished with the addition of 10 % KOH. |
| **Policy Statements** | This procedure applies to Microbiologists who perform culture set-up. |
| **Test Code** | WETP |
| **Materials** |  |  |  |  |
|  | **Reagents** | **Supplies** | **Equipment** |
|  | • 0.85% Saline (SLNE)• 10% KOH dropper | * Sterile disposable pipettes

• Glass slides• Coverslips, 22x22• Wooden applicator sticks | * Microscope
* Centrifuge
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| **Specimen** | 1. Acceptable specimens (Preferred specimens are on Amies or Stuart’s transport swabs or in a warm bacteriostatic saline tube)
* Vaginal discharge
* Urethral discharge
* Penile discharge
* Urethral mucosa scrapings
* First-voided urine
1. SDES codes/Specimen type
* PENI-DRA – penis drainage
* URE-DRA – urethra drainage
* VAGD – vaginal drainage
* VOID – voided urine with or without prostatic massage
1. [Refer to Lab Test Directory – Trichomonas Wet Prep](https://www.childrensmn.org/References/Lab/microbioviral/wet-prep.pdf) for specimen collection, transport and rejection criteria.
2. Special instructions
	* If specimen is collected in bacteriostatic saline, transport to the Microbiology Laboratory <=15 min at room temperature.
	* Do not refrigerate. Refrigeration inhibits the motility of *Trichomonas.*
	* If specimen cannot be examined within one hour of collection, place in Stuart’s or Amies transport medium, which will keep the organisms viable for approximately 24 hours.
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| **Special Safety Precautions** | Microbiologists are subject to occupational risks associated with specimen handling. Refer to the safety policies located in the safety section of the *Microbiology Procedure Manual***.**1. [*Biohazard Containment*](file:///G%3A%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CMCVI%203%20Safety%5CMCVI%203.1%20Biohazard%20Containment.docx)
2. [*Biohazardous Spills*](file:///G%3A%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CMCVI%203%20Safety%5CMCVI%203.4%20Biohazardous%20Spills.docx)
3. [*Safety in the Microbiology Laboratory*](file:///G%3A%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CMCVI%203%20Safety%5CMCVI%203.2%20Safety%20in%20the%20Microbiology%20Lab.docx)
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| **Procedure** | Specimen processing1. Voided urine
2. Centrifuge 10 ml of first voided urine at 500 X g for 10 min.
3. Examine the sediment.
4. Specimens received on swabs
5. Place swab in 0.5 – 1.0 mL of warm saline in a test tube and mix.
6. Examine suspension.
7. Examination
8. Place one drop of suspension on a glass slide and coverslip.
9. Examine the wet mount under low power objective (10X) and low light.
10. Examine the entire coverslip for motile flagellates.
11. Search on high power (40x) for yeast cells, pseudohyphae, Clue cells, WBCS and less motile trichomonads.
12. If no yeast is seen and specimen is thick or cellular, add I drop of specimen to a glass slide. Add one drop of 10% KOH to the specimen and mix with a wooden applicator stick. Add cover slip and heat slightly to speed up lysing of epithelial cells. (If cells are not lysed upon examination, let sit for up to 15 minutes and re-examine.)
13. Report the presence or absence of *Trichomonas vaginalis.*
14. Report the presence or absence of clue cells.
15. Report the presence or absence of yeast.
16. Report the number of WBCS per high power field (HPF). Average several fields and report as follows:

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| Many | ≥ 5 WBCS/HPF |
| Moderate | 1-4 WBCS/HPF |
| Few | <1 WBC/HPF |
| Negative | 0 WBC/HPF |

1. Occasionally, the physician will request a **WBC to epithelial cell ratio**. Count the number of WBCs and epithelial cells (including clue cells) per HPF. A gram stain of the wet mount saline may be needed in order to differentiate clue cell epi’s from non-clue cell epi’s.
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| **Interpretation/ Results** | 1. *Trichomonas vaginalis* is usually slightly larger than a PMN and a jerky flagellar motion should be detected. Examine the motile flagellate for an undulating membrane and axostyle.
2. Clue cells are epithelial cells whose borders appear irregular due to attached small bacteria giving a stippled or granular appearance. They can be seen in a saline prep, but will be dissolved in KOH.
3. Report yeast as being present if yeast cells, hyphae, or pseudohyphae are observed in the saline or KOH prep.
4. Quantitate WBCs.
5. If the physician requests WBC to epithelial cell ratio, report the number of WBCs to epithelial cells

**(**epithelial + clue cells) per high power field (HPF). |
| **Limitations** | 1. If the specimen is left at room temperature or held at refrigerated temperature for a prolonged period of time (> 1 hour), *Trichomonas vaginalis* will round up, lose their motility and eventually die.
2. If the patient has a *Pentatrichomonas hominis* intestinal infection and the specimen becomes contaminated with fecal material, a false-positive *T. vaginalis* result may be reported because *T. vaginalis* and *P. hominis* are very similar in shape.
3. Multiple specimens may have to be examined to detect *T. vaginalis.* There have been a large number of false-positive and false-negative results reported on the basis of stained smears, which strongly suggests the value of confirmation by culture or by molecular probes.
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| **Method Performance Specifications** | 1. It is very important that specimens be examined for *T. vaginalis* within 1 hour of collection.
2. Warming the specimen to 37ºC can enhance motility.
3. Calgiswabs are not recommended for collection because of the tight adherence of the specimen to the swab. Reject the specimen if it is collected on a Calgiswab.
4. The specimen may be gram stained if the presence of clue cells is questionable.
5. A fishy odor after adding KOH to the specimen is suggestive of Bacterial vaginosis or Trichomonas infection.
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| **Result Reporting** | 1. Report in Sunquest Microbiology Result Entry, Direct Exam tab.

Figure 1 Table of result codes

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| —there will be 4 lines of results |
|  | **Result** | **Code** | **Sunquest Key (F8)** |
| Trichomonas | Negative: No motile Trichomonas vaginalis seen | **NMTS** | R |
| Positive: Motile Trichomonas vaginalis seen | **MTS** | T |
|  Clue Cells | Negative | **NCLUE** | 7 |
| Positive |  **CLUE** | 8 |
|  Yeast | Negative | **NYST** | Y |
| Positive | **YSTP** | U |
|  WBCS-Quantitate per HPF.Use free text to add: ; /HPF | Negative | **NWBC** | ‘ |
| Few | **FEW WBCS** | 9 W |
| Moderate | **MOD WBCS** | 0 W |
| Many | **MANY WBCS** | - W |
| ONLY IF REQUESTED--  |
| WBC to Epithelial Cell Ratio | **Free text** the number of WBCS**:** number of epithelial cells (total of clue and non-clue cells)/hpf. (Example: ; 2 WBCs : 4 epithelial cells/hpf) |

Figure 2 Example of report1. If additional information is available after the exam has been finalized, remove the final status and send out a supplementary report using the code SRPT in SREQ or CULTURE RESULTS. Re-final the culture when identifications and/or testing is complete.
2. If an exam requires a correction, the code **CORR** (corrected report) must be used in CULTURE RESULTS. Refer [MCVI 5.0 Micro Computer Training](file:///G%3A%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CMCVI%205%20Computer%5CMCVI%205.0%20Micro%20Computer%20Training.docx) for instruction.
 |
| **References** | 1. Leber, A.L., Parasitology, 9.6.6 *Clinical Microbiology Procedures Handbook,* 2016 American Society for Microbiology, Washington, D.C.
2. Leber, A.L., 3.2.3 table 3.2.3-1 *Clinical Microbiology Procedures Handbook,* 2016 American Society for Microbiology, Washington, D.C.
3. Garcia, L.S., 2016, *Diagnostic Medical Guide to Parasitology*, 6th edition. ASM Press, American Society for Microbiology, Washington, D.C.
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| **Training Plan/ Competency Assessment** | **Training Plan** | **Initial Competency Assessment** |
| 1. Employee must read the procedure.
2. Employee will observe trainer performing the procedure.
3. Employee will demonstrate the ability to perform procedure, record results and document corrective action after instruction by the trainer.
 | 1. Direct observation.
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| **Historical Record** |  |  |  |  |
|  | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1.0 | Pat Ackerman | 1978 | Initial Version |
| 1.1 | Pat Ackerman | 02/06/1992 |  |
| 1.2 | Pat Ackerman | 09/19/2003 |  |
|  | 1.3 | Helen Stefan | 06/15/2008 | Added Stuart’s media for storage under special instructions. |  |  |
| 1.4 | Deb Judge | 12/17/2008 | Include identification and reporting of clue cells, yeast, and WBCs |
| 1.5 | Becky Carlson | 07/21/2009 | Added procedure to address the special request of WBC to epi cell ratio. |
|  | 1.6 | Jessica Craig | 06/29/2010 | Updated into online format. |
|  | 2 | Becky Carlson | 4/26/2015 | Re-numbered from MC 504 for CMS load |
|  | 3 | Susan DeMeyere | 5/10/2018  | Biennial Review updated logo and references.  |
|  | 4 | Susan DeMeyere | 8/3/2018 | Added table for quantitation of WBCS |
|  | 5 | Susan DeMeyere | 7/2/2020 | Removed quantitation of Clue cells |
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