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Wet Prep Procedure	**Attachments** ☐ Yes ☒ No
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Manual Clinic Laboratory Procedure Manual W@W Laboratory Procedure Manual Point of Care Procedure Manual	**Last Review Date** January 2015
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Level of Complexity Non-waived	**Contact** Laboratory Technical Consultants
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APPROVAL(S) Laboratory Medical Director	

I. PURPOSE/PRINCIPLE

The most common vaginal infections are bacterial vaginosis, trichomoniasis and vulvovaginal candidiasis. Some vaginal infections are transmitted through sexual contact but others, such as candidiasis (yeast infections) are not. The cause of vaginitis cannot be adequately determined solely on the basis of symptoms or a physical examination. Laboratory tests allowing the microscopic evaluation of vaginal fluid are required for a correct diagnosis.

Bacterial Vaginosis:

Bacterial Vaginosis (BV) is the most commons cause of vaginitis among women of childbearing age. Instead of the normal predominance of Lactobacillus bacteria, increased number of organisms such as Gardnerella vaginalis, Bacteroides, Mobiluncus and Mycoplasma hominis are found. Diagnosis is based on the presence of numerous “clue cells” (vaginal lining cells that are coated with G. Vaginalis and other BV organisms, a fishy odor and decreased acidity of the vaginal fluid.

Trichomoniasis:

Trichomonas vaginalis is a common sexually transimitted disease (STD) caused by a single-celled parasite called Trichomonas vaginalis. It is the most common cause of parasitic gynecological infections in women. In men, trichomonad infections of the urethra are usually asymptomatic. Trichomonas vaginalis appears as a pear-shaped flagellate. They have three to four anterior flagella and a singular posterior flagellum, with an undulating membrane that extends halfway down the length of the organism. They are motile in fresh wet preparations, and their characteristic flitting or jerky motions assists in identifying them. As the specimen ages the organisms will swell and lose the pear-shape, becoming round and non-motile.

Vulvovaginal Candidiasis:
Vulvovaginal Candidiasis (VVC) sometimes referred to as Candidal vaginitis, monilial infection or vaginal yeast infection is a common cause of vaginal irritation. VVC is caused by an overabundance or overgrowth of yeast cells, primarily Candida albicans, that normally colonize in the vagina. Diagnosis is usually made through the microscopic examination of vaginal secretions for evidence of yeast forms.

II. POLICY

Laboratory Staff will follow the approved techniques outlined in this procedure.

Specimen:

Direct Method: Vaginal discharge is collected and transported to the laboratory on a wooden collection stick in a tube container.

Saline Collection Method: A swab containing vaginal discharge is swished in 0.5 ml of 0.9% saline in a plastic tube. Discard the swab. Cap the tube and deliver to the laboratory.

- Specimens must be examined immediately upon arrival in the lab.

Reagents/Materials

- **0.9%** Saline. Single use ampules are used and must be discarded after each use. Store at room temperature.
- Plastic Tube with Cap transport container
- Wooden collection Stick
- Applicator Sticks
- Glass Slides
- Coverslips
- pH Paper 3.5-5.~~5~~
- 20% KOH vials (optional)- single use

III. PROCEDURES

Direct Method:

1. Perform a pH on the specimen using a wooden applicator stick and pH paper.
   - Use pH paper 3.5-5.5.
   - If specimen pH reads 5.5, when that number is resulted in the pH field, the computer will automatically result the pH as > 5.5.
2. Using an applicator stick, place specimen on a glass slide.
3. Add 1 drop of saline. Mix gently and coverslip.
4. Examine immediately under low power (10X) for Trichomonas. Examine a minimum of 20 fields.
   - Look for typical motile organisms that are pear-shaped, slightly larger than leukocytes.
   - Look for non-motile organisms with characteristic morphology but no longer motile due to time elapsed between collection and examination, lack of moisture, or stage of disease.
5. Examine further under high dry (40-50X)
   - Confirm trichomonas organism morphology. Look for motility in the undulating membrane and/or flagella located on one end in a tuft.
   - Look for yeast forms (budding or pseudohyphae)
   - Look for “clue cells” (vaginal lining cells which are coated with a small rod or cocco-bacilli). Most (but not all) of the cell surface should be covered with bacteria and the bacteria must extend past the cytoplasmic margins causing the cells to appear granular with undefined edges.
   - More than 20% of the epithelial cells should have the “clue” appearance to report the presence of clue cells.

6. Look at a minimum of 20 low power fields and confirm trichomonas under high power. Examine at least 20 high power fields for yeast and clue cells before reporting a negative result. Low numbers of organisms may be present in early and late stages of infections.
7. When completed, discard slides into the "sharps" container. The collection tube must be discarded into an orange biohazard bin.

Optional: If the fields are obscured by mucous or cellular debris, place specimen on a slide with an applicator stick and add one drop of 20% KOH to the specimen. This will allow yeast to be better visualized.

Saline Collection Method:

1. Do not pH a saline suspension of the specimen. The provider may perform a pH during collection and submit this information to the laboratory for computer documentation.
2. Mix the saline suspension and place a drop of the saline suspension on the slide and add a coverslip. Examine and report as listed in #4-6 under the Direct Method.

Expected Results:

Trichomonas: No Trichomonas seen
Clue: No Clue cells seen
Yeast: No Yeast found
pH 3.5-4.5

REPORTING RESULTS

Clinic Labs: see the Computer Entry section of this procedure

Well@Work Clinic:

1. enter results on test log sheet
2. fax test log sheet to Central Lab for result entry into the Laboratory Computer System

Provider-performed microscopy:

1. Providers must document point-of-care results on the worksheet provided.

PROCEDURE NOTES

1. In most cases, the following correlation can be made between pH and the cause of symptoms:
   - 4.0-4.5 (may be up to 5.0) Yeast
   - 5.0-6.0 Trichomonas vaginalis
   - 4.7-5.5 Gardnerella, Anaerobes (bacterial vaginosis)
   - >5.5 (may be as low as 5.0) Atrophic
2. The presence of RBC's may increase the pH.
3. The use of vaginal creams, jellies, etc. may alter the pH.
4. Positive yeast with only buds present may indicate an infection with Candida glabrata, as opposed to Candida albicans.
5. Atrophic vaginitis is most common in post-menopausal women.

REFERENCES

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Bertholf, ME and Stafford, MJ: An Office Laboratory Panel to Assess Vaginal Problems. Amer. Fam. Pract., Sept. 1985, pp 113-125.
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RELATED DOCUMENTS

APPENDIXES

AUTHOR/REVIEWER(S)

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IV. DEFINITIONS

V. COMPLIANCE

Failure to comply with this policy or the procedures may result in disciplinary action, up to and including termination.

VI. ATTACHMENTS

VII. OTHER RESOURCES

VIII. ENDORSEMENT

Laboratory Administration

Computer Order and Result Entry

(W@W Clinics are exempt)

WET PREP
Order Code: WET

RESULTING:

WORKSHEET:

Function MEM Worksheet WP_ _ (Wet Prep)

RESPONSE:

CODE	NAME	RESPONSE
WCLUE	Clue Cells	NCLUE (No clue cells seen) CLUE (Clue cells Present)
WTRIC	Trichomonas	NTRIC (No Trichomonas seen) TRIC (Trichomas Present)
WYST	Yeast	YN (No Yeast found) YSTP (Yeast Present)
WPH	pH	Enter number directly (1 Decimal place) If received in saline suspension: result with UPER If pH is 5.5, when entered, the computer will result as > 5.5
WCOM	Comment	VCRM (Vaginal Cream Present) Free text if RBCs present or if sperm present <Return> to HIDE

ADDITIONAL INFORMATION:

If some of the parameters cannot be resulted because of vaginal cream, use the code NOWP (Unable to Determine Due to the Presence of Vaginal Cream) to result the affected parameters.

CREDITING INFORMATION:

If none of the parameters can be resulted because of vaginal cream, credit the Wet Prep using the code NOWET (Unable to Perform Due to the Presence of Vaginal Cream).

If the specimen is QNS to perform any of the parameters, credit the test using QNSR (QNS, Unable to Perform).

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