Vaginal Fluids - objectives

1. Define and list at least three (3) symptoms of vaginitis.
2. Identify at least two (2) sources of error that can occur during the collection and processing of vaginal wet prep specimens.
3. List three (3) common causes of infectious vaginitis.
4. Describe “clue cells” and explain the significance of finding them in a vaginal wet prep.
5. Evaluate the test for estrogenic activity including the appearance of positive and negative results.

Vaginal Secretions

- Normal secretions
  - Clear mucus
    - May turn slightly white or pale yellow when exposed to air
  - Healthy vagina - *Lactobacillus species* predominates
    - pH: 4.5 (3.8-4.5)
    - Amount / volume varies through menstrual cycle
    - Normal microscopic exam
- Abnormal changes
  - Color
  - Consistency
  - Amount
Normal Wet prep

- No symptoms
- Lactobacillus (normal)
- Normal discharge

Vaginitis

- inflammation or infection of the vulva and vagina
- NOT a specific disease, but is a very common reason women seek medical attention
  - Estimated 1/3 to 1/2 outpatient visits by women
  - Can occur in all age groups, sexually active as well as sexually non-active.
- Common symptoms
  - Vaginal discharge
  - Foul smell
  - Itching
  - Spotting
  - Pain

Vaginitis - evaluation

- Patient history
  - Marital or relationship status
  - Timeline of when symptoms began, etc.
- Symptoms / complaint(s)
- Physical examination
- Tests
  - Physical properties
  - Vaginal pH
  - Microscopic exam / Wet Prep
  - Amine (Whiff) test
  - Cultures, if warranted
Vaginitis

- Two (major) types
  - Non-infectious
    - May be caused by soaps (no bubble baths ladies!), chemicals, foreign objects, allergies to condoms / lubricants etc.
  - Infectious (makes up 90% of all cases)
    - Fungal / yeast
    - Parasitic – Trichomonas vaginalis
    - Bacterial

Vaginitis

- Specimen - Vaginal Wet Prep
  - Sterile swab (moistened with normal saline)
    - Must process these immediately, ie within 5 minutes
    - Swab in tube with ½ mL normal saline, or Ringer’s lactate
      - Keeps organisms from drying out if delay is expected
    - Special collection procedures: Microbiology cultures for gonorrhea (GC) must be placed in special transport media immediately.
      - This microbiology testing being replaced by molecular diagnostics

- Collection / processing errors
  - Insufficient specimen / poor collection
  - Swabs / slide drying out

Vaginitis - testing

- Vaginal pH
  - Most important preliminary test
  - Normal (childbearing age) < 4.5
  - pH paper
Vaginitis - testing

- **Microscopic exam / Saline Wet Prep**
  - Sample mixed with saline examined microscopically to look for
    - Budding yeast with elongated pseudohyphae
    - Motile trichomonads & increased segmented neutrophils
    - PMNs & Clue cells

- **Limitations**
  - Skill of collection
  - Transport time
    - Trichomonas organisms die / become immotile
  - Skill of technician

- **New wave in laboratory testing**
  - Immunologic
  - Molecular diagnostic / PCR

Vaginitis - testing

- **Amine (Whiff) test**
  - Also called potassium hydroxide or KOH preparation
  - Vaginal fluid & 10% KOH placed on a slide
  - Fumes from the slide are smelled to detect presence of 'fishy odor' (trimethylamine).
  - Presumptive for bacterial vaginosis, though can also be positive for trichomonial vaginosis
Vaginitis - suspect yeast (candidiasis)

- **Candida albicans**
  - Commonly causes a majority of cases
  - Alteration of normal vaginal flora
    - Antibiotic regimens
    - Immunocompromised patients
  - Thick white, clumpy or "curd-like" discharge.
- Laboratory findings
  - Normal vaginal pH
  - Identification of yeast cells and elongated pseudohyphae (mycelia forms)
    - Saline wet mounts
    - 10% KOH wet prep
    - Gram stain

Vaginitis - suspect: *Trichomonas vaginalis*

- **Trichomonas vaginalis** - Free-living organism
- Parasitic vaginitis
  - Swimming / bathing in contaminated water
  - Sexually transmitted
  - Symptomatic - Yellow-Green frothy discharge; or may be asymptomatic
  - Organism seen in urine or on wet-prep
- In males - sexually transmitted urogenital infection
  - Usually asymptomatic
  - Organism may be detected in urine microscopic

*T. vaginalis* - testing / detection

- Laboratory findings
  - Wet-prep microscopic
    - Single celled flagellate demonstrating jerky movements
    - @ size of WBC, but no nucleus and actively motile - unless specimen is old, dry or cold.
    - May demonstrate WBCs
  - DNA and immunological tests
  - Elevated vaginal pH
  - Positive amine / "whiff" test
Vaginosis - suspect bacteria

- Healthy vagina - *Lactobacillus species* predominates
- Bacterial vaginosis
  - *Gardnerella vaginalis*
  - *Mobiluncus species*
  - *Prevotella species* (anaerobes)
- Characteristics (*Amsel criteria*)
  - Homogenous vaginal discharge
    - Amount & Color may vary, but often gray / off-white
    - Usually thin in consistency and malodorous
  - Lack of WBCs, but increased epithelial cell exfoliation
  - "Clue cells" (make up 20+%) - most reliable finding
  - Vaginal pH > 4.5
  - Positive amine test in the KOH prep

"Clue cells"

<table>
<thead>
<tr>
<th>Normal examples</th>
<th>Clue cells</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Normal example" /></td>
<td><img src="image2.png" alt="Clue cells example" /></td>
</tr>
</tbody>
</table>

Vaginitis - testing summary

<table>
<thead>
<tr>
<th>Observation / test</th>
<th>Candida vaginitis yeast</th>
<th>Trichomonas vaginalis</th>
<th>Bacterial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>Thick white, clumpy / curd-like</td>
<td>Green-yellow &amp; frothy</td>
<td>Thin, gray homogenous</td>
</tr>
<tr>
<td>pH</td>
<td>&lt; 4.5</td>
<td>&gt;4.5</td>
<td>&gt; 4.5</td>
</tr>
<tr>
<td>Wet Prep microscopic</td>
<td>Budding yeast and pseudohyphae</td>
<td>Motile trichomonads &amp; PMNs</td>
<td>&gt; 20% Clue cells identified</td>
</tr>
<tr>
<td>Amine (Whiff) test with 10% KOH</td>
<td>Negative</td>
<td>Negative, or Positive</td>
<td>Positive: fishy odor</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>DNA &amp; immunological tests available</td>
<td>Amsel criteria: at least 3 of 4 must be positive.</td>
<td></td>
</tr>
</tbody>
</table>
Summary

- From the lab's perspective - 3 main causes for vaginitis
  - Yeast infection / candidiasis
    - *Candida albicans* / other species possible
  - *Trichomonas vaginalis*
  - Bacterial
    - From disturbance of normal flora (ie decreased lactobacillus) that allows overgrowth of mixed flora, ie *Gardnerella vaginalis* and others
  - *Gardnerella* - results in 'clue cells'
  - Known pathogens, ie gonorrhea

Fern test

- Test for estrogenic activity
  - Cervical mucous smeared on glass slide and allowed to dry
  - Examine under the microscope - look for fern-like appearance / pattern
    - Seen during times of increased estrogen - as occurs at time of ovulation.
    - Also done to see if there has been premature leakage of amniotic fluid - as it will also make a fern pattern due to its protein and sodium chloride content.

Fern test - positive reactions
Fern test - negative reaction

Wet preps - 1
- No symptoms
- Lactobacillus (normal)
- Normal discharge

Wet preps - 2
- pH <4.5 (normal)
- KOH microscopic negative & Whiff test negative (no amine odor)
- Normal epithelial cells
- Predominately lactobacillus
- Rare WBC
Wet preps - 3

- Positive clue cells
- pH > 4.5
- Whiff test positive
- KOH microscopic negative
- Normal lactobacilli have been overrun by Gardnerella vaginalis and other organisms

Wet preps - 4

- Positive KOH microscopy
- Whiff test negative
- No amine odor when mixed with the KOH
- Vaginal pH < 4.5
- Moderate - increased discharge
- White to light yellow,
- Etiology - Candida albicans / Candida species

Wet preps - 5

- Microscopy - positive for motile 'trich'
- Whiff test often positive
- Vaginal pH > 5.0
- Discharge - greatly increased
- Green / yellow purulent, may appear foamy
- Etiology - Trichomonas vaginalis
Summary

• Yeast - Candidiasis
  • Candida albicans / other species possible
  • Microscopic shows mycelia forms

Summary

• Trichomoniasis
  • Trichomonas vaginalis
  • @ size of WBC (slide on Rt), but no nucleus and actively motile
  • Unless specimen is old, dry or cold

Reference Listing

- Lillian Mundt & Kristy Shanahan, Graff’s Textbook of Urinalysis and Body Fluids, 2nd Ed.
- Susan Streezinger & Marjorie Di Lorenzo, Urinalysis and Body Fluids, 5th Ed.
- Wikipedia, the free encyclopedia
  - www.wikipedia.org
- eMedicine from Webb MD
- Family Practice notebook.com