به نام خداوند جان و خردد

کزین برتر اندیشه بر نگذرد
The laboratory diagnosis of female genital tract infections and Chlamydia

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Normal Person

- The vagina produces a normal discharge
- The discharge contains many cells and mucus
- The vagina contains a rich growth of commensal
Characteristics of normal vaginal secretions

- Heterogeneous suspension of desquamated vaginal epithelial cells
- Lactobacilli dominate the microbial flora
- PH of 3.5 to 4.1
- Odorless
- Do not cause itching or irritation
The normal flora
adult female genital tract

- **Lactobacilli**
  - Women with normal flora benefit from the bi-products of *Lactobacillus*.
  - Lactic acid helps maintain a low vaginal pH
  - Hydrogen peroxide *in vitro* inhibits HIV.
  - Women colonized with H2O2 producing *Lactobacillus* are less likely to have a shift in flora

- **Diphtheroids**
- **Staphylococcus epidermidis**
- **Streptococci**
Diseases Characterized by Vaginal Discharge

- Bacterial Vaginosis (BV)
- Trichomoniasis
- Candidiasis
Replacement of the normal vaginal flora by an overgrowth of anaerobic microorganisms

- *Gardnerella vaginalis*
- *Mobiluncus spp*
- *Prevotella spp*
- *Bacteroides spp*
- *Peptococci*
- *Mycoplasmas hominis*
- *Ureaplasma urealyticum*
Clinical manifestation

- Dysuria and dyspareunia are rare
- Women complain of vaginal odor
- Abdominal discomfort is occasionally present
- Vagina usually contains a grayish, thin, homogenous discharge
Methods for Diagnosis of Bacterial Vaginosis

- Clinical Diagnosis
  This will be used to clinically manage patients

- Gram stain
  Centralized and standardized lab based method
**Specimen Collection**

- Obtain secretions from the mucosal membrane of the vaginal wall with a sterile swab
- Swab the lateral or posterior fornices of the vagina
- Place the swab in a sterile tube containing saline
Laboratory Identification

- Examination of wet mount
  - The relative absence of PMNs
  - Many PMNs should prompt a search for simultaneous infection
  - The predominant flora is coccobacilli (Clue cells)
- Gram stain
- Routine culture is not recommended
  - SBA
  - Chocolate agar
  - bilayer-Tween agar (HBT agar)
Clinical Diagnosis

1. Discharge
   ✓ The typical BV discharge is a thin white fluid with a consistency resembling sour milk
   ✓ There must not be any granular elements
   ✓ The fluid must be completely homogenous

Limitations
Use of microbicide or placebo gels may make evaluation of discharge more difficult
Clinical Diagnosis

2. pH of vaginal fluid
   - Touch the strip to the wall of the vagina or use a swab of the vagina to moisten the pH paper
   - Avoid the cervical mucus, which has a higher pH than the vagina
   - Normally, vaginal pH values lie between 3.5 and 4.1
   - pH >4.5 is consistent with BV

Limitations:
   - blood, sperm, and cervical mucus can elevate the pH; acid gels may lower the pH
Clinical Diagnosis

3. Whiff test. Amine odor

✓ Gardnerella vaginalis produce amines
✓ Transfer a small amount of the vaginal fluid onto a slide
✓ Add 1 drop of 10% KOH to one of the slides
✓ A fishy odor indicates the presence of amines

Limitation:
Technologist may not be able to smell the odor
Clinical Diagnosis

4. Clue cells: **Wet Mount**

- Transfer a small amount of the vaginal fluid onto a slide
- Cover slip the slide
- A large number of bacteria (thousands) adhere to the cell surface
Scoring

Gram Stain for Diagnosing BV
¬Based on the quantity of 3 morphotypes Lactobacilli, Gram variable rods, and curved rods
¬Sensitivity 86%-89% and specificity 94%-96%
Trichomoniasis

- Purulent vaginal discharge
- Vulvar irritation
- Dysuria
- Abnormal vaginal odor
- Copious, yellow-green or gray frothy discharge, adherent to vaginal walls
- Punctate cervical microhemorrhages seen in 25%
T. vaginalis Diagnosis

- Vaginal pH is almost always greater than 4.5
- A positive whiff test is not unusual (KOH) +/-
- Wet mount
  - An abundance of leukocytes
  - Motile flagellated trichomonads, twitching motility
- Culture

  Modified Dimond’s medium
  InPouch TV
T. vaginalis, wet mount
20% of wet mounts initially positive for *T. vaginalis* become negative within 10 minutes
Other Tests to Identify Trichomonas

- Polymerase chain reaction (PCR)
- Enzyme-linked immunoassay (ELISA)
- Direct fluorescence antibody assay (DFA)
- Enzyme immunoassay (EIA)
- Dot-immunobinding (DIBA) assay
- Indirect fluorescent antibody (IFA) assay
- Agglutination test (AT)
- Stained smear techniques
  (Gram stain, Papanicolaou smear)
Vulvovaginal Candidiasis (VVC)

- Abnormal vaginal discharge thick, white, vaginal discharge ("cottage cheese-like")
- Vaginal soreness
- Erythematous "satellite" lesion
- Vulvar pruritis
- Pain
- Vulvar edema
Uncomplicated VVC

- Non-persistent
- Non-pregnant women
- Sporadic
- No underlying disease
- Caused by *candida albicans*
- Mild or moderate severity
Complicated VVC

• Severe, persistent, recurrent VVC or underlying host abnormality
• Divided into:
  • 1 severe vulvovaginal candidiasis
  • 2 persistent non-albicans infection
  • 3 recurrent vaginal candidiasis
Diagnosis

- pH normal (≤4.5)
- Whiff test negative
- A wet preparation (saline, 10% KOH)
- Gram stain of vaginal discharge demonstrates yeasts or pseudohyphae
- Culture
  - *C. albicans* 85-90%
  - *C. glabrata* 5-10%
  - *Saccharomyces cervisiae*
  - Others: *C. tropicalis, C. krusei, C. kefyr*
cervicitis

A purulent or mucopurulent endocervical exudate visible in the endocervical canal or on an endocervical swab specimen

(commonly referred to as “mucopurulent cervicitis”)
cervicitis

✓ Finding of leukorrhea (>10 WBC per high power field on microscopic examination of vaginal fluid)
✓ Vaginal pH may be elevated
✓ There is no odor when the secretions are mixed with 10% KOH
✓ This might indicate endocervical inflammation caused specifically by
  *C. trachomatis* or *N. gonorrhoeae*
Chlamydia trachomatis

- Nonmotile
- Gram negative
- Obligate intracellular organisms
- A unique developmental cycle
Molecular Aspects

- It cannot synthesize ATP, so it needs living host cells to survive
  - Cannot grow on artificial media
- Two part life cycle
  - Non-replicating, infectious elementary bodies (analogous to a spore)
  - Replicating, non-infectious reticulate body (replicate inside host cell, in a membrane-bound inclusion)
  - When the bacterium gains entrance to the host cells elementary bodies germinate into reticulate bodies
Chlamydial Development

Developmental cycle of *C. trachomatis*

- **0** hours: Elementary body (EB)
- **0.5** hours: Spread of EBs
- **1-8** hours: Inclusion formation
- **12-24** hours: Inclusion development
- **24-30** hours: Reticulate body (RB)
- **40** hours: Further development
- **48-72** hours: Continued development

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Antigens

- The major common antigen (LPS)
- Species specific antigen (OmcB)
- Type specific antigen (MOMP)
Chlamydia serovars

- Serovars A, B, Ba and C associated with trachoma
- Serovars L1, L2, L3 associated with LGV
- Serovars D through K
  - Cervicitis
  - Urethritis
  - Endometritis
  - Salpingitis
Pathogenicity

- Genital tract infection
  - Asymptomatic in most cases
  - Symptoms
    - Painful urination
    - Lower abdominal pain
    - Unusual Discharge (yellow or cloudy mucoid)
    - Rectal pain or discharge
  - If left untreated, common cause
- Infertility
- Ectopic pregnancy
- Tubal scarring
- Newborns can contract the disease from infected mothers
Sampling

- The objective of specimen collection should usually be to include the host cells that harbor the organism.
- Specimen that contain secretions or exudate but lack the cells are not satisfactory.
- Dacron, cotton, rayon and calcium alginate swabs may be used.

- Transportation media
  - 2-sucrose phosphate (2SP)
  - Sucrose glutamate phosphate (SPG)
Sampling

- When the elapsed time between specimen collection and inoculation is \( \leq 24 \) hours, specimens should be stored at 4\(^\circ\)C.

- When specimens cannot be inoculated in \( \leq 24 \) hours, the specimens should be maintained at \( \leq -70 \)\(^\circ\)C.

- Specimens for culture should never be stored at --20\(^\circ\)C or in frost-free freezers.
Samples for non culture tests

- Collection of these samples should be performed as instructed by manufacturer
- Urine is a good sample
- A single endocervical or urethral swab specimen to be sufficient
Isolation in cell culture

- Cell culture
  - Cycloheximide-treated McCoy cells
    - HeLa 229 cells with DEAE-dextran and cycloheximide
  - Buffalo green monkey cells
  - Monkey kidney
  - HL
  - HEP-2
Identification

✓ Fluorescein-conjugated fluorescent monoclonal antibody stains (MOMP)
✓ Iodine staining (less specific)

Disadvantages

- low sensitivity
- long turnaround time
- Difficulties in standardization
- labor intensity
- Technical complexity
- Stringent transport requirements
- Relatively high cost

◊ Shell vial method (less specific)
Identification

• Non culture diagnostic tests
  Direct cytological examination
  – DFA procedure (MOMP or LPS molecule)
    • DFA is best suited for laboratories that test a limited number of specimens
      – Giemsa
      – Iodine staining
• Serological tests
  – Complement fixation (CF)(LPS)
  – IF
  – EIA
  – Microimmunofluorescence test (MIF)
Nucleic acid amplification tests (NAAT)

• Becton Dickinson BDProbeTec™ test uses
  ✔ DNA sequences in the cryptic plasmid

• The Gen-Probe APTIMA™ assay
  ✔ To detect a specific 23S ribosomal RNA target
Hybridization probes:
DNA sequences including:
✓ Genomic DNA
✓ Cryptic plasmid DNA

One of the advantages of the nucleic acid hybridization tests is the ability to store and transport specimens for \( \leq 7 \) days without refrigeration before receipt and testing by the laboratory.
**Chlamydia trachomatis**

**DIAGNOSIS**

**Culture**: high specificity BUT
- labor-intensive, expensive,
- variable sensitivity (50%-80%),
- not suitable for widespread screening

**Non-culture methods**:
- Serology: not very useful
- EIA, DFA, DNA probe : less sensitive(50-75%), nonspecific
- **Nucleic acid amplification tests (NAAT)**: PCR
  - more sensitive than culture (>80%-90%)
  - can use first void urine
  - can use self-obtained vaginal swab
Sero logic tests To screen for persistent 
C. trachomatis infections

- CRP levels between 1 and 10 mg/l reflect a low-grade inflammation
- IgG to *Chlamydia hsp 60*
  - is a *Chlamydia* genus-specific protein
  - have been suggested as markers of chronic inflammation

- Antichlamydial IgA
Screening Women or Men with Possible Rectal or Pharyngeal Exposure

- Culture isolation is acceptable
- *C. trachomatis*-MOMP--specific stain is acceptable
- DFA can be performed

Less-specific
- EIA
- Iodine
- Giemsa
- LPS-specific monoclonal antibodies are not recommended
Incidence rate of chlamydia

According to the WHO data:
- 92 million new cases of CI are diagnosed globally every year
- 5 million cases are diagnosed in Western Europe
- 5 million cases – in Eastern Europe and Central Asia

This is the most common bacterial STI in the world.
Genital chlamydial infection is the most common bacterial sexually transmitted infection worldwide.
Neisseria gonorrhoea

- Cervicitis
- PID
- Urethritis
- Pharyngitis
- Proctitis
- Disseminated infections
Clinical Manifestations

- Symptoms are non-specific:
  - Cervicovaginal discharge
  - Abnormal or intermenstrual bleeding
  - Abdominal pelvic pain
  - Presence of dysuria
- Clinical findings:
  - Mucopurulent or purulent cervical discharge
  - Induced cervical bleeding
- 50% of women with clinical cervicitis are asymptomatic
**GONORRHEA LAB DIAGNOSIS**

- Gram-stained smears (sensitivity of 50-70%)
  - Gram stain of
  - Endocervical specimens
  - Pharyngeal
  - Rectal specimens

  Are Not Sufficient

**Culture:**

- modified Thayer- Martin (MTM) medium
- Martin-Lewis (ML) medium
- GCLect medium
- New York City (NYC)
Neisseria gonorrhoea

- EIA
- Nucleic acid hybridization tests
- NAAT
Pelvic Inflammatory Disease

- Upper genital tract infections that involve the
  - Endometrium (endometritis)
  - Fallopian tubes (salpingitis)
  - Pelvic peritoneum (peritonitis)

- These infections result from ascending spread of lower genital tract infection
Common Etiologic Agents

✓ Bacteroides spp.
✓ Peptostreptococci
✓ E. coli
✓ N. gonorrhoeae
✓ C. trachomatis
✓ G. vaginalis
✓ Haemophilus influenzae

✓ Enteric Gram-negative rods
✓ Streptococcus agalactiae
✓ CMV
✓ M. hominis
✓ U. urealyticum
✓ M. genitalium
Symptoms suggestive of PID include:

- Abdominal pain
- Dyspareunia
- [abnormal] Vaginal discharge
- Dysuria
- Fever, and/or chills
- Nausea or vomiting
Diagnosis of Acute PID – CDC Criteria

- Oral temperature higher than 101°F
- Abnormal cervical or vaginal mucopurulent discharge
- Elevated erythrocyte sedimentation rate
- Elevated C-reactive protein level
- Laboratory documentation of cervical infection with *N. gonorrhoeae* or *C. trachomatis*
Diagnosis of PID – Laboratory Tests

1. Gram-stained endocervical smear (to quantify PMNs/1000x field)
2. Endocervical NAAT or endocervical (and rectal) cultures for *N. gonorrhoeae*
3. Culture of endocervical swab or NAAT for endocervical swab or first void urine for *C. trachomatis*
Genital Ulcer disease
Herpes Simplex Virus

- The vesicles that evolve into pustules and finally to shallow ulcers on an erythematous base, multiple lesions are common and may erupt in tightly grouped clusters
- More common in women than men
- Genital herpes is a recurrent, lifelong viral infection.
- Asymptomatic shedding occurs (Most sexual transmission occurs while source case is asymptomatic)
- Can be transmitted between sex partners, from mothers to newborns, and can increase a person's risk of becoming infected with HIV
**HSV Diagnosis**

- Isolation of HSV in cell culture
- PCR assays for HSV
- HSV Serologic Tests
  - HSV-specific glycoprotein G2 for HSV 2 infection and glycoprotein G1 for HSV 1
  - HerpeSelect™-2 ELISA IgG
  - HerpeSelect™ 1 and 2 Immunoblot IgG
  - HSV-2 ELISA
• **Lymphogranuloma Venereum**
  Genital and lymph node specimens (i.e., lesion swab or bubo aspirate) may be tested
  – culture
  – direct immunofluorescence
  – nucleic acid detection
  – complement fixation titers >1:64

• **Granuloma Inguinale (Donovanosis)**
  *Klebsiella granulomatis*
Chancroid
Haemophilus ducreyi

- The patient has one or more painful genital ulcers
- The patient has no evidence of *T. pallidum* infection
- A test for HSV performed on the ulcer exudate is negative

**Lab Diagnosis**

- A gram negative slender rod or coccobacillus
- Aligns in a pattern referred to as school of fish
- Culture
  - Mueller Hinton based chocolate agar supplemented with 1% IsoVitaleX and 3µg/ml vancomycin
Genital warts

Asymptomatic

Usually associated with HPV types 6 or 11

Other HPV types (16, 18, 31, 33, and 35)

Are strongly associated with cervical neoplasia
Inspection usually diagnostic of external warts:, biopsy if in doubt

Pap smear, biopsy for flat warts of cervix

HPV-DNA studies, PCR, hybrid capture

HPV cannot be cultured, and serologic tests are not available to test for HPV antibodies

Subclinical infections may be detected by applying 3% to 5% acetic acid solution for 5 to 10 minutes. The lesions then become visible
POSTPARTUM ENDOMETRITIS (PPE)  
MICROBIOLOGY

- Polymicrobial (GBS, enterococci, *G. vaginalis*, *E. coli*, *Prevotella bivia*, *Bacteroides spp*, peptostreptococci, *Ureoplasma urealyticum*, *Mycoplasma hominis*)

- *Chlamydia trachomatis* may cause a late form of PPE (>2 days to 6 wks postpartum, after vag delivery)

- Group A Strep PPE is rare
Genital tract infection in children

- Gonorrhea
- Chlamydia
- Syphilis
- HIV
- Genital warts
- BV
Thank you