Sexually Transmitted Diseases

Genitourinary Core
Computer Assisted Instruction
2007

Peter C. Iwen, PhD
piwen@unmc.edu
S. James Booth, PhD
jbooth@unmc.edu
Session Composition

- 10 Stations
  - Each contains a single disease
  - Questions for review
Stations

- **Viral Diseases**
  - #1 Genital herpes
  - #2 Genital warts
  - #3 HIV infection

- **Bacterial Diseases**
  - #4 Chlamydia
  - #5 Gonorrhea
  - #6 Syphilis
  - #7 Bacterial vaginosis

- **Fungal Disease**
  - #8 Vaginal candidiasis

- **Parasitic Diseases**
  - #9 Trichomoniasis
  - #10 Crab lice infestation
Viral STDs

- Genital herpes
- Genital warts
- HIV infection
Station #1
Genital Herpes

- Etiological agents
  - *Herpes simplex* viruses (HSV)
    - Type 1 and Type 2
  - DNA virus related to other herpes viruses
    - After infection, virus establishes latency in dorsal root ganglia
    - Reactivation may occur
    - Can be controlled but not cured
Genital Herpes

**Transmission**

- Direct contact with virus
  - Genital secretions
    - During active infection
  - About 25% of general population is sero-positive
Genital Herpes

Clinical features

- Primary infection
  - Vesicular lesion in genital tract
  - Fever and lymphadenopathy

- Recurrent infection
  - Frequency varies
    - Range from 0 to 12 or more infections per year
  - Generally milder symptoms and shorter duration of lesion healing
Genital Herpes

**Laboratory diagnosis**

- Specimen source
  - Lesion scrapping
  - Vesicular fluid
- Test methods
  - Direct exam or
  - Culture
  - Both are confirmatory tests

- Direct exam
  - Fluorescent antibody stain
    - Use fluorescein-labeled HSV-1 or HSV-2 antibody
  - Confirmatory test

Apple-green fluorescent HSV infected cells, 400x
Genital Herpes

– Culture
  ● Conventional tissue cell culture
  ● Characteristic CPE present
    – Cells round and subsequently detach from tube
    – Fluorescent staining of cells necessary to identify virus
  ● Confirmatory test

Normal tissue culture cells, 400x

Viral CPE suggesting herpes, 400x
Genital Herpes

Unique facts to consider

- *Herpes simplex* viruses are also associated with oral cold sores
- Herpes infection is extremely contagious in individuals with active lesions
- Herpes infection is controlled but not cured
**Station #1**

**Question**

- Which immunological test is commonly used on direct smears to identify *Herpes simplex* virus and to distinguish Type 1 from Type 2-caused disease?
  - Fluorescent antibody test
    - Use fluorescein-labeled HSC-1 and HSV-2 antibody
  - Considered a confirmatory test

- End Station #1
Genital Warts

- **Etiological agent**
  - Human papillomavirus (HPV)

- HPV is divided into two main types
  - **Cutaneous type**
    - Associated with warts on feet, hands, arms, and head
  - **Mucosa type**
    - Affect anogenital epithelium
    - > 40 mucosa types known
      - Some associated with epithelial cancers
      - Types 16, 18, 45, 56, 58, 59, & 68
      - Called high risk types
Genital Warts

Transmission

- Direct contact with virus
  - Through sexual exposure

- Genital warts is the most common viral sexually transmitted disease in US
  - Specific numbers unknown
    - Not a nationally reportable disease
Genital Warts

**Clinical features**

- Also called *condyloma acuminatum*
- Affects anogenital tract epithelium usually
  - May also affect upper respiratory tract
- Hyperkeratotic lesions of 2 types
  - Flat
  - Attached
    - By a broad stalk-like peduncle
- Gender specific symptoms
  - Males
    - Located on penile shaft or perianal area
  - Females
    - Perianal area, vagina, and cervix
    - Cervical infection frequently flat and not easily detected
Genital Warts

- **Laboratory diagnosis**
  - HPV cannot be grown in cell culture

- **Colposcopic exam**
  - The macroscopic appearance of warts are diagnostic
  - However, majority of genital HPV infections are the flat type
    - Not visible to the unaided eye
    - Usually found in the cervix

- **Specimens**
  - Exfoliated cell samples
    - From PAP smear in females
  - Tissue biopsy
Genital Warts

**Laboratory diagnosis**

- Direct exam
  - Cell scrapping or biopsy

- Biopsy
  - Koilocytes in tissue
    - Perinuclear clearing
    - Increase in density of surrounding rim of cytoplasm

Cervical bx, H & E, 400x
Genital Warts

- Cell scrapping
  - Papanicolaou (PAP) smear
  - Below see images of cervix using the PAP smear under low power objective

Normal

Moderate dysplasia

Typing of HPV:

Can be done using nucleic acid probes directed against specific viral types. Not currently routine
Genital Warts

Unique facts to consider

- Given the role specific HPV types play in pathogenesis of cervical cancer..........it is likely that typing will some day become standard of care to predict risk of disease
- A HPV vaccine is now available and recommended to begin immunization of females at age 11-12 years old.
Station #2
Questions

- Which cell type is characteristically observed in tissue infected with the human papillomavirus?
  - Koilocytes

- End Station #2
Station #3
HIV Infection

- Etiological agent
  - Human immunodeficiency virus types 1 and 2
    - HIV-1 and HIV-2
  - Classified as a Retrovirus
HIV Infection

Transmission

– Sexual intercourse
– Connately from mother to child
– Postnatally by breast feeding
– Parenteral inoculation

– Globally, most frequent route of transmission is vaginal infection of women by unprotected sexual intercourse
HIV Infection

- Clinical features
  - CD4 T lymphocytes represent the ultimate target
    - Culminates in severe immunosuppression, opportunistic infections, cancer, and ultimately death
  - Symptomatic disease
    - Called acquired immunodeficiency syndrome (AIDS)
  - Asymptomatic disease
    - Called HIV infection
  - Illness progresses over the course of many years
HIV Infection

- **Laboratory diagnosis**
  - Specimen source
    - Serum
      - Diagnosis is performed by serological testing
HIV Infection

Laboratory diagnosis

- Initial screen
  - Enzyme linked immunosorbent assay (ELISA)
    - Performed to detect HIV antibody in serum
    - Inexpensive test and automated for large volume testing
  - False-positive results possible
    - Thus requires confirmation testing
HIV Infection

Laboratory diagnosis

- Confirmation testing
  - Western blot test
    - Utilizes commercially prepared paper strips containing HIV protein antigens
      - The proteins have been electrophoresed and separated by size
    - Patient’s serum is placed over the strip
    - Specific antibodies if present will react with specific proteins on strip
      - Reactions are visualized as “bands”
    - Two or more bands of major proteins are diagnostic for HIV infection
      - Major proteins include: gp120, gp160, p41, p24
HIV Infection

Western blot examples

Each strip represents one patient….protein size is indicated on the left.

- Patient’s 1, 4, 9 & 11 have HIV infection
- Patient’s 3 & 8 are negative
- Patient’s 2 and 5 indeterminate (require repeat testing after waiting for 3-4 weeks)
Facts to consider

- Diagnosis of infection most frequently done by observing an IgG immune response
- Several companies offer kits that can detect both antibodies and antigen
  - Many “over the counter” test kits are available to the general public
- AIDS is considered the 3rd most common reportable disease in U.S. behind chlamydia and gonorrhea

  - 2004 totals (top 5)
    - Chlamydia: 929,462 cases
    - Gonorrhea: 330,132 cases
    - AIDS: 44,108 cases
    - Salmonellosis: 42,197 cases
    - Pertussis: 25,827 cases

(CDC, MMWR, Vol 53, June 16, 2006)
Station #3

Questions

• How is testing for HIV infection performed?
  – Detect IgG antibodies to HIV by first screening and then by confirmation of screen-positive results

• Name the tests most commonly used for screening and confirmation testing.
  – Screening, ELISA test
  – Confirmation, Western blot test

• End Station #3
Bacterial STDs

- Chlamydia
- Gonorrhea
- Syphilis
- Bacterial vaginosis
Station #4
Chlamydia

- **Etiological agent**
  - *Chlamydia trachomatis*
    - Small intracellular bacterium
    - 0.3 µ in diameter
      - Recognized as one of smallest bacterium known
  - Not part of the normal flora
    - True pathogen
Chlamydia

Transmission

- Sexual exposure
- Neonate during exposure to infected birth canal

- Reported by the CDC as most common reportable disease in U.S.
  - About 1 million new cases each year reported
    - Suspect this only represents 25% of actual cases
Chlamydia

**Clinical features**
- Bacteria attach to mucosal cells in urethra, cervix, rectum, eyes, and oral-pharyngeal area
- Nongonococcal urethritis or cervicitis
  - Males
    - Usually symptomatic
  - Females
    - Usually asymptomatic
- Clinically identical to gonorrhea
Chlamydia

- **Laboratory diagnosis**
  - Specimen sources
    - Urethral swab
      - Males
    - Cervical swab
    - Urine
      - Both females and males
      - Becoming more common because of the noninvasive collection procedure
  - Other sources
    - Throat, rectum, eyes
  - **Direct exam**
    - Not commonly done
    - Bacteria too small to be seen on Gram stain
Chlamydia

- **Laboratory diagnosis**
  - **Culture**
    - Difficult and requires cell culture
      - *C. trachomatis* acts like a virus and will only grow *in vitro* in living cells and not on standard bacteriological media
    - Required for the “other” sources
      - Throat, rectum, and eye
      - “Non-culture methods not FDA approved for these specimens
    - Confirmed in cells by FA staining using specific antibody
      - Detect chlamydial inclusions in cells

FA stained cell cultures

100x

Chlamydial inclusion

400x
Chlamydia

- **Laboratory diagnosis**
  - **Non-culture tests**
    - Mainstay of diagnosis
    - PCR-based automated testing
      - Useful for high volume testing
    - Approved to test urethral, cervical, and urine specimens
    - Highly sensitive (>95%) and highly specific (>99%)
      - <5% false negatives (sensitivity)
      - <1% false positives (specificity)
    - Dual testing is common practice
      - Detect for both chlamydia and gonorrhea at same time
Chlamydia

Facts to consider

– *Chlamydia trachomatis* is a bacterium with viral characteristics
  
  • Similarities to virus
    – Obligate intracellular parasite
    – Requires living cells to grow *in vitro*
    – Small size
  
  • Differences from virus
    – Contain both DNA and RNA
    – Can treat infections with antibacterial agents
Station #4
Questions

- When is culture most appropriate?
  - To evaluate throat, eye, and rectal specimens

- How common is chlamydia in the US?
  - Most common reportable disease

- End Station #4
Station #5
Gonorrhea

- **Etiological agent**
  - *Neisseria gonorrhoeae*
    - Gram negative coccobacillus
  - Not part of normal flora
    - True pathogen

Gram stain from culture,
1000x
Gonorrhea

- Transmission
  - Sexual exposure
  - Neonate in eyes during birth

- Reported by CDC as 2nd most common reportable disease in U.S.
  - About 400,000 cases reported per year
    - May represent less than half of the actual cases
Gonorrhea

- Clinical features
  - Clinically identical to chlamydia
  - Males
    - Symptomatic with urethritis
  - Females
    - Asymptomatic with cervicitis
Gonorrhea

Laboratory diagnosis
  – Specimen sources
    ● Urethral swab
      – Male
    ● Cervical swab
      – Female
    ● Urine
      – Female and male
    ● Other specimens
      – Throat, rectum, eyes

(Similar to chlamydia)
Gonorrhea

Laboratory diagnosis

- Direct exam
  - Unlike chlamydia, direct Gram stain is useful
  - Gram-negative intracellular diplococci
    - Pyogenic infection (PUS)
    - Diagnostic in males
    - Presumptive in females

Urethral exudate, Gram stain, 1000x
Gonorrhea

- **Laboratory diagnosis**
  - **Culture**
    - Fastidious organism
      - Plate specimen directly to medium after collection
      - Sensitive to cooling (keep at room temperature)
      - Sensitive to the atmosphere
        - CO$_2$-enriched environment during transport
        - Candle jar is a useful method
    - **Enriched medium**
      - Chocolate agar
        - Contains enrichments needed for growth
    - **Selective medium**
      - Medium containing antimicrobial agents
        - Useful to detect *N. gonorrhoae* from specimens contaminated with normal flora
      - Modified Thayer Martin agar
        - Chocolate agar base
        - Contains vancomycin (inhibit gram-positive bacteria), colistin (inhibit gram-negative bacteria), and nystatin (inhibit yeast)
Gonorrhea

- **Laboratory diagnosis**
  - Identification from culture
    - Fastidious growth requirement
      - Will only grow on chocolate agar
      - Will only grow in the presence of CO₂ enhanced atmosphere
    - Carbohydrate acidification biochemical tests
      - Look for the organisms ability to ferment a pattern of sugars
      - Classic method for culture ID
Gonorrhea

- Laboratory diagnosis
  - Non-culture tests
    - Test in combination with chlamydia detection
    - PCR-based assays are the mainstay of diagnosis
Gonorrhea

Facts to consider

- Nucleic acid amplification tests (NAATs) are the mainstay of diagnosis
- NAATs can be performed on urine which eliminates “invasive” procedures necessary for specimen collection
Neisseria gonorrhoea is a fastidious bacterium that is sensitive to which environmental conditions?
- Cooling and atmospheric air

Describe the classic Gram stain picture of gonorrhea.
- Gram-negative intracellular diplococci
- Many white blood cells (PMNs)

End Station #5
Station #6
Syphilis

- **Etiological agent**
  - *Treponema pallidum*
    - Spirochete
    - Up to 20 µ in length
  - Obligate parasites of humans
    - No known animal or environmental reservoirs
  - True pathogen
Syphilis

- **Transmission**
  - Sexual intercourse
    - Direct contact with active lesions
  - Transplacental
    - Infected mother to fetus
Syphilis

- Clinical features
  - Exhibits a wide variety of clinical manifestations
  - Primary syphilis
    - Occur at site of infection
      - Lesion characterized by ulceration
        - Generally painless
      - Lesions called chancre
Clinical features

- Secondary syphilis
  - Disseminated infection
    - Six weeks to 6 months after primary disease
    - Multiple papular lesions
      - On palms of hands, soles of feet and other locations
    - Patchy hair loss also common
Syphilis

**Clinical features**

- **Latent syphilis**
  - Interval between or following episodes of primary and secondary syphilis
  - About 75% of persons untreated will remain in this stage for life

- **Tertiary syphilis**
  - Occurs in about 25% of untreated cases
  - Characterized by chronic inflammatory granulomas (gumma)
  - Affect central nervous system, aortic valve, thoracic aorta, skin, and bone
Syphilis

Laboratory diagnosis

- Specimen
  - Primary syphilis
    - Aspirate from chancre
  - Secondary and tertiary syphilis
    - Serum to detect a serological response
Syphilis

- **Laboratory diagnosis**
  - Direct detection
    - Primary syphilis
      - Dark-field microscopic exam
        - Spirochete in lesion aspirate

Dark-field exam, chancre, 1000x
Syphilis

**Laboratory diagnosis**
- **Culture**
  - Organism cannot be cultured *in vitro*
- **Sero logical**
  - How most cases are diagnosed
    - Test for immunological response to infection
  - Usually involved secondary or latent syphilis
  - **Screen**
    - Using a non-treponemal test
    - Some false positive results occur
  - **Confirm**
    - Using treponemal test
Syphilis

- Laboratory diagnosis
  - Non-treponemal screening tests
    - Measure an antibody directed against a non-specific antigen
      - These antigens are called reagin
    - Tests are highly sensitive but have a low specificity
      - False positive results common
      - Requires that positive results be confirmed
  - Examples of tests used
    - Venereal Disease Research Laboratory (VDRL) test
    - Rapid Plasma Reagin (RPR) test
    - Elisa Immunoassay test
Syphilis

- **Laboratory diagnosis**
  - **Treponemal tests**
    - To confirm screen positive results
    - Detect anti-treponemal antibody and therefore are highly specific
      - Most common test
        - Fluorescent-treponemal antibody-absorption (FTA-ABS) test
      - Performed by overlaying slide containing commercially obtained *T. pallidum* with serum from patient
        - Subsequently stain with fluorescent-labeled antihuman reagent

FA, Shows fluorescing spirochetes to indicate specific antibody is present
400x
Syphilis

**Facts to consider**

- Syphilis is one of most common STDs in the U.S.
  - Over 20,000 new cases each year
- Diagnosis almost always by a serological test to detect an antibody response.
- *T. pallidum* cannot be cultured *in vitro*
Station #6
Questions

- **Screen for syphilis is done using which test method?**
  - VDRL, EIA, or RPR test

- **Why is confirmation testing required?**
  - Screening tests only detect a non-specific antibody associated with syphilis and thus may be a false-positive result

- **Which test is used to confirm a screen positive result?**
  - FTA-ABS test

- End Station #6
Station #7
Bacterial Vaginosis

- **Etiological agents**
  - *Gardnerella vaginalis*
    - Coryneform gram-positive rod
  - Various anaerobes
  - Unknown how these bacteria interact to produce infection
    - Decrease in normal flora vaginal *Lactobacillus* important as well
  - *G. vaginalis*
    - Considered normal vaginal flora, but
    - Overgrows in individuals with BV
Bacterial Vaginosis

Transmission
- BV is endogenous infection in females
- *G. vaginosis* may be recovered from urethra of males
  - Disease association is however questionable
Bacterial Vaginosis

Clinical features

- Inflammatory response
  - The production of a discharge with pungent fishy odor

Cervix covered by frothy discharge
Bacterial Vaginosis

- **Laboratory diagnosis**
  - Specimen
    - Vaginal secretions
  - Direct detection
    - Visualization of “clue cells”
      - Squamous epithelial cells peppered with *G. vaginalis*

Vaginal secretion wet mount, 400x;
Clue cells present
Bacterial Vaginosis

- **Laboratory diagnosis**
  - **Culture**
    - Not useful for diagnosis
  
  - **Clinical diagnosis**
    - Vaginal secretion pH > 4.5
    - Fishy amine odor following application of 10% KOH
      - Whiff test
    - Presence of “clue cells”
Bacterial Vaginosis

**Facts to consider**

- The gold standard for the diagnosis of BV is direct examination of vaginal secretions
  - Not the culture of *G. vaginalis*
    - *G. vaginalis* can be also be recovered from healthy women
Describe the major cell type observed in vaginal secretions of women with BV?

- Clue cells which are squamous epithelial cells with characteristic stripping caused by adherent *G. vaginalis*.

End Station #7
Fungal STD

- Vaginal candidiasis
Station #8
Vaginal Candidiasis

● Etiological agent
  - *Candida albicans*
    - Other *Candida* species may also be involved
    - Characterized as a yeast fungus
  
  - Present as normal flora
    - May overgrow during disruption of normal bacterial flora
      - Antibiotic usage
      - Pregnancy
      - Immune deficiency
      - Endocrine disturbance
Vaginal Candidiasis

- **Transmission**
  - Endogenous from normal flora
  - Transmission to male may occur
    - May lead to urethritis or balanitis
      (inflammation of the penis)
Vaginal Candidiasis

- Clinical features
  - Thick milky vaginal discharge
  - Inflammation
  - Disease also called vaginal thrush
**Vaginal Candidiasis**

- **Laboratory diagnosis**
  - Specimen source
    - Vaginal secretions
  - Direct exam
    - Wet preparation or Gram stain
      - Look for budding yeast, usually with pseudohyphae (characteristic of *Candida* species)

Wet preparation, budding yeast mixed with squamous epithelial cells, 400x
Vaginal Candidiasis

Laboratory diagnosis

- Direct exam
  - Gram stain
  - Budding yeast with pseudohyphae

Gram stain, 400x
Vaginal Candidiasis

Laboratory diagnosis

- Culture
  - Sabouraud dextrose agar
    - A classic fungal media used for culture
  - Culture not generally necessary for diagnosis

SAB agar culture
Vaginal Candidiasis

Facts to consider

– *Candida albicans* is by far the most common cause of candidiasis (>85% of cases)

– Yeast infections are most frequently diagnosed by direct exam without culture because of the reliability of direct detection
Station #8
Questions

- Which unique characteristic identifies *Candida* species in clinical specimens?
  - Presence of pseudohyphae with budding cells

- End Station #8
Parasitic STDs

- Trichomoniasis
- Crab lice infestation
Station #9
Trichomoniasis

- **Etiological agent**
  - *Trichomonas vaginalis*
    - Classified as a protozoal parasite
      - vs the helminths which are the true worms
  - Not normal flora
Trichomoniasis

Transmission

- Direct contact to infected individual

- Males are most frequently asymptomatic
  - May however develop prostatitis
Trichomoniasis

Clinical features
- Not reportable disease
  - Estimated that over 5 million men and women may be infected
- Most recognized disease occurs in women
  - Vaginal discharge
    - Described as greenish, frothy, and foul smelling
  - Intense vaginal and vulvar pruritus
Trichomoniasis

- **Laboratory diagnosis**
  - Must be differentiated from candidiasis and bacterial vaginosis

- **Direct exam**
  - Wet preparation of vaginal and urethral discharge
Trichomoniasis

- Direct exam
  - Examine immediately after collection under reduced light
    - Actively motile organism with movement of undulating membrane

![T. vaginalis at 100x magnification](image)
Trichomoniasis

- Laboratory diagnosis
  - Direct detection of the protozoan

Giemsa stain, trophozoite (adult organism called this)
Trichomoniasis

Facts to consider

- *T. vaginalis* is one of the few parasites that can be cultured *in vitro*
  - Culture however, is not cost effective
  - Direct detection is reliable and simple

- “Trich” is frequently diagnosed on-site after collection
  - Rarely are samples submitted to the laboratory for testing

- *T. vaginalis* does not produce a cyst form
  - Only a trophozoite
Station #9
Questions

- Which structure of *Trichomonas vaginalis* is detected in genital secretions from an infected individual?
  - Trophozoite

- End Station #9
Station #10
Crab Lice Infestation

- Etiological agent
  - *Phthirus pubis*
    - Also referred to as the crab louse
  - Classified as an arthropod “of the annoying” type
    - In contrast to those associated with disease transmission (vectors)
Crab Lice Infestation

Transmission
- Direct sexual contact with infected individual
- Males and females are equally affected
Crab Lice Infestation

Clinical features

- All lice suck blood intermittently
- Produce an unexplained dermatitis in the genital tract
  - Due to repeated feeding and chronic exposure to louse excreta
Crab Lice Infestation

- **Laboratory diagnosis**
  - Specimen
    - Pubic hair
  - Direct exam
    - Nits on the hair shafts
      - Also known as larval eggs
Crab Lice Infestation

- **Laboratory diagnosis**
  - Adults may be observed
    - on the skin or
    - on cloths or in the bed
  - Nits on the hair
Crab Lice Infestation

**Comparison**
- Head, body, and crab lice
- All three lice body site restricted
Crab Lice Infestation

**Fact to consider**

- Lice infestation can occur by
  
  • sharing infested clothing,
  
  • exposure to infested bedding, or
  
  • by direct contact
Name the three species of lice associated with lice infestation.

- Body louse, *Pediculus humanus var corporis*
- Head louse, *Pediculus humanus var capitis*
- Crab louse, *Phthirus pubis*

End Station #10
Conclusion

- This completes the computer-assisted instruction for the sexually transmitted diseases.

- For questions or comments about this educational experience please e-mail
  - Dr. Peter Iwen piwen@unmc.edu or
  - Dr. James Booth jbooth@unmc.edu