LABORATORY DIAGNOSIS
SEXUALLY TRANSMITTED DISEASES

LABORATORY MEDICINE COURSE
2004

CLINICAL MICROBIOLOGY SERVICE
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STD EPIDEMIC - USA
TIP OF THE ICEBERG

ININCIDENCE
DISCHARGE
- Chlamydia - 4 million
- Gonorrhea - 650,000
- Trichomoniasis - 180 million
- BV - Not reportable
- HPV - 5.5 million
- Herpes - 1 million
- Syphilis - 60,000

CLINICAL IMPACT
- INFERTILITY
- ECTOPIC PREGNANCY
- HIV TRANSMISSION
- PID
- CERVICAL CA
- ~$10 BILLION ANNUAL COST

STDs CLINICAL SYNDROME
THE HIDDEN EPIDEMIC

AT RISK
✓ ADOLESCENTS & MINORITIES
✓ MULTIPLE SEX PARTNERS
✓ PAST STDs

DISCHARGE DISEASES
✓ HIV ACQUISITION ✪ 5- FOLD

ULCERATIVE LESIONS/ GROWTHS
✓ HIV ACQUISITION ✪ 10- FOLD

WOMEN AT RISK THE X FACTOR

• STDs OFTEN ASYMPTOMATIC
  ✓ 75-90% WOMEN WITH CHLAMYDIA
  ✓ 50-70% MEN WITH CHLAMYDIA
  ✓ PID
  ✓ ≤ 40% UNTREATED CASES GC/CHLAMYDIA
  ✓ INFERTILITY
    • 1 IN 5 UNTREATED CASES
  • CERVICAL CANCER
  • AT- RISK PREGNANCIES

• PREMATURE LABOR & DELIVERY
  ✓ LARGEST RATE OF INFANT MORTALITY
  • PREMATURE RUPTURE OF MEMBRANES
  • ECTOPIC PREGNANCIES
  • NEONATES
    ✓ CONJUNCTIVITIS
    ✓ PNEUMONIA

TEENS & SEX
STD TESTS IN ADOLESCENT CLINICS

• AT-RISK GROUP
  ✓ MULTIPLE SEX PARTNERS
  ✓ UNPROTECTED SEX
  ✓ CONTRACEPTIVE USE INCONSISTENT

• THE X FACTOR: FEMALES
  ✓ GC HIGHEST IN 15-19 YRS
  ✓ 5-10% TEENS – CHLAMYDIA
  ✓ 28-46% <25 YRS – HPV
  ✓ SEX PARTNERS WITH OLDER MEN

DISCHARGE DISEASES

• MOST COMMON DISCHARGE DISEASE IN U.S.
  ✓ CHLAMYDIA TRACHOMATIS
  ✓ NEISSERIA GONORRHAEAE
  ✓ TRICHOMONAS VAGINALIS

• RISK FACTORS
  ✓ YOUNG AGE
  ✓ MULTIPLE SEX PARTNERS
  ✓ PAST STDs
GONORRHEA & CHLAMYDIA

- CLINICAL SYNDROME
  - SYMPTOMATIC OR ASYMPTOMATIC
  - DYSURIA & FREQUENCY
  - PURULENT DISCHARGE
  - INDISTINGUISHABLE

- GONORRHEA
  - OTHER SITES (PHARYNX, RECTUM, EYE)
  - NEWBORNS (PURULENT CONJUNCTIVITIS)
  - BACTEREMIA, ARTHRITIS

WHEN TO COLLECT ...........
ENDOCERVIX OR VAGINAL SPECIMENS

<table>
<thead>
<tr>
<th></th>
<th>ENDOCERVIX</th>
<th>VAGINA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal pH</td>
<td>7.0</td>
<td>&lt;4.5</td>
</tr>
<tr>
<td>Cell Type</td>
<td>COLUMNAR</td>
<td>SQUAMOUS</td>
</tr>
<tr>
<td></td>
<td>EPITHELIAL</td>
<td>EPITHELIAL</td>
</tr>
</tbody>
</table>

Pathogens
- Chlamydia trachomatis
- Neisseria gonorrhoeae
- Bacterial Vaginosis (BV)
- Trichomonas
- Candida sp

LAB DIAGNOSIS
C. TRACHOMATIS

- NA AMPLIFICATION
  - URINE SCREEN
- DIRECT FLUORESCENT AB
- DNA PROBES
  - URETHRAL SPECIMEN
- TISSUE CULTURE
  - McCoy Cells
    - INTRACYTOPLASMIC INCLUSIONS WITH IODINE
    - IMMUNOFLUORESCENCE

CT/GC LABORATORY DX

<table>
<thead>
<tr>
<th></th>
<th>GRAM STAIN</th>
<th>CULTURE</th>
<th>URINE NAA</th>
</tr>
</thead>
</table>
| GC             | GRAM – DIPLOCCCI
    (bean-shaped) |
    SENSITIVITY - MALES
    - 95-99%
    Symptomatic
    - 69%
    Asymptomatic
    SENSITIVITY - FEMALES
    - 45-65%
| CALCium ALGINATE Swab (Cotton-toxic) CHOCOLATE AGAR CO₂ TAT- 2 Days |
|                | SENSITIVITY 85-98% SPECIFICITY >98% TAT- 4 hrs |
| CT             | N/A        | McCoy Cells |
|                |            | TAT- 2-3 Days |

CHLAMYDIA/GC URINE NAAT SCREEN

- NON-INVASIVE, PAINLESS!!!
- AUTOMATION
- TURNAROUND TIME
  - 4 hrs
- SCREENING IS RECOMMENDED
  - CDC, AMA, DOH
  - MALES & TEENS
  - PREGNANCY

STD GUIDELINES - GC TESTING
CHILD ASSAULT CASES

- CULTURE - THE GOLD STANDARD
  - PHARYNX & ANUS IN MALES & FEMALES
  - VAGINA FEMALES
  - URETHRA MALES
- NOT RECOMMENDED
  - GRAM STAINS FOR DX
  - NON-CULTURE TESTS
  - NAAT – DATA WON’T HOLD UP IN COURT
  - CERVICAL SPECIMENS FOR PRE-PUBERTAL GIRLS
- PHARYNGEAL SPECIMENS
  - LACKS SENSITIVITY

NAAT = NUCLEIC ACID AMPLIFICATION TEST
**CDC GUIDELINES ADULTS – CT & GC TESTING**

- Cultures from all sites of penetration
- FDA approved NAAT
  - 2 NAATs (different targets)
  - Both must be positive
- Assays not approved
  - EIA, non-amplified probes, DFA
  - Insensitive - False negatives

**CLINICAL SYNDROME COMPARISON**

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Discharge</th>
<th>Odor</th>
<th>Presentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trichomonias</td>
<td>Watery, thin, gray, alkaline homogeneous</td>
<td>FISHY</td>
<td>Itching, vaginal erythema, dysuria, vaginal erosions, &amp; petechiae (strawberry cervix)</td>
</tr>
<tr>
<td>Bacterial vaginosis</td>
<td>Watery, gray, homogeneous alkaline</td>
<td>FISHY</td>
<td>Cervix normal, Often asymptomatic</td>
</tr>
<tr>
<td>Candidiasis</td>
<td>Thick, white, non-homogeneous</td>
<td>SOUR</td>
<td>Itching, vaginal erythema, dysuria, labial &amp; vulval swelling</td>
</tr>
</tbody>
</table>

**THE FISHY ODOR IN VIVO**

- Normal vaginal flora
  - H₂O₂-producing Lactobacilli ⇒ lactic acid
  - pH (<4.5)
- Bacterial vaginosis
  - Anaerobes increase ⇒ proteolytic enzymes act on vaginal peptides
  - Release of polyamines and trimethylamine
  - Trimethylamine in alkaline pH ⇒ fishy odor
  - Polyamines ⇒ exfoliation of epithelial cells (clue cell)
  - Polyamines ⇒ fishy discharge

**BRIEF CASES**

**BV DIAGNOSED**

- Facts to consider
  - Pregnant women
    - Affected 15-20% pregnant women
    - Amniotic fluid infection
    - Postpartum endometritis
    - Premature rupture of membranes
    - Preterm delivery
    - Low birth weight
  - Treat pregnant women
    - Metronidazole
    - Clindamycin

**TRICHOMONAS VAGINITIS LAB TESTS**

- Wet mount
  - Urine or vaginal secretion must be viewed immediately
  - Transport instability
  - <50% Sensitivity/Specificity
  - Time to results: 5-10 minutes
- In-pouch culture
  - No specimen transport problem
  - Direct inoculation into pouch & parasite grows in media
  - >95% Sensitivity/Specificity
  - Time to results: 18-48 hrs
TRICHOMONAS DX
FACTS TO CONSIDER
• THIRD MOST COMMON OF THE VAGINITIDES
• THOUGHT TO FACILITATE HIV TRANSMISSION
• PREVALENCE
  ✓ COMMERCIAL SEX WORKERS, 50-75%
  ✓ (TRICKY BUSINESS)
  ✓ STD CLINICS, 32-54%
  ✓ OB CLINICS, 10-26%
• TREATMENT
  ✓ METRONIDAZOLE

VULVOVAGINAL CANDIDIASIS
• ETIOLOGIC AGENT
  ✓ 85-95% CANDIDA ALBICANS
  ✓ CANDIDA GLABRATA
  ✓ LESS SUSCEPTIBLE TO AZOLES
  ✓ HIV INFECTED
• FREQUENCY
  ✓ 70-75% AT LEAST ONCE
  ✓ 40-50% RECURRENCE
• PREDISPOSING FACTORS
  ✓ ANTIBIOTICS, DIABETES
  ✓ ORAL CONTRACEPTIVES
• WHAT TESTS SHOULD BE ORDERED?
  ✓ GRAM STAIN
  ✓ CULTURE ONLY WHEN RECURRENT INFECTIONS
  ✓ YEAST IS NORMAL FLORA
  ✓ DNA PROBE TEST

ULCERATIVE DISEASES
• INCIDENCE
  ✓ >20 MILLION CASES
• ETIOLOGIC AGENTS
  ✓ HERPES SIMPLEX VIRUS
  ✓ TREPONEMA PALLIDUM
  ✓ HUMAN PAPILLOMA VIRUS
  ✓ CHANCROID
• CONTRIBUTE TO HIV TRANSMISSION
  ✓ ULCER IS PORTAL OF ENTRY

SYPHILIS
• Incidence
  ✓ Increase in NYC among men who have sex with men (MSM)
  ✓ WHITE MEN & THOSE IN MANHATTAN
  ✓ LARGELY HIV + INDICATING EROSION OF SAFE SEX
  ✓ Higher in the south
  ✓ HETEROSEXUAL BLACK MEN
• Primary Syphilis – CHANCRE
  ✓ Single, Painless, Smooth edges, Indurated, LA
• Secondary Syphilis
  ✓ Palmar rash
  ✓ 6 wk-6 mth after infection

LAB DX - SYphilis
• MICROSCOPIC (primary)
  ✓ DARK FIELD
  ✓ TREPONEMA PALLIDUM
  ✓ MOTILE SPIROCHETE
  ✓ (6-14 SPIRALS)
• SEROLOGY (secondary)
  ✓ RPR (rapid plasma reagin)
  ✓ FTA (fluorescent treponemal Ab)
  ✓ Confirmatory test
  ✓ VDRL - CSF
  ✓ UNCULTURABLE

LAB DX - SYphilis
<table>
<thead>
<tr>
<th>TEST</th>
<th>ANTIGEN</th>
<th>INTERPRETATION</th>
</tr>
</thead>
</table>
| VDRL (CSF)   | CARDIOLIPIN   | TRUE POSITIVE
| Screen       | CHOLESTEROL   | RPR TITER >1:2
| RPR Screen   | CAROTID       | VDRL TITER >1:2
|              | CHOLESTEROL   | FALSE POSITIVE
|              | CARDIOLIPIN   | Autoimmune diseases
|              | T. pallidum   | Infectious mono
| FTA-AB       | T. pallidum   | Pregnancy, Old age
| confirmatory | + FLUORESCENCE|
**SEROLOGIC TESTS FOR SYPHILIS**

<table>
<thead>
<tr>
<th>TEST</th>
<th>SENSITIVITY BY STAGE OF SYPHILIS (%)</th>
<th>SPECIFICITY (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1&lt;sup&gt;st&lt;/sup&gt;</td>
<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
</tr>
<tr>
<td>VDRL</td>
<td>78 (74-87)</td>
<td>100</td>
</tr>
<tr>
<td>RPR</td>
<td>86 (77-100)</td>
<td>100</td>
</tr>
<tr>
<td>FTA-ABS</td>
<td>84 (70-100)</td>
<td>100</td>
</tr>
</tbody>
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**HERPES SIMPLEX**

**LAB DX**

- DIRECT IMMUNOFLUORESCENCE
  - SCRAPE CELLS OFF BASE OF ULCER & STAIN
  - DIFFERENTIATES TYPES 1 & 2
- CULTURE
  - SHELL VIAL
  - CYTOPATHOGENIC EFFECT
  - SENSITIVITY 70-99%, SPECIFICITY 99%
- CYTOLOGY & H & E STAIN
  - MULTI-NUCLEATED GIANT CELLS
  - SENSITIVITY & SPECIFICITY <60%
- PCR
  - INVESTIGATIONAL
  - DIFFERENTIATES TYPES 1 & 2

**LAB DX - HPV**

- HYBRID CAPTURE PROBE
  - Sensitivity: 84-100%
  - Specificity: 74-95%
- PCR FROM CERVICAL SPECIMENS
- DX IS OFTEN CLINICAL

**HUMAN PAPILLOMA VIRUS**

- INCIDENCE
  - MOST PREVALENT STD IN COLLEGE WOMEN
- CLINICAL IMPRESSION
  - VISIBLE GENITAL WARTS CALLED CONDYLOMA ACUMINATA
    - CONDYLOMA = “KNUCKLES”, ACUMINATA = “POINTED”
  - SMOOTH OR FINGERLIKE FLESH-COLORED PROJECTIONS ★ ROUGH
  - MULTIPLE PAPULES MAY BECOME CONFLUENT, OR MultilObED MASSES
  - CAN BE PAINFUL, FRIABLE &/OR PRURITIC
- INVASIVE CANCER
  - 16, 18, 31, 33 & 35 TYPES CARRY HIGH TO MODERATE RISK