Sexually Transmitted Diseases: State Of The Art In Laboratory Diagnostics And More!

Sukantha Chandrasekaran, PhD, M(ASCP)CM
UCLA Health Systems
March 17th, 2018
Educational Objectives

• Identify the primary etiologic agents responsible for STDs
• Compare and contrast the clinical manifestation of STDs
• List methods for detecting agents that cause STDs
• Discuss treatment options and challenges for STDs
Topics for Discussion

- Human Immunodeficiency Virus (HIV)
- Herpes Simplex Virus (HSV)
- Syphilis
- *Trichomonas vaginalis*
- *Neisseria gonorrhoeae*
- *Chlamydia trachomatis*
- *Mycoplasma hominis*
- *Ureaplasma sp.*
Human Immunodeficiency Virus

YOU CAN GET HIV VIA...

- Sex without a condom
- Passed from mother to baby
- Sharing injecting equipment
- Contaminated blood transfusions & organ transplants

Avert.org
HIV: Retroviridae

- Single-stranded positive sense RNA virus, non-segmented
- Subfamily
  - Lentivirus – HIV, SIV and FIV
  - Alpharetrovirus, Betaretrovirus, Deltaretrovirus, Episilonretrovirus, Gammaretrovirus, Spumavirus
- Attacks CD4 T Cells
- Integrate their genome into ours
Human Immunodeficiency Virus

• HIV-1
  • M group – major group; N, O, P are uncommon (Cameroon, Gabon and Equatorial Guinea)
    • M has 8 genetic subtypes (A-H)

• HIV-2
  • Uncommon and limited to West Africa
  • Subtypes A to E (Less than 5 transmissions in the US)
US Stats

- CDC 2015 - An estimated 1,122,900 adults and adolescents were living with HIV
  - Of those, 162,500 (15%) had not received a diagnosis.
  - 30% of new HIV infections are transmitted by people living with undiagnosed HIV

- Transmission to infants in the US has decreased but adolescent transmission is on the rise
HIV Diagnostic Testing

- Primary Infection
- Acute HIV syndrome
  Wide dissemination of virus
  Seeding of lymphoid organs
- Clinical Latency
- Constitutional Symptoms
- Opportunistic Diseases
- Death

CD4+ T Lymphocyte Count (cells/mm³)

Weeks

Years

HIV RNA Copies per ml Plasma

10^7

10^6

10^5

10^4

10^3

10^2

0

100

200

300

400

500

600

700

800

900

1000

1100

1200
Clinical Manifestation

• 40-90% have flu-like symptoms within 2-4 weeks
  • Fever
  • Chills
  • Rash
  • Night sweats
  • Muscle aches
  • Sore throat
  • Fatigue
  • Swollen lymph nodes
  • Mouth ulcers

• Progression to AIDS
  • Rapid weight loss
  • Recurring fever/profuse night sweats
  • Swelling of the lymph glands in the armpits, groin, or neck
  • Diarrhea
  • Sores of the mouth, anus, or genitals
  • Pneumonia
  • Red, brown, pink, or purplish blotches on skin and mucous membranes
  • Neurologic disorders
### Table 1. Major HIV Proteins of Diagnostic Significance

<table>
<thead>
<tr>
<th>HIV Gene and Products</th>
<th>Viral Protein/Glycoprotein Molecular Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HIV-1</td>
</tr>
<tr>
<td><strong>Env</strong></td>
<td></td>
</tr>
<tr>
<td>Precursor</td>
<td>gp160</td>
</tr>
<tr>
<td>External glycoprotein</td>
<td>gp120</td>
</tr>
<tr>
<td>Transmembrane glycoprotein</td>
<td>gp41</td>
</tr>
<tr>
<td><strong>Pol</strong></td>
<td></td>
</tr>
<tr>
<td>Reverse transcriptase</td>
<td>p66</td>
</tr>
<tr>
<td>Reverse transcriptase</td>
<td>p56</td>
</tr>
<tr>
<td>Integrase</td>
<td>p31</td>
</tr>
<tr>
<td><strong>Gag</strong></td>
<td></td>
</tr>
<tr>
<td>Precursor</td>
<td>p55</td>
</tr>
<tr>
<td>Core</td>
<td>p24</td>
</tr>
<tr>
<td>Matrix</td>
<td>p17</td>
</tr>
</tbody>
</table>
Spectrum of HIV Tests

• HIV diagnosis (Antibody/Antigen testing)
  – Enzyme Immunoassays (EIAs)
  – Rapid tests

• Confirmatory Testing – Western Blot based

• Early diagnosis in infants
  – DNA PCR

• Monitoring of infection and response to ART
  – Viral Load
CDC Recommendation For HIV Screening

HIV-1/2 antigen/antibody combination immunoassay

(+)

(-)

Negative for HIV-1 and HIV-2 antibodies and p24 Ag

HIV-1/HIV-2 antibody differentiation immunoassay

HIV-1 (++)
HIV-2 (-)

HIV-1 antibodies detected

HIV-1 (-)
HIV-2 (+)

HIV-2 antibodies detected

HIV-1 (+)
HIV-2 (+)

HIV antibodies detected

HIV-1 (-) or indeterminate
HIV-2 (-)

HIV-1 NAT

HIV-1 NAT (+)
Acute HIV-1 infection

HIV-1 NAT (-)
Negative for HIV-1

(+): indicates reactive test result
(-): indicates nonreactive test result
NAT: nucleic acid test
4th generation HIV antibody and p24 ELISA (Combo)

1. HIV-1/2 protein antigen
2. p24 capture antibody
3. HIV-1/2-specific IgM antibody
4. p24 antibody conjugate
5. HIV-1/2 protein antigen conjugate
6. p24 protein
7. Colour reaction product
8. Light emission wavelength
HIV 1/2 Antibody Confirmation

Results:

- **HIV-1 positive**: antibodies to HIV-1 (2 of 4 HIV-1 lines positive)
- **HIV-2 positive**: antibodies to HIV-2 (2 of 2 HIV-2 lines positive)
- **Negative**: no antibodies detected – follow up with HIV-1 NAAT
- **HIV-1 indeterminate**: only 1 of 4 antibodies to HIV-1 detected – follow up with HIV-1 NAAT
- **HIV-2 indeterminate**: only 1 of 2 antibodies to HIV-2 detected – follow up with HIV-1 NAAT, retesting in a few weeks, if still indeterminate, HIV-2 NAAT (University of Washington)

BioRad Geenius

Test line 1: gp36 (HIV-2 envelope peptide)
Test line 2: gp140 (HIV-2 envelope peptide)
Test line 3: p31 (HIV-1 polymerase peptide)
Test line 4: gp160 (HIV-1 full length envelope recombinant protein)
Test line 5: p24 (HIV-1 fill length core recombinant protein)
Test line 6: gp41 (Group M & O HIV-1 envelope peptides)
Test line 7: Control band (Protein A)
HIV-1 RNA Viral Load Test

Viral load or viral burden is the quantity of HIV-RNA that is in 1ml of blood

PCR test that is quantitative from 10 – 10,000,000 copies/ml
PCR is qualitative (Detected vs not detected from 1-10 copies/ml

Possible Results: Not detected: No virus present
Detected <10: There are less than 10 copies/ml of HIV virus
Detected: A number from 10-10,000,000 copies/ml
Detected: >10,000,000 copies/ml
Challenges of HIV Testing

• Early detection of seroconversion
• Early detection in infants born to HIV positive mothers
• Effect of HIV subtypes on test performance
• Impact of other health conditions on test performance
• Product specific equipment
Antiretroviral Therapy (ART)

- Antiretroviral (ARV) classes:
  - nucleoside reverse transcriptase (RT) inhibitors
  - non-nucleoside RT inhibitors
  - protease inhibitors
  - fusion inhibitors
  - integrase inhibitors
  - CCR5 inhibitors

- For the most up-to-date guidelines for adults and peds:
  http://www.aidsinfo.nih.gov
Herpes Simplex Virus 1 and 2
Herpesviruses

• The eight known herpes viruses are divided by genomic and biologic behavior into 3 groups:

• Alphaherpesviruses
  • HSV-1, HSV-2 and Varicella-Zoster

• Betaherpesviruses
  • HHV-6, HHV-7 and CMV

• Gammaherpesviruses
  • EBV, Kaposi’s sarcoma-associated herpes viruses(HHV-8)
HSV 1 and 2

- Range from mild stomatitis to fatal disease
  - Oral ulcers
  - Genital ulcers
  - Keratitis
  - Encephalitis
  - Neonatal herpes

- HSV 1
  - Thought to be primarily oral ulcers = “cold sores”

- HSV 2
  - Originally associated with anogenital ulcers
Life cycle

- HSV infects epithelial cells in the mucosa
  - Replicates and causes cell death

- Latency
  - Enters sensory neurons
  - Reactivates and travels back to the epithelial cell
Methods of Detection

• Culture
  • Previous Gold Standard
    • Cell culture – Cytopathic effects
    • Takes several days

• Direct Fluorescent Assay
  • Cell culture

• Nucleic Acid Detection

• Serology
## Detection of Herpes Infection

<table>
<thead>
<tr>
<th>Assay</th>
<th>Target</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture lesion (Active Disease)</td>
<td>Virus</td>
<td>70%</td>
<td>100%</td>
</tr>
<tr>
<td>PCR lesion (Active Disease)</td>
<td>DNA</td>
<td>90%</td>
<td>100%</td>
</tr>
<tr>
<td>DFA lesion (Active Disease)</td>
<td>Capsid</td>
<td>80%</td>
<td>99%</td>
</tr>
<tr>
<td>EIA serum (Exposure)</td>
<td>IgG antibody</td>
<td>95%</td>
<td>95%</td>
</tr>
<tr>
<td>Western blot serum (Exposure)</td>
<td>IgG antibody</td>
<td>95%</td>
<td>100%</td>
</tr>
</tbody>
</table>
HSV 1/2 PCR Testing

CDC: PCR is the preferred HSV test for persons seeking medical treatment for genital ulcers or other mucocutaneous lesions

Failure to detect HSV by PCR in the absence of active lesions, does not indicate an absence of HSV infection due to intermittent shedding

HSV 1/2 IgM and IgG ELISA Testing

CDC: The presence of type specific HSV-2 antibody implies anogenital infection

This test is useful in the following scenarios:
1. Recurrent genital symptoms or atypical symptoms with negative HSV PCR
2. Clinical diagnosis of genital herpes without laboratory confirmation
3. A patient whose partner has genital herpes
Syphilis

Stage 1: 3-90 days after exposure
Stage 2: 4-10 weeks after initial infection
Stage 3: 3-15 years after initial infection
Syphilis - *Treponema*

- *Treponema pallidum* subsp. *pallidum*
  - Sexually and vertically transmitted syphilis

- Spirochete family

- Obligate human pathogen with no known animal reservoir

- 16s rRNA sequencing places spirochetes in a single phylum w/ five clusters
  - *Treponema*
  - *Spirochaetae*
  - *Borrelia*
  - *Brachyspira*
  - *Leptospira*
Syphilis - *Treponema*

- Cannot Gram stain or be visualized by conventional light microscopy
- Not Easily cultured
  - Can only be cultured reliably in rabbits
- Fragile does not tolerate, desiccation, elevated temperatures or high oxygen tension
- Reliant on host for fatty acids, nucleotides, cofactors, and most amino acids
- The outer membrane lacks LPS and has low protein density
Clinical Features of Treponematoses

- **Endemic syphilis** (*T. pallidum* subsp. *endemicum*)
  - Africa and Middle East
  - Nonsexual contact with infected skin and mucous membranes
  - Lesions of skin, bone and cartilage

- **Yaws** (*T. pallidum* subsp. *pertenue*)
  - Tropical regions
  - Nonsexual contact with exudative lesions in childhood/adolescence
  - Destructive lesions of bone and cartilage

- **Pinta** (*T. pallidum* subsp. *carateum*)
  - Central and South America
  - Nonsexual contact with exudative lesions in childhood/adolescence
  - Skin lesions and hyper pigmentation
Disease Progression - Venereal syphilis (*T. pallidum* subsp. *pallidum*) - Global

**Infection** → **Primary (chancre)** → **Secondary (rash)** → **Latent syphilis (no signs of disease)** → **Tertiary**

- **Incubation period**: 9–90 days
- **Early syphilis**: Single or multiple painless sores lasting 3-6 weeks
- **Late syphilis**: Fever, sore throat, swollen LN, patchy hair loss, headache, muscle ache, fatigue
- **Tertiary syphilis**: Many years to a lifetime
- **Late Stage**: Difficulty coordinating muscle, paralysis, numbness, blindness, dementia, death
Congenital Syphilis

- Many infected infants are asymptomatic at birth

- Symptoms
  - Early (1st 5 weeks)
    - Hepatosplenomegaly
    - Snuffles
    - Cutaneous lesions
    - Anemia
    - Jaundice
    - Periostitis
  - Late
    - Frontal bossing
    - Short maxilla
    - High palatal arch
    - Hutchinson Teeth
    - 8th nerve deafness
    - Saddle Nose
    - Perioral fissures

- Nasal secretions and lesions are highly infectious

- May not manifest until months or years after birth (e.g. Hutchinson Triad)

- Can be prevented if the mother's infection is treated prior to second trimester (28 weeks)
Epidemiology

• WHO estimates 5.6 million new cases (15-49 yr old)

• CDC: U.S. – 1.4 million since 2013
  • 2015 - 23,872 cases of primary and secondary syphilis were reported
    • Up by 19% since 2014
    • Men account for 90% (MSM)
  • Congenital syphilis rates have been rising since 2012 (12.4/100,000 live births)

• Morbidity and Mortality due to congenital syphilis
  • 350,000 adverse pregnancy outcomes worldwide
    • 143,000 fetal deaths/stillbirths
    • 62,000 neonatal deaths
    • 102,000 infected infants
Laboratory Diagnostics

Specimens collected from lesions contain high number of treponemes

- Culture – Rabbit infectivity test (RIT) - research settings
  - Intratesticular inoculation
  - High sensitivity and specificity but long turn around time

- Dark Field Microscopy
  - Scrapings from chancrets

- Immunofluorescence – DFA-TP
  - mAB of anti-T. pallidum
  - Tissue or fluids
Darkfield Microscopy

- Blocks central beam of light, captures oblique rays reflected from the specimen
- Examination of unstained specimens
- Greater resolution than conventional light microscopy
- Dependent on sample and technician
  - Sensitivity: 74-86%
  - Specificity: 85-97%
- Definitive for early syphilis – lesion exudate

T. pallidum
Leptospira
Laboratory Diagnostics - Serologic Tests

**Nontreponemal – RPR and VRDL (CSF)**
- Detect antibodies against lipoidal antigens (cardiolipid, lecithin, cholesterol)
- Used for screening and evaluation of therapy (Good for 2° phase)
- Lack specificity, antibodies can cross react from other conditions
- Prozone effect can occur

**Treponemal – TP-PA, FTA-ABS, EIAs, TPI**
- Tests incorporate Treponemal specific antigens
- Have higher specificity than non-treponemal tests
- Confirmatory tests for syphilis infection
- False positives (HIV, Toxo, *H.pylori*)
- Cannot monitor therapy

Additional conditions:
- EBV, hepatitis, VZV, measles, lymphoma, TB, malaria, endocarditis, pregnancy
- Specificity is high but lacks sensitivity
RPR (Rapid Plasma Reagin)

- Floccuation test – IgM and IgG to cardiolipin
  - Cardiolipin attached to a carbon molecule detects reagin
- Qualitative test followed by titer of reactive specimens
- Pro- Fast, easy inexpensive
- Can follow treatment
- Cannot be used for CSF
- Need to consider prozone effect
- Biologic false positives
- May be nonreactive in primary syphilis repeat 1 week, 1 month, 3 months
- 4X fold decrease in titer 3 months after therapy

- Specificity 93-99%
- Sensitivity
  - Primary Syphilis 60-86%
  - Secondary Syphilis 100%
  - Teritary 98%
VDRL (Venereal Disease Research Lab) Test

- Flocculation test
  - cardiolipin, cholesterol and lecithin antigens
- Detects IgG and IgM to lipoidal material from damaged cells
- Quantitative
- Can be performed on serum or CSF
- Requires microscopic examination to read (10X)
- More technically demanding
- Not diagnostic, biologic false positives
- Prozone effect

- Specificity 96-99%
- Sensitivity
  - Primary Syphilis 67-78%
  - Secondary Syphilis 96-100%
  - Tertiary Syphilis 85-95%
T. Pallidum Particle Agglutination Assay (TP-PA)

- Gel beads are sensitized with *T. pallidum* antigen
  - Sonicated *T. pallidum* cultures
  - All *T. pallidum* antigens, not specific
- Specific for the serologic detection of all *Treponema* species
- Agglutinated particles spread out if patient specimen contains anti-Treponemal antibodies
- Can be performed on sera, cannot be used with CSF
- Is specific for *Treponema* and is confirmatory (Pos ≥ 1:80)

- Specificity 98-100%
- Sensitivity
  - Primary Syphilis 85-98%
  - Secondary Syphilis 100%
  - Tertiary Syphilis 100%
Algorithms

**TRADITIONAL SYPHILIS SCREENING ALGORITHM**

- RPR Screening Test
  - Treponemal Test
    - Positive for Syphilis
    - Negative for Syphilis
  - Negative for Syphilis
- Non-treponemal tests are inexpensive, easy, follow therapy

**SYPHILIS REVERSE SEQUENCE SCREENING ALGORITHM**

- Treponemal Screening Test
  - Positive for Syphilis
  - Negative for Syphilis
- Reverse Advantage: Automated methods and fewer false positives

Theel et al. AACC Reverse Sequence Screening for Syphilis
Other Treponemal Assays

- FTA-ABS (Fluorescent Treponemal Antibody Absorption)
  - Indirect fluorescent antibody test using a non-pathogenic strain of *T. pallidum* subsp. *phagdensis* to detect IgG or IgM

- Microhamaagglutination test – MHA-TP – replaced by TP-PA
  - Uses erythrocytes instead of gelatin particles

- Western Blot
  - IgM western is useful in diagnosing congenital syphilis

- TPI – *Treponema pallidum* immobilization test
  - Use patient’s serum to inactivate live spirochetes (if Abs are present)

- EIAs – Use *T. pallidum* ag to detect IgM or IgG
Treatment

• Penicillin G
  • Single IM dose

• Jarisch-Herxheimer Reaction
  • Acute febrile reaction – headache, myalgia and fever
  • Occurs within the 1st 24 hours after treatment
  • Early syphilis – bacterial burden higher

• Management of partners
  • Transmission occurs when mucocutaneous syphilitic lesions are present
  • Sexual contact with a person who has received a diagnosis within 90 days
Trichomonas vaginalis

- Parasite
- Flagellate
History & Background

- *Trichomonas vaginalis* was first discovered in 1836 after it was found in genital secretions of both women and men.

- Most common, curable non-viral sexually transmitted disease in the world:
  - WHO estimates 7.4 million new cases of trichomoniasis each year in US
  - Affects both women AND men, despite what the name may suggest.

- Initially thought to be non-pathogenic.

- In 1942, 70 pregnant volunteers were inoculated with *T. vaginalis* culture (10,000 – 120,000 trichomonads per inoculum).
  - 13% developed trichomoniasis shortly after inoculation
  - Two patients were negative prior to giving birth but positive after...
Lifecycle & transmission

- Lifecycle consists of only a trophozoite stage
- Transmitted by direct contact during sexual intercourse
- Nonsexual transmission is extremely rare - usually confined to urogenital tract
- Asymptomatic infected individuals are important vectors
- Incubation period is 4-28 days in ~50% of women
Sites of infection

**Women**

- Vagina, cervix, Bartholins glands, bladder, urethra and occasionally the upper urinary tract
  - Over 95% of infections were isolated from the vagina
- Trichomonads have been shown to migrate to the fallopian tubes and peritoneal cavity

**Men**

- Urethra is most common site
- Trichomonads can also be detected in the epididymis, semen, urine and prostatic fluid
Pathogenesis

- Pathogenicity is not thoroughly understood
- Adherence to epithelial cells is dependent on specific parasite surface proteins
- Hemolysis is also correlated with virulence
- Trichomoniasis shown to increase in severity during or slightly after menstruation
Cause & risk factors

• Sexually spread by penis-to-vagina intercourse or vulva-to-vulva contact
• Women can get the disease from men or women, but men usually only get from infected women

Risk factors:
• Multiple sex partners
• Being of non-Hispanic African decent
• Past or present infection with other STDs
• Bacterial vaginosis
• High vaginal pH levels
  • vaginal pH < 4.5 reduces motility of *T. vaginalis*
<table>
<thead>
<tr>
<th>Sign/Symptom</th>
<th>% of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asymptomatic</td>
<td>≤ 50%</td>
</tr>
<tr>
<td>Vaginal/vulvar erythema (redness)</td>
<td>75%</td>
</tr>
<tr>
<td>Frothy, yellow/green discharge</td>
<td>25%</td>
</tr>
<tr>
<td>Vulvar itching</td>
<td>20-50%</td>
</tr>
<tr>
<td>Strawberry cervix</td>
<td>&lt; 2%</td>
</tr>
<tr>
<td>Strong, foul odor</td>
<td>60%</td>
</tr>
<tr>
<td>pH &gt; 5</td>
<td>60-90%</td>
</tr>
<tr>
<td>Dyspareunia (pain during sexual intercourse)</td>
<td>&lt; 25%</td>
</tr>
<tr>
<td>Dysuria (pain during urination)</td>
<td>&lt; 25%</td>
</tr>
</tbody>
</table>

**Symptoms in Men**

- Likely to be asymptomatic
  - Urethral discharge
  - Dysuria
  - Urethral pruritus
Complications in women

• Risk factor for HIV
• Associated with HSV-2 acquisition
• Contributor to female infertility
• Pelvic Inflammatory Disease (PID)
  • Significantly higher rate of PID in women with trichomoniasis compared with uninfected women
• Cervical Neoplasia
  • *T. vaginalis* associated with 2-fold increased risk, even after controlling for HPV infection
• Preterm birth
  • Preterm labor, delivery and low birth weight associated with *T. vaginalis* infection
PID

• Results from the ascension of organisms from the vagina/cervix to the uterus and fallopian tubes

• Significant burden and long term sequelae from tubal infections
  • Tubal factor infertility, pelvic adhesions, ectopic pregnancy, tubo-ovarian abscess

• Data from the National Health and Nutrition Examination Study 2014
  • 4.4% of women aged 18-44 in the U.S. have had PID
  • 2.5 million women ever having PID in their lifetime

• 1/3 of cases are caused by *C. trachomatis* or *N. gonorrhoeae,*
Complications in men

- Risk factor for HIV
  - 6-fold increase in semen HIV concentration in men with *Trichomonas* urethritis than men without *Trichomonas*

- Contributor to male infertility
  - In *Trichomonas* infections, significant decrease in sperm motility and viability

- Nongonococcal urethritis (NGU)
  - One study showed 19.9% of men with NGU were infected with *Trichomonas*
  - CDC STD treatment guidelines recommend inclusion of *Trichomonas* therapy for men with NGU

- Chronic prostatitis
  - One study showed the prevalence of *Trichomonas* exceeded 85% in men with symptoms of prostatitis
Prevalence

Prior to 2007, true prevalence of trichomoniasis among general population of women in the US was unknown

In 2007, CDC published results of their analysis of women aged 14-49 (left graph) - 4474 women

National Prevalence of Trichomoniasis in Women Aged 14-49 Years

![Graph showing prevalence of Trichomoniasis](image)
Diagnosis

• Wet mount
• Culture/InPouch™
• OSOM® Trichomonas Rapid Test
• PCR

Other tests
• Potassium hydroxide (KOH) “Whiff test”
• Vaginal pH test
• Papanicolaou test (Pap smear)
Diagnosis

Wet mount

- A microscope slide is prepared by suspending a specimen in saline solution
- Visual examination for trichomonads is the most common method used for diagnosis

Culture/InPouch™

- Specimen is placed in culture medium for 2-7 days
- Culture is considered gold standard - more sensitive than wet mount & pap smear
- InPouch™ system is a 2-chambered bag that allows one to perform a wet mount using the upper chamber and a culture using the lower chamber

Decreasing shelf-life of *T. vaginalis* on wet mount preps
Diagnosis

OSOM® Trichomonas Rapid Test

Point-of-care (CLIA-waived), antigen-detecting diagnostic test for trichomoniasis

Fast TAT - results in less than 15 min

Specimens may be held up to 24 hrs at room temp or stored at 2-8°C for up to 36 hrs

1. Fill Line
2. 10x
3. Allow to soak for 1 min
4. Incubate for 10 min
5. POSITIVE
   - Positive: A blue Test Line and a red Control Line.
   - Negative: A red Control Line but no blue Test Line.
   - Invalid: If no red Control Line appears or background color makes reading the red Control Line impossible.

Add Sample Buffer
Fill the dropper to the line indicated on the barrel and expel entire contents into tube.

Mix Swab in Buffer
Add swab to tube and mix vigorously (approx. 10 times).

Squeeze Liquid from Swab
Squeeze side of tube to express as much liquid from swab as possible.

Add Test Stick and Incubate
Place absorbent end of test stick into the solution.
Diagnosis

PCR - FDA approved test by Hologic

Other tests

Potassium hydroxide (KOH) “Whiff test”
- Conducted by mixing a swab of vaginal fluid with 10% KOH
- Fishy smell may indicate BV or Trichomoniasis

Vaginal pH test
- Touching pH paper to vaginal wall or to vaginal swab specimen
- Elevated pH may be an indication of trichomoniasis

Pap Smear
- May occasionally detect trichomonads but has a high diagnostic error rate
- Not suitable for screening unless used in conjunction with a more sensitive test
Detection of *T. vaginalis* infection

<table>
<thead>
<tr>
<th>Assay</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet mount</td>
<td>44-68%</td>
<td>100%</td>
</tr>
<tr>
<td>Culture</td>
<td>44-75%</td>
<td>100%</td>
</tr>
<tr>
<td>Antigen assay (swab)</td>
<td>77-98%</td>
<td>99-100%</td>
</tr>
<tr>
<td>PCR (Aptima TV)</td>
<td>88-100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Hobbs et al. Sex Transm Infect. 2013
Treatment & prevention

- Trichomoniasis can usually be cured in both women and men
  - Treatment of sexual partners is advised

- Medicines that will cure:
  - Tinidazole
  - Metronidazole

- Medicines that won’t cure:
  - Over-the-counter drugs
  - Intravaginal treatment with prescription medicines (i.e. metronidazole gel)

- Use of latex condoms can help prevent infection and re-infection
Neisseria gonorrhoeae

- *Neisseria* sp. are normal flora
  - 2 Pathogenic Species
    - *N. gonorrhoeae*
    - *N. meningtidis*
- Gram Negative diplococci
- Oxidase +
- Survives within neutrophils
- Fastidious
Pathogenesis

- Antigenic variation
- Lipooligosaccharides
- IgA protease

Symptoms
- Burning with urination
- Increased urge to urinate
- Purulent discharge
Clinical

- Transmission is through genital, oral, or anal sex
- Infect genitals, throat and eyes
- Asymptomatic carriage is common in both males and females
- Untreated infection
  - Pelvic Inflammatory Disease
  - Disseminate to the joints
Prevalence: U.S. 1941-2016

Rate (per 100,000 population)

Year

CDC
Diagnosis

- PCR
  - Now the preferred method

- Culture
  - Specimens: Urethral, urine, cervical, vaginal, rectal, oropharyngeal, conjunctiva, sterile joint fluid
  - Thayer Martin Agar
    - Chocolate Agar w/ vancomycin, nystatin and trimethoprim
  - 35°C
  - 3-7% CO2
CDC Threat Level Urgent!

DRUG-RESISTANT NEISSERIA GONORRHOEAE

246,000 DRUG-RESISTANT GONORRHEA INFECTIONS

820,000 GONOCOCAL INFECTIONS PER YEAR

THREAT LEVEL URGENT
This bacteria is an immediate public health threat that requires urgent and aggressive action.
Treatment

• Resistance is a serious problem

• Growing resistance to the treatment of choice led to a change in the CDC guidelines
  • Cefixime
  • Ciprofloxacin

• CDC Guidelines
  • Ceftriaxone + Azithromycin
  • Double coverage due to rising resistance to both
History of Antimicrobial Resistance in *N. gonorrhoeae*: Are We Fighting A Losing Battle?

- Time to emergence of widespread resistance:
  - Sulfonamides: <10 years
  - Penicillin: <20 years
  - Ciprofloxacin: ~5 years
Chlamydia trachomatis

• Obligate intracellular bacteria

• Biphasic Life cycle
  • 48-72 hours
  • Elementary body
    • Infectious particle
    • Enters host cells
  • Reticulate body
    • 8 hour
Prevalence

- Most common reportable STD in the U.S.

- Though often asymptomatic

- 2016
  - 1.59 million case
  - 4.7% increase from 2015

CDC Surveillance 2016
Clinical

- Trachoma
  - Leading cause of blindness worldwide – irreversible
  - 190.2 million people in endemic areas
    - Africa, Central and South America, Asia, Australia, and Middle East
- Non-gonococcal urethritis
  - Epididymitis
- Arthritis
- Lymphogranuloma venereum (LGV)
  - Tender inguinal and/or femoral lymphadenopathy
  - Rectal exposure - proctocolitis
  - Serovars L1, L2, L3

WHO July 2107
Diagnosis

- Women: first-catch urine or collecting swab specimens from the endocervix or vagina.
- Men: urethral swab or first-catch urine specimen.

- PCR
  - CDC recommended

- Culture
  - Tissue culture – Utilized immunofluorescence
  - Not typically performed anymore

- ELISA for antigen
Treatment

- Azithromycin or Doxycycline

- Alternatives: Erythromycin, Levofloxacin or Ofloxacin

- Sex partners should be evaluated and presumptively treated if they had sexual contact during the 60 days preceding partner’s symptoms
Ureaplasma and Mycoplasma species
Background

• Class of Mollicutes
  • 8 genera
    *Mycoplasma*, *Ureaplasma*, *Acholeplasma*, *Anaeroplasma*, and *Asteroloplasma*
  • More than 200 known species exist
  • 6 species are established or presumed human pathogens
    • Genitourinary tract
      • *M. genitalium* • *U. urealyticum*
      • *M. hominis* • *U. parvum*
      • *M. fermentans*
    • Respiratory tract
      • *M. pneumoniae*
Mollicutes

- Distinguished by the lack of a cell wall
  - Naturally resistant to $\beta$-lactams
  - Pleomorphic

- Smallest self-replicating organism both in cellular dimension and in genome size

- Limited biosynthetic capabilities
  - Requires complex media, sterols

- Slow growing and fastidious
Pathogenesis

- Attachment to epithelial cells within mucosal areas
  - Adherence occurs through antigenically variable proteins
    - *M. genitalium* has a flask shaped morphology with terminal attachment organelles
    - *Ureaplasma* and *M. hominis* lack prominent attachment tips
      - Erythrocytes, sperm, urethral epithelial cells
      - Resides intracellularly after invasion of host cells
  - Colonization can lead to pathological lesions with or without internalization
Pathogenesis

- *Ureaplasma* species
  - Urease activity
  - Release NH$_3$
  - Elevate urinary pH leading to magnesium ammonium phosphate $\Rightarrow$ calculi
  - Immunoglobulin A protease

- *Mycoplasma hominis*
  - Arginine metabolism
  - ATP + NH$_3$ + CO$_2$
  - Vaa Antigen
    - Variable Adhesion protein

- *M. genitalium*
  - Antigenic variation
Routes of transmission

- *Ureaplasma* sp. / *M. genitalium*
  - Sexually Transmitted
  - Vertical Transmission, *in utero* or peripartum

- *M. hominis*
  - Obstruction or Instrumentation of the urinary tract
  - Sexual contact
  - Vertical Transmission, *in utero* or peripartum
Epidemiology

- Worldwide
- Associated with high risk behaviors
- Colonizes mucosal surfaces of the cervix or vagina of sexually mature women
  - *Ureaplasma* sp.: 40-80%
  - *M. hominis*: 21-53%
- Incidence is lower in the urethra of men
- *M. genitalium* – typically does not colonize urethra but found in the rectum
  - Discovered in the 1980s
  - 1% of the screening population, up to 50% in high risk depending on the study

Waites et al. 2005. Clinical Microbiology Reviews
Manifestations

Non-Genital Tract Infections

Genital Tract Infections
The forgotten bugs

• This class of pathogens is often overlooked because their clinical manifestations are attributed to other more common organisms

• Differential Diagnosis is shared with:
  • *Chlamydia trachomatis*
  • *Neisseria gonorrhoeae*
  • *Trichomonas vaginalis*
  • GBS
  • *E. coli*
### Clinical Presentation – Genital Tract

<table>
<thead>
<tr>
<th>Clinical</th>
<th>Ureaplasma sp.</th>
<th>M. hominis</th>
<th>M. genitalium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Gonococcal Urethritis</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Chorioamnionitis</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Preterm labor/miscarriage</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Bronchopulmonary disease</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Pyelonephritis</td>
<td></td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Cervicitis</td>
<td></td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Pelvic Inflammatory Disease</td>
<td>✓</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Neonatal complications</td>
<td>✓</td>
<td></td>
<td>✓</td>
</tr>
</tbody>
</table>
Reproductive Tract Infections

• **Urethritis** – frequent/urgent need to urinate, discharge, blood in urine
  • Common causes: *E. coli*/*enterics*, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*

• **Cervicitis** – Inflammation that causes the tissue to become tender, swollen, discharge mucus or blood
  • Common causes: *N. gonorrhoeae*, *C. trachomatis*, Herpes, *Trichomonas vaginalis*

• **Pelvic Inflammatory Disease** – abdominal pain, fever, discharge, bleeding between menses
  • Common causes: *Neisseria gonorrhoeae*, *Chlamydia trachomatis*
Mother to Baby Transmission

• 3 Main routes
  • 1) Ascending intrauterine infection where the organisms gain access through the amniotic sac
  • 2) Hematogenous route through the placenta from the mother
  • 3) Through the passage of the birth canal which leads to colonization of the skin, mucosal membranes and respiratory tract
Adverse Pregnancy Outcomes

- Associated with preterm labor, premature rupture of membranes, and chorioamnionitis
  - 15/22 studies with a control group of uneventful pregnancies showed a significant association with the presence of *U. urealyticum*
    - Culture/PCR of cervicovaginal, amniotic fluid, and placentas
  - Of 11 studies regarding *M. hominis*, a significant association was found in 6
    - Culture/PCR of cervicovaginal samples
In Utero

• **Chorioamnionitis** - inflammation of the inner and outer fetal membranes, amniotic fluid, fetus, umbilical cord, or placenta
  - 1-4% of all births are complicated by this
  - Common causes: Group B *Streptococcus*, *E. coli*, *Listeria monocytogenes*, *Ureaplasma* and *M. hominis*

• The fetus inspires, swallows, and is bathed in amniotic fluid

• Enter the lung and multiply (congenital pneumonia)

• Major predictor of preterm birth
  - 40-70% of pre-term births associated with chorioamnionitis
In utero

- Preterm birth/miscarriage – occurs prior to 37 weeks of gestation
  - Greater risks with gestation < 32 weeks
  - Earlier the birth, the higher association of intrauterine infection
- Infectious Causes: *N. gonorrhoeae*, *C. trachomatis*, *Trichomonas vaginalis*, Bacterial vaginosis

- Babies born prematurely:
  - Developmental delay, cerebral palsy, vision and hearing problems

Neonates

- Pre-term and low birth weight neonates are at risk of acquiring these pathogens from their mother during birth
  - Greater risk with very low birth weight
    - Higher rate with < 1000 grams (60%) vs ≥ 1500g (15.3%)

- Bronchopulmonary Dysplasia (BPD)
  - Inflammation leads to reduced alveoli
  - Oxygen requirements and mechanical ventilation
  - 10,000 babies develop this in the U.S. per year
  - Significant correlation between *Ureaplasma* in respiratory specimens and BPD

- Other complications include bacteremia, meningitis and pneumonia
Infertility

- Contribution to infertility is controversial

- *Ureaplasma* sp. and *M. hominis* have been found in both fertile and infertile couples who were otherwise healthy

- Chronic Asymptomatic carriage
  - Damage Sperm
    - *M. genitalium* has been found to attach to sperm causing a reduction in motility
    - *Ureaplasma* has been found to reduce sperm count, motility and alter morphology
  - DNA damage
    - *U. urealyticum*

**M. genitalium**

- CDC 2015 STD Guidelines
- Emerging Issue
- More fastidious than *Ureaplasma* sp. or *M. hominis*
  - Hard to recover (culture can take up to 6 months)
  - Fewer studies
- Prior to 2003, all isolated strains of *M. genitalium* were susceptible to macrolides
- The median cure rate is declining – 85% down to 40%

Sethi et al. Infect Drug Resist. 2017
Prevalence Study

- Hologic, Inc.
- 1368 Females (Aged 14 to 70)
  - 16.1% positive for *M. genitalium*
- 599 Males (Aged 18 to 78)
  - 17.2% positive for *M. genitalium*
- Dual infections
  - With *Trichomonas vaginalis*: 6.3%
  - With *Chlamydia trachomatis*: 3.1%
- 48% had the macrolide resistance marker

Diagnosis

• Culture
  • Expensive
  • Tedious

• PCR
  • Faster
  • Specific and Sensitive
Culture Algorithm at UCLA

Urine, Genital Swabs, Body fluids, tissue, wounds, respiratory

M5
Antibiotics and protein stabilizers

10B Arginine broth
Urea and Arginine

Transport Time
1 to 24 hrs

Day 1-2

Days 2-14

Nutrient Rich

Mycoplasma hominis (ATCC® 23114)
Culture

- *M. hominis:*
  - Fried egg appearance
  - 20-300 μM

- *Ureaplasma* sp.:
  - Brown granular colonies
  - 15-60 μM
Treatment

• Mollicutes are resistant to all β-lactams, bactrim, and rifampin
  • 10-40% are resistant to tetracyclines
• *M. hominis* is resistant to 14-15 membered macrolides (i.e. erythromycin and azithromycin)
  • Susceptible to clindamycin
• *Ureaplasma* sp. can be resistant to clindamycin but susceptible to macrolides
• Treatment of partners is also recommended to prevent re-infection

CLSI M43-A 2011
# Treatment of Sexually Transmitted Infections

<table>
<thead>
<tr>
<th>Organism</th>
<th>Primary treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syphilis</td>
<td>Penicillin</td>
</tr>
<tr>
<td><em>N. gonorrhoea</em></td>
<td>Ceftriaxone + Azithromycin</td>
</tr>
<tr>
<td><em>C. trachomatis</em></td>
<td>Azithromycin or Doxycycline</td>
</tr>
<tr>
<td>UTIs</td>
<td>Nitrofurantoin, Bactrim, Fluoroquinolones, β-lactams</td>
</tr>
<tr>
<td><em>Ureaplasma sp.</em></td>
<td>Azithromycin, Fluoroquinolones, Doxycycline</td>
</tr>
<tr>
<td><em>M. hominis</em></td>
<td>Clindamycin, Fluoroquinolones, Doxycycline</td>
</tr>
<tr>
<td><em>M. genitalium</em></td>
<td>Azithromycin, Doxycycline</td>
</tr>
</tbody>
</table>
Mix and Match

Chancre

Most common non-viral STD

Rash/Malaise in the initial phase

Oral and genital ulcers

Underappreciated cause of NGU

HIV

Mycoplasma/Ureaplasma

Syphilis

HSV

T. vaginalis
References


• Waites et al. 2005. Mycoplasmas and Ureaplasmas as Neonatal Pathogens. Clinical Microbiology Reviews

• Manhart et al. 2007. M. genitalium among young adults in the United States: an emerging STI


• Getmen et al. M. genitalium Prevalence, Coinfection, and Macrolide resistance frequency in a multicenter clinical study cohort in the United States. 2016. JCM


• CDC Pelvic Inflammatory Disease (PID) - CDC Fact Sheet https://www.cdc.gov/std/pid/stdfact-pid-detailed.htm#ref5


• Bharat et al. Disseminated Ureasplasma infection as a cause of fatal hyperammonemia in human. 2015. Science Translation Medicine
Questions