Laboratory Diagnostic Testing for Chlamydia, Gonorrhea, and Syphilis

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SPEAKER DISCLOSURE

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“The Three Amigos”

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Objectives

• Review the epidemiology of Chlamydia, Gonorrhea, and Syphilis; e.g. the signs, symptoms, clinical disease, screening recommendations/risk factors, treatment, and diagnostic tests.

• Review the Laboratory Guidelines for the Detection of Chlamydia, Gonorrhea, and Syphilis.

• Discuss future directions and recommendations for CT/GC/Syphilis.
Chlamydia Infections

- Most common of all the “bacterial” STD’s.
- Estimated 3-4 million new cases yearly (US).
- One of the leading causes of PID and infertility.
- Most common cause of neonatal conjunctivitis, pneumonia, and the leading cause of preventable blindness.
- Direct and indirect costs exceed 2.4 billion yearly (US).
- Up to 80% of women and 40% of men may be asymptomatic.
Chlamydia Infections
(Signs and Symptoms)

**WOMEN**
- Vaginal and/or cervical discharge
- Bleeding between periods after sex
- Pain in the abdomen, sometimes with fever and nausea
- Burning or pain when urinating (Dysuria)
- Frequent Urination or urge to urinate more frequently
- Up to 80% of women are asymptomatic

**MEN**
- Burning or pain when urinating (Dysuria)
- Watery, white drip from the penis
- Up to 40% of men are asymptomatic

Photo: STD/HIV Prevention Training Center at the University of Washington/Connie Celum and Walter Stamm
Chlamydia Infections
(Clinical Disease)

**WOMEN**
- Cervicitis
- Urethritis
- Salpingitis
- Endometritis
- Pelvic Inflammatory Disease (PID)
- Perihepatitis
- Pharyngitis

**MEN**
- Postgonococcal Urethritis (PGU)
- Epididymitis
- Reiter’s syndrome
- Proctitis
- Pharyngitis

Photo: Seattle STD/HIV Prevention Training Center at the University of Washington/UW HSCER Slide Bank
Chlamydia Infections
(Clinical Disease)

- INFANTS
  - Inclusion conjunctivitis
  - Pneumonia

Photo: CDC
Screening Recommendations
(Risk Factors)

• All sexually active women ≤25 years
• Women >25 with risk factors
  – New or multiple sex partners
  – Pregnant
  – History of sexually transmitted infections (STI)
  – Lack or inconsistent use of condoms
  – Engage in commercial sex work
  – Report the use of illegal drugs

¹Based on Universal Screening
Uncomplicated Treatment

• Adults and Adolescents
  • Azithromycin – 1 gm orally in a single dose
    OR
  • Doxycycline - 100 mg; 2x day; 7 days

• Infants
  • Erythromycin base or ethylsuccinate
    50 mg/kg/day orally divided into
    4 doses daily; 14 days
Chlamydia Laboratory Methods

- Culture (Cell Culture)
Advantages/Disadvantages
Cell Culture

ADVANTAGES
• Used for many types of specimens, e.g. endocervical, urethral, rectal, ocular, etc.
• Meets medico-legal standards (specificity)
• Used for strain studies (DNA fingerprinting)
• Susceptibility testing possible

DISADVANTAGES
• Comparatively expensive
• Many variables involved, e.g. cell culture, medium, etc.
• Technically more difficult than many non-culture tests
• Delayed turn around time
• Lack of sensitivity (compared to amplified tests)
Chlamydia Laboratory Methods
Non-culture

- **ANTIGEN DETECTION**
  - Direct Fluorescent Antibody (DFA)
  - Enzyme Immunoassay (EIA)

- **NUCLEIC ACID DETECTION METHODS**
  - QIAGEN digene HC2 CT-GC Dual ID DNA Test
Advantages/Disadvantages
Non-culture

ADVANTAGES
• Used for many types of specimens, e.g. endocervical, urethral, rectal, ocular, etc. (DFA)
• Effective for large scale screening (EIA/NAP)
• Viable organism not required
• Evaluate quality of specimens (DFA)
• Inexpensive
• Rapid turn around time

DISADVANTAGES
• Not suitable for large volume testing (DFA)
• Lack of sensitivity (compared to amplified tests)
• Not FDA cleared for alternate specimens, e.g. urine, etc.
Chlamydia Laboratory Methods
Non-culture

• Nucleic Acid Amplification Tests (NAATs)
  • Hologic GenProbe APTIMA COMBO 2 (TMA)
  • Hologic GenProbe APTIMA CT and GC (TMA)
  • BD ProbeTec™ ET (SDA)
  • BD ProbeTec™ *Chlamydia trachomatis* (CT) (GC)
  • Qx Amplified DNA Assay (SDA)
  • Roche Cobas CT/NG v2.0 Test (PCR)
  • Abbott RealTime CT/NG (PCR)
  • Cepheid GenXpert™ CT/NG (PCR)
Advantages/Disadvantages (NAATs)

ADVANTAGES
• Most sensitive and specific tests
• Effective for large scale screening
• Rapid turn around time
• NAATs are FDA cleared for urine specimens
• Some may be used with other alternate specimens, e.g. vaginal swabs

DISADVANTAGES
• Some versions not suitable for large volume screening
• High technical skill required
• Special facilities or clean area required
• Expensive
Chlamydia Laboratory Methods
Point-of-Care Tests (POCTs)

• Point-of-Care Tests
  • Clearview (Inverness)
  • QuickVue (Quidel)

Recommendation for Single Use Devices for Point-of-Care Chlamydia Tests
http://www.region8ipp.com/Docs/02%20NCLC%20POC.pdf
Advantages/Disadvantages
POCTs

ADVANTAGES
• Rapid turn around time
• Allows treatment of patient while in clinic

DISADVANTAGES
• Expensive
• Not suitable for large volume screening
• Poor sensitivity with some POCTs
• Complexity non-waived
Chlamydia Serologic Tests

“Serology has little, if any, value in testing for uncomplicated genital *C. trachomatis* infection. It should not be used for screening because previous chlamydial infection might or might not elicit a systemic antibody response. Infections caused by LGV serovars of *C. trachomatis* tend to invade to the draining lymph nodes resulting in a greater likelihood of detectable systemic antibody response and might aid in diagnosis of inguinal (but not rectal) disease.”

Recommendations for the Laboratory-Based Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* Infections – 2014 MMWR, March 14, 2014/Vol. 63/No. 2
Gonorrhea Infections

• Over 300,000 cases are reported annually in the U.S. It is estimated that up to an additional million may go unreported.

• Direct and indirect costs associated with infections exceed $1 billion annually.

• Asymptomatic infections in women are common, men less common.
Gonorrhea Infections

- One of the leading causes of pelvic inflammatory disease (PID).
- \(^1\text{,}^2\text{,}^3\) Gonorrhea infections are becoming increasingly resistant to routine antibiotic treatment.

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\(^3\)National Action Plan for Combating Antibiotic-Resistant Bacteria. March 2015
Gonorrhea Infections (Signs and Symptoms)

WOMEN
• Yellowish vaginal discharge
• Bleeding between periods or after sex
• Pain in the abdomen, sometimes with fever and nausea
• Burning or pain when urinating

MEN
• Severe burning or pain when urinating
• Discharge from the penis
Gonorrhea Infections
(Clinical Sequelae)

**WOMEN**
- Cervicitis
- Urethritis
- Salpingitis
- Pharyngitis
- Endometritis
- Pelvic Inflammatory Disease (PID)
- Premature delivery
- Perihepatitis

**MEN**
- Urethritis
- Epididymitis
- Proctitis
- Pharyngitis
Gonorrhea Infections
(Clinical Sequelae)

• INFANTS
  • Conjunctivitis
Screening Recommendations (Risk Factors)

• Targeted Screening of Women ≤25 years
  – All sexually active women
  – Pregnant
  – History of sexually transmitted infections (STI)
  – New or multiple sex partners
  – Lack or inconsistent use of condoms
  – Engage in commercial sex work
  – Report the use of illegal drugs
  – Based on local data

Centers for Disease Control and Prevention. Sexually Transmitted Diseases Treatment Guidelines, 2010. MMWR 2010;59 (No. RR-12)
Uncomplicated Treatment  
(Cervix, Urethra, and Rectum)

• **Recommended Regimen**
  – Ceftriaxone - 250 mg IM in a single dose
    
      PLUS

  – Azithromycin - 1 g orally in a single dose

• **Alternative Regimen (if Ceftriaxone is not available)**
  – Cefixime 400 mg orally in a single dose
    
      PLUS

  – Azithromycin - 1 g orally in a single dose
Gonorrhea

Laboratory Methods

Culture
  • Thayer Martin, etc.
  • Genetic transformation (Gonostat)

Direct Microscopic Exam
  • Gram Stain
Advantages/Disadvantages
Bacterial Culture

ADVANTAGES
• All types of specimens, e.g. endocervical, urethral, rectal, pharyngeal, ocular, etc.
• Meets medico-legal standards (specificity)
• Used for strain studies fingerprinting
• Susceptibility testing possible
• Inexpensive

DISADVANTAGES
• Some variables, e.g. various culture media, etc.
• Delayed turn around time
• Lack of sensitivity (compared to amplified tests)
Gonorrhea
Laboratory Methods

Non-Culture

• Nucleic acid detection method
  • QIAGEN digene HC2 CT-GC Dual ID DNA Test
Advantages/Disadvantages
NAPSA

**ADVANTAGES**
- Effective for large scale screening
- Viable organism not required
- Rapid turn around time
- Inexpensive
- Moderate technical skill required

**DISADVANTAGES**
- Lack of sensitivity (compared to amplified tests)
- Not FDA cleared for alternate specimens, e.g. urine, etc.
Gonorrhea Laboratory Methods
Non-culture

- **Nucleic Acid Amplification Tests (NAATs)**
  - Hologic GenProbe APTIMA COMBO 2 (TMA)
  - Hologic GenProbe APTIMA CT and GC (TMA)
  - BD ProbeTec™ ET (SDA)
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  - Qx Amplified DNA Assay (SDA)
  - Roche Cobas CT/NG v2.0 Test (PCR)
  - Abbott RealTime CT/NG (PCR)
  - Cepheid GenXpert™ CT/NG (PCR)
Advantages/Disadvantages
Nucleic Acid Amplified Tests (NAATs)

ADVANTAGES
• Most sensitive and specific tests
• Effective for large scale screening
• Rapid turn around time
• NAATs are FDA cleared for urine specimens
• May be used with some alternate specimens

DISADVANTAGES
• Some versions not suitable for large volume screening
• High technical skill required
• Special facilities or clean area required
• Expensive
Recommendations for the Laboratory-Based Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* — 2014 (Highlights)

- All culture and non-culture tests may generate false-positive and false-negative results
- Nucleic acid amplification tests (NAATs) are recommended for genital, rectal, and oropharyngeal infections.
  - Urine for Men
  - Vaginal swabs for Women
- Culture is still useful in certain circumstances
  - GC susceptibility testing
  - Detect mutant strains
- Pooling specimens for testing with NAATs is an acceptable method to reduce costs without compromising performance.
Syphilis

• Total number of cases of syphilis in the U.S. increased 13.1% during 2012-2013, from 49,915 cases to 56,471 cases.

• MSM accounted for 75% of the P/S syphilis cases in 2013.

• While the rate of P/S syphilis cases increased 12% among men during 2012-2013 the rates remained unchanged in women.

• Untreated early syphilis in pregnant women results in perinatal death in up to 40% of the cases.
Syphilis Infection

- **Infection**: Incubation period 9 – 90 days
  - **Primary Chancre**: 6 weeks to 6 months
    - **Secondary Rash**: ~ 18 months
      - **Latent Asymptomatic**: Many years to a lifetime
        - **Tertiary**: Late Syphilis Many years to a lifetime
          - **Benign gumatous Cardio-vascular syphilis Neurosyphilis**
Uncomplicated Treatment

• Adults:
  • Benzathine penicillin G 2.4 million units IM in a single dose.

• Infants/Children:
  • Benzathine penicillin G 50,000 units/kg IM, up to the adult dose of 2.4 million units in a single dose.
Syphilis Laboratory Methods

- Diagnosis
  - Direct tests
    - Dark Field
    - DFA-TP
    - NAATs (PCR)
      - No FDA cleared test available
      - Useful for chancre, csf (neurosyphilis), and congenital syphilis
      - More sensitive than Dark Field or DFA
Syphilis Laboratory Methods
(Serologic Diagnosis)

• Non-treponemal – screening tests, useful for monitoring efficacy of treatment.
• Treponemal – more sensitive and specific than non-treponemal tests, increase positive predictive value (PPV) of “reactive” or “positive” non-treponemal screening tests; reactive for lifetime.
Syphilis Laboratory Methods (Serologic Diagnosis)

- Non-treponemal tests
  - Rapid Plasma Reagin (RPR) test
  - Venereal Disease Research Laboratory (VDRL) test
  - Toluidine Red Unheated Serum Test (TRUST)
  - Point of Care Tests (POCTs)

- Treponemal tests
  - Fluorescent Treponemal Antibody Absorbed (FTA-ABS) test
  - *Treponema pallidum* Particle Agglutination (TP-PA) test
  - Enzyme Immunoassays (EIAs)
    - Trep-Chek
    - Trep-Sure
  - Chemiluminescence Immunoassay (CLIA)
    - Liaison
  - Point of Care Tests (POCTs)
    - Syphilis Health Check (SHC) (VEDALAD – France)
  - Microbead Immunoassays (MBIA)
    - BioPlex 2200 Syphilis IgG
Syphilis Laboratory Methods
(Non-Treponemal Screening)

PROS
• High Sensitivity
• Rapid
• Low cost
• Does not detect past infections
• Requires little equipment for testing
• Usually requires only one reflex test
• Useful for treatment monitoring

CONS
• Lower specificity
  – False positives
• Lower sensitivity
  – Primary infections
• Labor-intensive
• Subjective results
• Manual data manipulations
Syphilis Laboratory Methods (Treponemal Screening)

**PROS**
- High Sensitivity
- High Specificity
- Objective results
- Automation / high throughput
- Interface with LIS
- Useful as a confirmatory test
- Useful as a large volume screening test – Reverse algorithm

**CONS**
- Cannot distinguish between active and previously treated disease
- Potential for over diagnosis and over treatment
- May require more resources for investigations
- Specific, potentially costly instrumentation
- May require multiple reflex tests for resolving discrepant results
Syphilis Laboratory Methods
(Recommendations)

• The selection of specific tests for screening depends on the prevalence of disease in the population, purpose of the test (screening or confirmation), subjectivity of the test and experience of the technologist, equipment, cost, etc.

• More than one algorithm may be necessary
  – One using a non-treponemal test as initial screen
  – One using a treponemal test as initial screen

• A combination of treponemal and non-treponemal tests must be used; a single treponemal test cannot be relied upon for syphilis diagnosis
Traditional Testing Algorithm Using Non-Treponemal Initial Screen

Non-Treponemal Test
  RPR, VDRL, TRUST

  Reactive
  Nonreactive
  Not syphilis (or early syphilis)

Titer

Treponemal Test
  FTA-ABS, TP-PA, POCT?

  Reactive
  Nonreactive
  Syphilis - Treat
  False positive
  Non-Treponemal Test

Pope Infect Med 2004
Testing Algorithm Using EIA or CLIA as Initial Screen

A1
Syphilis EIA or CLIA

A1+
Negative for Syphilis antibodies

A1-
Quantitative Nontreponemal (i.e. RPR)

A2

A1+ A2+
Consistent with Syphilis (past or current infection)

A1+ A2-
Possible Syphilis infection; Requires further historical and clinical evaluation

A1+ A2- A3+
Unconfirmed EIA; Unlikely to be Syphilis; If patient is at risk for syphilis, re-test in 1 month

A1+ A2- A3-

* Laboratory should report the results of all three assays (if applicable) within 7 days
Syphilis Testing Guidelines

• Use of Treponemal Tests to Screen for Syphilis - Infect. Med. 21(8):399-404, 2004

• Meeting proceedings from Syphilis Expert Consultation held in 2009 are available on the Association of Public Health Laboratories (APHL) website:

• Discordant Results from Reverse Sequence Syphilis Screening - Five Laboratories, United States, 2006 - 2010 - CDC Morbidity and Mortality Weekly Report (MMWR) February 11, 2011/60(5);133-137

• Sexually Transmitted Diseases Treatment Guidelines, 2015 - CDC Morbidity and Mortality Weekly Report (MMWR) June 5, 2015/64(03);34-50
Future Directions

• New Tests/Technology
  – *Mycoplasma genitalium*

• Real Time and Random Access Assays

• Multiplex
  – Syphilis (GUD)

• Alternate Specimens
  – More testing
  – FDA clearance

• Home Collection

• Point of Care Technology
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Questions