 Advances in molecular diagnosis of GUD and rectal LGV

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Overview

- Background – GUD, including LGV
- Challenges with syphilis diagnosis
- Molecular tests for syphilis
- Evaluation of CDC Real-Time M-PCR for GUD
- Molecular tests for LGV & prevalence data
- Summary
Reported cases of bacterial STDs in US - 2017

- **Syphilis**
  - 30,644 cases of P&S syphilis - 76% increase since 2013
  - Congenital syphilis - 918 cases; 154% increase since 2013
  - Ocular syphilis – Cluster of 12 cases in Seattle & San Francisco in Dec 2014 – Mar 2015; cases reported in multiple states

- **Chancroid**
  - 7 cases in 5 states
  - *H. ducreyi* not detected in reference specimens sent to CDC in the past decade

- **Lymphogranuloma venereum (LGV)**
  - National prevalence is unknown because LGV-specific Dx tests are not widely available and cases are not differentiated from Chlamydia reporting

STD surveillance, CDC 2017
Herpes simplex virus (HSV) - 2008 data

- **Herpes simplex virus**
  - HSV-2 – 776,000 new infections
  - HSV-1 infections are typically orolabial but appears to be increasing among young adults in the US
Trend of proportion of genital ulcers caused by infections with *H. ducreyi*, 1979–2010
Cutaneous *H. ducreyi*

<table>
<thead>
<tr>
<th>Country</th>
<th>Prevalence by PCR (%)</th>
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<tbody>
<tr>
<td>Ghana</td>
<td>27.4</td>
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<tr>
<td>Solomon Islands</td>
<td>32</td>
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<tr>
<td>Vanuatu</td>
<td>38.6</td>
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<tr>
<td>Papua New Guinea</td>
<td>60</td>
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- 2 isolates resistant to penicillin (Ghana & PNG)
- *Tet(B)* and *catS* resistance determinants in Ghana strains
- CS strains have all virulence genes
Haemophilus ducreyi genital & cutaneous lesions
Syphilis – US Rates of Reported Cases by Stage of Infection, 1941–2017

STD surveillance, CDC 2017
Primary and Secondary Syphilis — Rates of Reported Cases by Sex and Male-to-Female Rate Ratios, US, 1990–2017
Primary and Secondary Syphilis
Reported Cases by Sex and Sexual Behavior from 37 States, 2013–2017

STD surveillance, CDC 2017
Challenges with syphilis diagnostics

- No FDA-cleared NAAT test
- Combination of tests are required to diagnose infection (NAATs, treponemal, non-treponemal tests)
- Nontreponemal and treponemal serological tests are insensitive in early primary syphilis and may be nonreactive in up to 47% of patients
- *T. pallidum* cannot be grown on routine lab media
- Primary lesions are painless and may go unnoticed in women and MSM
- Treponemal tests usually remain positive for life after successful treatment vs nontreponemal titers (decline slowly or remain serofast) - difficult to distinguish between treated and new infections in high-risk individuals
Challenges with syphilis diagnostics

- Darkfield microscopy (DFM) is the only routinely available direct detection test for moist lesions of primary & secondary syphilis
  - DFM is on the decline in STD clinics
  - Test relies on an adequately trained microscopist - testing proficiency needs to be maintained
  - Test must be done while treponemes are still motile (within 20 min. of collection)
  - Sensitivity of DFM is about 88% compared to PCR
Sensitivity and Specificity of syphilis PCR

- **Sensitivity***
  - lesion exudate of primary syphilis: 72% to 95%
  - secondary lesions swabs: 20% to 86%
  - lesion biopsies of secondary syphilis: 26% to 75%**
  - CSF from neurosyphilis patients: 50% to 77%
  - whole blood or its components (serum/plasma): 12% to 55% (1\textsuperscript{o}), 15% to 47% (2\textsuperscript{o}), 0% to 62% (latent syphilis)
  - amniotic fluid: 75% to 100%
  - neonatal CSF: 60% to 75%
  - neonatal whole blood or serum: 67% to 94%

- **Specificity***: 98%-100%
  - lesion exudate of primary and secondary syphilis; lesion biopsy of secondary syphilis; CSF from neurosyphilis cases; whole blood, serum, and plasma from primary, secondary, and latent

*based on a number of published studies

**fresh frozen tissue

Theel E; APHL/CDC syphilis consult 2017
Molecular tests for syphilis and Genital Ulcer Disease

- Quest Diagnostics offers an LDT PCR as a CLIA regulated test for use on CSF, blood, serum, and lesion swabs
- Medical Diagnostics Laboratories, LLC: GUD Panel (HSV-1 & HSV-2, H. ducreyi, T. pallidum) - OneSwab, ThinPrep - CLIA test
- GUD multiplex PCR (T. pallidum, HSV 1 & 2, H. ducreyi) available at CDC for reference testing—test code: CDC-10174 - CLIA test
- Hologic TMA Assay for T. pallidum - RUO
- ? LDTs in use at PHLs
- A number of FDA-cleared tests for HSV 1&2 are available
CE Mark tests – GUD

Seegene - Allplex Genital Ulcer Assay

- **7 pathogens:**
  - HSV-1 & 2
  - H. ducreyi
  - T. pallidum
  - Lymphogranuloma venereum (LGV)
  - Cytomegalovirus (CMV)
  - Varicella-zoster virus (VZV)

- **Specimens:** Genital swab, urine, liquid-based cytology specimen

- **Instrument:** Biorad CFX96 Real-time PCR System

SpeeDx - PlexPCR® VHS

- **4 pathogens:**
  - HSV-1 & 2 specific
  - T. pallidum
  - Varicella-zoster virus (VZV)

- **Specimens:** Genital & non-genital swabs

- **Instrument:** Roche LightCycler 480 Instrument & Cobas z 480 analyzer
Evaluation of CDC Real-Time GUD M-PCR at PHLs (CLIA regulated test)

- Collaboration between APHL and the Laboratory Reference & Research Branch (LRRB) within DSTDP, CDC

- Sites: 4 PHLs participated in the evaluation - Dallas County HHS Lab; City of Milwaukee Health Department Lab; Maryland DOH Lab; Michigan DHHS, Bureau of Labs

- Specimens: residual swab specimens collected for HSV testing

- Aliquot of specimens sent to LRRB for PCR testing; results will be compared to participating labs

- Testing is ongoing at CDC and 3 of the 4 sites
Real-Time M-PCR for GUD Diagnosis - AB7500 and QS

- CDC GUD M-PCR has been validated on the RotorGene-Q instrument
- Assay modified for use on Applied Biosystems 7500 Fast Dx and QuantStudio Dx
- Dyes used for detection of GUD organisms
  - Channel 1: FAM-QSY, HSV-1/-2 (Glycoprotein D)
  - Channel 2: VIC-QSY, H. ducreyi (Hemolysin, hhdA)
  - Channel 3: ABY-QSY, T. pallidum (47 Kd lipoprotein)
  - Channel 4: JUN-QSY, Human DNA control (RNase P)
Real-time M-PCR for GUD

- **T. pallidum pos - ROX**
- **HSV pos - FAM**
- **T. pallidum pos control**
- **Human DNA Control, RNP – Cy5**

*HEX dye for HD*
Lymphogranuloma venereum - LGV

- Infection with L1, L2, or L3 serovars of *C. trachomatis* may result in a disease characterized by hemorrhagic proctitis, genital lesions, tender inguinal and/or femoral lymphadenopathy

- Outbreak of LGV proctocolitis among HIV+ MSM caused by the L2 genotype was reported in Western Europe in 2003

- Sporadic cases or clusters of inguinal and anorectal LGV have been reported in the US but national surveillance data is lacking

- Accurate diagnosis is important because an extended period of treatment vs non–LGV CT is required - current recommendation is 21 vs 7 days of doxycycline (100 mg orally twice a day) or erythromycin (500 mg orally four times a day)
Molecular tests for LGV

- FDA-cleared NAATs for CT detection in rectal & pharyngeal specimens - Aptima Combo 2 Assay CT/NG; Xpert CT/NG
- Commercial NAATs for CT cannot differentiate LGV from non-LGV infection
- Commercial labs offering LGV testing:
  - BioReference Laboratories: LGV PCR, ThinPrep Vial
  - Medical Diagnostics Laboratories, LLC: LGV Real-Time PCR, OneSwab, UroSwab, ThinPrep
  - ARUP Laboratories: LGV PCR, Vaginal, rectal, cervical, urethral, genital, or penile swab with APTIMA Unisex Swab Specimen Collection kit; VTM, urine
- CLIA regulated testing using LDTs is available at a few PHLs
Molecular tests for LGV

- CE Mark tests: Seegene - Allplex Genital Ulcer Assay
- LGV PCR available at CDC for research or epidemiological purposes – test code: CDC-10523 (non-CLIA test)
LGV Real-Time Quadriplex PCR – CDC Lab

Channel 1 (FAM): TaqMan Probe 1 (Non-LGV)
Channel 2 (VIC): TaqMan MGB Probe (LGV)
Channel 3 (ROX): Cryptic plasmid (+CT Control)
Channel 4 (Cy5): RNase P (Human DNA Control)

Chen et al. STI 2008
LGV Real-Time Quadriplex PCR

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<tr>
<th>LGV</th>
<th>FAM</th>
<th>VIC</th>
<th>ROX</th>
<th>CY5</th>
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<tr>
<td>LGV</td>
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<tr>
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<tr>
<td>Negative</td>
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CDC LGV assay – outbreak investigation

- Michigan
  - Cluster of suspect cases identified among MSM in 2015
  - Specimens from patients with signs or symptoms compatible with LGV (proctocolitis, inguinal/femoral lymphadenopathy, or genital or rectal ulcers) with other causes of LGV-like symptoms ruled out & and positive culture or NAAT
  - 21/38 (55%) cases confirmed as LGV by real-time quadriplex PCR
    - 19 rectal- & 2 penile lesion swabs
  - L2b genotype determined by sequencing VD2 of \textit{ompA}

- Chicago
  - Cluster of suspect cases identified among MSM in 2016
  - Specimens – same criteria as Michigan
  - 19/47 (40%) cases confirmed as LGV by real-time quadriplex PCR
  - L2b genotype identified

\text{de Voux et al. MMWR 2016}
Molecular surveillance of LGV: 7 sites across the US

**Aim:** Estimate the prevalence of LGV among CT-positive rectal specimens using real-time PCR and to determine the genotypes of CT strains

**Methods**
- 172 rectal swabs from men and women – CT-pos by Hologic APTIMA CT/NG NAAT between Sept 2015 - Feb 2017
- Participating PHLs in 7 states - Alabama, Indiana, Massachusetts, Nevada, New Jersey, Michigan, and Tennessee
- Two real-time duplex PCRs used: 1st detects CT, 2nd differentiates LGV and non-LGV
- Genotyping was performed by nested PCR and sequencing of the outer membrane protein A gene (ompA)
Results

- 132 (76.7%) of the 172 specimens were positive for CT by real-time PCR
- **13.6% (18)** of 132 were positive for LGV and 86.4% (114) were non-LGV
- Of the remaining 40, 21.5% (37) tested negative for CT by real-time PCR and 1.7% (3) were invalid - due to PCR inhibition or no human DNA detected
- All 14 specimens from women – negative for LGV
- L2 genotype was identified in all specimens
- Serovars D, E, G, and J accounted for ~95% of non-LGV CT

- Overall 8.7% LGV+ (114/1317) over 3.5 years (2008 – mid-2011) - CT-pos samples (12.3% CT-pos) reflexed to LGV testing
- Overall 16.6% LGV+ (398/2396) between 2012-2015 - batched random sampling

Pathela et al. 2019, STD
Summary

- FDA-cleared NAAT is needed for patients presenting with moist lesions of primary & secondary syphilis
- PCR testing for *syphilis* & *LGV* is being done at a few commercial labs under CLIA regulations
- LDTs for *LGV* are being used in some PHLs
- Two FDA-cleared CT/NG tests are now available for use on rectal & pharyngeal specimens
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GUD MPCR sites

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