Update on STI diagnostic tests

STI UPDATE AND NEW WHO POST-EXPOSURE PROPHYLAXIS GUIDELINES

9TH INTEREST WORKSHOP
Harare, ZIMBABWE
5 – 8 MAY 2015

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• Chairperson, Harare Central Hospital Management Board
• International consultant on STIs and gonococcal antimicrobial resistance (GC AMR)
• Member of the WHO Strategic and Technical Advisory Group on Antimicrobial Resistance (WHO AMR-STAG)
• Executive Member of International Union against STI (IUSTI)
Highlights of the talk

- Where we are now in STI diagnosis
- What can be done to improve STI diagnosis
- What to wish for, or expect, in the future
Approaches to STI case management

- **AETIOLOGIC**
  - laboratory isolation of the causative organism

- **CLINICAL ASSESSMENT:**
  - “diagnosis” based on clinical appearance

- **SYNDROMIC**
  - syndromes – clinical symptoms, signs, (risk assessment), rapid and cost-effective tests

- **PRESUMPTIVE**
  - Synonymous (sometimes) with syndromic management or as a means to treating presumed infections in sex partners, sex workers, etc. without symptoms of STIs
Clinical diagnosis approach

Identify the STI causing symptoms based on clinical experience

- even experienced STI providers often misdiagnose STIs
- miss mixed infections
- difficult for surveillance
Aetiologic Diagnosis Approach

The ideal!
Identifies the organism causing the symptoms with microscopy and/or laboratory tests and equipment

BUT
- tests can be time consuming and expensive
e.g. cultures cost US$12-40 & take up to 3-6 days
- even rapid tests (RPR) require equipment to obtain and separate venous blood – c. US$7 per test
- May miss mixed infections
- Dependent on skill of technician & laboratory accuracy
Syndromic case management

1. Main features

• Classify main causative agents by the clinical syndromes to which they give rise

• Use flow charts to determine causes of a given syndrome

• Treat patient for *all* the important causes of the syndrome

• Ensure that partners are treated - ?presumptively
Syndromic case management

2. Disadvantages of syndromic management

- Overtreatment in some patients
- Not a good tool for gonococcal and chlamydial detection in vaginal discharge syndrome in settings of low GC/CT prevalence
- Partner notification may be a concern without a definitive aetiological diagnosis

NB Syndromic approach was never intended for asymptomatic infections, because if there are no symptoms or signs there is no syndrome
Commonly encountered STI-related syndromes

1. Urethral discharge
2. Genital ulcer disease (M & F)
3. Vaginal discharge
4. Pelvic inflammatory disease (PID)
5. Scrotal swelling
6. Inguinal swelling
7. Ophthalmia neonatorum
Flowchart for the management of Urethral Discharge Syndrome in men

Patient complains of urethral discharge or dysuria

- Take history and examine
- Milk urethra if necessary

Urethral discharge present?

Yes → Use appropriate flowchart and/or treat appropriately

No → Any other STI or genital condition?

Yes → ± Trichomonas vaginalis treatment
± Mycoplasma genitalium treatment

No → No

• Reassure patient
• Educate and counsel
• Offer HIV counselling and testing
• Promote condom use and provide condoms

TREAT FOR GONORRHOEA AND CHLAMYDIA TRACHOMATIS

± Trichomonas vaginalis treatment
± Mycoplasma genitalium treatment
2nd Generation Syndromic Diagnosis Approach

Symptom + sign
Particularly urethral discharge, vaginal discharge or genital ulcer disease

Take history & examine

Decision

Risk assessment
Incorporate rapid diagnostic test

Action

Condom use
Partner notification
Other action
Dwindling treatment options for *Neisseria gonorrhoeae*

2006–2008 change to Cephalosporins

- Penicillins
- Tetracyclines
- Aminoglycosides (*gentamicin, kanamycin*)
- Quinolones (*norfloxacin, ciprofloxacin*)
- Macrolides (*azithromycin*)
- Spectinomycin
- Cephalosporins
  - *ceftriaxone* (IM or IV)
  - *cefixime* (oral)
Countries reporting increased MIC of Cefixime and Ceftriaxone in *N. gonorrhoeae* (2010)

Source: WHO Gonococcal antimicrobial susceptibility programme
The technology exists

Price $22.95

$24.95
The technology exists

Test Kit Price $155.95
Multi-test Price $142.95

Packaged: 2 tests per kit (10 tests in total)
Procedure: Simple/Moderate
Results In: 5-15 Minutes
Specimen: Endocervical Swab, Whole Blood
Performance of Rapid GC Tests in women

STI clinic in Brazil
N = 236; 15% prevalence

Sensitivity*  60%
Specificity*  91%
PPV   56%
NPV   93%

* vs. culture on cervical swabs
(Benzaken et al STI 2006; 82 Suppl V: v26-8)

STI clinic in Benin
N = 1084; 5% prevalence

Sensitivity*  70%
Specificity*  97%
PPV   55%
NPV   99%

* vs. PCR on cervical swabs
(Alary et al STI 2006; 82 Suppl V: v29-32)
# Cost and performance of the rapid tests for STIs

<table>
<thead>
<tr>
<th>STI</th>
<th>Tests available</th>
<th>bedside format</th>
<th>Cost</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syphilis</td>
<td>Trep. test RPR</td>
<td>√</td>
<td>$ .19 -3.0</td>
<td>Active vs. past infection</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±</td>
<td>$ .20</td>
<td></td>
</tr>
<tr>
<td>Chlamydia trachomatis</td>
<td>ICS</td>
<td>√</td>
<td>$5-10</td>
<td>Low sensitivity</td>
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<tr>
<td>Vaginal infections</td>
<td>pH Whiff test</td>
<td>√</td>
<td>$ .20</td>
<td>? Syndromic mx</td>
</tr>
<tr>
<td></td>
<td>Tests for TV</td>
<td>√</td>
<td>$ .50</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>√</td>
<td>$ 3.0</td>
<td></td>
</tr>
<tr>
<td>Herpes</td>
<td>serology</td>
<td>√</td>
<td>$35</td>
<td>Expensive</td>
</tr>
</tbody>
</table>
The Cepheid GeneXpert Assay

- Is a modular cartridge-based platform for testing samples by nucleic acid amplification (NAAT)
- Can detect the DNA of *C. trachomatis* and *N. gonorrhoeae* in endocervical/urethral swabs, vaginal swabs and urine with closed extraction system
- Results can be ready in less than 2 hours and can process from 1 to 96 specimens
- Can be used in an on-site laboratory, but not quite point-of-care test
Performance of the Cepheid’s GeneExpert real-time NAAT test for Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*

**Multicenter trial of 1,722 female and 1,387 male volunteers**

Comparison assay – Patient Infected Status (at least 2 specimens positive)
- Gen-Probe Hologic Aptima Combo 2 assay (AC2)
- Becton Dickinson ProbeTec CT and GC

**Specimens:**
- **GeneXpert:** endocervical & vaginal swabs, male and female urine
- **AC2 and ProbeTec:** endocervical swabs, male and female urine

Performance of the Cepheid’s GeneExpert real-time NAAT test for Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*

Multicenter trial of 1,722 female and 1,387 male volunteers

Results compared to Patient-Infected Gold Standard: Chlamydia

<table>
<thead>
<tr>
<th>GeneXpert</th>
<th>Female vaginal swab: <em>Chlamydia trachomatis</em></th>
<th>Male urine: <em>Chlamydia trachomatis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity: 98.7%</td>
<td>Sensitivity: 97.5%</td>
<td></td>
</tr>
<tr>
<td>Specificity: 99.4%</td>
<td>Specificity: 99.9%</td>
<td></td>
</tr>
<tr>
<td>PPV: 88.6%</td>
<td>PPV: 98.7%</td>
<td></td>
</tr>
<tr>
<td>NPV: 99.9%</td>
<td>NPV: 99.8%</td>
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<th>Female endocervical swab: <em>Chlamydia trachomatis</em></th>
<th>Female urine: <em>C. trachomatis</em></th>
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<td>Sensitivity: 97.4%</td>
<td>Sensitivity: 97.6%</td>
<td></td>
</tr>
<tr>
<td>Specificity: 99.6%</td>
<td>Specificity: 99.8%</td>
<td></td>
</tr>
<tr>
<td>PPV: 91.6%</td>
<td>PPV: 96.4%</td>
<td></td>
</tr>
<tr>
<td>NPV: 99.9%</td>
<td>NPV: 99.9%</td>
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Performance of the Cepheid’s GeneExpert real-time NAAT test for Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*

Multicenter trial of 1,722 female and 1,387 male volunteers

Results compared to Patient-Infected Gold Standard: *N. gonorrhoeae*

**GeneXpert**

**Female vaginal swab: *N. gonorrhoeae***

- Sensitivity: 100%
- Specificity: 99.9%
- PPV: 91.7%
- NPV: 100%

**Male urine: *N. gonorrhoeae***

- Sensitivity: 98%
- Specificity: 99.9%
- PPV: 98%
- NPV: 99.9%

**GeneXpert**

**Female endocervical swab: *N. gonorrhoeae***

- Sensitivity: 100%
- Specificity: 100%
- PPV: 100%
- NPV: 100%

**Female urine: *N. gonorrhoeae***

- Sensitivity: 95.6%
- Specificity: 99.9%
- PPV: 95.6%
- NPV: 99.9%

Performance of the Cepheid’s GeneExpert real-time NAAT test for Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*:

Experience from the Zimbabwe multi-site testing, 2015
136 patients

- Testing by GeneXpert®, Probetec™, and M-PCR completed for 136 patients with genital discharge syndromes
- Concordance of positive or negative results across all 3 platforms:
  - Gonorrhoea: 133/136 (97.8%)
  - Chlamydia: 132/136 (97%)

Courtesy of Dr Kees Rietmeijer
Performance of the Cepheid’s GeneExpert real-time NAAT test for Detection of Chlamydia trachomatis and Neisseria gonorrhoeae:

Experience from the Zimbabwe multi-site testing, 2015
136 patients

• GeneXpert® CT/NG compared to concordant results on Probetec® and M-PCR platforms:

  • N. gonorrhoeae
    • Sensitivity: 100%
    • Specificity: 98.8%
  • Chlamydia trachomatis
    • Sensitivity: 100%
    • Specificity: 100%

Courtesy of Dr Kees Rietmeijer
Cepheid’s GeneExpert real-time NAAT test in detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*:

**Summary conclusions**

- In the multicentre study by Gaydos et al, patients were symptomatic and asymptomatic, thus GeneXpert
  - Can be used for diagnosis of symptomatic patients
  - Can be used for screening of asymptomatic patients

- In the Zimbabwe study, some patients with GUD but asymptomatic for genital discharge were found to have significant gonococcal & chlamydial infections
  - Can be used for case finding in patients presenting with other STIs
Genital ulcer diseases syndrome

Impossible to say what the pathogen is by looking; therefore group as genital ulcer disease (GUD) syndrome

Squamous cell carcinoma
Testing for STI pathogens associated with genital ulcer disease: is it feasible?

- Darkfield Microscopy
- Syphilis serology test (as baseline test)
- PCR for *Treponema pallidum*
- Culture for *Haemophilus ducreyi*
- PCR for *Haemophilus ducreyi*
- PCR for Herpes simplex virus (HSV)
- Type-specific HSV serology not helpful & not available
- PCR for *C. trachomatis* (L-serovars)
- Chlamydial serology (CFT or micro-IF)
- PCR for *Klebsiella granulomatis*
HSV-2 dominates as cause of GUD

ACCRA 2005 (N = 83 women with GUD)
- HSV-2: 56%
- Unknown: 42%
- EBV: 2%

BANGUI 2005 (N = 46 women with GUD)
- HSV-2: 50%
- Unknown: 33%
- EBV: 17%
- Multiple: 10%

UGANDA
- None: 32%
- Herpes: 40%
- Other: 13%
- Syphilis: 2%
- Chancroid: 3%

SRI LANKA (N = 261 men with GUD)
- HSV-2: 63%
- Unknown: 14%
- EBV: 13%
- Other: 3%

CHINA
- None: 34%
- HSV: 19%
- TP: 35%
- TP+HSV: 12%
- Non-venereal: 3%
- Syphilis: 14%
- Candidiasis: 2%
- Drug allergy: 2%
- Other: 5%
Current Rapid Tests for Syphilis  
(Equivalent to TPHA)

Procedure:
1. Use dropper provided, dispense 1 drop of serum/whole blood to sample well S
2. Add 2 drops of diluent buffer to sample well S
3. Read results at 15 minutes
Bioline Treponemal Point-of-care (POC) Test
Ideal, conventional diagnostic algorithm for the diagnosis of active syphilis – RPR+TPHA

- Serum
  - RPR
    - +ve
      - Treponemal test
        - +ve
          - Confirmed syphilis
            - TREAT
        - -ve
          - No treatment necessary
    - -ve
Span ‘Signal Spirolipin’
Flow-through Syphilis Dual Test Platform

Control Spot

Cardiolipin Antigen Spot (RPR equivalent)

Treponemal Antigen Spot (TPHA equivalent)
Addition of wash buffer

Addition of serum

Addition of wash buffer

Addition of conjugate

Addition of wash buffer
Rapid Simultaneous Detection of Reagin and Treponemal Antibodies Using the ‘Signal Spirolipin’ Flow-through Test

NON-REACTIVE TESTS

ONLY THE NON-SPECIFIC CARDIOLIPIN TEST REACTIVE

CONFIRMED REACTIVE TEST

ONLY THE TREPONEMAL TEST REACTIVE
Field testing by CDC-USA in Madagascar

(Courtesy Prof Ron Ballard)
1. Add 5 µl of serum/blood to the sample+buffer well.

2. Add 2 drops of buffer to the sample+buffer well.

3. Add five drops of buffer to the buffer well when the colored lines disappear.

4. Read the results at 15 minutes

Negative result: one line or
Positive result: two lines
Chembio Lateral Flow-Test Reader

DPP™ Handheld Reader

1. TEST1 POSITIVE
2. TEST2 POSITIVE
3. T1=109.6
   T2=113.7
Fluorescence latex technology with a simple UV light instead of digital reader

Shelf life of at least 2 years at Room Temperature
Rapid diagnostic tests for diagnosis of syphilis and HIV infections

• Single rapid tests - existing
  • Syphilis
    ▪ Rapid treponemal tests
  • HIV
    ▪ Rapid HIV-1/2 antibody tests
    ▪ Rapid HIV antibody and p24 antigen tests

• Dual rapid tests - new
  • Syphilis
    ▪ Dual treponemal and non-treponemal tests
  • HIV/syphilis
    ▪ Rapid dual HIV (HIV-1/2 antibody) and syphilis (treponemal) tests

• Triple rapid tests – future development
  ▪ Rapid HIV, HBV and HCV tests
  ▪ Rapid HIV, treponemal, non-treponemal (not yet available)
## Dual rapid HIV and syphilis tests – performance characteristics

<table>
<thead>
<tr>
<th>Manufacturer (Country)</th>
<th>Sensitivity (%)*</th>
<th>Specificity (%)*</th>
<th>Commercially available?</th>
<th>WHO prequalified?</th>
<th>Estimated cost?</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Standard Diagnostics (Korea)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>99</td>
<td></td>
<td></td>
<td>US$ 1.20 (Single HIV 0.80, single syphilis 0.50)</td>
</tr>
<tr>
<td><strong>Chembio (USA)</strong></td>
<td>99.1-100</td>
<td>95.7-100**</td>
<td>99.6-100</td>
<td>98.2-100</td>
<td>Ongoing</td>
</tr>
<tr>
<td><strong>Medmira (Canada)</strong></td>
<td>99.8</td>
<td>94.4</td>
<td>99.7</td>
<td>100</td>
<td>Ongoing</td>
</tr>
<tr>
<td><strong>BioLytical (Canada)</strong></td>
<td>99.0-99.6</td>
<td>?</td>
<td>99.3-100</td>
<td>?</td>
<td>Ongoing</td>
</tr>
<tr>
<td><strong>Mbio (USA)</strong></td>
<td>?</td>
<td></td>
<td>?</td>
<td></td>
<td>No application yet</td>
</tr>
</tbody>
</table>

*Sensitivity and specificity estimates as provided by manufacturer at time of application of WHO PQDx programme.

**Compared with ELISA followed by RPR confirmation.
Currently available dual rapid HIV and Syphilis tests

SD BIOLINE Duo HIV/syphilis
- Immunochromatographic IgA, IgM, IgG assay

Chembio DPP® HIV-Syphilis
- Dual path platform

MedMira Multiplo
- Immunoreactive test membrane comprised of TP recombinant antigens (15 kDa, 17 kDa, 47 kDa) and synthetic HIV peptides to gp36, gp41, gp120 and HIV-group O
# Field evaluation of Dual Rapid Diagnostic Tests for Syphilis/HIV Screening in ANC

Performance of dual HIV/syphilis tests in laboratory evaluation in China and Nigeria (N=1,514 specimens)

<table>
<thead>
<tr>
<th></th>
<th>SD Bioline</th>
<th>Chembio</th>
<th>MedMira</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>99.0 (98.0-99.5)</td>
<td>99.6 (98.8-99.9)</td>
<td>99.5 (99.4-99.8)</td>
</tr>
<tr>
<td>Specificity</td>
<td>99.0 (98.0-99.5)</td>
<td>97.9 (96.7-98.7)</td>
<td>98.3 (97.2-99.0)</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>96.6 (95.0-97.7)</td>
<td>97.0 (95.5-98.0)</td>
<td>94.2 (92.3-95.7)</td>
</tr>
<tr>
<td>Specificity</td>
<td>99.1 (98.2-99.6)</td>
<td>99.6 (98.9-99.9)</td>
<td>97.2 (95.8-98.1)</td>
</tr>
</tbody>
</table>

Source: Preliminary results from National Center for STD Control, China; University College Hospital, Nigeria; Ahmadu Bello University Teaching Hospital, Nigeria
The future: dual testing for HIV and syphilis

The mChip: The “Credit Card” that can tell you if you have HIV and syphilis within minutes and costs just $1

Reproduces all the steps of a lab-based immunoassay in minutes

Laboratory-based vs Rapid Tests: trade-off between sensitivity and speed

<table>
<thead>
<tr>
<th>Lower Detection Limit (Bacteria)</th>
<th>Time to Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^2$</td>
<td>4 h</td>
</tr>
<tr>
<td>$10^3$</td>
<td>30 min</td>
</tr>
<tr>
<td>$10^4$</td>
<td>Lab on a chip</td>
</tr>
</tbody>
</table>

Nucleic Acid Amplification Test

ELISA
The future: Multiplex technology for diagnosis of urethral discharge & asymptomatic urethritis

- Amplified nucleic acid based testing
  - for *N. gonorrhoeae*
  - for *C. trachomatis*
  - for *M. genitalium*
  - ? for *U. urealyticum*
  - for *T. vaginalis*
The future: Multiplex technology for diagnosis of vaginal discharge syndrome

- Amplified nucleic acid based testing
  - for *T. vaginalis*
  - for *N. gonorrhoeae*
  - for *C. trachomatis*
  - for *M. genitalium*
Criteria for selection of tests for procurement

- Cost
- Test Performance [accuracy, reproducibility]
- Stability
- Need for additional supplies, e.g. micropipette
- Format: dipstick vs cassette
- Training required
- Ease of interpretation of results
- Regulatory approval in country
Quality Assurance

- Quality control of tests (QC)
  - Rapid tests have no internal QC
  - Need to monitor transport and storage conditions (temperature and humidity)
    - Temperature spiking in transit
    - Storage at central stores
    - Storage at health-care facilities at all levels

- Quality of testing – EQA (proficiency)
  - Quality system throughout health-care infrastructure
  - National reference labs, regional, district, health centre
The Future Goal

- The development of integrated diagnostic platforms that are rapid, easy to use, sensitive and specific to detect multiple targets for diagnosis of infectious diseases
- Oral rapid tests to screen for HIV/syphilis, other viral STIs
- Determination of antimicrobial resistance without culture
- For monitoring treatment and prevention
- For use in peripheral point-of-care settings
- Access ensured in low resource settings
THANK YOU