New diagnostic tests for sexually transmitted infections

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Introduction

- Data from our national microbiological labs suggest STIs are an important clinical issue
- Correlation with clinical data is crucial to document real clinical burden
- Figures are focusing on the ‘big three’

STI: sexually transmitted disease

Surveillance report of STI’s Sciensiano, 2018
The ideal STI test

- Detects multiple STIs simultaneously with a high sensitivity and specificity
- Has a rapid turn-over and low cost
- Rapports presence of human cells in test sample
- Permits sexual history-based screening of multiple anatomic sites
- Rapports resistance of relevant pathogens
- Detects emerging (potential) STI as *M. genitalium*, *U. urealyticum* and *M. hominis*
- Is available at my local institution/nearby lab
Diagnostic tools for STI: old stuff

- (Culture)
  - Relevant to determine antimicrobial resistance
- Serology
  - Cornerstone for diagnosis of syphilis, HIV and viral hepatitis
  - ‘Windows phase’
  - Historic sample needed for proper interpretation
- Combotest (antigen/antibody) for viruses
  - Enhanced sensitivity as test becomes positive during viremia
  - Available for HIV and HCV
Diagnostic tools for STI: better stuff

• Nuclear acid amplification tests (NAATs)
  – Available since the 90’s for chlamydia and gonorrhoea
  – Different detection techniques, including PCR, SDA, LCR and TMA
  – More sensitive than culture, especially for extra-genital sites
  – Also more sensitive (20-35%) than other non-culture direct tests, including EIA, DAT and nucleic acid hybridization tests
  – Permits less invasive collection of specimens (first-voided urine and vaginal swabs)
  – Feasible transport conditions

EIA: enzyme immunoassay  DAT: direct fluorescent antibody test
PCR: polymerase chain reaction  SDA: strand displacement amplification
LCR: Ligase Chain Reaction  TMA: transcription-mediated amplification

Papp JR et al, 2014
NAAT: PCR principle

Cycle

1. DNA sample is heated
2. DNA strands separate
3. Primers bind
4. Primers extended
Diagnostic tools for STI: new stuff

- Micro-array rtPCR
  - ‘In-house’
  - Semi-quantification through rtPCR ($C_t$ value)
  - Tests a battery of STI
  - Resistance testing by detecting resistance-mediating mutations

rtPCR: real-time PCR $C_t$: threshold value

Courtesy of M. Reynders
Diagnostic testing: rtPCR

The micro-array Taqman® amplification card allows performing multiple rtPCR reactions simultaneously. Each channel contains 48 reaction wells, each with a pre-spotted assay. Sample extracts are added to the wells, and the card is loaded into a PCR machine for amplification. Each well represents 1 μl reaction volume, corresponding to 1 Real Time PCR reaction.

Courtesy of M. Reynders
Multiplex testing for STI

- STI TAC AZ Sint-Jan detects 13 potential pathogens allowing a syndromic approach
  - Adenovirus, CMV, HSV-1 and HSV-2
  - *Chlamydia trachomatis*, *Mycoplasma genitalium*, *Mycoplasma hominis*, *Haemophilus ducreyi*, *Neisseria gonorrhoeae*, *Treponema pallidum*, *Ureaplasma parvum* and *Ureaplasma urealyticum*
  - *Trichomonas vaginalis*
  - Macrolide resistance-mediating mutations of *M. genitalium* (mutations in region V of the 23S rRNA gene (A2058G, A2059G, A2058T))
  - Controls: 18S, SHV control, Human Rnase P gen, Hs04260458_s1

- Reporting is semiquantitatively (low/medium/high load), potentially discerning infection from asymptomatic carriership/shedding

- Oral swabs, vaginal swabs, first-voided urine or rectal swabs

Courtesy of M. Reynders
Syndromic approach of STI

• Acute urogenital symptoms
• Genital ulcer disease
• Anorectal symptoms
• Screening after risk contact(s)
• Pelvic inflammatory disease or Fitz-Hugh-Curtis syndrome
• Chorio-amnionitis and/or premature contractions
• Fertility problems
• Respiratory failure and/or sepsis of the neonate after chorio-amnionitis
F.V., 35-year old male, MSM, HIV negative, presenting with persisting urethritis despite treatment with azithromycin 1 g and ceftriaxone 500 mg IM by his GP. Symptoms resolved after moxifloxacin 400 mg dd during 10 days, test of cure was negative.
M. genitalium: key features

- First identified in 1981 as a cause of nongonococcal urethritis
- In the 90’s NAATs were introduced in research labs
- Moderate to strong association with PID and cervicitis in women, but frequently asymptomatic
- Infection raises risk for HIV infection
- First choice treatment is azithromycin (500 mg on d1, followed by 250 mg dd during 4d)
- Mutations in region V of the 23S rRNA gene confer resistance to macrolides
  - Moxifloxacin 400 mg dd during 10d

NAAT: nucleic acid amplification test, PID: pelvic inflammatory disease
Syndromic approach: screening after risk contacts

• Attestation of Truvada® for PrEP denotes:
  ‘ik verbind mij ertoe de volgende andere aangeraden testen uit te voeren/je m’engage à effectuer les autres tests recommandés suivants: ‘HIV, syphilis, gonorrhea, chlamydia, hepatitis C, others…’

• Patients starting on PrEP were screened by means of oral and rectal swabs, and collection of first-voided urine

• Urine, self-collected oral and rectal swabs were pooled from month 3

• In total 12% of screenings were positive for *M. genitalium* (first year)

• 43% of the strains were resistant for macrolides

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<th>Negative</th>
<th>Wild-type</th>
<th>Macrolide-R</th>
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PrEP: pre-exposure prophylaxis
Take home messages

- Serology and NAAT are not the ideal STI tests
- Micro-array rtPCR allows syndromic approach of STI
- Commercial STI field is growing
- AMR testing is possible by detecting resistance-mediating mutations
- *M. genitalium* is frequently detected in patients taking PrEP
- Prospective clinico-microbiological studies will learn us more on management of *Ureaplasma spp.* and *Mycoplasma spp.* in different clinical contexts

NAAT: nuclear acid amplification tests, STI: sexually transmitted disease, PrEP: pre-exposure prophylaxis
AMR: antimicrobial resistance