Chlamydia trachomatis & Mycoplasma genitalium—Microbiology and diagnostic tests.

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Aims

• Chlamydia trachomatis
  • Replication and principles (problem) of culture
• Mycoplasma genitalium
  • Replication and principles (problem) of culture
• Principles of Nucleic Acid Amplification techniques (NAAT)
• What is the best genital tract specimen?
• Antimicrobial resistance
**Chlamydia trachomatis**

- Ocular trachoma recognised since antiquity
- Early 20<sup>th</sup> C: identical cytoplasmic inclusions seen in neonatal conjunctivitis and genital tract cells from both mother and father of a case
- Now known to cause NGU, PID, ectopic pregnancy, infertility, infantile pneumonitis

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**C. trachomatis – life cycle**

Genetically ~stable  
Low mutation rate  
Intracellular niche

EB = elementary body (infectious) : RB = Reticulate body (replicative)
Tissue Culture
Intracellular requires continuous cell line

Iodine stain of *Chlamydia trachomatis*.
Chlamydia culture

- Sensitivity < 70%
- Not validated to UKAS standard
- No longer available in UK

Direct Immunofluorescence - stained with fluorescein conjugated specific monoclonal antibody
**C. trachomatis – life cycle**

**EB** = elementary body (infectious) ; **RB** = reticulate body (replicative)

**Unfavourable growth conditions:**
- Interferon gamma
- Penicillin

**Chlamydia intracellular growth**

- Cell death
- Production pro-inflammatory cytokines & chemokines
  - Interleukins: IL-8, IL-6,
  - Granulocyte-macrophage colony stimulating factor (GM-SF)
  - Tumour necrosis factor α (TNF α)
- Recruitment and activation immune response
  - Resolution infection vs collateral inflammatory damage tissue
- Persistence
  - Antimicrobial resistance

*Menon S 2015 Clin Micro Rev*
**Mycoplasma genitalium**

- One of eight mycoplasma species found in human genital tract
- First identified in 1983
- 1993: Proposed as cause of NCNGU
- Also causes cervicitis, PID
- 2015: third National Survey of Sexual Attitudes and Lifestyles strengthens evidence that *M. genitalium* is an STI
Mycoplasma genitalium

- Smallest known bacteria

M. genitalium life cycle

- Very slow growing
  - Epithelial cells – endocervical and ectocervical
    - Non-keratinized?
- Replication
  - Extracellular
  - Intracellular
- High mutation rate
- Antimicrobial resistance
M. genitalium intracellular growth

- Cell death – only at high infectious loads
- Production pro-inflammatory cytokines & chemokines
  - Interleukins: IL-8, IL-6,
  - granulocyte-macrophage colony stimulating factor (GM-SF)
  - ?Less marked than with chlamydia (no TNF )
- ?Recruitment and activation Immune response
  - ?Resolution infection vs collateral inflammatory damage tissue

McGowin C. Inf & Immun 2012; 80:3842

Human fallopian tube explant culture

Baczynska A. Hum Repro 2007; 22:968–979
Nucleic Acid Amplification Techniques (NAAT) for STI diagnosis

- Test of choice for detecting bacterial, protozoal and viral STIs
- Multiple technologies
- Point of care NAATs
- Genotypic antimicrobial resistance tests beginning to become available

<table>
<thead>
<tr>
<th>Commercially available NAAT kits.</th>
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<tbody>
<tr>
<td><strong>PCR</strong></td>
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<td><strong>SDA</strong></td>
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<td><strong>TMA</strong></td>
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</table>
Commercial NAATs

- *Chlamydia trachomatis* (usually combined with *Neisseria gonorrhoeae*)
  - Laboratory – Roche, Hologic, BD, Abbott
  - Point of care - Cepheid
- *M. genitalium*
  - Laboratory – Hologic, SpeeDx, Biorad
  - Point of care – Cepheid (soon!)

Commercial NAATs

- *M. genitalium* macrolide resistance (23S rRNA)
  - Laboratory – SpeeDx, Seegene
  - Point of care – Cepheid (soon!)
Components of a NAAT test

• Three steps:
• Nucleic acid extraction

DNA/RNA extraction

• Heat
• Lysis/Chemical
• Mechanical
• +/- Purification/extraction step (Aptima)
  • Removes inhibitors (blood, protein, mucus, some spermicidal agents, feminine powder sprays)
• Automated vs manual – high throughput or small numbers
Components of a NAAT test

• Three steps:
  • Nucleic acid extraction
  • Nucleic Acid Amplification
    • Transcription-mediated amplification
    • Polymerase chain reaction
    • Strand displacement amplification
PCR Amplifies a Targeted Sequence

DNA Structure

Hydrogen Bonds

Cytosine (C)
Adenine (A)
Thymine (T)
Guanine (G)
Deoxyribose (Sugar molecule)

Phosphoric Acid (Phosphate molecule)
PCR Cycle – Step 1 - Denaturation by Heat

Target Sequence

PCR Cycle – Step 2 - Biotinylated Primer Pair Anneals to Ends of Target Sequence

Target Sequence

Primer 1

Primer 2

Biotin

Target Sequence
PCR Cycle - Step 3 - *Taq* DNA Polymerase Catalyses Primer Extension as Complementary Nucleotides are Incorporated

End of the 1st PCR Cycle - Results in Two Copies of Target Sequence
Target Amplification

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<th>No. of Amplicon</th>
<th>No. of Cycles</th>
<th>Copies of Target</th>
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Components of a NAAT test

- Three steps:
  - Nucleic acid extraction
  - Nucleic Acid Amplification
    - Transcription-mediated amplification
    - Polymerase chain reaction
    - Strand displacement amplification
  - Detection of amplification products
    - Real-time PCR e.g. Taqman probes
Classic Nucleic Acid Amplification Tests e.g. PCR

One way flow:

- Sample preparation ↓
- Amplification ↓
- Detection ↓

Step 4 - Denaturation and Hybridisation
eg conventional PCR
Detection stage and Measurement of absorbance: Conventional PCR

1. Temperature is raised to 94°C to denature strands
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2. As temperature is lowered, the probe binds to the target at around 70°C. The quencher stops the reporter from fluorescing.
1. Temperature is raised to 94°C to denature strands.

2. As temperature is lowered the probe binds to the target at around 70°C. The quencher stops the reporter from fluorescing.

3. When the temperature reaches 60°C the primers bind.

4. Taq polymerase extends the new strand at 60°C.

5. Taq polymerase degrades the probe releasing the reporter from the quencher. The reporter fluoresces. The process then repeats in the next cycle.

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Applied Biosystems Prism 7000/7500

TaqMan PCR

Delta Rn vs Cycle

Cycle Number

Delta Rn
Real-Time PCR - quantification

High organism load

Ct = cycle threshold
What is the best specimen?

- Women
  - Vulvovaginal better than endocervical
    - Chlamydia - time since last sexual intercourse may be relevant - 20% women detection negative after ~2 weeks
    - *M. genitalium* can infect vaginal epithelial cells
  - Historically first void urine (FVU) less sensitive
    - Chlamydia - good performance with 2nd generation assays
- Men
  - FVU equivalent to intra-urethral swab

NAATS and menstruation

- Blood potential inhibitor NAATs
  - Roche, Abbott and BD assays
- Tampon may reduce chlamydia load in vagina
  - Unclear if an FVU specimen if menstruating would perform better as a non-invasive specimen.
Extra-genital sites - NAATS

- Originally not FDA approved or CE marked
- Now rectal swabs and throat swabs are validated on several commercial platforms

Testing low prevalence populations.

- Sensitivity
  Proportion of true positives correctly identified.
- Specificity
  Proportion of true negatives correctly identified.
Testing low prevalence populations

Prevalence = 1%, 1,000 screened

Sensitivity = 99%
10 true positives
0 false negative

Specificity = 99%
980 true negatives
10 false positives

Testing low prevalence populations

Prevalence = 1%, 1,000 screened

Sensitivity = 99%
10 true positives
0 false negative

Specificity = 99.9%
989 true negatives
1 false positive
Prevalence 1%, 1,000 screened

- If Sensitivity = 99%, specificity = 99%
  Total number of positives = 20
  True positives = 10
  Predictive value positive = 50%

- If Sensitivity = 99%, specificity = 99.9%
  Total number of positives = 11
  True positives = 10
  Predictive value positives = 91%

Useful references

- Horner PJ. Azithromycin antimicrobial resistance and genital Chlamydia trachomatis infection: duration of therapy may be the key to improving efficacy. *Sex Transm Infect* 2012; 88(3):154-156.