Controls for *Chlamydia trachomatis* and *Neisseria gonorrhoea*

*Mark Manak, Ph.D.*,  
SeraCare Life Sciences  
Gaithersburg, MD USA

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Chlamydia
- Most common reported STD
- Most infections are asymptomatic
- 2-17% incidence rate
  >5% sexually-active women in UK
- Causes urogenital infections leading to PID and infertility

Gonorrhoeae
- Second most common reported STD
- Widely variable incidence among countries
- 40% co-infected with C. trachomatis
- Mucosal infection leads to cervicitis, prostatitis, PID, sterility
- Increase in antibiotic resistance noted
**C. trachomatis Infection**

Elementary Body (EB) is infectious form
- 0.3-0.4µM
- Environmentally resistant
- Metabolically inert

Columnar epithelium
- Endocytosis
- Reticulate Body (metabolically active)
- Binary fission replication (100-500)
- Converted to EB
- Released by reverse endocytosis

15 Serovars
- A, B, Ba, C →Trachoma
- D-K →Genital infection
- LGV 1-3 →Lymphogranuloma venerum
**N. gonorrhoeae Infection**

Targets non-ciliated mucous epithelium
- Endocytosis
- Exocytotic release to sub-epithelial tissue
- Present in infectious exudate

Human only known natural host
- Almost exclusively by sexual contact
- 35% transmission efficiency ♀→♂
- 50-60% efficiency ♂→♀
- 90% ♂ develop symptoms
- <50% ♀ develop symptoms

Antibiotic Resistance
- Chromosomal and plasmid-derived
Nucleic Acid Amplification Testing (NAAT)

More sensitive than culture for screening and diagnosis

Noninvasively collected specimens

Urine (men or women)
  - First-Catch Urine samples (20-30 ml)
  - Transport in Sample Transport Media
  - Unprocessed Specimen container

Swab
  - Female Endocervical swabs,
  - Male urethral swab
  - Transport Dry or in Sample Transport Media
### C. trachomatis N. gonorrhoeae assays

#### Commercial C. trachomatis/ N. gonorrhoeae NAATs

<table>
<thead>
<tr>
<th>Technology</th>
<th>Roche Amplicor&lt;sup&gt;1&lt;/sup&gt;</th>
<th>BD ProbeTec SDA&lt;sup&gt;2, 3&lt;/sup&gt;</th>
<th>Gen-probe APTIMA&lt;sup&gt;4&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene target CT</td>
<td>Cryptic plasmid</td>
<td>Cryptic plasmid</td>
<td>23S ribosomal RNA gene</td>
</tr>
<tr>
<td>Gene target NG</td>
<td>Cytosine DNA methyltransferase gene</td>
<td>Multicopy pilin gene-inverting protein homologue</td>
<td>16S ribosomal RNA gene</td>
</tr>
</tbody>
</table>

#### Analytical Sensitivity of Commercial N. gonorrhoeae NAATs (Manufacturer data)

<table>
<thead>
<tr>
<th>Assay</th>
<th>Vol/assay</th>
<th>Analytical sensitivity (Limit of Detection)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gen-Probe Aptima Combo&lt;sup&gt;1&lt;/sup&gt;</td>
<td>400μl</td>
<td>50 cells/assay; 125 cells/ml</td>
</tr>
<tr>
<td>Roche COBAS Amplicor&lt;sup&gt;2&lt;/sup&gt;</td>
<td>50μl</td>
<td>100 CFU/ml urine; 400 CFU/ml swab</td>
</tr>
<tr>
<td>BD ProbeTec ET&lt;sup&gt;3&lt;/sup&gt;</td>
<td>200μl</td>
<td>10 cells/assay; 50 cells/ml</td>
</tr>
</tbody>
</table>

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1. Gen-Probe APTIMA Combo2 Product Insert, IN0156 rev. B
2. Roche COBAS Amplicor CT/NG Test for N. gonorrhoeae Product Insert, 10/2004, Rev. 5.0
3. BD ProbeTec ET CTNG Amplified DNA assay Product Insert. Rev. 0606
Urine vs. Swab Processing: COBAS

Roche Urine Protocol (COBAS Amplicor System)

- 500 µl sample
  - Centrifugation/Particle collection
  - Lysis and Dilution
  - Final volume after extraction = 500 µl
  - Take 50 µl for Amplification & Analysis

Roche Swab Protocol (COBAS Amplicor System)

- 100 µl sample
  - Lysis and Dilution
  - Final volume after extraction = 400 µl
  - Take 50 µl for Amplification & Analysis
ACCURUN Controls

Control Design

- Resemble clinical specimen
- Control for all aspects of processing and detection
- Non-Infectious
- Validated for Swab and Urine protocols

Intended Use

- Accuracy: Can the test produce the correct result?
- Reproducibility/precision: Can it do this consistently?
- Robustness: Can all techs run this method reliably?
- QC Monitoring: Can errors be detected when they occur?
Accurun 341 CT/NG Positive Controls

**C. trachomatis**
- Purified Elementary Bodies
- Serovar L2, strain 424
- rDNA homology across serovars

**N. gonorrhoeae**
- (Zopf 1885) Trevisan 1885
- NCTC 8375/ATCC 19424

- Multi-Analyte Control
- Compatible with all assay platforms
- Suspended in stabilized transport medium
- 2°C to 8°C storage
- >5 yr real-time stability (Roche COBAS Amplicor)
- CE Mark
Quantitation Standard

Plasmid Design

- Neisseria gonorrhea (NG) porA pseudogene (pap)
- Chlamydia trachomatis (CT) MOMP gene sequences.

Quantitated in SeraCare CT/NG TaqMan assay
Quantitation of ACCURUN 341 Controls

Prepare individual dilutions of CT and NG stocks
Run dilutions on each platform following assay instructions
Bracket additional sub-dilutions for continuity

Convert dilutions to theoretical particle counts

- CT: TEM
- NG: A$_{550}$
- Quantitated against standardized plasmid

CT Stock: $6.0 \times 10^8$ Copies/ml = $1.1 \times 10^8$ EB/ml (EM)
NG Stock: $1.6 \times 10^8$ Copies/ml = $1.0 \times 10^8$ cells/ml (A550)

Determine C$_{50}$ and C$_{95}$ levels in each assay
Gen-Probe APTIMA Combo2 Assay

Gen-Probe Aptima Combo CT LOD

Gen-Probe Aptima Combo NG LOD

Ferrer et al. 2002 IUSTI
100% Positive at 1 EB/mL

Gen-Probe APTIMA Combo2 Package Insert
<10 EB/mL

Gen-Probe APTIMA Combo2 Package Insert
125 NG cells/mL
Roche COBAS Amplicor CT/NG Assay

Roche Amplicor CT EB LOD

Roche Amplicor NG LOD

Roche COBAS Amplicor CT/NG N. gonorrhoeae Package Insert
100 CFU/mL Urine
400 CFU/mL Swab
BD ProbeTec ET CT and NG Amplified DNA assay

**BD ProbeTec CT LOD**

<table>
<thead>
<tr>
<th>Particle count</th>
<th>% Positive Score</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>200</td>
<td>20%</td>
</tr>
<tr>
<td>400</td>
<td>40%</td>
</tr>
<tr>
<td>600</td>
<td>60%</td>
</tr>
<tr>
<td>800</td>
<td>80%</td>
</tr>
<tr>
<td>1,000</td>
<td>100%</td>
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**BD ProbeTec GC LOD**

<table>
<thead>
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<th>Particle count</th>
<th>% Positive Score</th>
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<tr>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>25</td>
<td>20%</td>
</tr>
<tr>
<td>50</td>
<td>40%</td>
</tr>
<tr>
<td>125</td>
<td>60%</td>
</tr>
<tr>
<td>250</td>
<td>80%</td>
</tr>
<tr>
<td>500</td>
<td>100%</td>
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**EB/mL % Pos**

<table>
<thead>
<tr>
<th>EB/mL</th>
<th>% Pos</th>
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<tbody>
<tr>
<td>3</td>
<td>0%</td>
</tr>
<tr>
<td>10</td>
<td>8%</td>
</tr>
<tr>
<td>50</td>
<td>17%</td>
</tr>
<tr>
<td>125</td>
<td>33%</td>
</tr>
<tr>
<td>625</td>
<td>50%</td>
</tr>
<tr>
<td>1,000</td>
<td>67%</td>
</tr>
<tr>
<td>1,250</td>
<td>89%</td>
</tr>
<tr>
<td>2,500</td>
<td>100%</td>
</tr>
<tr>
<td>5,000</td>
<td>100%</td>
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Chalker et al., J Clin Microbiol 43, 2005

**BD ProbeTec ET Package Insert LOD**

BD ProbeTec ET Package Insert LOD$_{50}$: 50 cells/ml
Low-Positive CT and NG Nucleic Acid Controls

Controls designed to challenge each type of assay
Different sensitivities prevents use of a single control for all assays

<table>
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<tr>
<th>Assay</th>
<th>C. trachomatis</th>
<th>N. gonorrhoeae</th>
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<tbody>
<tr>
<td></td>
<td>$C_{50}$</td>
<td>$C_{&gt;95}$</td>
</tr>
<tr>
<td>Aptima</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>Amplicor</td>
<td>38</td>
<td>75</td>
</tr>
<tr>
<td>ProbeTec</td>
<td>625</td>
<td>2500</td>
</tr>
</tbody>
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3 Series of A341 Multi-Analyte CT and NG NAT Controls
Targeted at $5 \times C_{50}$ values for each major platform

- Series 100 for Gen-Probe APTIMA Combo2
- Series 200 for Roche COBAS Amplicor CTNG
- Series 400 for BD ProbeTec ET CTNG Amplified DNA Assay
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mmanak@seracare.com