

Performance of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* nucleic acid amplification assays using low level controls

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New Product Development Group
SeraCare Life Sciences, Milford, Massachusetts, USA

SeraCare Life Sciences

Acquired BBI (Boston Biomedica Inc.) and BBI Biotech Research Labs in 2004



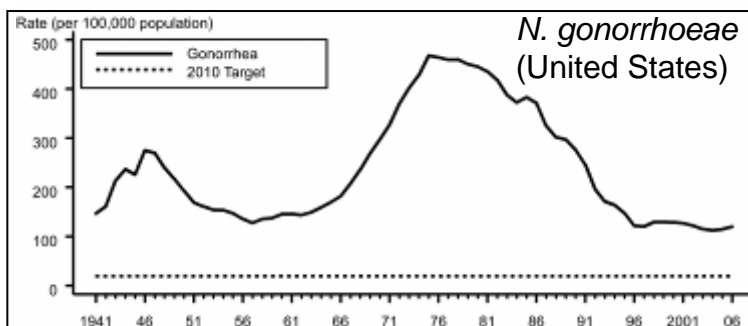
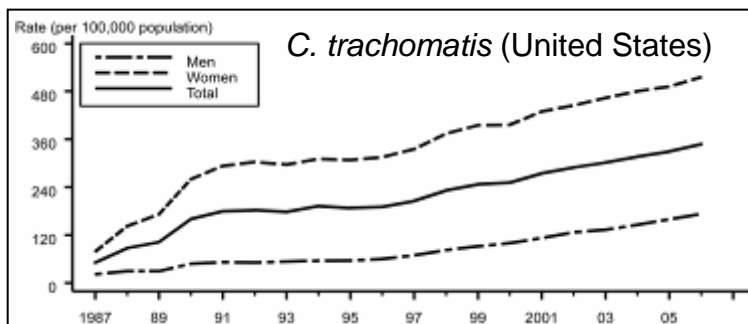
Corporate Headquarters, Manufacturing
Milford, MA



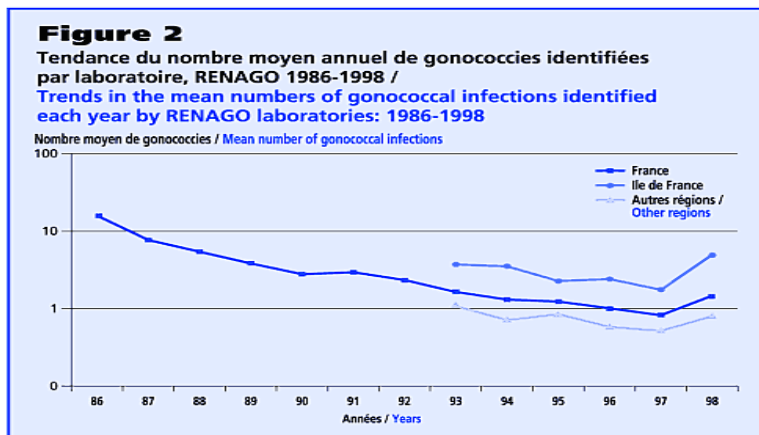
BioServices, Manufacturing
Gaithersburg, MD



BioServices
Frederick, MD



U.S. Centers for Disease Control and Prevention; www.cdc.gov



Goulet et al., Eurosurveillance 5(1),2000

European Incidence

Chlamydia

- Most common reported STD
- Not a notifiable infection in most European countries
- 2-17% incidence rate (asymptomatic♀) >5% sexually-active women in UK
- Causes urogenital infections, leading to PID and infertility
- New variant (nvCT) identified in Sweden
 - Not detectable by PCR-based assays
 - Evidence of spreading

Gonorrhoeae

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

C. trachomatis Infection

Elementary Body (EB) is infectious form

- 0.3-0.4 μ M
- Environmentally resistant
- Metabolically inert

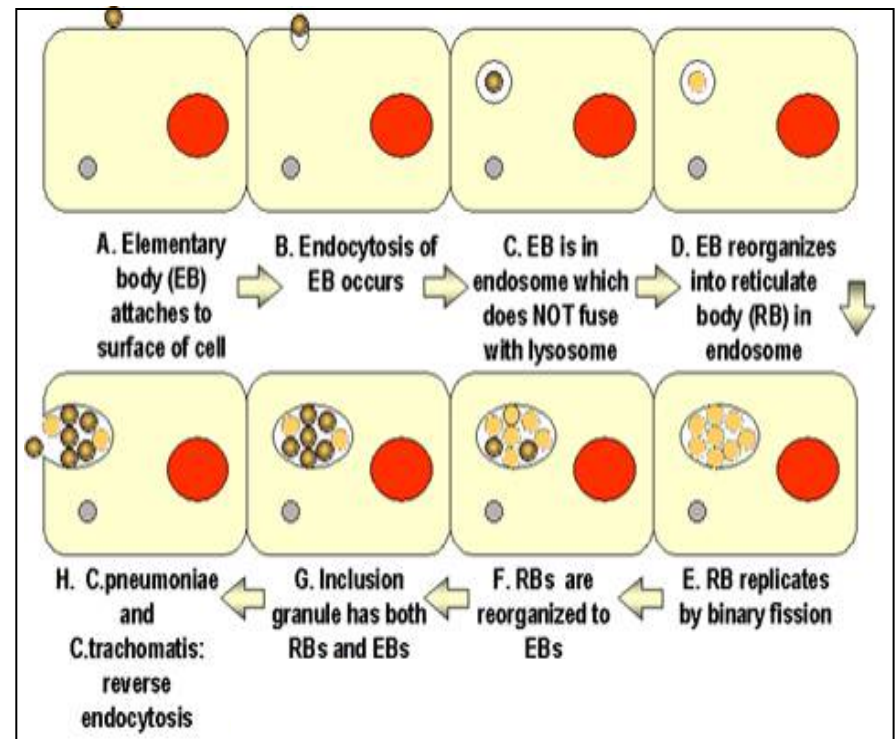
Columnar epithelium

- Endocytosis
- Reorganizes to Reticulate Body (metabolically active)
- Binary fission replication
- Reorganize to EB
- 100-500 EB's released by reverse endocytosis

15 Serovars

- A, B, Ba, C → Trachoma
- D-K → Genital infection
- LGV 1-3 → Lymphogranuloma venerum

Humans only known natural host



N. gonorrhoeae Infection

Not part of normal flora

Targets non-ciliated mucous epithelium

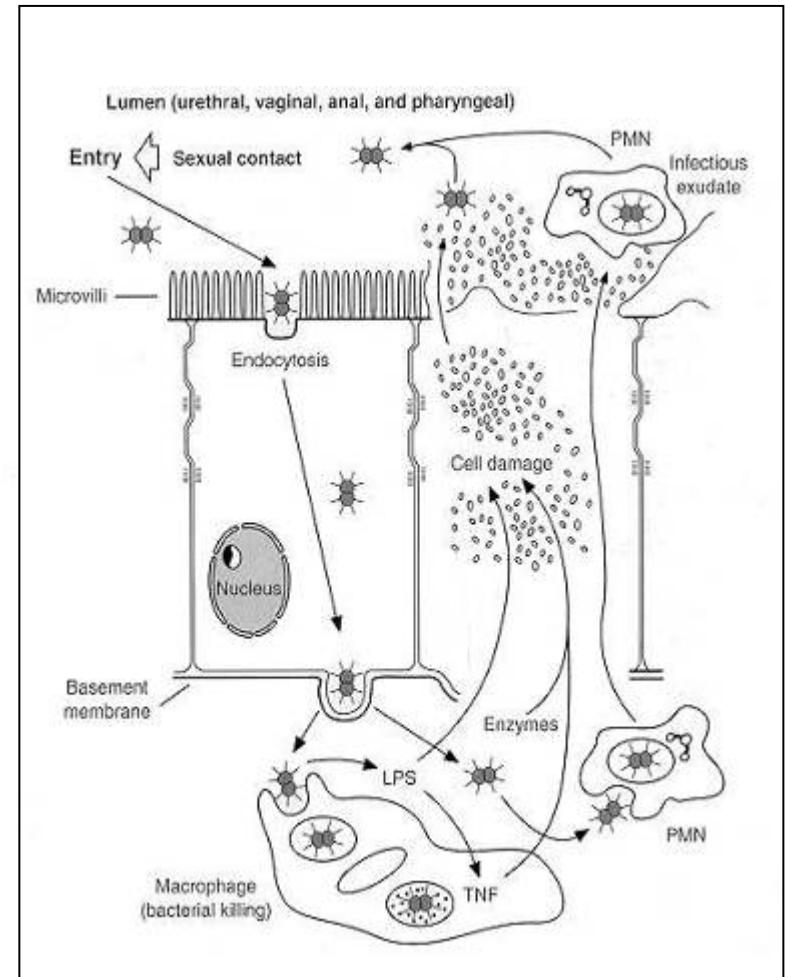
- Endocytosis
- Exocytotic release to sub-epithelial tissue
- Produces lipopolysaccharide endotoxin
- TNF-induced cell damage
- Macrophage/PMN phagocytosis
- Present in infectious exudate

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



Specimens and Assay Controls

Swab

- Endocervical swab
- Male urethral swab
- Transport
 - Dry
 - Sample Transport Media

Urine

- First Catch (20-30mL)
- Processed
 - Sample Transport Media
- Unprocessed
 - Specimen container

Controls

- Appropriate Target type
 - Intact *C. trachomatis* EB's
 - *N. gonorrhoeae* cells
- Nucleic Acids
 - DNA (plasmid and chromosomal) and rRNA
- Control for Sample Processing
- Validated for Swab and Urine specimens

Analytical Sensitivity of *C. trachomatis* assays

Specimen	Dilution (EB/ml ^a)	Result by:			
		BDProbeTecET		Roche AMPLICOR CT/NG	
		No. pos/total	% Pos	No. pos/total	% Pos
L2 strain CT001					
6680	1:200 (5,000)	59/59	100	66/66	100
6788	1:500 (2,000)	65/65	100	66/67	98.5
6157	1:1,000 (1,000)	30/32	93.8	39/39	100
6962	1:1,200 (833)	11/35	31.4	60/71	84.5
6424	1:1,500 (667)	40/51	78.4	52/53	98.1
6233	1:2,000 (500)	32/40	80	42/44	95.5
6681	1:2,000 (500)	36/57	63.2	56/63	88.9
6346	1:2,500 (400)	26/47	55.3	51/52	98.1
6547	1:3,000 (333)	27/59	45.8	54/58	93.1
Clinical isolate 6498					
6963	1:40 (85,750)	47/47	100	35/35	100
6964	1:400 (8,575)	47/47	100	35/35	100
7112	1:400 (8,575)	54/54	100	41/41	100
7113	1:250,000 (14)	18.54	33.3	13/41	31.7
7115	1:1,200 (2.858)	54/54	100	41/41	100

^a Estimated EB per milliliter are extrapolated from IF data for a 1:1,000 dilution (4 x 10- μ l wells)

Chalker et al., *J Clin Microbiol* 43, 2005

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Performance of *C. trachomatis* assays

Clinical Sensitivity of Commercial <i>C. trachomatis</i> NAATs			
	Roche Amplicor¹	ProbeTec SDA^{2, 3}	Gen-probe APTIMA⁴
Gene target	Cryptic plasmid	Cryptic plasmid	23S ribosomal RNA gene
Technology	PCR	SDA	TMA
Sensitivity (Swab)	91.3% (85.8-96.7)	93.7% (92.8-94.6)	96.5% (92.9-96.9)
Specificity (Swab)	98.1% (97.3-98.9)	96.2% (98.1-94.2)	97.6% (96.8-98.2)
Sensitivity (Urine)	87.9% (81.4-94.3)	96.0% (88.8-99.2)	96.5% (94.5-98.0)
Specificity (Urine)	97.2% (96.2-98.2)	100% (99.1-100)	98.7% (98.2-99.2)
Serovars Detected	All 15; not nvCT	All 15 incl. nvCT	All 15 incl. nvCT

¹ Roche COBAS Amplicor CT/NG Test for *N. gonorrhoeae* Product Insert, 10/2004, Rev. 5.0

² BD ProbeTec ET CTNG Amplified DNA assay Product Insert. Rev. 0606

³ Gaydos et al. J Clin Microbiol. 42, 2004)

⁴ Gen-Probe APTIMA Combo2 Product Insert, IN0156 rev. B

Performance of *N. gonorrhoeae* assays

Analytical Sensitivity of Commercial <i>N. gonorrhoeae</i> NAATs (Manufacturer data)		
Assay	Vol/assay	Analytical sensitivity (Limit of Detection)
Gen-Probe Aptima Combo ¹	400µl	50 cells/assay; 125 cells/ml
Roche COBAS Amplicor ²	50µl	100 CFU/ml urine; 400 CFU/ml swab
BD ProbeTec ET ³	200µl	10 cells/assay; 50 cells/ml
Gen-Probe PACE 2 ⁴	100µl	650 CFU/assay; 6500 CFU/ml

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

⁴ Gen-Probe PACE 2C Product Insert, 103906 rev. L

Clinical Sensitivity of Commercial <i>N. gonorrhoeae</i> NAATs			
	Roche Amplicor	ProbeTec SDA	Gen-probe APTIMA
Gene target	Cytosine DNA methyltransferase gene	Multicopy pilin gene-inverting protein homologue	16S ribosomal RNA gene
Technology	PCR	SDA	TMA
Sensitivity	64.8 to 100%	84.9 to 100%	91.3 to 98.5%
Specificity	93.9 to 100%	98.4 to 100%	98.7 to 99.3%
PPV	31.3 to 100%	54.8 to 100%	88.1 to 97.4%
NPV	99.5 to 100%	95.2 to 100%	99.2 to 99.9%
Cross-reactivity with other <i>Neisseria</i> species	<i>N. cinerea</i> , <i>N. flavescens</i> , <i>N. lactamica</i> , <i>N. sicca</i> , <i>N. subflava</i>	<i>N. flavescens</i> , <i>N. lactamica</i> , <i>N. subflava</i> , <i>N. cinerea</i>	None identified

Verification Studies

Purpose: to assess error inherent in the test, so that you can recognize unacceptable changes

Studies:

- Accuracy
 - comparison of methods experiment
 - sensitivity, specificity, PPV, NPV
- Linearity
- Precision
 - within-run (repeatability)
 - between-run (reproducibility)
- Analytical sensitivity (detection limits)
- Analytical Specificity

Verifying test performance in your lab

Accuracy: can the test produce the correct result?

Reproducibility/precision: can it do this consistently?

Robustness: can all our techs run this method reliably on our schedule?

Reference range/reportable range: was the manufacturer's range established with a population similar to ours?

Calibration plan: what does the manufacturer recommend, and can we do this?

QC plan: how do we detect errors when they occur?

Precision verification

Repeatability: results of multiple observations (tests) under identical conditions (often in one run)

- Estimates **random error** of a test
- Use **clinically relevant analyte levels**
 - low/high clinical decision points
- Use **clinically relevant sample matrix**
 - more than one?
- Typically involves ~20 replicates
 - calculate mean, sd, CV
- QC samples customarily used

Accurun 341 CT/NG Positive Controls

C. trachomatis

- Purified Elementary Bodies
- Serovar L2, strain 424
- Derived from LGV
- Heat-inactivated

Compatible with all assay platforms

- Contains non-deleted cryptic plasmid
- rDNA homology across serovars

N. gonorrhoeae

- (Zopf 1885) Trevisan 1885
- NCTC 8375/ATCC 19424
- Heat-inactivated

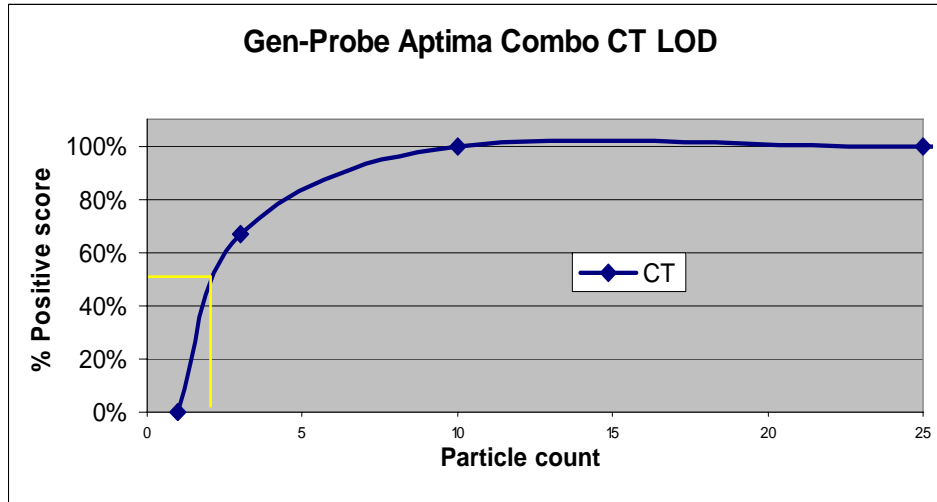
Compatible with all assay platforms

-
- Multi-Analyte Control
 - Suspended in stabilized trypticase soy broth (TSB) media
 - Non-interfering with assay platforms
 - >5 yr real-time stability (Roche COBAS Amplicor)
 - 2°C to 8°C storage

C. trachomatis/N. gonorrhoeae Assay Comparison

1. Prepare CT and GC dilutions individually
2. Run same dilutions on each platform, following assay instructions
3. Bracket additional sub-dilutions for continuity
4. Convert dilutions to theoretical particle counts
 - NG: A_{550}
 - CT: TEM
 - qPCR values ~ 10-fold higher for both
5. Determine C_{50} and C_{95} levels

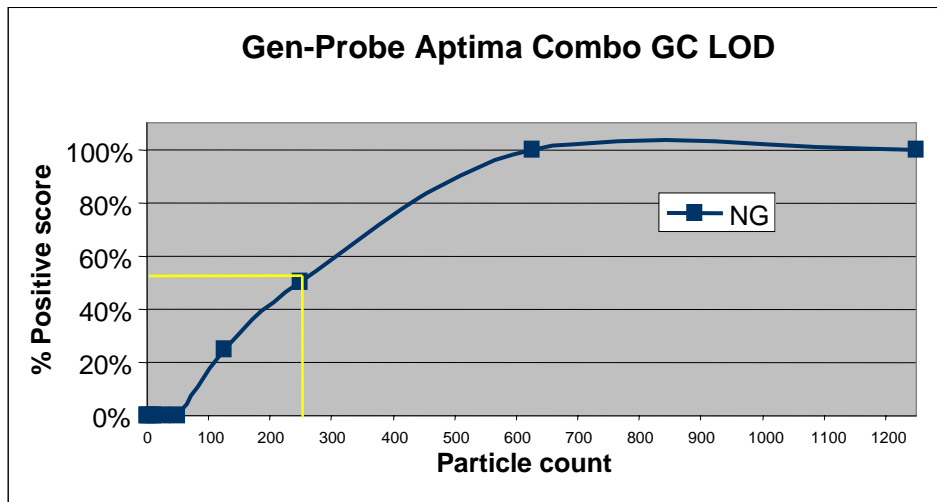
Gen-Probe APTIMA Combo2 Assay



EB/mL	% Pos
1	0%
3	67%
10	100%
25	100%
50	100%
125	100%
250	100%
625	100%
1250	100%
2500	100%

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

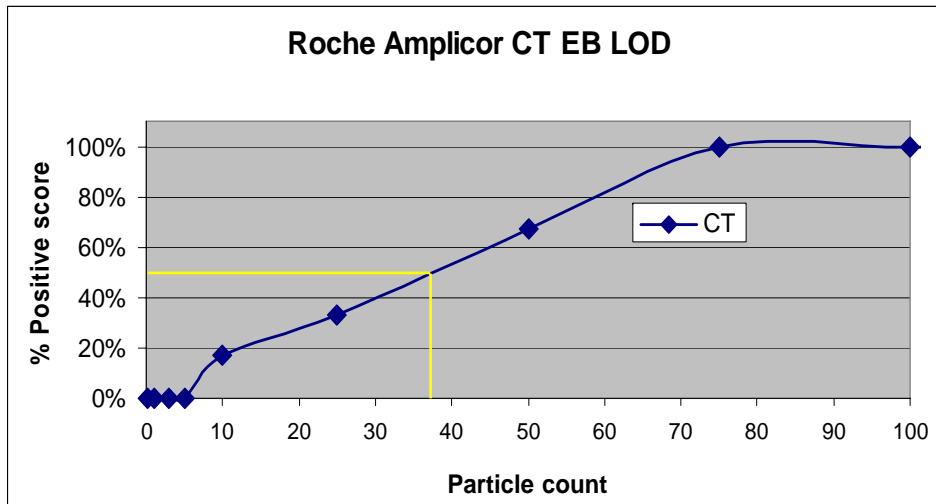
**Gen-Probe APTIMA Combo2
Package Insert**
<10 EB/mL



Cell/mL	% Pos
1	0%
3	0%
10	0%
25	0%
50	0%
125	25%
250	50%
625	100%
1250	100%
2500	100%

**Gen-Probe APTIMA Combo2
Package Insert**
125 NG cells/mL

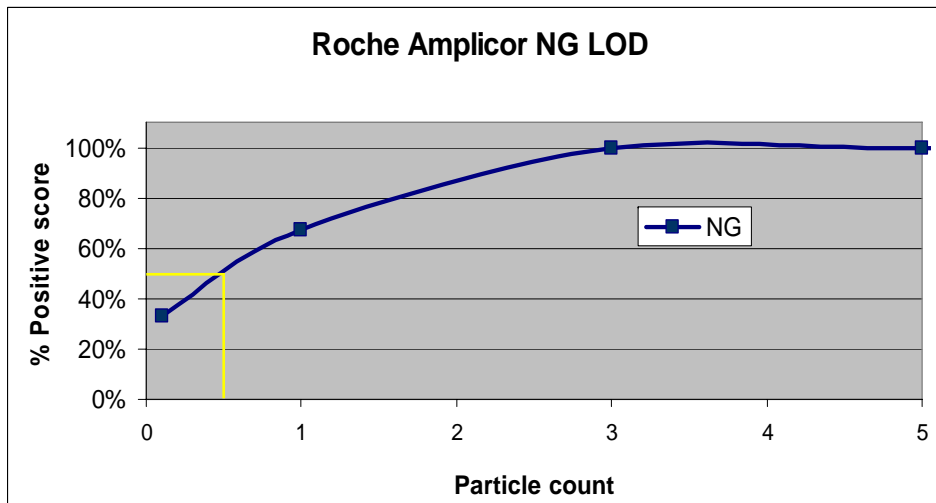
Roche COBAS Amplicor CT/NG Assay



EB/mL	% Pos
0.1	0%
1	0%
3	0%
5	0%
10	17%
25	33%
50	67%
75	100%
100	100%

EB/mL	% Pos
333	93.1%
400	98.1%
500	88.9%
500	95.5%
677	98.1%
833	84.5%
1,000	100%
2,000	98.5%
5,000	100%

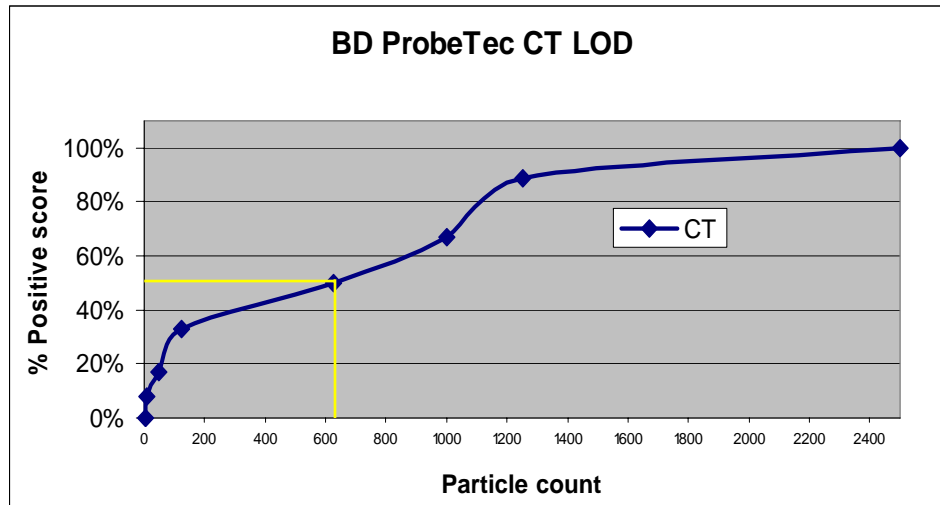
Chalker et al., J Clin Microbiol 43, 2005



Cell/mL	% Pos
0.1	33%
1	67%
3	100%
5	100%
10	100%
25	100%
50	100%
75	100%
100	100%

**Roche COBAS Amplicor
CT/NG *N. gonorrhoeae*
Package Insert**
100 CFU/mL Urine
400 CFU/mL Swab

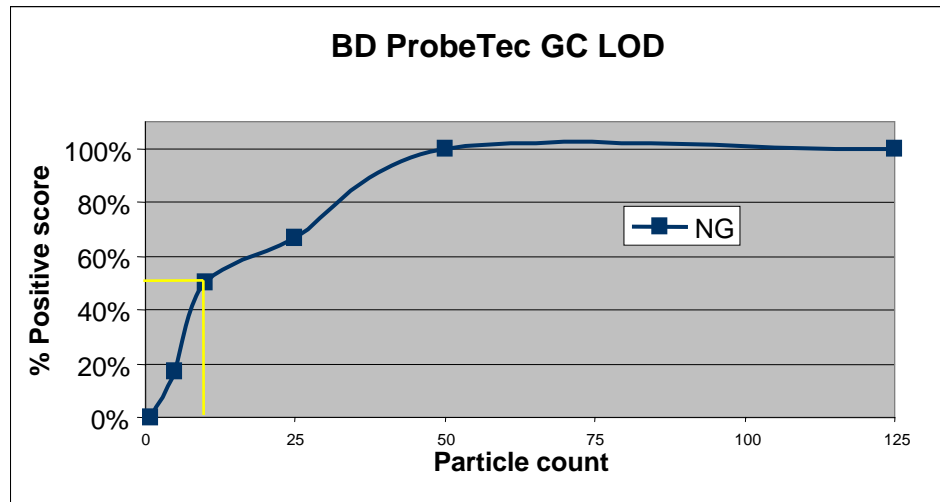
BD ProbeTec ET CT and NG Amplified DNA assay



EB/mL	% Pos
3	0%
10	8%
50	17%
125	33%
625	50%
1000	67%
1250	89%
2500	100%
5000	100%

EB/mL	% Pos
333	45.8%
400	55.3%
500	63.2%
500	80%
677	78.4%
833	31.4%
1,000	93.8%
2,000	100%
5,000	100%

Chalker et al., J Clin Microbiol 43, 2005



EB/mL	% Pos
1	0%
5	17%
10	50%
25	67%
50	100%
125	100%
625	100%

BD ProbeTec ET Package Insert LOD₉₅: 50 cells/ml

Low-Positive CT and NG Nucleic Acid Controls

Purpose of controls is to challenge each type of assay

Different assay sensitivities prevents use of a single control for all assays

Assay	C. trachomatis		N. gonorrhoeae	
	C ₅₀	C _{>95}	C ₅₀	C _{>95}
Aptima	3	10	250	625
Amplicor	38	75	1	3
ProbeTec	625	2500	10	50

Solution: Prepare Low-Positive Multi-Analyte CT and NG Nucleic Acid Controls targeted to each major assay platform

- A341 Series 100 for Gen-Probe APTIMA Combo2
- A341 Series 200 for Roche COBAS Amplicor CTNG
- A341 Series 400 for BD ProbeTec ET CTNG Amplified DNA Assay

Acknowledgements

SeraCare Life Sciences

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 - Diane McCoy
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 - Jim Doherty
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