



Evaluation of

## a novel *Chlamydia trachomatis* assay

for detection and genotyping of the different serovars in clinical samples

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- Pharma projects: Phase II & III, vaccine, antivirals, ....

# *Chlamydia trachomatis* (Ct)

- Most prevalent sexually transmitted bacterial pathogen
- 90 mln new cases per year worldwide

Indicators 2009 (Europe)	Chlamydia	Gonorrhoea	Syphilis
Rate per 100,000 population*	185.0	11.3	4.5
Number of countries reporting	23	28	29
Trends over 2006 - 2009	+ 42%	- 11%	- 14%
Male-to-female ratio in reported cases	0.7	2.5	3.1
Percentage in young people <25 years**	75%	45%	18%

\*Only calculated for countries with comprehensive surveillance systems

\*\*Based on countries with known information regarding the indicators

# Spectrum of diseases and sequelae in adults and maternally related neonatal infections with oculogenital serovars of *C. trachomatis*

	Men	Women	Neonates and infants
Disease	urethritis epididymitis proctitis prostatitis	urethritis cervicitis endometritis salpingitis PID <sup>1</sup> perihepatitis / FHC <sup>2</sup>	conjunctivitis pneumoniae Pharyngitis
Sequelae	urethral stricture infertility Reiter's syndrome	ectopic pregnancy infertility Reiter's syndrome	obstructive lung disease

<sup>1</sup>PID: pelvic inflammatory disease

<sup>2</sup>FHC: Fitz-Hugh-Curtis syndrome

# *C. trachomatis* serovar classification and tissue tropism

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Biovar	Serovars	Sites of infection
Trachoma	A, B, Ba, C	Conjunctivae; urogenital tract (rare)
Oculogenital	D, Da, E, F, G, Ga, H, I, Ia, J, K	Urogenital tract; conjunctivae; respiratory tract (rare)
LGV	L1, L2, L2a, L3	Inguinal lymph nodes, urogenital tract, rectum

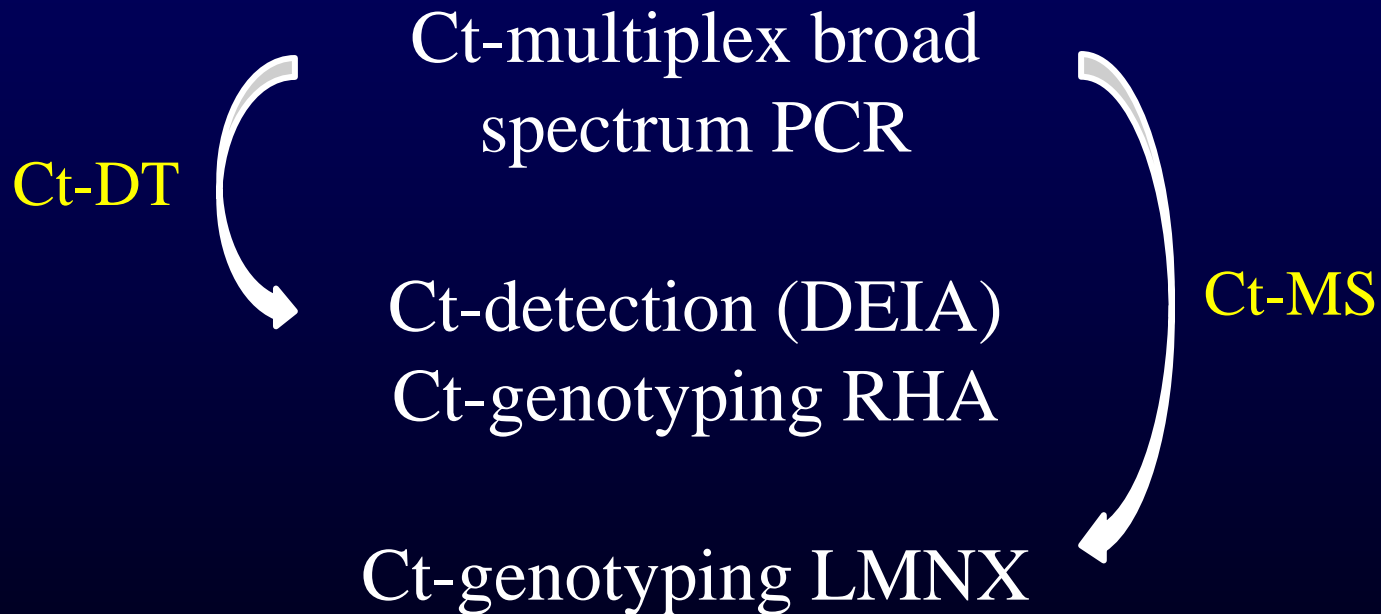
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Serogroup B	Serogroup C	Serogroup I
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# Why Ct genotyping?

- Treatment:
  - LGV proctitis
- Research:
  - Epidemiology
  - STI contact tracing
  - Analysis of cofactor role in cervical cancer
  - Analysis of role in reactive arthritis
- Vaccination trials

# *Chlamydia trachomatis* detection and genotyping assays

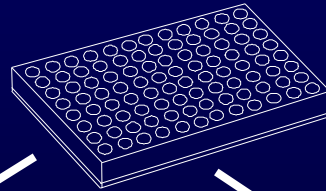


# Algorithm in Ct detection and genotyping

Cervical scrape  
First void urine  
Biopsy

DNA isolations

Detection in microtiter plate (DEIA)



CT-negative

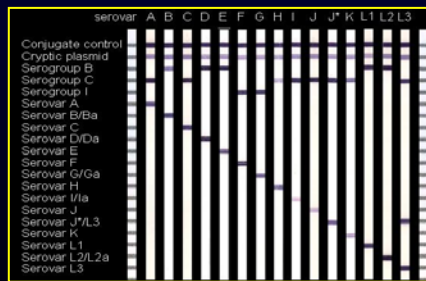
CT-positive

CT genotypes

Ct amplification step

Ct detection step

Ct genotyping step



RHA

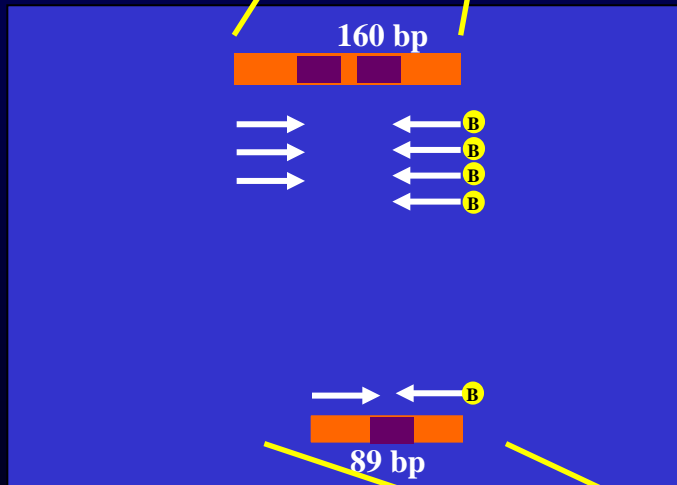
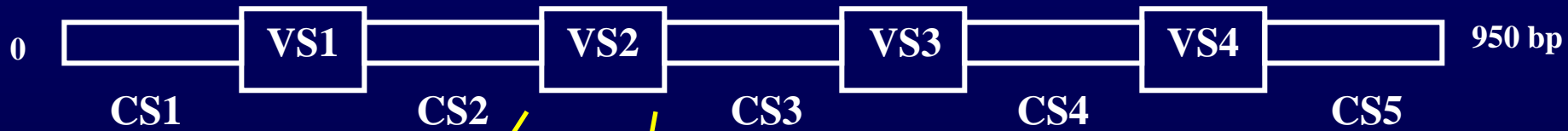
Table 1. Type specificity of the Ct-MS assay for 15 Ct reference strains\*

Probe	<i>Chlamydia trachomatis</i> genotypes/serovars														
	Group B					Group I					Group C				
	B	D	E	L1	L2	F	G	A	C	H	I	J	J <sup>III</sup>	K	L3
Comp	322	377	74	676	349	273	358	422	328	234	359	429	328	287	409
Ct-Cp <sup>a</sup>	2524	2476	2511	2742	2796	2566	2381	2376	2144	2663	2409	1331	2187	2187	2566
Netgroup probes															
Ct-IgH	1744	3278	1992	2488	1786	1	1	1	0	1	1	1	1	1	10
Ct-IgI	7	2	2	2	2	2	363	2888	2	3	2	1	2	2	2
Ct-IgC	7	7	7	7	7	7	2	3574	3176	3017	4107	3204	2414	2073	2701
Netovar probes															
Ct-B/Ba	5258	2	2	1	3	1	1	1	1	0	1	1	1	1	1
Ct-B/Da	1	1980	1	1	1	1	2	1	1	1	4	1	1	1	1
Ct-E	1	1	1876	1	1	1	1	1	1	1	1	1	1	1	1
Ct-L1	1	1	2	2679	2	1	2	2	1	1	1	2	1	1	2
Ct-L2/L2a	4	3	3	45	3889	3	3	4	3	3	3	3	2	3	4
Ct-F	2	3	3	2	3	4712	2	2	2	2	2	3	3	2	2
Ct-G/Ga	3	3	3	2	3	4	1360	2	3	3	3	3	2	3	2
Ct-A	2	2	2	2	2	2	2	2	2	3	2	2	2	2	2
Ct-C	1	1	1	2	1	1	3	1221	1	1	2	2	1	1	4
Ct-H	1	1	1	1	1	2	2	2	2	1	1979	1	1	2	1
Ct-I/a	2	2	2	2	2	3	4	4	2	4	2	2769	4	2	3
Ct-J	2	2	2	2	2	3	2	3	4	2	2	1732	2	5	2
Ct-J/L3	2	2	2	2	2	2	2	2	2	3	4	3	754	2	408
Ct-K	2	2	2	2	2	2	2	3	2	2	2	2	2	2	2447
Ct-L3	2	2	2	2	2	2	2	2	2	2	2	2	2	2	712

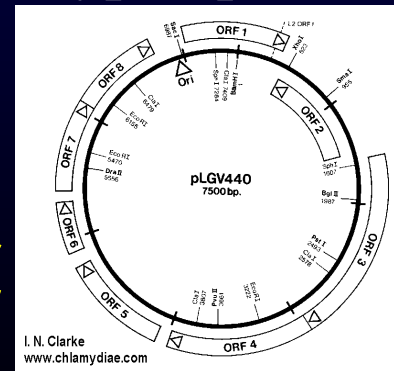
LMNX

# Ct amplification step with multiplex broad-spectrum PCR

Bacterial chromosome, *ompI*



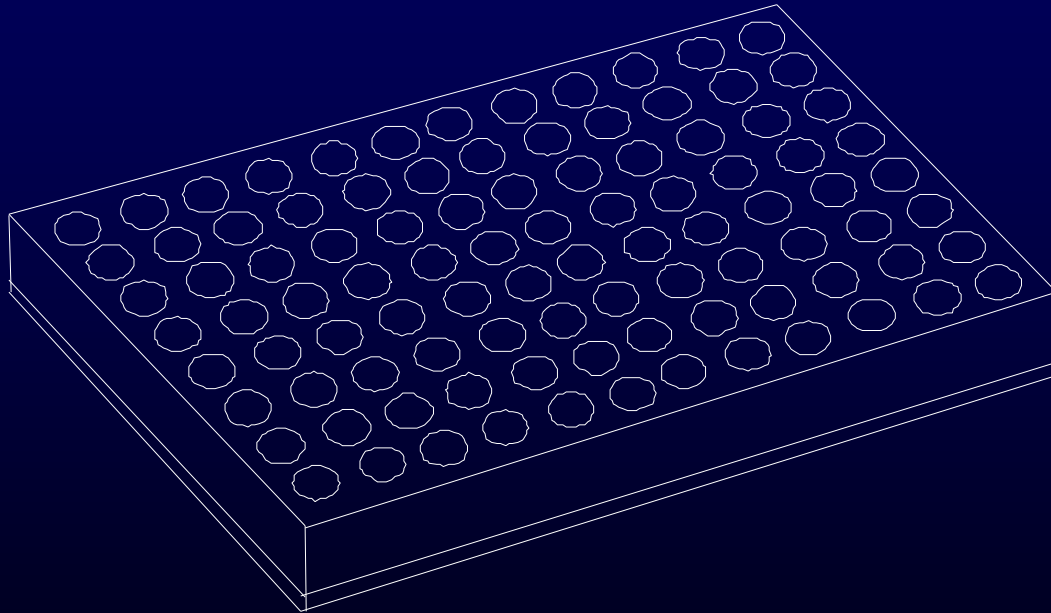
Cryptic plasmid



*ompI*: outer membrane protein I  
CP: Sweden variant detectable

# Ct-DT

Ct Detection (DEIA)



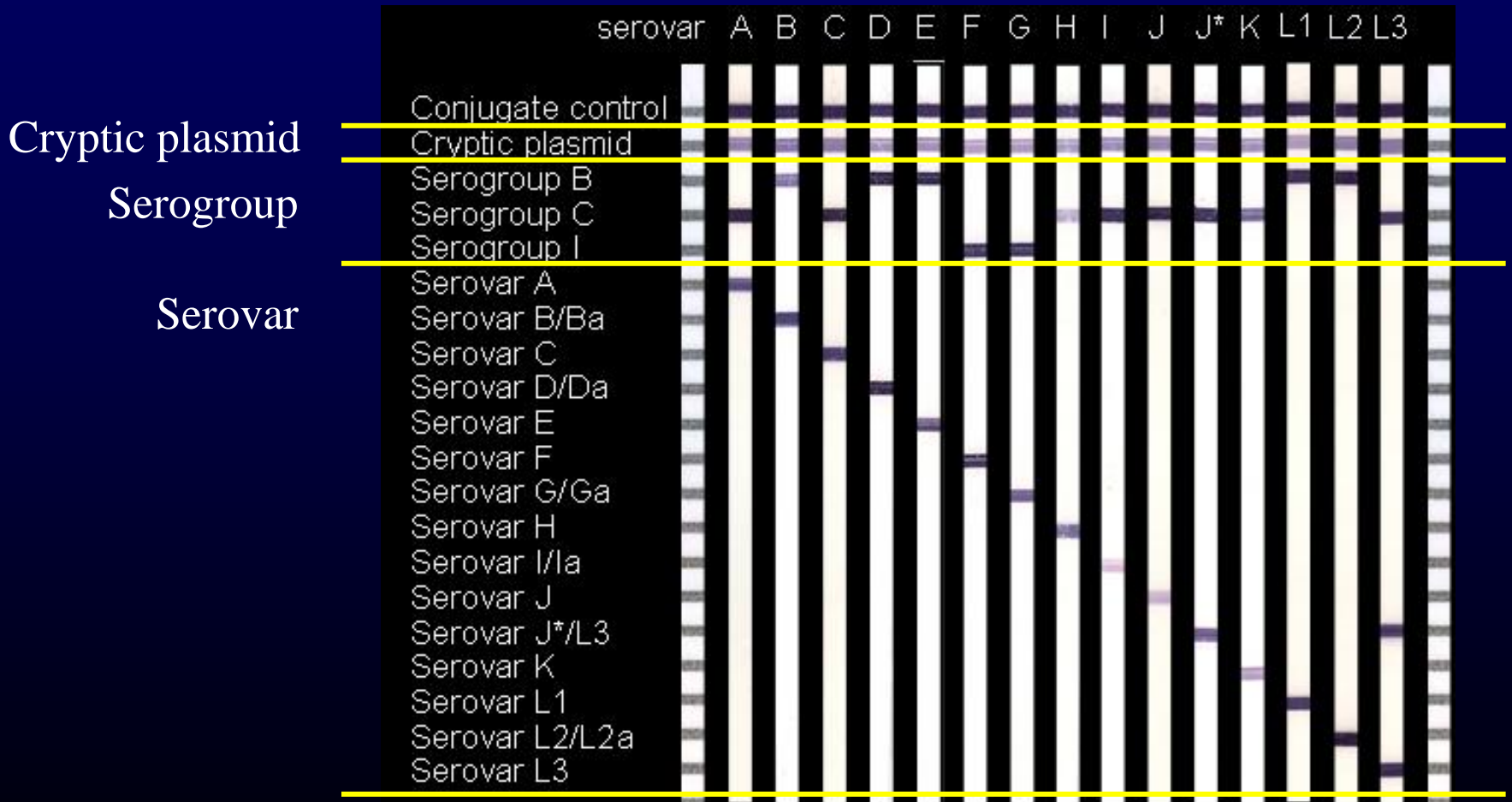
# Comparison Ct-DT assay with Cobas Taqman (Roche)

**Table 1.** *C. trachomatis* Detection Results of a Panel of 290 Cervical Swabs from Uganda and the Netherlands Tested with Both the Cobas TaqMan and the Multiplex PCR-DEIA Ct-Detection Step (Ct-DT Assay)

		Cobas TaqMan						
		The Netherlands ( <i>n</i> = 99)		Uganda ( <i>n</i> = 191)		Total	Total ( <i>n</i> = 290)	
		Positive	Negative	Positive	Negative		Positive	Negative
Multiplex PCR- DEIA	Positive	97	2	51	2	152	148	4
	Negative	0	0	3	135	138	3	135
	Total	97	2	54	137	290	151	139
		Not determined		$\kappa = 0.93$ (95% confidence interval = 0.88 to 0.99)			$\kappa = 0.95$ (95% confidence interval = 0.92 to 0.99)	

# Ct-DT

## Ct Genotyping (RHA)



# Ct-MS

## Ct Genotyping (LMNX)

Table 1. Type specificity of the Ct-MS assay for 15 Ct reference strains<sup>a</sup>

<i>Chlamydia trachomatis</i> genotypes/serovars															
Probe	Group B				Group I				Group C						
	B	D	E	L1	L2	F	G	A	C	H	I	J	J <sup>var</sup>	K	L3
<b>Conj</b>	<b>8574</b>	<b>8572</b>	<b>7444</b>	<b>8795</b>	<b>8640</b>	<b>8273</b>	<b>8555</b>	<b>8227</b>	<b>7858</b>	<b>8234</b>	<b>8694</b>	<b>8224</b>	<b>9238</b>	<b>9187</b>	<b>8668</b>
<b>Ct-CP<sup>#</sup></b>	<b>2624</b>	<b>2496</b>	<b>2511</b>	<b>2742</b>	<b>2916</b>	<b>2635</b>	<b>2666</b>	<b>2581</b>	<b>2376</b>	<b>2414</b>	<b>2663</b>	<b>2409</b>	<b>1931</b>	<b>2539</b>	<b>2465</b>
<b>Serogroup probes</b>															
<b>Ct-gB</b>	<b>1764</b>	<b>3290</b>	<b>1992</b>	<b>2480</b>	<b>1786</b>	1	1	1	0	1	1	1	1	1	10
<b>Ct-gI</b>	7	2	2	2	2	<b>3363</b>	<b>2888</b>	2	3	2	3	2	2	2	2
<b>Ct-gC</b>	2	2	2	2	2	2	2	<b>3574</b>	<b>3176</b>	<b>3017</b>	<b>4107</b>	<b>3204</b>	<b>2514</b>	<b>2073</b>	<b>2702</b>
<b>Serovar probes</b>															
<b>Ct-B/Ba</b>	<b>5250</b>	2	2	1	3	1	1	1	1	0	1	1	1	1	1
<b>Ct-D/Da</b>	1	<b>1980</b>	1	1	1	1	2	1	1	1	4	1	1	1	1
<b>Ct-E</b>	1	1	<b>1876</b>	1	1	1	1	1	1	1	1	1	1	1	1
<b>Ct-L1</b>	1	1	2	<b>2679</b>	2	1	2	2	1	1	1	2	1	1	2
<b>Ct-L2/L2a</b>	4	3	3	6	<b>3030</b>	3	3	5	4	3	3	3	2	3	4
<b>Ct-F</b>	2	3	3	2	3	<b>4712</b>	2	2	2	2	2	3	3	2	2
<b>Ct-G/Ga</b>	3	3	3	2	3	4	<b>1360</b>	3	3	3	3	3	2	3	2
<b>Ct-A</b>	2	2	2	2	2	2	2	<b>5645</b>	2	3	2	2	2	2	2
<b>Ct-C</b>	1	1	1	2	1	1	1	3	<b>1221</b>	1	1	2	2	1	4
<b>Ct-H</b>	1	1	1	1	1	2	2	2	1	<b>1979</b>	1	1	1	1	1
<b>Ct-I/Ia</b>	2	2	2	2	2	2	3	4	4	2	<b>2769</b>	4	2	3	3
<b>Ct-J</b>	2	2	2	2	2	3	2	3	4	2	2	<b>1732</b>	2	5	2
<b>Ct-J/L3</b>	2	2	2	2	2	2	2	3	2	3	4	3	<b>754</b>	2	<b>400</b>
<b>Ct-K</b>	2	2	2	2	2	2	2	3	2	2	2	2	2	<b>2447</b>	2
<b>Ct-L3</b>	2	2	1	1	2	1	2	1	2	1	2	1	2	2	<b>7129</b>

<sup>a</sup> Analytical type-specificity of the Ct-MS assay. Indicated are the MFIs read outs of the Ct-DT PCR amplicons generated from Ct reference strains (listed on top) in relation to the bead-bound capture probes (listed on the left). The conjugate control (conj) serves as the positive control for correct incubation with the detection conjugate for each separate serovar.

# Comparison Ct-MS with Ct-DT assay (Detection)

Table 3. Comparison of Ct detection by the Ct-DT DEIA assay and the Ct-MS assay (n=712)

	Ct-MS assay	
Ct-DT assay	+	-
DEIA+	670	2
DEIA-	2	38

Kappa = 0.947 (95% CI = 0.895-0.999)  
McNemar's p= 1.000

Cut-off Ct-MS assay = 100 MFI

# Comparison Ct-MS with Ct-DT assay (Genotyping)

Table 4. Comparison of genotyping findings of the Ct-DT assay and the Ct-MS assay in 712 Ct positive samples

	MFI threshold 30					MFI threshold 100						
	MS and RHA positive	RHA positive Only	MS positive only	MS and RHA negative	Kappa (95% CI)	McNemar p- value	MS and RHA positive	RHA positive only	MS positive only	MS and RHA negative	Kappa (95% CI)	McNemar p- value
Probe cp	668	1	2	41	0.962 (0.920-1.000)	1.000	668	1	2	41	0.962 (0.920-1.000)	1.000
<b>Serogroup probes</b>												
Probe gB ●	308	→ 2	0	402	0.994 (0.986-1.000)	0.500	305	5	0	402	0.986 (0.973-0.998)	0.063
Probe gI	214	→ 1	0	497	0.997 (0.990-1.000)	1.000	214	1	0	497	0.997 (0.990-1.000)	1.000
Probe gC ●	108	→ 5	0	599	0.973 (0.950-0.997)	0.063	107	6	0	599	0.968 (0.942-0.993)	<b>0.031</b>
<b>Serovar probes</b>												
Probe B ●	10	0	0	702	1.000	1.000	10	0	0	702	1.000	1.000
Probe D	60	2	0	650	0.982 (0.957-1.000)	0.500	58	4	0	650	0.964 (0.928-0.999)	0.125
Probe E	231	2	0	479	0.994 (0.985-1.000)	0.500	227	6	0	479	0.981 (0.965-0.996)	<b>0.031</b>
Probe L1	0	0	0	712	-	-	0	0	0	712	-	-
Probe L2	6	0	0	706	1.000	1.000	6	0	0	706	1.000	1.000
Probe F	114	0	0	598	1.000	1.000	114	0	0	598	1.000	1.000
Probe G	100	0	0	612	1.000	1.000	99	1	0	612	0.994 (0.983-1.000)	1.000
Probe A ●	0	0	0	712	-	-	0	0	0	712	-	-
Probe C	0	0	0	712	-	-	0	0	0	712	-	-
Probe H ●●	10	→ 1	0	701	0.952 (0.857-1.000)	1.000	9	2	0	701	0.899 (0.758-1.000)	0.500
Probe I ●	10	→ 0	0	702	1.000	1.000	10	0	0	702	1.000	1.000
Probe J ●	44	→ 1	1	666	0.976 (0.943-1.000)	1.000	44	1	1	666	0.976 (0.943-1.000)	1.000
Probe J/L3	10	1	0	701	0.952 (0.857-1.000)	1.000	10	1	0	701	0.952 (0.857-1.000)	1.000
Probe K ●	33	0	0	679	1.000	1.000	32	1	0	679	0.984 (0.952-1.000)	1.000
Probe L3 ●	0	0	3	709	-	-	0	0	0	712	-	-
<b>Total probes</b>	<b>1926</b>	<b>16</b>	<b>6</b>	<b>11580</b>	<b>0.993 (0.977-1.000)</b>	<b>0.053</b>	<b>1913</b>	<b>29</b>	<b>3</b>	<b>11583</b>	<b>0.990 (0.974-1.000)</b>	<b>&lt;0.001</b>

Bold p values are considered significant

- Optimized probes in LMNX system
- Degradation of amplimers tested
- Different amounts tested
- cut-off multiple infections

# Comparison Ct-MS with Ct-DT assay (Genotyping multiple infections)

Table 5. MFI values of the Ct-MS assay among the 20 multiple infections determined with the Ct-DT assay (MFI threshold = 30)

<i>Multiple serovar Ct infections determined with the Ct-DT RHA</i>																				
probe	F&K <sup>®</sup>	E&gI	E&G <sup>®</sup>	E&K <sup>®</sup>	E&G	E&G	E&K	D&G	E&I	E&K	F&gC <sup>#</sup>	E&J	H&K <sup>§</sup>	E&F	E&J	H&K <sup>§</sup>	G&gC	E&J	F&K	E&G
Ct-CP	5290	5041	5357	5337	5667	4876	5291	5298	5368	6515	6159	6044	5596	5621	5825	5643	4221	3272	6837	4142
Serogroup probes																				
Ct-gB	1	1427	53	84	826	103	154	165	1240	163	1	800	1	268	1863	1	1	16*	1	22*
Ct-gI	257	27*	2245	2	1407	2219	2	1496	3	2	2976	2	2	1996	2	2	506	2	1346	224
Ct-gC	76	1	1	1900	2	2	2002	2	3441	1098	443	2240	1717	2	2600	197	2*	155	1382	2
Serovar probes																				
Ct-B/Ba	1	2	1	1	1	2	1	1	2	0	1	1	1	1	2	1	0	1	2	1
Ct-D/Da	0	1	1	1	1	1	1	95	3	1	1	1	1	1	1	1	1	1	1	1
Ct-E	1	1501	30	44	454	55	79	1	1112	134	2	565	3	143	1995	2	2	15*	5	20*
Ct-L1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	1	1	1	1	1	2
Ct-L2/L2a	2	3	3	3	3	2	2	3	3	3	3	2	4	3	3	3	3	3	3	3
Ct-F	3427	2	2	2	2	3	2	2	2	2	2	2	2	2409	2	2	2	2	1869	2
Ct-G/Ga	4	9	1019	3	675	879	2	624	2	2	3590	3	2	4	2	2	157	3	3	49
Ct-A	3	3	3	2	3	4	3	3	3	4	3	4	4	4	3	4	3	5	7	2
Ct-C	1	2	1	1	1	2	1	2	1	1	2	2	1	2	5	2	1	2	1	2
Ct-H	2	1	1	1	1	2	1	1	2	2	1	1	69	2	1	2*	1	1	2	1
Ct-I/Ia	2	2	2	4	3	2	2	2	1769	3	3	3	3	2	5	3	2	3	4	2
Ct-J	2	2	3	4	1	2	4	2	3	4	446	854	5	3	1624	4	2	3	6	2
Ct-J/L3	1	2	2	2	2	2	2	2	2	2	3	2	2	2	3	2	2	12*	3	2
Ct-K	69	2	2	1562	2	3	1664	2	2	2	2163	2	2	2042	2	2	1304	2	2467	2
Ct-L3	1	2	2	1	1	2	1	2	3	1	1	1	2	2	2	2	2	2	2	1

\* Determined positive with the Ct-DT RHA, but negative with the Ct-MS assay at a MFI threshold of 30

# Determined with the Ct-DT assay as a multiple infection with serovar F and serogroup C. The Ct-MS assay revealed serovar J, belonging to serogroup C.

§ Sequencing revealed a genovariant of serovar K. So also for the Ct-MS assay it is recommended to sequence multiple serovars belonging to one serogroup

@ Recognized as double serovar infection at a threshold of 30 MFI, while not recognized as double infection at a threshold of 100 MFI

Bold numbers indicate a positive MFI value

# Conclusions


- At detection level Ct-DT (and Ct-MS) assays are highly comparable with TaqMan Cobas ( $\kappa = 0.95$ )
- At detection ( $\kappa = 0.947$ ) and genotyping level ( $\kappa = 0.993$ ) Ct-DT and Ct-MS assays are highly comparable
- Ct-DT requires upgrade to Ct-MS probes (on going)
- In multiple infected samples occasionally a very weak signals is observed for the second genotype

# Serovar distribution in cervical swabs for different countries

Ct serovar distribution and percentage of multiple Ct infections. Distribution was determined with the Ct-genotypings assay.

Country	Most prevalent serovar	Second most prevalent serovar	Third most prevalent serovar	Percentage Multiple infections
The Netherlands (n=94)	E (40%)	F (25%)	G/Ga (10%)	4%
Uganda (n=53)	G/Ga (45%)	E (23.6%)	F (9%)	4%
Costa Rica (n=806)	E (31%)	F and D/Da (21%)	I/Ia (15%)	1.7%
Russia (n=180)	E (34%)	G/Ga (24%)	J (9%)	3.9%

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  - UvA:
    - H. de Vries
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    - W. Melchers
  - Labo Biomedical Products:
    - J. Lindeman
- 
- Assay design, development, & validation
  - Epidemiology
  - Clinical trials
  - Samples
  - Scientific input
  - Comparison with RFLP
  - Samples
  - Scientific input
  - Comparison with COBAS Taqman
  - Providing assays