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High-Resolution Genotyping of *Chlamydia trachomatis* by Use of a Novel Multilocus Typing DNA Microarray▽†

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Typing of *Chlamydia trachomatis* is important to understanding its epidemiology. Currently used methods such as DNA sequencing of the *ompA* gene and multilocus sequence typing (MLST) either offer limited epidemiological resolution or are laborious and expensive, or both. DNA microarray technology using the ArrayStrip format is an affordable alternative for genotyping. In this study, we developed a new multilocus typing (MLT) DNA microarray, based on the target regions of a high-resolution MLST system as well as software for easy analysis. Validation of the array was done by typing 80 previously MLST-typed clinical specimens from unselected adolescents in school. The MLT array showed 100% specificity and provided 2.4-times-higher resolution than *ompA* sequencing, separating the commonly predominating *ompA* E/Bour genotype into 7 MLT array genotypes. The MLT array reproduced epidemiological findings revealed by the MLST system and showed sufficient sensitivity to work with clinical specimens. Compared to MLST analysis, the expenses needed for testing a sample with the MLT array are considerably lower. Moreover, testing can be completed within 1 working day rather than 3 or 4 days, with data analysis not requiring highly specialized personnel. The present MLT array represents a powerful alternative in *C. trachomatis* genotyping.

Urogenital infection with *Chlamydia trachomatis* is one of the most common sexually transmitted infections worldwide (28). *C. trachomatis* also causes lymphogranuloma venereum (LGV), which is a less common but more severe sexually transmitted disease, and trachoma, which is a major cause of preventable blindness in the developing world. Severe sequelae from urogenital chlamydia include ectopic pregnancies and infertility (9). In spite of testing, treatment, partner notification, and counseling, huge public health efforts have not been able to control urogenital chlamydia. Current knowledge about the role of repeated infections and transmission in sexual networks is still limited and needs to be extended to achieve a reduction in the number of infections.

In this context, it is important to have adequate tools for genotyping to understand the epidemiology of *C. trachomatis*. Such tools should preferably be highly affordable, fast, and easy to use and need to have enough resolution to discriminate among closely related clinical strains to allow outbreak tracing and epidemiological surveillance.

Traditional typing of *C. trachomatis* was done by serotyping of the major outer membrane protein (MOMP), which is a time-consuming process requiring several passages in eukaryotic cell culture and large panels of antibodies. Later on, typing included PCR to amplify the *ompA* gene, which encodes MOMP, followed by restriction fragment length polymorphism analysis or DNA sequencing. However, neither MOMP nor *ompA* sequencing provides sufficient epidemiological resolution (21). In Sweden, for example, almost half of all urogenital chlamydia infections are of serotype E, and within this serotype, the *ompA* E/Bour genotype predominates (14, 17).

Multilocus sequence typing (MLST) relies on PCR amplification and DNA sequencing of several genomic loci. There are three such systems described for *C. trachomatis*. Two of them are based on housekeeping genes, have a resolution similar to that of *ompA* sequencing, and are suitable for evolutionary studies (4, 20). The third system was developed by Klint et al. (15) and is intended for short-term clinical epidemiology and outbreak investigations. The latter system is based on five highly variable but stable genomic loci (*hctB*, CT058, CT144, CT172, and *pbpB*), which give the system up to 5-fold-higher resolution than that of *ompA* sequencing (15; K. Gravningen, L. Christerson, A. S. Furberg, G. S. Simonsen, K. Ödman, A. Ståhlsten, and B. Herrmann, submitted for publication). Since its creation in 2006, the system has been applied to a variety of clinical specimens, including urogenital chlamydia (3, 11, 13), LGV (2), and trachoma (10). There is also a multilocus variable-number tandem repeat (VNTR) analysis (MLVA) system (22) which offers a resolution similar to that of the MLST system by Klint et al. (12, 15). MLST and MLVA, however, are laborious techniques requiring several days of work until final results can be achieved.

Diagnostic DNA microarray technology has emerged as an alternative in microbial genotyping. While most of the commercially available equipment is still too expensive for smaller laboratories, the ArrayStrip platform represents an easy-to-

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TABLE 1. Primers used in the MLT array

Locus	Primer	Sequence (5′ → 3′)[a]	Product size(s) (bp)	Reference
CT144	CT144-248F	Bio-ATGATTAACGTGATTTGGTTTCCTT	799–808	15
	CT144-1046R	Bio-GCGCACCAAAACATAGGTACT		15
CT172	CT172-268F	Bio-CCGTAGTAATGGGTGAGGGA	376–685	15
	CT172-610R	Bio-CGTCATTGCTTGCTCGGCTT		15
*pbpB*1	pbpB1F	Bio-TATATGAAAAGAAAACGACGCACC	843	15
	pbpB823R	Bio-CAGCATAGATCGCTTGCCTAT		13
*pbpB*2	pbpB1455F	Bio-GGTCTCGTTTTTGATGTTCTATTC	920	13
	pbpB2366R	Bio-TGGTCAGAAAGATGCTGCACA		15
CT058	CT222F	Bio-CTTTTCTGAGGCTGAGTATGATTT	1474–1500	15
	CT1678R	Bio-CCGATTCTTACTGGGAGGGT		15
hctB	hctB39F	Bio-CTCGAAGACAATCCAGTAGCAT	579–904	15
	hctB794R	Bio-CACCAGAAGCAGCTACACGT		15
ompA	Trach-VD1-fw	Bio-ACCAAGCCTTATGATCGAC	326	23
	Trach-VD1-rv	Bio-AGAATACATCAAAACGATCCCA		23
	Trach-VD2-rv	Bio-TTGAGCATATTGGAAAGAAGC	572[b]	23
	Trach-VD4-fw	Bio-CTTACATTGGAGTTAAATGGTCT	231	23
	Trach-VD4-rv	Bio-CTACTGCAATACCGCAAGA		23

[a] Bio, biotin.
[b] With primers Trach-VD1-fw and Trach-VD2-rv.

handle and affordable solution. It features microarrays implanted on the bottoms of standard 8-well microtiter strips and allows high throughput, as 96 samples can be analyzed in parallel. All steps of the hybridization reaction can be conducted in the vessel carrying the array, without the need for a separate hybridization chamber or other expensive laboratory equipment. It has been shown that specific hybridization patterns can be obtained from a single PCR-amplifiable target copy (5). This technology helps to avoid extensive sequencing and has been used in both research and different routine diagnostic applications (1, 19, 26).

The aim of the current study was to develop a multilocus typing (MLT) DNA microarray for *C. trachomatis* based on the target regions of the MLST system by Klint et al. (15) and to validate it using previously MLST-typed clinical specimens.

MATERIALS AND METHODS

Specimens. Reference strains ($n = 18$) and clinical specimens ($n = 109$) for optimization and for the database came from heterosexual populations in different countries in Europe, from men who have sex with men (MSM) in Europe and North America, and from trachoma cases in the Gambia and Senegal. Clinical specimens for evaluation of the optimized array came from Finnmark in Norway ($n = 80$) and were collected from adolescents in school but were otherwise unselected (Gravningen et al., submitted). All specimens were previously MLST genotyped.

DNA extraction. DNA was extracted from culture, urine, or rectal specimens using a MagAttract DNA mini-M48 kit (Qiagen, Hilden, Germany) on a BioRobot M48 workstation (Qiagen), according to the manufacturer's instructions.

Multiplex biotinylation PCR. Amplification of the five MLST regions and *ompA* was conducted in three separate reactions using the Qiagen multiplex PCR kit (Qiagen, Hilden, Germany) and 5′-biotinylated primers (Table 1). All reaction mixes included 10 μl Qiagen multiplex mastermix and 2 μl DNA template. Reaction mixture 1 contained 1 μl of a 10 μM solution (final in-tube concentration of 0.5 μM) of each forward and reverse primer for CT144, CT172, *pbpB1*, and *pbpB2*. Reaction mixture 2 contained 1 μl each of the CT058 primers (same concentration as above). Reaction mixture 3 contained 1 μl each of *hctB* primers and Trach-VD4-fw/rv, as well as 3 μl of primer Trach-VD1-fw, 1 μl of Trach-VD1-rv, and 2 μl of Trach-VD2-rv. All three reactions used the same PCR cycling conditions, as follows: initial denaturation at 95°C for 15 min, 40 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 90 s, and elongation at 72°C for 90 s, with a final elongation at 72°C for 10 min, followed by cooling at 4°C. PCR products were analyzed using gel electrophoresis and a 1.5% agarose gel containing ethidium bromide and visualized on a UV transilluminator (G:Box imager and GeneSnap software obtained from Syngene, Cambridge, United Kingdom). After amplification, reaction mixtures 1 to 3 were combined into a final PCR product mixture, of which 3 μl was used per hybridization reaction.

Probe and MLT array design. The design of oligonucleotide probes was based on data generated with the MLST system by April 2009. At that time, the database (http://mlstdb.bmc.uu.se/) included 385 specimens: 16 reference strains; 287 urogenital specimens from heterosexuals, mainly from Sweden ($n = 177$) and the Netherlands ($n = 70$), and from MSM ($n = 38$); 30 trachoma specimens from the Gambia and Senegal; and 52 LGV specimens from Europe ($n = 42$) and the United States ($n = 10$). In total, 153 unique sequence variants had been identified for the five MLST regions.

The probe design was done using BioEdit 7.0.9 (Ibis Therapeutics, Carlsbad, CA), Microsoft Office Excel 2003 together with Visual Basic for Applications 6.3 (Microsoft, Redmond, WA), and Basic Local Alignment Search Tool (BLAST) analysis. All together, the final version of the array harbored 271 probes: 35 for the *hctB* locus, 58 for CT058, 39 for CT144, 16 for CT172, 62 for *pbpB*, and 61 for *ompA*. The *ompA* probes were designed previously using a different process (23).

The probes were spotted onto microarray chips as previously described (24) and assembled in an 8-well microtiter strip format called ArrayStrip (Alere Technologies GmbH, Jena, Germany). Out of the 271 probes, 124 were spotted 3-fold, and 147, as well as biotinylated oligonucleotide staining controls, were spotted 2-fold, thus bringing the total number of active spots to 668.

ArrayStrip hybridization. Hybridization was conducted using the hybridization kit (Alere Technologies) by following the instructions of the manufacturer, as described previously (23). Briefly, 3 μl of the final PCR product mixture was used, the hybridization buffer was modified by adding 10% (vol/vol) dimethyl sulfoxide (DMSO; Roth, Karlsruhe, Germany), and all washing and hybridization steps were performed at 45°C, using a heatable horizontal tube shaker (BioShake iQ; Quantifoil Instruments, Jena, Germany). Hybridization signals were measured using an ArrayMate transmission reader (Alere Technologies).

Processing of ArrayStrip hybridization data. The hybridization data were processed using the Iconoclust software, version 3.3 (Alere Technologies). Normalized signal intensities were calculated automatically by the software as described previously (23), subsequently transferred into Microsoft Office Excel

2003 (Microsoft), and analyzed using the newly developed MLT Line software, version 2.0. The software was written in Visual Basic for Applications 6.3 (Microsoft) and integrated into Excel. More detailed information about the software can be found in Appendix S1 in the supplemental material.

RESULTS

Probe design. When *in silico* work on probe selection was conducted, 153 sequence variants of the five MLST regions were known, based on all available sequences from our database (http://mlstdb.bmc.uu.se/). A total of 210 probes were defined (see Table S1 in the supplemental material) to identify these variants (for details, see Appendix S1 in the supplemental material). The probes were designed to minimize the mean variation in the melting temperatures (T_m), resulting in a mean T_m of 57.8°C (minimum, 56.5°C; maximum, 59.1°C). The average length was 25 nucleotides (nt) (minimum, 17 nt; maximum, 36 nt), and the average GC content was 43% (minimum, 23%; maximum, 68%). Self-annealing was kept as low as possible, and all probes were checked against all 153 sequence variants to ensure specificity and avoid cross-reactions.

The *ompA* probes had been designed previously (23). However, more *ompA* sequences had been published in GenBank since the time of that study, so the *in silico* analysis had to be updated. This resulted in 86 unique *ompA* variants covering the VD1 to VD4 regions (by July 2010), compared to 54 in the previous study. This set of probes could theoretically discriminate among 67 variants.

Optimization. The approach consisted of identifying experimental conditions leading to hybridization patterns that came as close to the theoretically expected ones as possible, i.e., representing hybridization at high stringency. In total, 68 experiments were conducted to find the following optimal hybridization conditions: a temperature of hybridization and wash steps of 45°C, a 10% (vol/vol) DMSO content in hybridization buffer, and an amount of 3 μl of PCR product in the hybridization mix. To verify reproducibility, the experiments were conducted in duplicate in two different laboratories.

Building the database. To test the MLT array, 18 type and reference strains representing the MOMP-based serovars of *C. trachomatis* were examined. Initial results showed discrepancies between the empirical data and the theoretical binding patterns (data not shown). Therefore, an additional panel of 109 specimens representing the majority of the variation observed in the MLST system was tested. Together with the 18 *ompA* reference strains, these 127 specimens were analyzed in 139 experiments, which were entered into a database and used to adapt the theoretical binding patterns. For a few sequence variants for which strains were not available, the binding patterns were manually corrected in accordance with the empirical data from similar strains. This procedure solved most specificity problems in all but the CT172 region, which therefore was excluded from further analysis.

The experiments in the database were processed in a sequential manner, in which one experiment at a time was removed from the database and then analyzed so as not to affect its own analysis. The 139 experiments could be divided into 31 *ompA* genotypes, 80 MLST genotypes, and 63 MLT array genotypes, giving the MLT array 2.0-times-higher resolution than *ompA* sequencing. Three of the experiments were incorrectly genotyped, resulting in a specificity of 98%. These three experiments were redone, resulting in one of them being correctly genotyped and two being marked as having inconclusive results by the MLT Line software.

Validation of clinical specimens. The MLT array was applied to 80 specimens from Finnmark in Norway. In the first run, 16 out of 80 specimens (20%) were marked by the MLT Line software as having inconclusive results. These 16 specimens were reanalyzed, in multiplex or simplex reactions, after which only 2 specimens (2.5%) remained inconclusive. The remaining 78 specimens could be divided into 7 *ompA* genotypes, 19 MLST genotypes, and 17 MLT array genotypes (Table 2). This gave the MLT array 2.4-times-higher resolution than *ompA* sequencing. The *ompA* E/Bour genotype ($n = 20$) could be separated into 7 MLT array genotypes. Specimen F-31 was correctly identified as having a genotype unique to the new variant *C. trachomatis* (nvCT). Specimen F-74 was correctly identified as having a genotype previously found only among men who have sex with men (MSM). None of the 78 specimens were incorrectly genotyped, yielding a specificity of 100%.

Software. The newly developed MLT Line software, used for analysis, was integrated with Excel (Microsoft) for ease of use. The software was tested to work with Excel 2003, 2007, and 2010. The software (http://mlstdb.bmc.uu.se/) includes a database with 217 array experiments from specimens with known MLST genotypes. Analysis is done by simply pasting the normalized hybridization signal intensities into Excel and then clicking a button. The data are processed with MLT Line and then automatically stored and displayed in a separate Excel file.

DISCUSSION

Up until recently, genotyping of *C. trachomatis* was entirely based on analysis of *ompA*, which provided only a limited insight into the genetic variability of naturally occurring clinical strains (2, 13). More recent genotyping methods based on several genomic loci are expensive and time-consuming (15, 22), while the resolution of some of them is not significantly higher than that for *ompA* alone (4, 20).

ArrayStrip and ArrayTube DNA microarrays have been successfully used in a number of applications, including detection and extensive genotyping of *Staphylococcus aureus* (18), *Chlamydiaceae* spp. (24), *Chlamydia psittaci* (25), *Escherichia coli* (8), *Burkholderia* spp. (27), and avian influenza viruses (6, 7). The ArrayStrip platform has proved economical while allowing high throughput and has been shown to be suitable for routine diagnosis (1, 19) because of its rapidity, robustness, and high sensitivity (5).

In the present study, we wanted to improve the methodology of *C. trachomatis* genotyping by using the ArrayStrip technology. The aim was to develop a MLT DNA microarray based on the high-resolution MLST system by Klint et al. (15) and to validate it by typing a panel of clinical specimens.

Initial experiments showed that using theoretical binding patterns resulted in too-low specificity. In particular, the CT172 region contains a single nucleotide repeat, and the probes covering this repeat were too unstable to achieve adequate specificity. As most of the variation in CT172 is confined

TABLE 2. MLT array results of 80 specimens from Finnmark in Norway[b]

Specimen	Genovar	MLST region result[a]				Group[c]
		hctB	CT058	CT144	*pbpB*	
F-1	G	10	5, 7, 8, 10, 30	1	5, 18	1
F-10	G	8, 10	5, 7	1	5	1
F-14	G	10	5, 7, 8, 10, 30	1	5, 16, 18	1
F-15	G	8, 10	5, 7	1	5	1
F-17	G	8, 10	5, 7, 8, 10, 30	1	5	1
F-21	G	8, 10	5, 7, 8	1	5	1
F-25	G	8, 10	5, 7	1	5	1
F-26	G	8, 10	5, 8	1	5	1
F-29	G	8, 10	5, 7	1	5	1
F-3	G	8, 10	5, 7	1	5	1
F-39	G	8, 10	5, 7	1	5	1
F-4	G	8, 10	5, 7, 8, 30	1	5, 18	1
F-40	G	8, 10	5, 7	1	5	1
F-51	G	8, 10	5, 7	1	5	1
F-53	G	8, 10	5, 8	1	5	1
F-55	G	8, 10	5, 7	1	5	1
F-58	G	8, 10	5, 8	1	5	1
F-6	G	8, 10	5, 7, 8	1	5, 18	1
F-64	G	8, 10	5	1	5	1
F-65	G	8, 10	5, 7	1	5, 18	1
F-75	G	8, 10	5, 8	1	5	1
F-76	G	8, 10	5, 8	1	5	1
F-78	G	10	5, 7, 8, 10, 30	1	5, 16, 18, 23, 42	1
F-79	G	8, 10	5, 8	1	5	1
F-80	G	8, 10	5, 7	1	5, 18	1
F-9	G	8, 10	5	1	5	1
F-11	E	5	1	7	1, 2	2
F-12	F	5	1	15	4	3
F-73	F	5	1	15	4	3
F-13	F	5	1	7	4	4
F-18	F	5	1	7	4	4
F-24	F	5	1	7	4	4
F-27	F	5	1	7	4	4
F-28	F	5	1	7	4	4
F-32	F	5	1	7	4	4
F-33	F	5	1	7	4	4
F-34	F	5	1	7	4	4
F-37	F	5	1	7	4	4
F-45	F	5	1	7	4	4
F-50	F	5	1, 3, 25	7	2, 4, 5, 28, 29, 41	4
F-56	F	5	1	7	4	4
F-59	F	5	1	7	4	4
F-61	F	5	1	7	4	4
F-63	F	5	1	7	4	4
F-7	F	5	1	7	4	4
F-16	E	1, 4	1	7	2	5
F-49	E	1, 4	1	7	2	5
F-54	E	1, 4	1	7	2	5
F-19	K	8, 10	6	22	8	6
F-35	K	8, 10	6	22	6, 8	6
F-52	K	8, 10	6	22	6, 8	6
F-69	K	8, 10	6	22	8	6
F-23	E	7	1	7	1, 2	7
F-68	E	7	1	7	1	7
F-30	K	10	5, 7, 8, 10, 30	9	6, 8	8
F-31	E	14	1	1	1, 2	9
F-38	H	12	12	9	6	10
F-44	E	1	1	7	1	11
F-47	D	5	1	7	4	12
F-48	D	5	1	7, 18	2, 4	12
F-67	D	5	1	7	4	12
F-66	E	4, 14	1	7	1	13
F-71	E	7	1	14	1	14
F-72	D, Da	12	6	1	23	15
F-74	G	10	5, 8, 30	5	6	16

Continued on following page

TABLE 2—*Continued*

Specimen	Genovar	MLST region result[a]				Group[c]
		hctB	CT058	CT144	*pbpB*	
F-77	G	8, 10	36	22	6	17
F-5	E	1, 4	1	7	1	11 or 13
F-57	E	1, 4	1	7	1	11 or 13
F-70	E	1, 4	1	7	1	11 or 13
F-20	F	5	1	7, 14, 15, 18	4	3 or 4
F-60	F	5	1	7, 15, 18	4	3 or 4
F-42	E	1	1	7	1, 2	5 or 11
F-46	E	1	1	7	1, 2, 29	5 or 11
F-8	E	1	1	7	1, 2	5 or 11
F-2	E	1, 4	1	7	1, 2	5, 11, or 13
F-22	E	1, 4	1	7	1, 2	5, 11, or 13
F-41	E	1, 4	1	7	1, 2, 29	5, 11, or 13
F-43	E	1, 4, 14	1	7	1, 2	5, 11, or 13
F-36	—	—	—	—	—	N/A
F-62	G	8, 10	—	—	—	N/A

[a] The numbers refer to MLT array genotypes.
[b] Gray shading indicates closely related genotypes that have been clustered together due to the highly stringent settings in the MLT Line software. —, no signals or inconclusive results.
[c] The group numbers are automatically generated and show the minimum resolution, i.e., the minimum number of genotypes into which the specimens can be divided. If specimens belong to different groups, they have different genotypes. N/A, not applicable.

to this repeat, this locus was therefore excluded from the MLT array. Furthermore, the *hctB* gene contains one to four repetitive elements, each 108 nucleotides long, which in turn are comprised of smaller repetitive units (16). Such a repetitive structure causes problems with a probe-based system because there is no possibility of counting the number of elements or deducing their order. Finally, the possible locations of probes were constricted due to the nature of the MLST regions, where most of the strain discrimination comes from single-nucleotide polymorphisms. This led to nonoptimal probe design and resulted in the failure of several probes.

These three major constraints prevented the use of theoretical hybridization patterns as references, as done previously (23), and they provide an explanation for the 11 to 21% loss in resolution compared with that of the MLST system. The specificity problem was solved by excluding the CT172 locus and creating a database from empirical hybridization data.

The MLT array achieved 100% specificity and 2.4-times-higher resolution than *ompA* sequencing when applied to the 80 clinical specimens from Finnmark in Norway. The MLT array provided more resolution, especially within the commonly predominating *ompA* E/Bour genotype.

Interestingly, 1 of the 80 specimens was identified as having a genotype previously found only among men who have sex with men (MSM). In accordance with the results, the male patient reported having sex with men. Another specimen was identified as having a genotype unique to the new variant *C. trachomatis* (nvCT). The nvCT was detected in 2006 in Sweden and has since then spread quickly within the country (11). Identification of the MSM genotype and the nvCT was not possible using *ompA* genotyping, which illustrates the MLT array's capability to reveal additional epidemiological details.

A total of 2 (2.5%) of the 80 specimens could not be PCR amplified and genotyped with the MLT array, even though they had been successfully MLST genotyped. This might be explained by DNA degradation caused by additional freeze-thawing following a delayed transport. As the present 80 specimens were collected from adolescents in school but otherwise were unselected, the findings of this study show that the MLT array is sensitive enough to work on clinical specimens.

Analysis of a further 127 specimens in 139 hybridization experiments resulted in 98% specificity, which is sufficient for strain discrimination in most applications.

This MLT array can now be used in studies in which rapid genotyping of *C. trachomatis* is needed to achieve epidemiological information. The MLT array is a powerful alternative to *ompA* sequencing and MLST analysis (Table 3). The MLT array provides >2-fold-higher resolution than *ompA* sequenc-

TABLE 3. Comparison of the MLT array to *ompA* sequencing and MLST analysis

Typing method	Time per assay	Resolution	Throughput	Cost per specimen (consumables)	Cost of equipment
ompA sequencing	2 days (in-house), 2–4 days (commercially)	Moderate	Moderate	Moderate (ca. US$14 in-house, ca. US$23 commercially)	High
MLST+*ompA*	3–4 days (in-house), 4–5 days (commercially)	High (3 times higher than *ompA* sequencing)	Low	High (ca. US$64 in-house, ca. US$107 commercially)	High
MLT array	8 h	High (2 times higher than *ompA* sequencing)	High (up to 96 specimens in parallel)	Moderate (ca. US$30)	Moderate

ing and attains about 79 to 90% of the resolution of MLST. Analysis can be performed within a single working day, compared to 3 or 4 days for MLST. The hybridization can be conducted at high throughput with up to 96 samples in parallel. Including the cost of equipment, the MLT array analysis is considerably cheaper than *ompA* sequencing and MLST analysis. Processing of the measured hybridization data and assignment of genotypes is easily done. In contrast, DNA sequencing must be followed by thorough data analysis, requiring extensive expertise of laboratory staff.

In conclusion, the MLT array developed here represents a considerable improvement to previous methods of *C. trachomatis* genotyping.

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REFERENCES

1. **Borel, N., et al.** 2008. Direct identification of *Chlamydiae* from clinical samples using a DNA microarray assay: a validation study. Mol. Cell. Probes **22:**55–64.
2. **Christerson, L., et al.** 2010. Typing of lymphogranuloma venereum *Chlamydia trachomatis* strains. Emerg. Infect. Dis. **16:**1777–1779.
3. **Christerson, L., H. J. de Vries, M. Klint, B. Herrmann, and S. A. Morre.** 2011. Multilocus sequence typing of urogenital *Chlamydia trachomatis* from patients with different degrees of clinical symptoms. Sex Transm. Dis. **38:** 490–494.
4. **Dean, D., et al.** 2009. Predicting phenotype and emerging strains among *Chlamydia trachomatis* infections. Emerg. Infect. Dis. **15:**1385–1394.
5. **Ehricht, R., P. Slickers, S. Goellner, H. Hotzel, and K. Sachse.** 2006. Optimized DNA microarray assay allows detection and genotyping of single PCR-amplifiable target copies. Mol. Cell. Probes **20:**60–63.
6. **Gall, A., et al.** 2009. Rapid and highly sensitive neuraminidase subtyping of avian influenza viruses by use of a diagnostic DNA microarray. J. Clin. Microbiol. **47:**2985–2988.
7. **Gall, A., et al.** 2009. Rapid haemagglutinin subtyping and pathotyping of avian influenza viruses by a DNA microarray. J. Virol. Methods **160:**200–205.
8. **Geue, L., et al.** 2010. Rapid microarray-based genotyping of enterohemorrhagic *Escherichia coli* serotype O156:H25/H-/Hnt isolates from cattle and clonal relationship analysis. Appl. Environ. Microbiol. **76:**5510–5519.
9. **Haggerty, C. L., et al.** 2010. Risk of sequelae after *Chlamydia trachomatis* genital infection in women. J. Infect. Dis. **201**(Suppl. 2)**:**S134–S155.
10. **Harding-Esch, E. M., et al.** 2010. Multi-locus sequence typing: a useful tool for trachoma molecular epidemiology, p. 55–58. *In* A. Stary, G. I. et al. (ed.), Chlamydial infections. Proceedings of the 12th International Symposium on Human Chlamydial Infections, Salzburg, Austria.
11. **Herrmann, B., et al.** 2008. Emergence and spread of *Chlamydia trachomatis* variant, Sweden. Emerg. Infect. Dis. **14:**1462–1465.
12. **Ikryannikova, L. N., M. M. Shkarupeta, E. A. Shitikov, E. N. Il'ina, and V. M. Govorun.** 2010. Comparative evaluation of new typing schemes for urogenital *Chlamydia trachomatis* isolates. FEMS Immunol. Med. Microbiol. **59:**188–196.
13. **Jurstrand, M., et al.** 2010. Characterisation of *Chlamydia trachomatis* by *ompA* sequencing and multilocus sequence typing in a Swedish county before and after identification of the new variant. Sex Transm. Infect. **86:**56–60.
14. **Jurstrand, M., et al.** 2001. Characterization of *Chlamydia trachomatis omp1* genotypes among sexually transmitted disease patients in Sweden. J. Clin. Microbiol. **39:**3915–3919.
15. **Klint, M., et al.** 2007. High-resolution genotyping of *Chlamydia trachomatis* strains by multilocus sequence analysis. J. Clin. Microbiol. **45:**1410–1414.
16. **Klint, M., et al.** 2010. Mosaic structure of intragenic repetitive elements in histone H1-like protein Hc2 varies within serovars of *Chlamydia trachomatis*. BMC Microbiol. **10:**81.
17. **Lysen, M., et al.** 2004. Characterization of *ompA* genotypes by sequence analysis of DNA from all detected cases of *Chlamydia trachomatis* infections during 1 year of contact tracing in a Swedish County. J. Clin. Microbiol. **42:**1641–1647.
18. **Monecke, S., et al.** 2007. Comparative genomics and DNA array-based genotyping of pandemic *Staphylococcus aureus* strains encoding Panton-Valentine leukocidin. Clin. Microbiol. Infect. **13:**236–249.
19. **Monecke, S., P. Slickers, and R. Ehricht.** 2008. Assignment of *Staphylococcus aureus* isolates to clonal complexes based on microarray analysis and pattern recognition. FEMS Immunol. Med. Microbiol. **53:**237–251.
20. **Pannekoek, Y., et al.** 2008. Multi locus sequence typing of *Chlamydiales*: clonal groupings within the obligate intracellular bacteria *Chlamydia trachomatis*. BMC Microbiol. **8:**42.
21. **Pedersen, L. N., B. Herrmann, and J. K. Moller.** 2009. Typing *Chlamydia trachomatis*: from egg yolk to nanotechnology. FEMS Immunol. Med. Microbiol. **55:**120–130.
22. **Pedersen, L. N., L. Podenphant, and J. K. Moller.** 2008. Highly discriminative genotyping of *Chlamydia trachomatis* using *omp1* and a set of variable number tandem repeats. Clin. Microbiol. Infect. **14:**644–652.
23. **Ruettger, A., et al.** 2011. Genotyping of *Chlamydia trachomatis* strains from culture and clinical samples using an *ompA*-based DNA microarray assay. Mol. Cell. Probes **25:**19–27.
24. **Sachse, K., H. Hotzel, P. Slickers, T. Ellinger, and R. Ehricht.** 2005. DNA microarray-based detection and identification of *Chlamydia* and *Chlamydophila* spp. Mol. Cell. Probes **19:**41–50.
25. **Sachse, K., et al.** 2008. Genotyping of *Chlamydophila psittaci* using a new DNA microarray assay based on sequence analysis of *ompA* genes. BMC Microbiol. **8:**63.
26. **Sachse, K., et al.** 2009. DNA microarray-based genotyping of *Chlamydophila psittaci* strains from culture and clinical samples. Vet. Microbiol. **135:**22–30.
27. **Schmoock, G., et al.** 2009. DNA microarray-based detection and identification of *Burkholderia mallei*, Burkholderia pseudomallei and *Burkholderia* spp. Mol. Cell. Probes **23:**178–187.
28. **World Health Organization.** 2001. Global prevalence and incidence of selected curable sexually transmitted infections: overview and estimates. WHO/HIV_AIDS/2001.02 and WHO/CDS/CSR/EDC/2001.10. World Health Organization, Geneva, Switzerland.