JOURNAL OF CLINICAL MICROBIOLOGY, July 2004, p. 2935–2939 Vol. 42, No. 7
0095-1137/04/$08.00+0 DOI: 10.1128/JCM.42.7.2935–2939.2004

Combination of PCR Targeting the VD2 of *omp1* and Reverse Line Blot Analysis for Typing of Urogenital *Chlamydia trachomatis* Serovars in Cervical Scrape Specimens

Monica Molano,[1,2] Chris J. L. M. Meijer,[1] Servaas A. Morré,[1] Rene Pol,[1] and Adriaan J. C. van den Brule[1,3]*

Department of Pathology, Vrije Universiteit Medical Center, Amsterdam,[1] and Labs for Pathology and Medical Microbiology, PAMM Institute, Eindhoven,[3] The Netherlands, and División Epidemiología, Instituto Nacional de Cancerología, Bogota, Colombia[2]

Received 9 December 2003/Returned for modification 26 January 2004/Accepted 4 April 2004

In this study we developed and evaluated a new PCR-based typing assay, directed to the VD2 region of the *omp1* gene, for the detection and typing of urogenital *Chlamydia trachomatis* infections. A nested VD2 PCR-reverse line blot (RLB) assay was developed for the typing of nine different urogenital serovars of *C. trachomatis*. The assay developed was tested with reference strains of *C. trachomatis* serovars and cervical scrapes of 86 Colombian women previously found to be positive for *C. trachomatis* by using plasmid PCR. Two sets of primers directed to the VD2 region of the *omp1* gene of *C. trachomatis* were designed, and fragments of 220 and 166 bp were generated in the primary and nested PCRs, respectively. In addition, an RLB assay was developed to identify nine different urogenital serovars of *C. trachomatis* (Ba, D, E, F, G, H, I, J, and K) and group controls, including group B (Ba, D, and E), group C (I, J, K, and H), and an intermediate group (F and G). Using this assay, we were able to type 81 of the 86 samples (94.2%). Of these samples, 91.3% were single *C. trachomatis* infections, and 8.7% were multiple infections. The most common serovars identified were serovars D (22.2%), F (18.5%), G (13.6%), and E (12.3%). Of the women with multiple *C. trachomatis* infections, >50% contained both serovars D and E. The nested VD2 PCR-RLB developed is a simple, fast, and specific method for the identification of individual urogenital *C. trachomatis* serovars previously detected by using plasmid PCR. Moreover, it is an appropriate method for studying multiple *C. trachomatis* infections and for use in large epidemiological studies.

Chlamydia trachomatis is one of the most common pathogens of sexually transmitted diseases worldwide.

Initially, 15 prototypic serovars labeled A to K and L1, L2, and L3 were identified based on immunoepitope analysis of the major outer membrane protein (MOMP) with monoclonal and polyclonal antibodies (7, 10). On the basis of the pathogenic potential, the serovars A, B, Ba, and C are commonly associated with trachoma, D to K are commonly associated with urogenital infections, and L1, L2, and L3 are commonly associated with lymphogranuloma venereum. In addition, based on amino acid similarity, the serovars have been grouped in three different serogroups: group B (B, Ba, D, Da, E, L1, L2, and L2a), group C (A, C, H, I, Ia, J, Ja, K and L3), and an intermediate group (F and G) (20).

The typing of *C. trachomatis* infection is important for epidemiological and vaccination studies. In addition, the knowledge of pathogenesis and transmission rates of *C. trachomatis* serovars are important topics in clinical and basic research (1, 3, 14).

To study the epidemiology of *C. trachomatis* infections, different laboratory techniques for *C. trachomatis* serovar identification have been developed in the last few years. These techniques include standard MOMP serotyping, restriction fragment length polymorphism (RFLP) analysis of the PCR-amplified *omp1* gene, and nucleotide sequencing of the *omp1* gene (3, 4, 12, 13, 19). Compared to immunotyping, the genotyping methods are more sensitive and specific for *C. trachomatis* serovar identification. In addition, the multiple passages in cell culture and the need for a large panel of monoclonal antibodies are the principal disadvantages of MOMP serotyping (1, 9).

Although nucleotide sequencing and RFLP analysis of the *omp1* gene are good methods for *C. trachomatis* identification, they are still very laborious and not suitable for typing of a large number of clinical samples and for the detection and typing of multiple *C. trachomatis* infections. In addition, the primers generally used in the RFLP-PCR analyses generate a quite large fragment (1.1 kb) of the *omp1* gene. Therefore, samples without good DNA quality are frequently negative and cannot be typed.

Here we report the development of a nested VD2 PCR, followed by an easy and rapid reverse line blot (RLB) assay for identification of the most common urogenital serovars of *C. trachomatis*. In addition, we used this assay to type the *C. trachomatis* infections found in cervical scrapes by using plasmid PCR from women from the general population of Colombia.

MATERIALS AND METHODS

***C. trachomatis* reference strains and cervical scrapes.** To evaluate the sensitivity and specificity of the *C. trachomatis* VD2 PCR and RLB typing, 12 different reference serovars were used (D, D1, E, Ba, I, J, Jv, K, H, G, G1, and F) as previously described in the *omp1* RFLP-PCR method (9).

In addition, DNAs from cervical scrapes of 86 women that were initially *C. trachomatis* positive by *C. trachomatis* plasmid PCR in a cohort study from

* Corresponding author. Mailing address: Laboratory for Pathology and Medical Microbiology, PAMM Institute, Michelangelolaan 2, 5623 EJ Eindhoven, The Netherlands. Phone: 31-40-2396100. Fax: 31-40-2396109. E-mail: a.van.den.brule@pamm.nl.

Colombia were purified by using a filter tube-based isolation method (High Pure PCR Template Preparation kit; Roche), suspended in 200 μl of elution buffer according to the manufacturer's instructions, and used for PCR analysis.

Informed consent was obtained from all participants included in the cohort study and the ethical committee of the International Agency for Cancer Research (Lyon, France) approved the study protocol.

PCR amplification. Plasmid PCR was performed as previously described (9). With respect to the *omp1* VD2 PCR, the primary PCR amplification was performed in a final volume of 50 μl containing 50 mM KCl, 1.5 mM $MgCl_2$, a 0.2 mM concentration of each deoxynucleoside triphosphate, 25 pmol of each of the MCTV2S and MCTV2AS primers (primer sequences are given in Results) (Eurogentec, Seraing, Belgium), 1 U of *Taq* polymerase (AmpliTaq; Cetus/Perkin-Elmer), and 10 μl of isolated nucleic acid. Amplification was performed in a Perkin-Elmer 9600 thermocycler. The optimal amplification conditions for the PCR were as follows: denaturation for 4 min at 95°C, followed by 50 cycles of amplification. Each cycle consisted of 1 min at 95°C, 2 min at 55°C, and 1.5 min at 72°C. The final elongation step was extended for another 4 min. The amplified DNA was analyzed by 1.5% agarose gel electrophoresis and ethidium bromide staining.

Then, 2 μl of the primary PCR product was used for the nested PCR, which was done with the same reagents and conditions (50 cycles) as the primary PCR, except for the primers, which were MCTV2N and MCTV2AN (primer sequences are shown in Results).

The stocks of 12 different reference *C. trachomatis* serovars were amplified by the same protocol. Negative controls, with all of the reaction components but void of DNA, and positive controls, with *C. trachomatis* serovar D, were included in each experiment.

RLB analysis. RLB analysis was performed by using a system that has been described previously (5, 6, 11, 16). In this method, oligonucleotide probes containing a 5′ amino group are covalently attached to a membrane in parallel lines by using a miniblotter. After binding of the oligonucleotides, the membrane is removed from the miniblotter and rotated 90°. The slots of the miniblotter, which now are perpendicular to the oligonucleotide lines, are filled with biotin-labeled target DNA (usually biotinylated PCR products). Hybridization takes place in the miniblotter and is visualized by using peroxidase-labeled streptavidin, which interacts with the biotin of the probe, followed by enhanced chemiluminescence (ECL) detection.

Probes. Twelve different oligonucleotide probes (Isogen, Maarssen, The Netherlands) directed against the VD2 region of MOMP gene of 12 different urogenital serovars of *C. trachomatis* were selected by using a primer/probe selection software (Oligo). In addition, three different probes were identified to use as controls for the three principal groups of *C. trachomatis* serovars: group B (D, D, E, and Ba), group C (I, J, Jv, K, and H), and the intermediate group (F and G). The probes were designed to have similar melting temperatures (T_m) in such a way that the T_m was ca. 55°C and the length of the oligonucleotide probes was ~20 bp. The sequences of the RLB probes are shown in Table 2.

Covalent coupling of oligonucleotide probes to the membrane. All oligonucleotide probes were synthesized with a 5′-terminal amino group, which was used to covalently link the oligonucleotides to an activated negatively charged Biodyne C membrane (5, 16).

Hybridization with PCR products and detection. Briefly, 10 μl of the PCR products were added to 150 μl of 2× SSPE (1× SSPE is 0.18 M NaCl, 10 mM NaH_2PO_4, and 1 mM EDTA [pH 7.7])–0.1% sodium dodecyl sulfate (SDS) (Roche, Mannheim, Germany), and the PCR products were then denatured for 10 min at 99°C and cooled in ice. The membrane (with the oligonucleotide probes) was incubated for 5 min at room temperature in 2× SSPE–0.1% SDS and placed in a miniblotter in such a way that the slots were perpendicular to the line pattern of the applied oligonucleotides. The slots were filled with the diluted PCR products (160 μl) and hybridized for 60 min at appropriate temperatures. The samples were removed by aspiration, and the membrane was washed twice in 2× SSPE–0.5% SDS for 10 min at 51°C. Subsequently, the membrane was incubated with 1:4,000-diluted peroxidase-labeled streptavidin conjugate in 2× SSPE–0.5% SDS for 1 h and washed in 2× SSPE–0.5% SDS. For chemiluminescent detection of hybridized DNA, the membrane was incubated for 2 min in 20 ml of ECL detection liquid (Amersham, Buckinghamshire, England) and exposed to a film (Hyperfilm; Amersham, Buckinghamshire, England) for 10 min. Films were subsequently developed (5). For repeated use, the membranes were stripped and stored at 4°C until further use.

RESULTS

Selection of VD2-PCR primers and probes. In order to detect areas with a high degree of conservation in the VD2 region, multiple sequence alignments from the VD2 region of 12 different strains available in GenBank (Table 1) were performed by using the CLUSTAL W (1.81) program.

TABLE 1. *C. trachomatis* serovars analyzed in the present study

Serovar	Source	GenBank accession no.
Ba/Apache-2	Conjunctiva	AF063194
B/IU-1226	Urine	AF063208
D/IC-Ca18	STD[a]	X62920
E/UW5	Cervix	X52557
F/IU-1552	Urine	AF063212
G/UW57	Cervix	AF063199
H/UW4	Cervix	X16007
I/UW12	Urethra	AF063200
J/IU-1553	Urine	AF063206
Jv	Urine	AF202458
K/IU-1619	Urine	AF063207

[a] STD, sexually transmitted disease.

On the basis of these alignments, one region with a high degree of conservation between the different serovars was identified. This region showed sufficient homology between the different serovars to allow for the design of two sets of primers (general and nested primers) to amplify segments of 220 and 166 bp, respectively. The following primers were used for the VD2 PCR of *C. trachomatis*: MCTV2S (5′-GTATT YTGTACAYTRGGAGCM-3′ biotin) and MCTV2AS (5′-CCYCARTCCCASAYAGCTGC-3′) for the primary PCR and MCTV2N (5′-AGGAAAYTCNGCWTCYTTCAA-3′ bio) and MCTV2AN (5′-CTGCNCGAGCNCCNACYCT-3′) for the nested PCR. The reaction conditions were optimized by changing the annealing temperature in the interval from 40 to 60°C and by altering the cycle number. The highest amplification sensitivity and efficiency was obtained with an annealing temperature of 55°C for 50 cycles.

In addition, the region between these primers was sufficiently heterogeneous to allow for the identification of the different urogenital *C. trachomatis* serovars by hybridization of type-specific oligonucleotide probes.

Plasmid PCR and VD2 PCR. In order to compare and determine the sensitivity of the VD2 PCR with the plasmid PCR, a dilution assay with the different serovars was performed. This assay showed that the plasmid PCR was able to detect until 0.01 inclusion-forming units (IFU) by using a nonradioactive enzyme immunoassay (EIA) from all of the different serovars, whereas the primary PCR was able to detect ~0.1 IFU at the gel level. With the nested VD2 PCR the level of *C. trachomatis* detection increased to 0.01 IFU, which was similar to the results obtained with the plasmid PCR.

Development of the RLB. All oligoprobe sequences selected for RLB showed a complete matching with the nucleotide sequence of the corresponding urogenital *C. trachomatis* serovar. Each oligoprobe sequence had at least two mismatches with a different urogenital *C. trachomatis* serovar and one mismatch between variants (i.e., between D and D^-, J and Jv, and G and Ga) (Table 2).

To determine the optimal concentration of the oligoprobes to be used in the RLB, different oligoprobe concentrations ranging from 100 to 400 pmol/μl were applied to the membranes. Subsequently, the membranes were hybridized with

TABLE 2. *C. trachomatis* type-specific oligoprobes used in RLB typing

Serovar[a]	Sequence
D	GATAATGAAAATCAAAAAACG
D$^-$	GATAGTGAAAATCAAAAAAC
E	CAAAGCACGGTCAAAACGAATT
B	AATGGTGCGTTTGTACCAA
Ba	AAATAGTACGTTTGTACCAAT
I	CACAATCTTCTAACTTTAATAC
J	TCTTTTTCCTAACACTGCTTT
Jv	CTTTTTCCTAACACTGCATT
K	AACACTGCTTTGGATCGAG
H	ATCTTCTGATTTTAATACAGC
G	GCCACGCAGCCTGCTGCAACA
Ga	AACGTGCAGATAAATCAGTCT
F	ACGAAACCTGCTGCAGATA
Group B	AGCTTNGATCAATCTGTTGTT
Group C	AACTTAGTTGGATTATTCGGA
Intermediate group	CTGTGGTGGAACTGTATACA

[a] Group B (the oligoprobe detected Ba, D, and E serovars), group C (the oligoprobe detected I, J, K, and H serovars), and the intermediate group (the oligoprobe detected F and G serovars) are included.

VD2 PCR products from the different serovars studied. The optimal concentrations of oligoprobes to be used in the RLB were 100 pmol/μl for the D, E, K, and G oligoprobes, 200 pmol/μl for the I, J, and F oligoprobes, and 400 pmol/μl for the B and H oligoprobes. In addition, the concentration of the three probes used as positive controls for the B, C, and intermediate groups was 100 pmol/μl.

Although this method was developed to identify urogenital *C. trachomatis* serovars, additional sequence alignments to select probes specific for the remaining *C. trachomatis* serovars (A to C and L1 to L3) were performed and showed no cross-reaction in silico with urogenital serovars. These probes can be tested further in future RLB experiments (data not shown).

Sensitivity and specificity of *C. trachomatis* typing assay. Plasmid PCR showed similar detection level as the nested VD2-PCR with the different urogenital serovars studied. We made dilution series of the serovar D to compare the level of sensitivity between the RLB and the plasmid PCR-nonradioactive EIA assay. Both RLB and EIA reached the same detection level (0.01 IFU). Figure 1 shows dilution series of serovar D and the level of detection with RLB.

Although in silico investigations did not reveal any cross-reaction between *C. trachomatis* probes and nucleotide sequences of microorganisms, we also tested the specificity with a panel of urogenital microorganisms (hemolytic group B streptococci, *Lactobacillus* spp., *Gardnerella vaginalis*, *Enterobacter cloacae*, *Klebsiella oxytoca*, *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Candida albicans*, *Candida tropicalis*, herpes simplex virus types 1 and 2, *Mycoplasma genitalium*, *Mycoplasma hominus*, *Ureaplasma urealyticum*, *Neisseria gonorrhoeae*, *Chlamydia pneumoniae*, and *Chlamydia trachomatis*). Although some bacteria yielded fragments with RLB primers in nested PCR, these fragments did not have the right size and, indeed, after RLB hybridization with specific *C. trachomatis* oligoprobes no signals were detected, even after long exposure.

The specificity of RLB was evaluated further by using VD2 PCR products of the 12 different urogenital serovars studied. Serovars (D, E, Ba, I, J, K, H, G, and F) showed specific hybridization, and no cross-reaction was observed between them even after longer ECL exposure times (see Fig. 1). However, in our assay it was not possible to differentiate between variants that had only one mismatch (i.e., D and D$^-$, J and Jv, and G and Ga). In addition, the oligoprobes designed to differentiate the three principal groups of *C. trachomatis* serovars (group B, group C, and the intermediate group) were specific and could be used as internal control in the specific serovar identification (Fig. 1).

Typing of *C. trachomatis*-positive cervical scrapes. Eighty-six cervical scrapes initially positive for *C. trachomatis* plasmid PCR were subjected to the primary VD2 PCR developed to identify *C. trachomatis* infections. The amplified product obtained was used in an RLB for the identification of nine different serovars of *C. trachomatis*.

Forty-six percent of the samples were typed with this primary VD2-PCR. To increase the level of detection, the samples that were negative in the primary PCR were reamplified by using a nested VD2 PCR, and another 48.22% of the samples could be identified. In all, 81 of 86 *C. trachomatis*-positive samples could be typed (94.2%) by the nested VD2 PCR. A representative RLB result is shown in Fig. 2. Of the samples identified by the nested VD2 PCR, 91.3% had single *C. trachomatis* infections and 8.7% had multiple infections.

The most common serovars identified were serovar D (22.2% of the positive samples), serovar F (18.5%), serovar G (13.6%), and serovar E (12.3%) (Table 3). Of the multiple infections, the most common serovars detected were serovars D and E (4.95%).

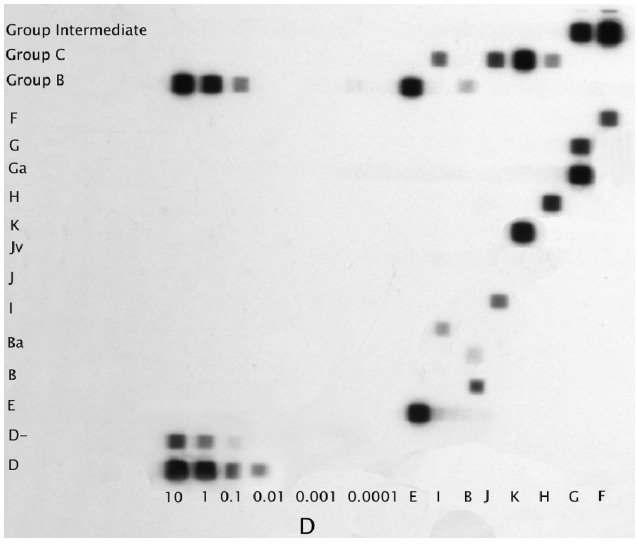

FIG. 1. Standarization of RLB. *C. trachomatis* type-specific detection. *C. trachomatis* serovars amplification by nested VD2 PCR and detection by RLB. The results for a dilution series of serovar D (10^1 to 10^{-4}) and specific detection of serovars E, I, B, J, K, H, G, and F are shown. Probes are indicated on the *y* axis, and serovars are indicated on the *x* axis.

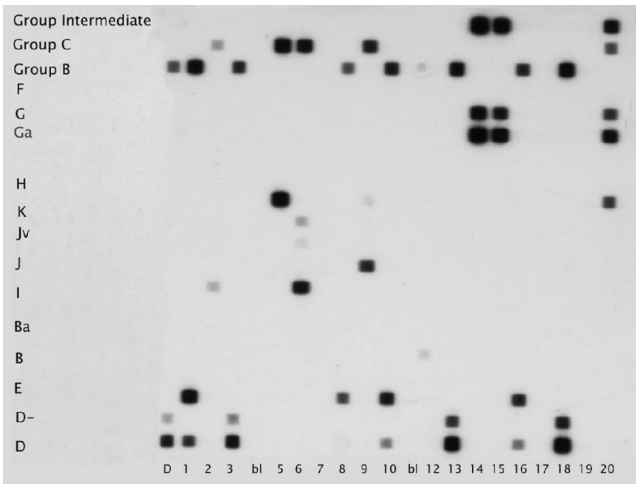

FIG. 2. Representative example of RLB typing of *C. trachomatis* nested VD2-PCR products derived from cervical scrapes. Numbers indicate the different amplified products of cervical scrapes positive for *C. trachomatis* serovars. Samples with multiple infections are shown (lanes 1, 6, 9, 10, 16, and 20). D, control positive; bl, blank.

DISCUSSION

We developed a novel, sensitive, and simple VD2 PCR-RLB assay for the typing of urogenital *C. trachomatis* infections. This assay is an excellent alternative method for the identification of individual and multiple *C. trachomatis* infections and has several advantages over other *C. trachomatis* methods to identify the most common serovars in terms of speed, usability, sensitivity, and specificity.

The sensitivity of the nested VD2 PCR is similar to that of the commonly used *C. trachomatis* plasmid PCR, which has a detection limit of 0.01 IFU (9). The nested VD2 PCR results in a rather small product (166 bp), which is adequate to amplify samples without high DNA quality from archival smears and paraffin-embedded biopsies. In addition, the combination of VD2 PCR and RLB gives an ideal accordance between efficient small-fragment amplification and specific *C. trachomatis* typing.

The nested VD2 PCR-RLB showed the same detection level (0.01 IFU) as the *C. trachomatis* plasmid PCR when dilution series of serovar D were used. However, when the cervical scrapes were analyzed, 5.8% still could not be typed with the new VD2 PCR-RLB. The false-negative results might be due to either DNA degradation, low *C. trachomatis* load, or the presence of inhibitors in the samples that could not be typed. Therefore, VD2 PCR-RLB typing can be most reliably used in samples that are already identified as *C. trachomatis* positive, as is the case in the present study with plasmid PCR, which has been shown to be diagnostically valid.

Initially, we tried to type the samples of the Colombian cohort by using the *omp1* RFLP assay, but we could only type 40% of the samples. The size of the fragment generated in this PCR is rather large (1.1 kb), which makes this *omp1* RFLP assay sensitive for DNA degradation, thus explaining the low level of detection with this technique. Therefore, due to the small size of amplified product, the VD2 PCR-RLB assay is suitable for the analysis of less-preserved material such as archival smears and paraffin-embedded tissues, as previously also shown for the typing of human papillomaviruses with PCR-RLB (15).

At present the VD2 PCR-RLB technique can identify nine different urogenital serovars (D, E, Ba, I, J, K, H, F, and G) and three group-specific probes used as internal control groups: group B (Ba, D, and E), group C (H to K), and the intermediate group (F and G). This method cannot differentiate between closely related variants (i.e., D and D$^-$, J and Jv, and G and Ga). The probes selected for the specific identification of these variants had only a single mismatch that resulted in cross-reaction with related serovars. Even if we used more stringent hybridization temperatures, the cross-reaction remained. Although RLB is a highly specific method, potentially able to differentiate between samples with a single mismatch, we were only able to differentiate between closely related serovars when they differed in two or more nucleotides in sequence. Alternatively, additional probes could be designed in other regions of the *omp1* gene to subsequently subtype and identify these variants.

Although the focus of the present study was the identification of urogenital *C. trachomatis* serovars, this RLB typing method can be easily extended to the identification of other serovars of *C. trachomatis*.

In the cervical material derived from a population-based study in Colombia, serovars D, F, G, and E were the most frequently observed serovars (74.1%). This is in agreement with other studies performed in different parts of the world (8, 15, 17, 18). With the RLB developed we found that 91.3% of the women showed single *C. trachomatis* infections and 8.7% showed multiple infections. The percentage of multiple infections identified in our study is in line with those reported in other studies, where 2 to 15% of the infections contain two or more serovars (2, 3, 8). The use of this RLB assay facilitates the detection of multiple infections that are difficult to identify by the most commonly used methods, such as RFLP and sequencing. In addition, the identification of multiple infections

TABLE 3. *C. trachomatis* serovar distribution in positive samples from the general population from Colombia as determined by using RLB

Infection type and serovar(s)	*n*	% Positive
Single infections (total)	74	91.33
Ba	0	
D (D and D$^-$)	18	22.22
E	10	12.35
F	15	18.51
G (G and Ga)	11	13.58
H	3	3.70
I	7	8.64
J (J and Jv)	6	7.40
K	4	4.93
Multiple infections (total)	7	8.67
D and E	3	3.71
D and I	1	1.24
K and H	1	1.24
D, E, and F	1	1.24
G, J, and K	1	1.24
Total	81	100

might be important for advancing our knowledge of the natural history of *C. trachomatis* infections and for determining whether there are differential effects for *C. trachomatis* serovars on infectivity, immune response, and treatment response.

Finally, the RLB method developed is useful for use in large epidemiological studies due its advantage to analyze large number of clinical samples (44 per blot), the comfort to perform the assay without requirement of specialized instruments and the short time required for testing compared to conventional methods (e.g., sequencing and RFLP). For example, the use of two miniblotters allow typing of up to 84 samples in less than 1 day, whereas analysis of the conventional RFLP maximum of 22 samples is more labor-intensive, and it takes at least 1 week to identify a similar number of samples. In addition, because the RLB method uses a nonprecipitating ECL substrate, it has the advantage that nylon filters can be stripped and reused at least 10 times (16). Based on the fact that the VD2 primers are located within the *omp1* gene, our RLB system has a further advantage in that it can also be used for typing of the PCR-amplified *omp1* gene used for RFLP (4, 8, 12, 13).

In conclusion, the nested VD2 PCR-RLB developed is a simple, fast, and specific method for the identification of individual urogenital *C. trachomatis* serovars and is an appropriate assay for use in large epidemiological studies to study the prevalence and persistence of specific *C. trachomatis* serovars.

ACKNOWLEDGMENTS

We thank all of the study participants. We also thank R. van Andel and D Kramer for technical support.

M. Molano obtained a fellowship from Colciencias of the Colombian Government. Additional financial support was received from the Department of Pathology, Vrije Universiteit Medical Centre, Amsterdam, The Netherlands; the Department of Epidemiology, Instituto Nacional de Cancerologìa, Bogota, Colombia; the World Health Organization, Geneva, Switzerland; and the International Agency for the Cancer Research, Lyon, France.

REFERENCES

1. **Bandea, C. I., K. Kubota, T. M. Brown, P. H. Kilmarx, V. Bhullar, S. Yanpaisarn, P. Chaisilwattana, W. Siriwasin, and C. M. Black.** 2001. Typing of *Chlamydia trachomatis* strains from urine samples by amplification and sequencing the major outer membrane protein gene (*omp1*). Sex. Transm. Infect. **77:**419–422.
2. **Brunham, R. C., J. Kimani, J. Bwayo, G. Maitha, I. Maclean, C. Yang, C. Shen, S. Roman, N. J. D. Nagelkerke, M. Cheang, and F. A. Plummer.** 1996. The epidemiology of *Chlamydia trachomatis* within a sexually transmitted disease core group. J. Infect. Dis. **173:**950–956.
3. **Dean, D., E. Oudens, G. Bolan, et al.** 1995. Major outer membrane protein variants of *Chlamydia trachomatis* are associated with Severe upper genital tract infections and histopathology in San Francisco. J. Infect. Dis. **172:**1013–1022.
4. **Frost, E. H., S. Deslandes, S. Veilleux, and D. Bourgaux-Ramoisy.** 1991. Typing *Chlamydia trachomatis* by detection of restriction fragment length polymorphism in the gene encoding the major outer membrane protein. J. Infect. Dis. **163:**1103–1107.
5. **Kamerbeek, J., L. Schouls, A. Kolk, M. van Agterveld, D. van Soolingen, S. Kuijper, A. Bunschoten, H. Molhuizen, R. Shaw, M. Goyal, and J. van Embden.** 1997. Simultaneous detection and strain differentiation of *Mycobacterium tuberculosis* for diagnosis and epidemiology. J. Clin. Microbiol. **35:**907–914.
6. **Kaufhold, A., A. Podbielski, G. Baumgarten, M. Blokpoel, J. Top, and L. Schouls.** 1994. Rapid typing of group A streptococci by the use of DNA amplification and nonradioactive allele specific oligonucleotide probes. FEMS Microbiol. Lett. **119:**19–26.
7. **Kuo, C. C., S. P. Wang, K. K. Holmes, and J. T. Grayston.** 1983. Immunotypes of *Chlamydia trachomatis* isolates in Seattle, Washington. Infect. Immun. **41:**865–868.
8. **Morre, S. A., L. Rozendaal, I. G. M. van Valkengoed, A. J. P. Boeke, P. C. van Voorst Vader, J. Schirm, S. de Blok, J. A. R. van den Hoek, G. J. J. van Doornum, C. J. L. M. Meijer, and A. J. C. van den Brule.** 2000. Urogenital *Chlamydia trachomatis* serovars in men and women with a symptomatic or an asymptomatic infection: an association with clinical manifestations? J. Clin. Microbiol. **38:**2292–2296.
9. **Morre, S. A., P. T. Sillekens, M. V. Jacobs, et al.** 1998. Monitoring of *Chlamydia trachomatis* infections after antibiotic treatment using RNA detection by nucleic acid sequence based amplification. Mol. Pathol. **51:**149–154.
10. **Ossewaarde, J. M., M. Rieffe, A. de Vries, R. P. Derksen-Nawrocki, H. J. Hooft, G. J. J. van Doornum, and A. M. van Loon.** 1994. Comparison of two panels of monoclonal antibodies for serovar determination of *Chlamydia trachomatis*. J. Clin. Microbiol. **32:**2968–2974.
11. **Rijpkema, S. G., M. J. Molkenboer, L. M. Schouls, F. Jongejan, and J. F. Schellekens.** 1995. Simultaneous detection and genotyping of three genomic groups of *Borrelia burgdorferi* sensu lato in Dutch *Ixodes ricinus* ticks by characterization of the amplified intergenic spacer region between 5S and 23S rRNA genes. J. Clin. Microbiol. **33:**3091–3095.
12. **Rodriguez, P., A. Vekris, B. de Barbeyrac, B. Dutilh, J. Bonnet, and C. Bebear.** 1991. Typing of *Chlamydia trachomatis* by restriction endonuclease analysis of the amplified major outer membrane protein gene. J. Clin. Microbiol. **29:**1132–1136.
13. **Sayada, C., E. Denamur, J. Orfila, F. Catalan, and J. Elion.** 1991. Rapid genotyping of the *Chlamydia trachomatis* major outer membrane protein by the polymerase chain reaction. FEMS Microbiol. Lett. **83:**73–78.
14. **Stothard, D. R., G. Boguslawski, and R. B. Jones.** 1998. Phylogenetic analysis of the *Chlamydia trachomatis* major outer membrane protein and examination of potential pathogenic determinants. Infect. Immun. **66:**3618–3625.
15. **van de Laar, M. J. W., J. Lan, Y. T. H. P. van Duynhoven, J. S. A. Fennema, J. M. Ossewaarde, A. J. C. van den Brule, G. J. J. van Doornum, R. A. Coutinho, and J. A. R. van den Hook.** 1996. Differences in clinical manifestations of genital chlamydial infections related to serovars. Genitourin. Med. **72:**261–265.
16. **van den Brule, A. J. C., R. Pol, N. Fransen-Daalmeijer, L. M. Schouls, C. J. L. M. Meijer, and P. Snijders.** 2002. $GP5^{+}/6^{+}$ PCR followed by reverse line blot analysis enables rapid and high throughput identification of HPV genotypes, suitable for mass screening. J. Clin. Microbiol. **40:**770–787.
17. **van Duynhoven, Y. T., J. M. Ossewaarde, R. P. Derksen-Nawrocki, et al.** 1998. *Chlamydia trachomatis* genotypes: correlation with clinical manifestations of infection and patients' characteristics. Clin. Infect. Dis. **26:**314–322.
18. **Wagenvoort, J. H. T., R. J. Suchland, and W. E. Stamm.** 1988. Serovar distribution of urogenital *Chlamydia trachomatis* strains in The Netherlands. Genitourin. Med. **64:**159–161.
19. **Yang, C. L., I. Maclean, and R. C. Brunham.** 1993. DNA sequence polymorphism of the *Chlamydia trachomatis* OMP1 gene. J. Infect. Dis. **168:** 1225–1230.
20. **Yuan, Y., Y. X. Zhang, N. G. Watkins, and H. D. Caldwell.** Nucleotide and deduced amino acid sequences for the four variable domains of the major outer membrane proteins of the 15 *Chlamydia trachomatis* serovars. Infect. Immun. **57:**1040–1049.