

Lymphogranuloma venereum variant L2b-specific polymerase chain reaction: insertion used to close an epidemiological gap

S. P. Verweij¹, A. Catsburg², S. Ouburg¹, A. Lombardi³, R. Heijmans¹, F. Dutly⁴, R. Frei⁵, S. A. Morré¹ and D. Goldenberger⁵

1) Laboratory of Immunogenetics, Department of Pathology, 2) Department of Medical Microbiology and Infection Control, VU University Medical Center, Amsterdam, the Netherlands, 3) Department of Clinical Microbiology, University Hospital L. Sacco, Milano, Italy, 4) IMD, Institute for Medical and Molecular Diagnostics Ltd, Zurich and 5) Division of Clinical Microbiology, University Hospital Basel, Basel, Switzerland

Abstract

The management of the ongoing *lymphogranuloma venereum* epidemic in industrialized Western countries caused by *Chlamydia trachomatis* variant L2b still needs improvements in diagnosis, therapy and prevention. We therefore developed the first rapid *C. trachomatis* variant L2b-specific polymerase chain reaction to circumvent laborious *ompA* gene sequencing.

Keywords: *Chlamydia trachomatis*, diagnostics, L2b, LGV, lymphogranuloma venereum, MSM, RT-PCR, *OmpA*, *PmpH*, sequencing

Original Submission: 21 October 2010; **Revised Submission:** 12 January 2011; **Accepted:** 23 January 2011

Editor: G. Greub

Article published online: 1 February 2011

Clin Microbiol Infect 2011; **17**: 1717–1726

10.1111/j.1469-0691.2011.03481.x

Corresponding author: S. A. Morré, VU University Medical Center, Department of Pathology, Laboratory of Immunogenetics (LoI), De Boelelaan 1117, 1081 HV, Amsterdam, the Netherlands
E-mail: samorretravel@yahoo.co.uk

Lymphogranuloma venereum (LGV) is caused by *Chlamydia trachomatis* serovars L1–L3. LGV is more invasive than infections caused by the oculo-genital *C. trachomatis* serovars D–K. It classically manifests itself as an inguinal syndrome,

with genital ulceration, inguinal lymphadenopathy (buboes), and subsequent suppuration. But it can also cause a severe anorectal syndrome, with proctocolitis and hyperplasia of intestinal and perirectal lymphatic tissue [1]. LGV is endemic in Africa, southeast Asia and the Caribbean. It is a sporadic disease in Europe and North America.

In 2003, an LGV outbreak was reported in the Netherlands and other Western European countries among men who have sex with men (MSM). The European Surveillance of Sexually Transmitted Infections (<http://www.essti.org>) and the Centers for Disease Control and Prevention (<http://www.cdc.gov>) instigated warning and response systems to increase the awareness and the management of the LGV outbreak, but the outbreak is still ongoing [1–8].

In 2005, we identified a unique single-nucleotide mutation in the *ompA* gene of the LGV L2 serovar circulating among MSM. This serovariant was designated L2b [2]. Subsequently we developed a specific LGV real-time polymerase chain reaction (PCR) exploiting a unique deletion (36 bp) in all LGV serovars within the polymorphic membrane protein H (*pmpH*) gene [3].

In our laboratories in Amsterdam and Basel we get frequent epidemiology-based requests to identify the aetiological LGV serovar as the L2b variant. To identify the L2b mutation in the variable segment 2 of the *ompA* gene, we have to amplify and sequence this fragment. To avoid the high costs of sequencing and the need for sophisticated equipment, we developed an L2b-specific primer/probe set using fast and reliable real-time PCR techniques for identification of the L2b variant.

The Study

We sequenced the *pmpH* gene (2952 bp) of two serovariant L2b-containing clinical specimens (accession numbers EF534758 and EF612788), which were 100% identical, and compared them with a reference sequence from serovar L2. To our surprise we identified several unique differences (Figs 1 and 2). First, L2b has a single-nucleotide polymorphism at the second base where the previously developed LGV-specific probe binds, just besides the 36-bp deletion (Fig. 1). Based on this finding we adjusted our previously published [3] probe: instead of a C base, we incorporated a degenerated base (C/T) to be able to detect all LGV serovars and variants L1, L2, L2a, L2b and L3 adequately. Additionally, we identified a 9-bp insertion unique for L2b resulting in a repeat sequence (TCT AGT AGT)₂ (Fig. 2). These two sequence heterogeneities were then confirmed in another ten L2b-positive samples by sequencing. We verified whether the insertion is unique

	475	485	495	505	515	525	
L2b	AACTCCGC	T T	GC-----	-----	-----	-----TC	CAACAGTTAG
L1	AACTCCGC	C T	GC-----	-----	-----	-----TC	CAACAGTTAG
L2	AACTCCGC	C T	GC-----	-----	-----	-----TC	CAACAGTTAG
L3	AACTCCGC	C T	GC-----	-----	-----	-----TC	CAACAGTTAG
Probe	C	C T	GC			TC	CAACAGT
A	AACTCCGC	C T	GCTCTAGATC	CATCCCTAC	CGTTCAAGC	TCTTCATCTC	CCACAGTCAG
B	AACTCCGC	C T	GCTCCAGATC	CATCCCTAC	CGTTCAAGC	TCTTCATCTC	CCACAGTCAG
Ba	AACTCCGC	C T	GCTCCAGATC	CATCCCTAC	CGTTCAAGC	TCTTCATCTC	CCACAGTCAG
C	AACTCCGC	C T	GCTCCAGATC	CATCCCTAC	CGTTCAAGC	TCTTCATCTC	CCACAGTCAG
D	AACTCCTC	C A	GCACCAGCAC	CAGTCCTGC	TGTTCAAGC	TCTTTATCTC	CAACAGTTAG
Da	AACTCCGC	C T	GCTCCAGATC	CATCCCTAC	CGTTCAAGC	TCTTCATCTC	CCACAGTCAG
E	AACTCCTC	C A	GCACCAGCAC	CAGTCCTGC	TGTTCAAGC	TCTTTATCTC	CAACAGTTAG
SW-E	AACTCCTC	C A	GCACCA----	--GCTCCTGC	TGTTCAAGC	TCTTTATCTC	CAACAGTTAG
F	AACTCCTC	C A	GCACCAGCAC	CAGTCCTGC	TGTTCAAGC	TCTTTATCTC	CAACAGTTAG
G	AACTCCTC	C A	GCACCAGCAC	CAGTCCTGC	TGTTCAAGC	TCTTTATCTC	CAACAGTTAG
H	AACTCCTC	C A	GCACCAGCAC	CAGTCCTGC	TGTTCAAGC	TCTTTATCTC	CAACAGTTAG
I	AACTCCTC	C A	GCACCA----	--GCTCCTGC	TGTTCAAGC	TCTTTATCTC	CAACAGTTAG
Ia	AACTCCTC	C A	GCACCAGCAC	CAGTCCTGC	TGTTCAAGC	TCTTTATCTC	CAACAGTTAG
J	AACTCCTC	C A	GCACCA----	--GCTCCTGC	TGTTCAAGC	TCTTTATCTC	CAACAGTTAG
Ja	AACTCCTC	C A	GCACCA----	--GCTCCTGC	TGTTCAAGC	TCTTTATCTC	CAACAGTTAG
K	AACTCCTC	C A	GCACCA----	--GCTCCTGC	TGTTCAAGC	TCTTTATCTC	CAACAGTTAG

FIG. 1. LGV-specific probe: alignment of partial pmpH gene of *C. trachomatis* serovars. L2b sequence used as reference (accession numbers EF534758 and EF612788). Nucleotide numbers are relative to the start codon. The mutation in L2b is marked by the red rectangle. The first four lines represent the LGV serovars. Probe represents the LGV-specific MGB-probe, A–K represent the ocular and urogenital *C. trachomatis* serovars. SW-E is the Swedish variant of serovar E. The dashed lines within the LGV serovars represent the LGV-specific deletion sequence.

	1845	1855	1865	1875	1885	1895	1905	1915
L2b	TGGGATCGCT	GACGATTCCT	TTTGTACC	TATCTTCTAG	TAGTCTTAGT	AGTGCTAGTA	ACGGGGTTAC	AATGAAGCGT
Forward	TCGCT	GACGATTCCT	TTTGT					
Probe				CTTCTAG	TAGTCTTAGT	AGTGCT		
Reverse						TA	ACGGGGTTAC	AATGAAGCG
L1	TGGGATCGCT	GACGATTCCT	TTTGTACC	TATCTTCTAG	TAGT-----	---ACTAGTA	ACGGGGTTAC	AATGAAGCGT
L2	TGGGATCGCT	GACGATTCCT	TTTGTACC	TATCTTCTAG	TAGT-----	---ACTAGTA	ACGGGGTTAC	AATGAAGCGT
L3	TGGGATCGCT	GACGATTCCT	TTTGTACC	TATCTTCTAG	TAGT-----	---ACTAGTA	ACGGGGTTAC	AATGAAGCGT
A	TGGGATCGCT	GACAATTCCT	TTTGTACC	TATCTTCTAG	CAAT---AAT	ACCAATAGTA	ATGGGGTTGT	AAAAACAGCT
B	TGGGATCGCT	GACAATTCCT	TTTGTACC	TATCTTCTAG	CAAT---AAT	ACCAATAGTA	ATGGGGTTGT	AAAAACAGCT
Ba	TGGGATCGCT	GACAATTCCT	TTTGTACC	TATCTTCTAG	CAAT---AAT	ACCAATAGTA	ATGGGGTTGT	AAAAACAGCT
C	TGGGATCGCT	GACAATTCCT	TTTGTACC	TATCTTCTAG	CAAT---AAT	ACCAATAGTA	ATGGGGTTGT	AAAAACAGCT
D	TGGGATCGCT	GACAATTCCT	TTTGTACTC	TATCTTCTAG	TAGT-----	---GCTAGTA	ACGGGGTTAC	AAAAAATTCCT
Da	TGGGATCGCT	GACAATTCCT	TTTGTACC	TATCTTCTAG	CAAT---AAT	ACCAATAGTA	ATGGGGTTGT	AAAAACAGCT
E	TGGGATCGCT	GACAATTCCT	TTTGTACTC	TATCTTCTAG	TAGT-----	---GCTAGTA	ACGGGGTTAC	AAAAAATTCCT
SW-E	TGGGATCGCT	GACAATTCCT	TTTGTACTC	TATCTTCTAG	TAGT-----	---GCTAGTA	ACGGGGTTAC	AAAAAATTCCT
F	TGGGATCGCT	GACAATTCCT	TTTGTACTC	TATCTTCTAG	TAGT-----	---GCTAGTA	ACGGGGTTAC	AAAAAATTCCT
G	TGGGATCGCT	GACAATTCCT	TTTGTACTC	TATCTTCTAG	TAGT-----	---GCTAGTA	ACGGGGTTAC	AAAAAATTCCT
H	TGGGATCGCT	GACAATTCCT	TTTGTACTC	TATCTTCTAG	TAGT-----	---GCTAGTA	ACGGGGTTAC	AAAAAATTCCT
I	TGGGATCGCT	GACAATTCCT	TTTGTACTC	TATCTTCTAG	TAGT-----	---GCTAGTA	ACGGGGTTAC	AAAAAATTCCT
Ia	TGGGATCGCT	GACAATTCCT	TTTGTACTC	TATCTTCTAG	TAGT-----	---GCTAGTA	ACGGGGTTAC	AAAAAATTCCT
J	TGGGATCGCT	GACAATTCCT	TTTGTACTC	TATCTTCTAG	TAGT-----	---GCTAGTA	ACGGGGTTAC	AAAAAATTCCT
Ja	TGGGATCGCT	GACAATTCCT	TTTGTACTC	TATCTTCTAG	TAGT-----	---GCTAGTA	ACGGGGTTAC	AAAAAATTCCT
K	TGGGATCGCT	GACAATTCCT	TTTGTACTC	TATCTTCTAG	TAGT-----	---GCTAGTA	ACGGGGTTAC	AAAAAATTCCT

FIG. 2. L2b-specific PCR: alignment of partial pmpH gene of *C. trachomatis* serovars. L2b sequence used as reference (accession numbers EF534758 and EF612788). Nucleotide numbers are relative to the start codon. The unique insertion of the L2b serovariant is located at relative position 1885–1893. The newly developed probe covers the repeat (TCT AGT AGT)₂. Forward, Reverse and Probe represent the primers and MGB-probe of the L2b-specific PCR, L1–L3 and A–K represent the *C. trachomatis* LGV and oculogenital serovars, respectively. The reverse primer depicted here is the reverse complementary sequence of the actual reverse primer.

among all *C. trachomatis* serovars. Using BioEdit Sequence Alignment Editor we aligned the sequences of *pmpH* genes of 19 serovars/serovariants (NCBI accession number in brackets): A (AY184155), B (AY184156), Ba (AY184157), C (AY184158), D (AY184159), Da (AY967759), E (AY184160), Swedish variant E (SW-E; FN652779), F (AY184161), G (AY184162), H (AY184163), I (AY184164), Ia (AY967760), J (AY184165), K (AY184166), LI (AY184167), L2 (AY184168) and L3 (AY184169). The analysis confirmed the unique nature of the insert of L2b within the *pmpH* gene (Fig. 2). Our sequence findings have also been confirmed by the recent publication of the entire L2b genome [9] (accession no. NC_010280). The following primers and probe were selected based on this unique insertion: L2b-F 5' TCG CTG ACG ATT CCT TTT GTT 3', L2b-R 5' CGC TTC ATT GTA ACC CCG TTA 3', and L2b MGB-probe 5' VIC-CTT CTA GTA GTT CTA GTA GTG CT-MGB 3'. Standard TaqMan conditions with 45 cycles of 15 s at 95°C and 1 min at 60°C were used for PCR amplification.

We compared our old LGV probe with the adjusted LGV probe within the LGV-specific deletion region. We tested *C. trachomatis* L1, L2, L2a, L2b and L3 reference strains. Using titrated serial dilutions we found a slightly increased analytical sensitivity (factor 2–20) of our new LGV assay for the detection of L2b. Although we found this increased analytical sensitivity, every L2b case previously tested was detected by both the old and new LGV assay. No differences were observed for the other serovars tested, showing a well-defined LGV-specific PCR.

To determine specificity of the newly developed L2b-specific insertion probe, we firstly analysed different *Chlamydia* strains: *C. trachomatis* (serovars/serovariants, A, B, Ba, C, D, Da, D-, E, F, G, Ga, H, I, Ia, J, K, LI, L2, L2a, L3), *C. muridarum* (MoPn), *C. pneumoniae*, *C. pecorum* and *C. psittaci*. Secondly, we tested 31 microorganisms found in the perianal and urogenital regions and the oropharynx: *Bacteroides* spp. (*ao. fragilis*), *Clostridium* spp. (*ao. novyi*), *Faecalibacterium prausnitzii*, *Atopobium parvulum*, *Enterococcus faecalis*, *Propionibacterium acnes*, *Bordetella pertussis*, *Neisseria gonorrhoeae*, *Mycoplasma pneumoniae*, *Legionella pneumophila*, *Bartonella henselae*, *Mycobacterium tuberculosis*, *Streptococcus pyogenes*, *Staphylococcus aureus* and *Saccharomyces cerevisiae*. Thirdly, we isolated DNA from *C. trachomatis*- and LGV-negative rectal swaps obtained from ten healthy men. Isolated DNA of these samples included (unknown) microorganisms commonly found in the rectum. Finally, we included a series of 60 *ompA*-based L2b-positive clinical isolates from Switzerland, the Netherlands and Italy (see Table I for an overview of the results).

The results showed that only the samples that had been previously diagnosed as L2b by *ompA* sequencing tested posi-

TABLE I. Overview of the LGV L2b test results

	L2b RT-PCR test results	
	Positive with L2b RT-PCR	Negative with L2b RT-PCR
<i>C. trachomatis</i> serovars A–K	–	All
<i>C. trachomatis</i> serovars L1, L2, L2a, L3	–	All
<i>C. muridarum</i> (MoPn), <i>C. pneumoniae</i> , <i>C. pecorum</i> , <i>C. psittaci</i>	–	All
31 commonly found microorganisms*	–	All
Ten rectal swaps of healthy men	–	All
60 L2b-positive samples	All	–

*These 31 microorganisms are commonly found in the urogenital and anorectal tract, or in the oropharynx.

tive with the new insertion assay. There was no cross-reactivity with either the *Chlamydia* serovars/serovariants, including L2 and L2a, *Chlamydia* species or the other tested bacteria and clinical samples. In addition, we found equal sensitivity of the new L2b-specific and our adjusted LGV-specific PCR performing serial dilution tests. As expected, the general *C. trachomatis* plasmid PCR, targeting around ten cryptic plasmids, had a 10–50 times higher analytical sensitivity in serial dilutions.

Conclusions

The prevalence of LGV is still increasing in many European countries since the first reports of the LGV epidemic in 2003 [10]. Almost all strains causing this LGV epidemic are L2b [11–13]. Mapping the spread and prevalence of LGV serovariant L2b specifically may provide answers to important epidemiological questions that are needed for disease control and prevention. Therefore, a fast and highly accurate detection assay is a prerequisite. Our new LGV L2b test fulfills all these requirements and omits the laborious *ompA* sequencing step.

Acknowledgments

We thank Dries Budding, VU Medical Center Amsterdam, for providing the gastrointestinal samples to test specificity. We also thank Thomas Frey, medical practitioner, Zurich, for initiating the diagnosis of lymphogranuloma venereum in Zurich, Switzerland, in the year 2004. Josefine Mosimann, IMD, Institute for Medical & Molecular Diagnostics Ltd, Zurich, Switzerland, is acknowledged for support of parts of this study. We thank Rosangela Beretta, Giovanna Orlando and Maria Rita Gismondo for collection and processing of the specimens from Milano, Italy. The aims of the current study are in line with the European EpiGenChlamydia Consortium,

which is supported by the European Commission within the Sixth Framework Programme (FP6) through contract no. LSHG-CT-2007-037637. See <http://www.EpiGenChlamydia.eu> for more details about this Consortium.

Transparency Declaration

Conflicts of interest: nothing to declare.

References

1. Mabey D, Peeling RW. Lymphogranuloma venereum. *Sex Transm Infect* 2002; 78: 90–92.
2. Spaargaren J, Fennema HS, Morre SA, de Vries HJ, Coutinho RA. New lymphogranuloma venereum *Chlamydia trachomatis* variant, Amsterdam. *Emerg Infect Dis* 2005; 11: 1090–1092.
3. Morre SA, Spaargaren J, Fennema JS, de Vries HJ, Coutinho RA, Pena AS. Real-time polymerase chain reaction to diagnose lymphogranuloma venereum. *Emerg Infect Dis* 2005; 11: 1311–1312.
4. Vandenbruaene M, Ostyn B, Crucitti T et al. Lymphogranuloma venereum outbreak in men who have sex with men (MSM) in Belgium, January 2004 to July 2005. *Euro Surveill* 2005; 10: E050929.
5. Herida M, de BB, Sednaoui P et al. Rectal lymphogranuloma venereum surveillance in France 2004–2005. *Euro Surveill* 2006; 11: 155–156.
6. French P, Ison CA, Macdonald N. Lymphogranuloma venereum in the United Kingdom. *Sex Transm Infect* 2005; 81: 97–98.
7. van Weel J. Rare sexually transmitted disease hits Europe. *Lancet Infect Dis* 2004; 4: 720.
8. Gebhardt M, Goldenberger D. Lymphogranuloma venereum (LGV) serotype L2 in Switzerland, 2003–2005. *Euro Surveill* 2005; 10: E051222.
9. Thomson NR, Holden MT, Carder C et al. *Chlamydia trachomatis*: genome sequence analysis of lymphogranuloma venereum isolates. *Genome Res* 2008; 18: 161–171.
10. Nieuwenhuis RF, Ossewaarde JM, van der Meijden WI, Neumann HA. Unusual presentation of early lymphogranuloma venereum in an HIV-1 infected patient: effective treatment with 1 g azithromycin. *Sex Transm Infect* 2003; 79: 453–455.
11. Ward H, Martin I, Macdonald N et al. Lymphogranuloma venereum in the United Kingdom. *Clin Infect Dis* 2007; 1: 26–32.
12. Nieuwenhuis RF, Ossewaarde JM, Gotz HM et al. Resurgence of lymphogranuloma venereum in Western Europe: an outbreak of *Chlamydia trachomatis* serovar I2 proctitis in The Netherlands among men who have sex with men. *Clin Infect Dis* 2004; 7: 996–1003.
13. Spaargaren J, Schachter J, Moncada J et al. Slow epidemic of lymphogranuloma venereum L2b strain. *Emerg Infect Dis* 2005; 11: 1787–1788.