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

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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

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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	CATCCCCTAC	CGCTTCAAGC	TCTTCATCTC	CCACAGTCAG
B	AACTCCGC C T	GCTCCAGATC	CATCCCCTAC	CGCTTCAAGC	TCTTCATCTC	CCACAGTCAG
Ba	AACTCCGC C T	GCTCCAGATC	CATCCCCTAC	CGCTTCAAGC	TCTTCATCTC	CCACAGTCAG
C	AACTCCGC C T	GCTCCAGATC	CATCCCCTAC	CGCTTCAAGC	TCTTCATCTC	CCACAGTCAG
D	AACTCCTC C A	GCACCAGCAC	CAGCTCCTGC	TGCTTCAAGC	TCTTTATCTC	CAACAGTTAG
Da	AACTCCGC C T	GCTCCAGATC	CATCCCCTAC	CGCTTCAAGC	TCTTCATCTC	CCACAGTCAG
E	AACTCCTC C A	GCACCAGCAC	CAGCTCCTGC	TGCTTCAAGC	TCTTTATCTC	CAACAGTTAG
SW-E	AACTCCTC C A	GCACCA----	--GCTCCTGC	TGCTTCAAGC	TCTTTATCTC	CAACAGTTAG
F	AACTCCTC C A	GCACCAGCAC	CAGCTCCTGC	TGCTTCAAGC	TCTTTATCTC	CAACAGTTAG
G	AACTCCTC C A	GCACCAGCAC	CAGCTCCTGC	TGCTTCAAGC	TCTTTATCTC	CAACAGTTAG
H	AACTCCTC C A	GCACCAGCAC	CAGCTCCTGC	TGCTTCAAGC	TCTTTATCTC	CAACAGTTAG
I	AACTCCTC C A	GCACCA----	--GCTCCTGC	TGCTTCAAGC	TCTTTATCTC	CAACAGTTAG
Ia	AACTCCTC C A	GCACCAGCAC	CAGCTCCTGC	TGCTTCAAGC	TCTTTATCTC	CAACAGTTAG
J	AACTCCTC C A	GCACCA----	--GCTCCTGC	TGCTTCAAGC	TCTTTATCTC	CAACAGTTAG
Ja	AACTCCTC C A	GCACCA----	--GCTCCTGC	TGCTTCAAGC	TCTTTATCTC	CAACAGTTAG
K	AACTCCTC C A	GCACCA----	--GCTCCTGC	TGCTTCAAGC	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	TTTGTTACCC	TATCTTCTAG	TAGTTCTAGT	AGTGCTAGTA	ACGGGGTTAC	AATGAAGCGT
Forward	TCGCT	GACGATTCCT	TTTGTT					
Probe				CTTCTAG	TAGTTCTAGT	AGTGCT		
Reverse						TA	ACGGGGTTAC	AATGAAGCG
L1	TGGGATCGCT	GACGATTCCT	TTTGTTACCC	TATCTTCTAG	TAGT------	---ACTAGTA	ACGGGGTTAC	AATGAAGCGT
L2	TGGGATCGCT	GACGATTCCT	TTTGTTACCC	TATCTTCTAG	TAGT------	---ACTAGTA	ACGGGGTTAC	AATGAAGCGT
L3	TGGGATCGCT	GACGATTCCT	TTTGTTACCC	TATCTTCTAG	TAGT------	---ACTAGTA	ACGGGGTTAC	AATGAAGCGT
A	TGGGATCGCT	GACAATTCCT	TTTGTTACCC	TATCTTCTGT	CAAT---AAT	ACCAATAGTA	ATGGGGTTGT	AAAAACAGCT
B	TGGGATCGCT	GACAATTCCT	TTTGTTACCC	TATCTTCTGT	CAAT---AAT	ACCAATAGTA	ATGGGGTTGT	AAAAACAGCT
Ba	TGGGATCGCT	GACAATTCCT	TTTGTTACCC	TATCTTCTGT	CAAT---AAT	ACCAATAGTA	ATGGGGTTGT	AAAAACAGCT
C	TGGGATCGCT	GACAATTCCT	TTTGTTACCC	TATCTTCTGT	CAAT---AAT	ACCAATAGTA	ATGGGGTTGT	AAAAACAGCT
D	TGGGATCGCT	GACAATTCCT	TTTGTTACTC	TATCTTCTAG	TAGT------	---GCTAGTA	ACGGAGTTAC	AAAAAATTCT
Da	TGGGATCGCT	GACAATTCCT	TTTGTTACCC	TATCTTCTGT	CAAT---AAT	ACCAATAGTA	ATGGGGTTGT	AAAAACAGCT
E	TGGGATCGCT	GACAATTCCT	TTTGTTACTC	TATCTTCTAG	TAGT------	---GCTAGTA	ACGGAGTTAC	AAAAAATTCT
SW-E	TGGGATCGCT	GACAATTCCT	TTTGTTACTC	TATCTTCTAG	TAGT------	---GCTAGTA	ACGGAGTTAC	AAAAAATTCT
F	TGGGATCGCT	GACAATTCCT	TTTGTTACTC	TATCTTCTAG	TAGT------	---GCTAGTA	ACGGAGTTAC	AAAAAATTCT
G	TGGGATCGCT	GACAATTCCT	TTTGTTACTC	TATCTTCTAG	TAGT------	---GCTAGTA	ACGGAGTTAC	AAAAAATTCT
H	TGGGATCGCT	GACAATTCCT	TTTGTTACTC	TATCTTCTAG	TAGT------	---GCTAGTA	ACGGAGTTAC	AAAAAATTCT
I	TGGGATCGCT	GACAATTCCT	TTTGTTACTC	TATCTTCTAG	TAGT------	---GCTAGTA	ACGGAGTTAC	AAAAAATTCT
Ia	TGGGATCGCT	GACAATTCCT	TTTGTTACTC	TATCTTCTAG	TAGT------	---GCTAGTA	ACGGAGTTAC	AAAAAATTCT
J	TGGGATCGCT	GACAATTCCT	TTTGTTACTC	TATCTTCTAG	TAGT------	---GCTAGTA	ACGGAGTTAC	AAAAAATTCT
Ja	TGGGATCGCT	GACAATTCCT	TTTGTTACTC	TATCTTCTAG	TAGT------	---GCTAGTA	ACGGAGTTAC	AAAAAATTCT
K	TGGGATCGCT	GACAATTCCT	TTTGTTACTC	TATCTTCTAG	TAGT------	---GCTAGTA	ACGGAGTTAC	AAAAAATTCT

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 complimentary sequence of the actual reverse primer.