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Short Communication OMICS International

Implementation of *Lymphgranuloma venereum* Identification in *Chlamydia trachomatis* Testing by Nucleic Acid Amplification Tests

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Lymphogranuloma venereum (LGV) is caused by particular serovars/genotypes of *Chlamydia trachomatis* (L1-L3). In contrast to genotypes A-K, LGV genotypes are more invasive, as they can cross the epithelium and spread via the lymphatics. Until recently, LGV is rarely detected outside endemic areas in Africa, Asia, South America and the Caribbean, but since 2003 several outbreaks with genotype L2 were reported among men who have sex with men (MSM) in Europa, North America and Australia [1]. In 2013, more than 1.000 cases were reported to the ECDC [2]. The real number is probably higher, as in many European countries LGV is not recorded routinely.

Among MSM anorectal symptoms are the predominant clinical manifestation of LGV, in contrast to the classical inguinal syndrome of sporadic LGV [3]. Laboratory confirmation by C. trachomatis detection and typing is important because LGV requires longer antibiotic treatment (Doxycyclin 200 mg/day for at least 3 weeks in contrast to one week for non-LGV cases) [4]. Accordingly, identification of LGV by laboratory tests should always be considered when investigating MSM. Several currently available commercial NAATs allow sensitive and specific detection of *C. trachomatis*, but do not provide any information about the underlying genotype(s) [5]. Identification of LGV in specimens from MSM or other suspicious cases would require a separate analysis. Methods for typing include genotype-specific PCRs and RFLP or sequence analysis of appropriate *omp*1-gene regions [1,6,7]. LGV and non-LGV strains can also be differentiated by PCR tests based on *pmp*H or *omp*1 gene regions that contain specific deletions or single nucleotide polymorphisms [7-9].

In contrast to chlamydia diagnostics in MSM, other indications of *C. trachomatis* testing (screening of young women or pregnant women, testing of symptomatic heterosexual patients) generally do not require LGV typing in case of positive results, because LGV is still a very rare disease in these populations. The recognition of variant *C. trachomatis* strains that may escape diagnosis by NAATs based on single target regions [10] has led to recommendations to use a second target region for amplification. Indeed, some novel commercial NAATs are designed as dual target assays [11]. In addition, laboratories using in house assays for *C. trachomatis* testing are also advised to use two different target regions in order to prevent missed infections due to target sequence variation. In this context using a DNA sequence that differs between LGV and non LGV as a second target would kill two birds with one stone: detection of strains with variation in one of the target regions and identification of LGV genotypes.

Several years ago we introduced a duplex PCR assay in our laboratory to test for *C. trachomatis* and to differentiate between LGV and non LGV strains in a single reaction. The laboratory is hospital-based (Department of Microbiology, University Medical Center Hamburg-Eppendorf, Germany) and receives samples from patients of different clinical departments (urology, gynecology, and dermatology as well as outpatient center for infectious diseases). The assay targets a sequence from the cryptic plasmid and the *pmp*H sequence, based on the method described by Chen et al. [8] with some modifications. DNA was extracted from 200 µl E-swabs (Hain Lifesciences, Nehren, Germany) or first void urine using the MagNa Pure DNA and viral NA small volume kit on the MagNa Pure automated nucleic acids extraction system (Roche, Basel, Switzerland). 5 µl of the DNA eluate (representing 5% of the original sample volume) was combined with Quantifast Pathogen DNA Mastermix (Qiagen, Hilden, Germany), primers and probes (sequences given in Table 1) and water up to 25 µl total PCR volume. The mastermix contains HotStarTaq Plus DNA polymerase, dNTP mix and buffer, as well as an internal control DNA to check for efficient amplification. The PCR was performed on LC480 thermal cycler (Roche) by using the following temperature profile: 5 min 95°C (activation of the polymerase), 40 cycles of 15 sec 95°C and 30 sec 60°C (amplification), 30 sec 40°C (cooling).

Primer/probe	Sequence/fluorescence label
pmpH forward	GGATAACTCTGTGGGGTATTCTCCT
pmpH reverse	AGACCCTTTCCGAGCATCACT
pmpH non LGV probe	ROX-GCTTGAAGCAGCAGGAGCTGGTG-BHQ2
pmpH LGV probe	FAM-CCTGCTCCAACAGT-NFQ-MGB
Plasmid forward	GGATTGACTCCGACAACGTATTC
Plasmid reverse	ATCATTGCCATTAGAAAGGGCATT
Plasmid probe	Atto 647-TTACGTGTAGGCGGTTTAGAAAGCGG-BHQ650

Table 1: Sequences of primers and probes used for detection of *Chlamydia trachomatis* and differentiation of LGV and non LGV genotypes.

During routine testing over the last 2 years (1.12.14-30.11.16) we detected 156 *C. trachomatis* infections. In 148 cases (94.9%) we were able to differentiate between LGV and non LGV (38 LGV, 109 genotype D-K, 1 mixed infection with LGV and genotype D-K). In 8/156 samples (5.1%) only the plasmid sequence was amplified (Table 2). Lack of amplifying of the *pmp*H target probably resulted from low bacterial concentrations, as the threshold cycle of plasmid DNA detection is >36 in all these cases. In contrast to the cryptic plasmid,

which is present in about 10 copies per cell, *pmp*H is a single copy gene probably affecting the comparatively lower analytical sensitivity of *pmp*H amplification. Our results are similar to those of Chen et al. who failed to detect 3/39 (7.7%) non LGV infections [8]. Quint et al. reported a high sensitivity of the *pmp*H PCR for LGV, but they missed 24% of non LGV infections in their study [12].

	n	non-LGV	LGV	not identified
CT-positive	**156**	**110***	**39***	**8**
Females	**45**	**41**	**0**	**4**
Vaginal swab	33	29	0	4
Cervical swab	7	7	0	0
Urine	1	1	0	0
Conjunctival swab	4	4	0	0
Males	**111**	**69***	**39***	**4**
Genital swab	10	7	3	0
Urethral swab	28	26	0	2
Urine	5	5	0	0
Anal swab	67	30*	36*	2
Conjunctival swab	1	1	0	0

*one patient with mixed infection (LGV and non LGV genotype);
CT: *Chlamydia trachomatis*

Table 2: Distribution of LGV and non LGV genotypes in *C. trachomatis* positive samples.

The various sensitivity for non LGV types may be explained by methodological differences (the studies differed with respect to primer sequences, fluorescence labels, polymerases, thermal cycler and cycling conditions). In all studies the sensitivity of the *pmp*H based PCR assays was inferior to the comparator *C. trachomatis* NAATs. Thus, *pmp*H based assays are not suitable as a single primary test, but the gene represents a useful second target in dual target assays to take account of *Chlamydia trachomatis* sequence variability and to simultaneously allow identification of LGV strains.

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