

# Lymphogranuloma venereum diagnostics: from culture to real-time quadriplex polymerase chain reaction

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Since the end of 2003, an ongoing lymphogranuloma venereum (LGV) proctitis outbreak has been reported in industrialised countries, first in The Netherlands, followed by neighbouring European countries and the United Kingdom, and now in many other countries and continents including the United States, Canada and Australia.<sup>1,2</sup>

When we analysed the LGV strain identified in this outbreak on a nucleotide level, four nucleotide changes were found when compared with reference serovars (L2, L2a, and the variant L2') including one change that encoded the previously undescribed change at amino acid 162, AAT→AGT and we designated this strain L2b (GenBank accession number AY586530).<sup>2</sup> We have shown by retrospective epidemiological analyses that this L2b strain was the only strain present among men who have sex with men in Amsterdam and appeared to have been circulating in Amsterdam in 2000 (no retrospective samples were available before 2000).<sup>3</sup> Moreover, we showed that this L2b variant was already present in the 1980s in San Francisco, with exactly the same mutations in the complete *ompA* gene (previously *omp1*).<sup>3</sup>

Since the identification of this at least 28-year slowly evolving epidemic with an organism that has gone unnoticed in the

community, major advances have been made regarding the diagnostics for LGV. Correct diagnostics for LGV are essential because successful treatment of LGV (serovars L1–L3) proctitis requires a 3-week course of doxycycline, whereas in the case of *Chlamydia trachomatis* proctitis caused by serovars D–K, a 1-week course is given, which does not clear LGV-based proctitis.<sup>4,5</sup> Treatment is essential because it seems that the natural course of these LGV infections is much more severe compared with the natural course of anourogenital serovars D–K.<sup>5,6</sup>

In fig 1 are shown the most commonly used techniques to diagnose LGV infection. The first three approaches have serious disadvantages: cell culture is rarely available in routine diagnostic settings, PCR-based restriction fragment length polymorphism (RFLP) analysis needs post-PCR restriction enzyme profiling, and sequencing requires additional analyses of sequence data to identify the *C trachomatis* serovar responsible for infection.<sup>7,8</sup> In addition, all three techniques are time consuming (at least 1–4 days to get a result), laborious, and require specially trained personnel in a sophisticated laboratory setting. Sensitive, specific and fast real-time PCR approaches are now the standard for many infectious diseases including LGV (see fig 1).

In 2005 we developed the first LGV real-time PCR assay, to be used after a *C trachomatis*-positive test result in cases in which the patient belonged to a risk group for LGV infection.<sup>9</sup> In 2006, a more advanced test became available from Halse and colleagues,<sup>10</sup> who described a real-time multiplex PCR that is capable of detecting all *C trachomatis* serovars while simultaneously detecting serovar L2 in particular.<sup>10</sup> In addition, this assay includes a spiked internal plasmid control to monitor PCR inhibition. This test is unable to detect L1 and L3 serovars,

however, and also a mixed infection with a non-LGV strain would remain undetected if further molecular testing was not performed.

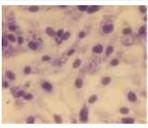
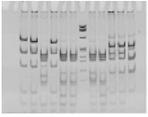
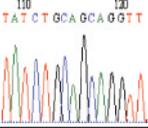
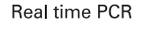
In 2007 Chen *et al*<sup>11</sup> developed a multiplex real-time PCR assay that simultaneously detects and differentiates all LGV strains from non-LGV strains, but this test was not capable of differentiating a co-infection caused by LGV and non-LGV strains. In 2007 the first assay was published that is able to distinguish multiple serovar infections in one sample including LGV strains.<sup>12</sup> This assay is very suitable for epidemiological purposes but too laborious for routine diagnostic purposes as is the case for other direct multiple serovar detection systems.<sup>13,14</sup>

In this issue (see page 273) Chen and colleagues<sup>15</sup> report how they further developed the test previously published by their group<sup>11</sup> into a quadriplex reverse transcriptase-PCR assay that detects individual LGV or non-LGV infection as well as mixed infections, making this a unique assay.<sup>15</sup> In addition, their assay is also able to identify specimens that either contain PCR inhibitors or are not adequately collected for PCR. This assay only requires additional molecular testing in order to differentiate among LGV serovars. For treatment purposes, however, differentiating between the different LGV strains is irrelevant, so the current format combines all variables for a perfect diagnostic test. As we have shown previously in double infections with LGV and non-LGV strains in the same sample, we always found that the LGV strain had a lower bacterial load (>10×) compared with the non-LGV strain, with the result that when using PCR-based RFLP, because of the exponential amplification in a PCR, one loses the LGV signal. The quadriplex RT-PCR has proved itself in that even double infections with bacterial load differences far above factor 10 can be detected, making this test a very robust and clinically relevant test.

US Food and Drug Administration approval has not been granted for LGV detection for any commercially available test and also the use of the specimen in which this LGV is mostly detected, a rectal swab, has not been approved by the US Food and Drug Administration or given CE certification. The fact that current LGV rates have not declined over the past 2 years in Amsterdam emphasises the need for ongoing public health awareness, and further validated implementation of the LGV test is warranted.

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Diagnostics for LGV infection	
	Chlamydia culture - Cell culture - Microscopic assessment - Mono/poly clonal antibody typing - MIF patterns
	Omp1 PCR RFLP analysis - Nested PCR - PAA gel - Restriction enzyme treatment - Profile determination
	Sequencing omp1 gene - PCR based - Sequencing of the complete gene or variable segment two (VS-2) - BLAST profiling
	Simplex (Morré <i>et al</i> <sup>9</sup> ) - LGV specific (L1, L2, L3), used after a positive general <i>C trachomatis</i> PCR
	Real time PCR Multiplex (Halse <i>et al</i> <sup>10</sup> ) - All CT serovars together and L2 specifically and monitoring of inhibition
	Multiplex (Chen <i>et al</i> <sup>11</sup> ) - Simultaneously detects and differentiates LGV from non-LGV strains
	Nested RT PCR (Jalal <i>et al</i> <sup>12</sup> ) - Multistep RT-PCR for specific genotyping of <i>C trachomatis</i> including mixed infections
	Quadriplex (Chen <i>et al</i> <sup>15</sup> ) - Detection of LGV and non-LGV serovars and detection of mixed infections - Human DNA control for monitoring sample adequacy and PCR inhibition

**Figure 1** Diagnostic approaches for the detection of LGV infections.

CT, *Chlamydia trachomatis*; LGV, lymphogranuloma venereum; MIF, Müllerian inhibiting factor; RFLP, restriction fragment length polymorphism; RT, real-time. The current figure is adapted with permission from fig 1 in Morr e *et al.*<sup>9</sup> (Morr e SA, Spaargaren J, Fennema JS, *et al.* Real-time polymerase chain reaction to diagnose lymphogranuloma venereum. *Emerg Infect Dis* 2005;**11**:1311–12).

Can we expect further improvements in the LGV test in the near future? For LGV and non-LGV diagnostic purposes the current quadriplex assay has it all, the only improvement envisioned would be to add other sexually transmitted diseases, beginning with *Neisseria gonorrhoeae*, to develop a multi-sexually transmitted disease multiplex real-time PCR.

**Competing interests:** None declared.

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