A comparison of two methods for the diagnosis of lymphogranuloma venereum

Sarah Alexander, Iona M. C. Martin and Catherine Ison

Sexually Transmitted Bacteria Reference Laboratory, Health Protection Agency, Centre for Infections, 61 Colindale Ave, London NW9 5HT, UK

A recent outbreak of lymphogranuloma venereum (LGV) within the men who have sex with men (MSM) community and their requirement for extended therapy has highlighted the need for laboratory tests that differentiate LGV- from non-LGV-associated serovars of Chlamydia trachomatis. Two previously described methods were evaluated against 495 clinical specimens referred to the Sexually Transmitted Bacteria Reference Laboratory (London, UK): (i) PCR amplification of a 1.1 kb region of the ompI gene followed by restriction enzyme digestion (ompI RFLP-PCR); and (ii) real-time PCR targeting a 36 bp deletion present within the polymorphic membrane protein H gene of LGV-associated serovars (pmpH real-time PCR). For specimens that could be categorized using both methods, a 94.7 % (390/412) concordance was achieved. Eighty-three specimens were found to be untypeable by ompI RFLP-PCR due to a failure to amplify the 1.1 kb fragment. Of these 83 untypeable specimens, 19 were determined to be an LGV-associated serovar by pmpH real-time PCR. Despite the high level of concordance, there were differences found in the technical complexity of the two methods. The pmpH real-time PCR exhibited greater sensitivity, a more rapid turnaround time and a lower technical requirement. Whilst the ompI RFLP-PCR was not as robust as a laboratory diagnostic method, it did enable serovar-level identification. LGV infection remains an important threat to the health of high-risk MSM in Europe. In conclusion, the two methods for the detection of LGV from clinical samples were found not only to have a high concordance (94.7 %) but also to be complementary, and could be used in an integrated way to aid LGV detection.

INTRODUCTION

A recent outbreak of lymphogranuloma venereum (LGV) within the men who have sex with men (MSM) community has raised awareness of this disease and highlighted it as a re-emerging public health problem within the UK (Ward et al., 2007). It was previously regarded as a tropical infection (LGV is endemic in parts of Africa, Asia and South America) and, until recently, cases in Europe remained rare and were almost exclusively confined to importation (Jebbari et al., 2007; Ward et al., 2007).

LGV is caused by specific serovars of Chlamydia trachomatis (L1, L2 and L3) and these strains are associated with a more chronic and invasive infection than other serovars. Although LGV symptoms can vary according to site of entry and stage of infection, genital ulceration and inguinal lymphadenopathy are the classical presentations of this disease. However, during the recent European MSM outbreak, anorectal symptoms were the most common presentation, making syndromic management problematic (van de Laar, 2006; Richardson & Goldmeier, 2007). LGV infection is also more complex than standard C. trachomatis infection, with patients requiring an extended course of therapy when compared with infection with serovars D–K. Because of this, the accurate laboratory diagnosis of LGV serovars of C. trachomatis is essential (BASHH, 2006; McLean et al., 2007).

The laboratory identification of LGV can be problematic, as routine culture of C. trachomatis for diagnostic purposes has largely been replaced with nucleic acid amplification techniques; consequently, traditional serotyping methods are now redundant. Two main molecular methods that enable the identification of LGV biovars of C. trachomatis have been described: (i) PCR amplification of a 1.1 kb region of the outer-membrane I (ompI) gene followed by restriction enzyme digestion (ompI RFLP-PCR; Lan et al., 1993, 1994); and (ii) real-time PCR targeting a 36 bp deletion present within the polymorphic membrane protein H (pmpH) gene of LGV-associated serovars (pmpH real-time PCR; Morre et al., 2005).

Clinical specimens referred to the Sexually Transmitted Bacteria Reference Laboratory (STBRL; London, UK) for LGV diagnosis were examined initially using a combina-
tion of a plasmid-based real-time PCR that detects all C. trachomatis serovars and ompI RFLP-PCR. These specimens were then examined retrospectively using the LGV-specific pmpH real-time PCR. Here, we report the first comparative data for the two major methods available for the detection of LGV from clinical specimens.

METHODS

Specimens examined. A total of 495 specimens from the following sites were examined in this study: rectal (411), urethral (14), other (49) and site unstated (21). All specimens were taken from high-risk symptomatic MSM and were referred to the STBRL during 2004–2005 for LGV testing.

A range of specimens were received, including fresh dry swabs, unprocessed swabs in transport buffer and residual processed material from nucleic acid amplification techniques. Dry swabs were hydrated with 500 μl PBS and agitated on an orbital shaker at 150 r.p.m. for 1 h.

DNA extraction. DNA extractions were performed using one of three methods: (i) manual extraction using a QiAamp Viral RNA Mini kit (Qiagen); (ii) automated extraction using a Corbett DNA X-Tractor with a Macherey-Nagel blood extraction kit; or (iii) MagNA Pure automated extraction (Roche). The three different methods of DNA extraction were employed to cope with increasing levels of clinical specimens, each representing an advancement in automation. All extractions were performed according to the manufacturer’s instructions and each clinical specimen was extracted using only a single DNA extraction method.

C. trachomatis detection. The Chlamydia status of all specimens was determined using the method of Chen et al. (2007). The PCR contained primers targeting an 88 bp region of the C. trachomatis cryptic plasmid and primers targeting the human RNase P gene, which acted as an internal control. All reactions were performed on a Corbett RotorGene-3000. Each 25 μl reaction contained the following primers and reagents: 200 nM CTF-008 (5'-GGATTGACTCCGA-CAACGTATTCT-3'), 300 nM CTR-009 (5'-ATCATTTGCCATTAG-AAGGGCATT-3'), 200 nM CTP-010 (5'-GGATTGACTCCGA-CAACGTATTCT-3'), 200 nM CTR-009 (5'-AGATTTGGACCTGGACAGCGG-3'), 200 nM RNPR-003 (5'-AGATTTGGACCTGGACAGCGG-3'), 80 nM RNPF-003 (5'-AGATTTGGACCTGGACAGCGG-3'), 80 nM RNPR-002 (5'-GACGGGCTGT-CTCCACAAGT-3'), 80 nM RNPF-001 (5'-CY5-TTCGAGCTCTTGAAGCCTGT9C-3'), 200 μM dATP, 200 μM dCTP, 200 μM dGTP, 400 μM dUTP, 1X PCR buffer (Applied Biosystems), 4 mM MgCl2, 5 U AmpliTaq Gold polymerase, 0.5 U UNG and 5 μl DNA template. Reaction conditions were: 2 min at 50 °C (denaturation of UNG) and 10 min at 95 °C (hot-start Tag activation), followed by 45 cycles of 25 s at 95 °C (denaturation) and 1 min at 60 °C (combined annealing and extension). Where ompI RFLP-PCR and pmpH real-time PCR produced discordant results, both tests were repeated, if sufficient sample was available, to confirm the results in an attempt to resolve the discordant status.

RESULTS

Comparison of ompI RFLP-PCR and pmpH real-time PCR

A total of 495 specimens sourced from high-risk patients were examined using both a combination of a C. trachomatis-specific in-house PCR and ompI RFLP-PC, and a pmpH LGV-specific real-time PCR assay (Table 1).

Table 1. Evaluation of the two different methods for the detection of LGV from clinical samples

Results are given as the number of samples. Three specimens produced pmpH real-time PCR discordant results that could not be resolved and therefore are not represented in Table 1, giving a total of 492 samples. An explanation of these three specimens is given in Results.

Results of ompI RFLP-PCR and C. trachomatis plasmid real-time PCR confirmation

<table>
<thead>
<tr>
<th></th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>LGV</td>
<td>163</td>
<td>5</td>
</tr>
<tr>
<td>Non-LGV associated</td>
<td>14</td>
<td>141</td>
</tr>
<tr>
<td>ompI failed to amplify</td>
<td>19</td>
<td>64</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>78</td>
</tr>
<tr>
<td>Equivocal</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Inhibited</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>
The two methods produced concordant results in 390 of the specimens examined: (i) 163 specimens were determined to be LGV using both methods; and (ii) 227 specimens were determined to be negative using the \textit{pmpH} real-time PCR, of which 78 specimens were negative for \textit{C. trachomatis}, six were equivocal for \textit{C. trachomatis} and 141 were determined to be non-LGV-associated by \textit{ompI} RFLP-PCR typing. Two specimens were found to be PCR inhibitory. Eighty-three specimens (which were determined to be \textit{C. trachomatis}-positive using the plasmid-based real-time PCR) were untypeable using the \textit{ompI} RFLP-PCR assay due to repeated failure to amplify the 1.1 kb fragment. Of the 83 untypeable specimens by \textit{ompI} RFLP-PCR, 19 were found to be positive for LGV using the \textit{pmpH} real-time PCR. This highlights the increased sensitivity that can be achieved when using a real-time PCR-based method. Ultimately, for specimens that could be categorized using both methods, a 94.7\% (390/412) concordance was achieved.

Twenty-two specimens (22/412; 5.3\%) were found to be discordant between the two methods. Five of these were negative by \textit{pmpH} real-time PCR but generated profiles that were consistent with L2 by \textit{ompI} RFLP-PCR. The retrospective analysis of the specimen collection using \textit{pmpH} real-time PCR is a plausible explanation for these discordant specimens, as DNA degradation could have occurred. However, it is also possible that some of these specimens were initially misidentified as LGV serovars by \textit{ompI} RFLP-PCR due to the subjective and complex nature of the interpretation of the banding profiles. In our laboratory, only LGV serovars (L1, L2 and L3) are included as positive controls, rather than a full set of all 14 \textit{C. trachomatis} serovars. This can sometimes make the interpretation of \textit{ompI} RFLP-PCR profiles difficult, as some digests of standard serovars of \textit{C. trachomatis} can closely resemble those of LGV strains.

Fourteen specimens were found to be positive using the \textit{pmpH} real-time PCR method but non-LGV-associated using \textit{ompI} RFLP-PCR. It is unlikely that false-positive \textit{pmpH} real-time PCR results can explain these discrepancies, as repeat testing of ten of these specimens confirmed that these samples were reproducibly positive by \textit{pmpH} real-time PCR (the four remaining specimens had insufficient volume for repeat testing). It should be noted that the specimens examined in this study were obtained from very high-risk patients and therefore the possibility of mixed-serovar \textit{C. trachomatis} infections cannot be overlooked. This could provide a highly likely explanation for these discrepant results, as the real-time PCR method only requires one LGV \textit{pmpH} target to generate a positive result, whereas the \textit{ompI} RFLP-PCR would generate either a mixed profile or a profile of the most abundant \textit{C. trachomatis} serovar, which could have resulted in a result for non-LGV-associated serovar of \textit{C. trachomatis} being generated. Again, it is also possible that the subjective nature of RFLP-PCR profiles could have resulted in some weak or partially digested LGV profiles being misidentified as non-LGV-associated serovars.

The remaining three clinical specimens produced discrepant results when examined by \textit{pmpH} real-time PCR and these could not be resolved. For all three specimens, two DNA extractions were performed from the original swab transport medium because the \textit{ompI} gene had failed to amplify in the initial RFLP-PCR. When retrospectively performing the \textit{pmpH} real-time PCR on both extractions from all three specimens, discordant results were produced, with one extraction producing a positive result and one producing a negative result. Attempts were made to resolve the discordant status of these specimens by performing a fresh DNA extraction (one specimen had insufficient volume for a repeat extraction); however, both specimens had completely degraded.

**DISCUSSION**

The recent outbreak of LGV throughout western Europe has highlighted the need for patients infected with LGV serovars of \textit{C. trachomatis} to be given extended antimicrobial therapy and the problems associated with trying to diagnose this condition syndromically. Consequently, rapid and reliable laboratory methods that can differentiate LGV from non-LGV-associated \textit{C. trachomatis} infections are extremely important. In this paper, we provide the first comparative data for the two major methods available for the diagnosis of LGV.

This study compared two methods for the detection of LGV from clinical samples. Whilst both methods showed high levels of concordance (94.7\%), they were found to differ in terms of technical complexity. The greater sensitivity, shorter turnaround time and lower technical requirement of the \textit{pmpH} real-time PCR test would make it the test of choice for diagnostic use. The \textit{ompI} RFLP-PCR is not a diagnostic test but rather a \textit{C. trachomatis} genotyping method, which enables serovar-level identification of a specific clinical specimen. However, this method lacks sensitivity, has a lengthy turnaround time and requires both experienced staff and specialist equipment, and therefore is almost exclusively confined to specialized centres. In the UK, all LGV infection is detected at a central facility (STBRL), where currently the LGV testing algorithm is to test any referred specimen for LGV using the more sensitive and rapid \textit{pmpH} real-time PCR. All \textit{pmpH} real-time PCR-positive specimens are then examined further using the \textit{ompI} RFLP-PCR to identify the precise serovar of LGV responsible and to confirm the original \textit{pmpH} result.

Ultimately, the detection of a LGV-positive clinical specimen using either of the methods examined in this study will always be technically demanding, as specimens first require a primary screen with a \textit{C. trachomatis} diagnostic test. Such an approach has been problematic historically, as not all commercial \textit{C. trachomatis} platforms are approved by the Food and Drug Administration for the examination of rectal specimens, although recent data suggest that these platforms exhibit high specificity when
presented with these specimens (Alexander et al., 2007). In addition, whilst a recent real-time PCR method has been described that enables the simultaneous detection of both C. trachomatis serovars and L2 strains specifically, such a method would clearly miss LGV infections caused by L1 and L3 serovars (Halse et al., 2006).

LGV infection remains an important threat to the health of high-risk MSM in Europe, with 492 cases of LGV confirmed within the UK to date (Jebbari et al., 2007). In the UK – unlike other European countries – the case definition of a confirmed LGV infection is one that has been detected in the laboratory using solely molecular methods. In this study, two methods were compared for the detection of LGV from clinical samples, both of which were found not only to have a high concordance (94.7 %), but also to be complementary, and could be used in an integrated way to aid LGV detection.

ACKNOWLEDGEMENTS

Authors would like to thank the microbiologists and GUM staff who referred specimens to STBRL for LGV detection and all members of the LGV UK Incident Group.

REFERENCES


