Anal lymphogranuloma venereum screening with IgA anti-\textit{C. trachomatis}-specific Major Outer Membrane Protein serology

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Abstract

**Background** Anal lymphogranuloma venereum infections, caused by *Chlamydia trachomatis* biovar L (Ct+/LGV+), are endemic among men who have sex with men (MSM). We previously showed that anal non-LGV biovar Ct infections (Ct+/LGV-) can be eradicated with 1 week doxycycline, whereas Ct+/LGV+ infections require a 3 week doxycycline regimen. Therefore it is important to differentiate anal Ct+/LGV+ from Ct+/LGV- infections in MSM and biovar specific chlamydia nucleic acid amplification technologies (NAATs’) are considered standard. However, these assays are expensive and laborious. We therefore evaluated 4 chlamydia specific serological assays to differentiate Ct+/LGV+ from Ct+/LGV- irrespective of symptoms, and additionally in an asymptomatic patient group.

**Methods** MSM visiting the Amsterdam STI clinic before January 2008 were diagnosed Ct+/LGV+ or Ct+/LGV- based on a commercial non-specific NAAT for anal chlamydia and confirmed with an in house biovar L specific NAAT. Serum samples were evaluated with chlamydia specific anti-Major Outer Membrane Protein (MOMP) and anti-Lipopolysaccharide assays of both IgA and IgG classes.

Asymptomatic patients were identified as: 1) no anal complaints or 2) no microscopic inflammation (i.e. <10 leucocytes per high power field in anal smears). The best differentiating assay was subsequently evaluated in 100 Ct+/LGV+ and 100 Ct+/LGV- MSM visitors using different cut off points.

**Results** From the 4 evaluated serologic assays, the anti-MOMP IgA assay was the most accurate to differentiate Ct+/LGV+ (n=42) from Ct+/LGV- (n=19) with 85.7% sensitivity (CI95%, 72.2-93.3) and 84.2% specificity (CI95%, 62.4-94.5). The anti-MOMP IgA identified LGV proctitis with similar accuracy in patients without anal complaints (23 proctitisCt/LGV+ and 15 Ct+/LGV-), and without microscopic inflammation (14 proctitisCt/LGV+ and 8 Ct+/LGV-). In a population comprising 98 Ct+/LGV+ and 105 Ct+/LGV- patients the anti-MOMP IgA assay scored most accurate when the cut off point was set to 2.0 with 75.5% (CI95% 65.8-83.6) sensitivity and 74.3% (CI95%, 64.8-82.3) specificity.

**Conclusions** In situations lacking a biovar L specific NAAT, the IgA anti-MOMP assay can be used as alternative test to identify anal LGV infections, even in asymptomatic patients.
Background

Lymphogranuloma venereum (LGV) is an invasive ulcerative STI caused by C. trachomatis biovar L.[1] The infection spreads beyond mucosal linings into connective tissue layers and via lymphatic vessels and causes destructive and systemic inflammatory reactions, usually with extensive production of pathogen specific antibodies. Acute anal LGV infections are characterised by anal cramps (tenismus), pain, bloody discharge, and constipation due to local oedema. If left untreated, chronic disease can lead to irreversible anal strictures causing soiling, pain, constipation and mega colon.[2] In contrast, anal C. trachomatis infections caused by biovars D-K do not spread beyond the mucosa and generally cause far less symptoms, and usually minimal antibody production.

Since 2003 an ongoing epidemic of anal LGV infections among Men who have Sex with Men (MSM) was first reported in the Netherlands, followed by other Western countries.[3] Anal LGV infections are associated with high risk behaviour reflected in numerous co-infections like hiv, syphilis, hepatitis C, and hepatitis B, and with the use of rectal enemas (douching).[4-6]

Routine screening of MSM with receptive anal contact on anal C. trachomatis infections is recommended in the United States CDC and in the European IUSTI/WHO guidelines.[7,8] Sequential LGV serovar confirmation is desirable since anal LGV infections require doxycycline for 21 days, where 7 days suffice for anal non-LGV (biovars D-K) chlamydia infections.[9]

In contrast to textbook reports, a considerable portion of the anal LGV infections in the current epidemic are asymptomatic (i.e. without patient reported complaints or without clinical signs) at the time of diagnosis. In a previous retrospective study we showed that, upon anoscopyc examination, mucosal membrane abnormalities were visible in only 47% of 87 cases with an anal LGV infection, and signs of microscopic inflammation (i.e >10 leucocytes counted in a high power field in a Gram stained rectal smear) was present in 61%.[5] In a prospective study including 32 cases with an anal LGV infection, only 44% reported anal complaints.[6] The cause of an asymptomatic presentation is unknown, but is possibly related to an altered immune response due to concurrent hiv infection (which is present in approximately 80% of the cases). Some national guidelines recommend LGV
screening only in cases with suspected symptoms. Consequently, asymptomatic infections will be missed.

The gold standard to diagnose anal LGV infections nowadays is the detection of LGV biovar specific chlamydia DNA through “in house” developed nucleic acid amplified tests, but these tests are expensive, and require specialised laboratory conditions. There is a need for less expensive, less laborious and less specialized screening methods to screen large patient groups at risk for LGV. Serological tests which detect antibodies against chlamydia specific membrane proteins could be an option for this purpose, but it is of importance that these serologic tests can also detect asymptomatic LGV infections. In this study we evaluated the diagnostic characteristics of 4 chlamydia specific serological assays to detect anal LGV infections. The study population consisted of MSM visiting the STI clinic with a confirmed anal chlamydia infection, either with or without symptoms.

Methods

Routine anal infection screening procedure

At the time of this study, all MSM reporting receptive anal sex in the preceding 6 months at the Amsterdam STI outpatient clinic, were routinely checked for anal chamydia (including LGV) and gonorrhoea infections by collection of mucosal swabs during anoscopy as described before. The following anal complaints were recorded: discharge, pain or itch, constipation and a sense of incomplete defecation. Upon anoscopy the following mucous membrane abnormalities were recorded: discharge, oedema, tissue fragility (bloody mucosal surfaces when swabs were obtained), ulceration and abscesses. Moreover, the number of leucocytes per high power field (leucos/hpf) were counted in Gram-stained anal mucosal smears. Patients were considered to have a symptomatic proctitis if they had anal complaints or mucous membrane abnormalities, and \( \geq 10 \) leucos/hpf. Symptomatic patients started a presumptive treatment of doxycycline 100 mg orally b.i.d, until the chlamydia nucleic acid test results became available. Moreover, if Gram negative diplococci in leucocytes were noticed in the anal smear, suggestive for anal gonorrhoea, a presumptive treatment of ceftriaxone 500 mg i.m. once, was administered. During anoscopic examination, swabs for chlamydia nucleic acid identification (Cobas Amplicor; Hoffman–La Roche) and gonorrhoea cultivation, were obtained from all patients. On all chlamydia positive (Ct+) anal samples,
additional biovar L confirmation was performed with a nucleic acid test, as described before [12]. The chlamydia test results were available within 7 days, and the biovar L confirmation within 10-13 days after the initial screening visit.

Serologic test evaluation upon time of diagnosis

For this part of the analysis we did not use any additional data or samples other than obtained in the routine screening procedure of the clinic. Therefore neither ethical approval, nor patient consent was considered necessary.

To evaluate the different chlamydia serologic assays, we compared patients with anal LGV infections (Ct+/LGV+ i.e. an anal swab positive for chlamydia L biovar) to patients with anal non-LGV chlamydia infections (Ct+/LGV- i.e. an anal swab positive for chlamydia non-L biovar). In a sub-analysis, the 4 assays were evaluated according to patient reported anal complaints (discharge, pain, itch, constipation or a sense of incomplete defecation) and to microscopic signs of inflammation (< 10 leucos/hpf in the anal smear).

Serodynamic evaluation after treatment of anal infections

For the evaluation of Chlamydia serologic dynamics after doxycycline therapy, a selection of participants with Ct+/LGV+ and Ct+/LGV- were followed up during one year. This part of the study was in accordance with the Helsinki Declaration and we obtained ethical approval from the Academic Medical Centre ethical committee, Amsterdam, The Netherlands.

Consent to participate in the study was obtained from patients with a symptomatic proctitis during the initial screening visit. Asymptomatic patients were asked for consent when they returned for doxycycline therapy for their anal chlamydia infection. Patients with Ct+/LGV+ were instructed to use 100 mg of doxycycline b.i.d. for a minimum duration of 21 days. Patients with Ct+/LGV- were treated with a minimum of 7 days doxycycline 100mg b.i.d. Serum was collected on the day doxycycline treatment was commenced (t₀) and at subsequent visits during weeks 1, 2, 3, 6, 12, 24, 36 and 52. Patients with the most complete set of serology samples during the follow up period were selected and used to perform the assays on.

Chlamydia specific serologic tests
Serologic assays for IgA anti-MOMP (Chlamydia trachomatis-IgA-pELISA medac, Hamburg, Germany), IgG anti-MOMP (Chlamydia trachomatis-IgG-pELISA medac), IgA anti-LPS (Chlamydia-IgA-rELISA medac) and IgG anti-LPS (Chlamydia-IgG-rELISA medac) were performed on serum samples in micro titer plate wells according to the manufacturer’s guidelines. The test results were given as optical density, calibrated to a positive and negative control sample per microtiter plate and expressed as cut-off index (COI) per sample.

For the IgA anti-MOMP and IgG anti-MOMP assays (and according to the manufacturer’s guidelines) a negative cut-off titer corresponded with COI<0.9, an equivocal titer with COI≥0.9 but ≤1.1 and a positive titer with COI>1.1. In the test evaluation, negative and equivocal titers were considered not indicative for LGV proctitis. For the IgA anti-LPS and IgG anti-LPS assay titers <1:200 were considered not indicative for LGV proctitis. For the IgA anti-LPS this corresponded to a COI <3.61 and for the IgG anti-LPS assay to a COI <1.81.

**Statistical analysis**

Per assay at t₀, sensitivity, specificity, and the diagnostic odds ratio were calculated with 95% confidence intervals (CI95%) to differentiate Ct+/LGV+ from Ct+/LGV-. Comparing the performance of competing tests with paired indicators such as sensitivity and specificity can have a disadvantage, especially if one test does not outperform the other on both indicators. The diagnostic odds ratio is the equivalent of (true positives/false negatives)/(false positives/true negatives).[13] As a single indicator of diagnostic performance, it overcomes the disadvantage of paired indicators and allows the comparison of various diagnostic tests for one indication. A value of 1 indicates that a test does not discriminate between patients with the disorder and those without it.

The 4 assays were then evaluated with respect to symptoms (i.e. patient reported complaints and leucos/hpf). Data were presented as mean COI with CI95%. A Student t-test was used and a p-value <0.01 was considered significant. Moreover, the 4 assays were analyzed in time during the one year follow-up after treatment. Intra-individual correlation was accounted for in a random effects model. For this analysis the R statistical package was used.[14] Data were presented as fitted mean COI trends with CI95%.
All 4 assays were first evaluated in a limited number of patients. The test with the highest diagnostic odds ratio was then evaluated on an expanded serum panel of the last retrospective 100 MSM visitors diagnosed with Ct+/LGV+ and 100 with Ct+/LGV- from June 26 2009 on. Based on the results of the expanded serum panel a continuous sensitivity, specificity analysis and an ROC analysis was performed to determine the optimal cut-off point to differentiate between Ct+/LGV+ and Ct+/LGV- infections.

**Results**

**Serologic test performance to diagnose anal LGV infections**

The first evaluation of the four assays was performed in 61 MSM diagnosed with an anal Chlamydia infection (42 had Ct+/LGV+, and 19 had Ct+/LGV-) in the period from August 2004 until April 2006 with oversampling of visitors with an anal LGV infection for whom the inclusion period was extended until January 2008.

Overall, the IgA anti-MOMP assay had the most optimal test characteristics to differentiate Ct+/LGV+ from Ct+/LGV- with 85.7% sensitivity (CI95%, 72.2%-93.3%), 84.2% specificity (CI95%, 62.4%-94.5%), and a diagnostic odds ratio of 32.0 (CI95%, 7.1-144.3) (table 1). The other 3 assays performed worse. The IgG anti-MOMP and IgG anti-LPS lacked specificity (both 31.6% with CI95%, 15.4-54.0) and the IgA anti-LPS lacked sensitivity with 47.6% (CI95% 33.4-62.3).

**Performance of the serologic assays in asymptomatic patients**

In a sub-analysis, the 4 assays were evaluated in asymptomatic participants. The population consisted of 38 patients without anal complaints (23 with Ct+/LGV+ and 15 with Ct+/LGV-), and 22 patients with <10 leucocytes/hpf in anal smears (14 Ct+/LGV+ and 8 Ct+/LGV-). Again, the anti-MOMP IgA identified Ct+/LGV+ cases best of all 4 assays in the group reporting no complaints. The mean COI of cases reporting no anal complaints with Ct+/LGV+ was 3.7 (CI95%, 2.3-5.5) and in cases with Ct+/LGV- 0.6 (CI95%, 0.4-0.9, figure 1). In cases with <10 leucos/hpf in anal smears, only the IgA anti-MOMP assay showed significant COI differences between
Ct+/LGV+ and Ct+/LGV- cases, respectively 5.0 (CI95%, 3.3-6.8) and 1.4 (CI95% 0.6-2.4).

In the patient group with no complaints, the IgA anti-MOMP assay showed 73.9% sensitivity (CI95%, 53.5%-87.5%), 93.3% specificity (CI95%, 70.2%-99.7%), and a diagnostic odds ratio of 39.7 (CI95%, 4.3-369.7), and in cases with <10 leucocytes/hpf in anal smears respectively 85.7% (CI95% 60.1%-96.0%), 75.0% (CI95%, 40.9%-92.9%), and 18.0 (CI95%, 2.0-161.1, table 2).

**Serodynamics after treatment**

For the serodynamic analysis 20 patients with Ct+/LGV+ were selected (based on the maximal number of follow up sera) and all 19 patients with Ct+/LGV-. With all 4 assays a consistent significant downward trend in the serologic response was observed in the Ct+/LGV+ group (figure 2). The IgA anti-MOMP assay showed the largest decrease in the serologic titer one year after treatment. However, with all assays the mean COI values remained above the cut-off titer considered positive for an anal LGV infection.

**Performance of the IgA anti MOMP assay in the expanded serum panel**

In a small number of individuals (n=61), the IgA anti-MOMP assay performed best in differentiating Ct+/LGV+ from Ct+/LGV-. We therefore evaluated this assay in a panel of 203 serum samples from successive MSM with either Ct+/LGV+ (n=98) or Ct+/LGV- (n=105) who visited in the period from January 2008 to July 2009.

The area under the curve was 80.2. Sensitivity and specificity were calculated for a continuous range of cut-off points from 0 to 12 COI (figure 3). At the COI considered positive by the manufacturer (positive if > 1.1), the sensitivity was 78.6 (CI95% 69.5-85.5), the specificity 61.0 (CI95% 51.4-69.7), and the diagnostic odds ratio 5.7 (CI95% 3.1-10.7, table 3). Based on the diagnostic odds ratio, the optimal cut-off point was found if the COI was considered positive > 2.0 with a sensitivity of 75.5 (CI95% 66.1-83.0), a specificity of 74.3% (CI95% 65.2-81.7), a positive predictive value of 73.3 (63.5-81.6), a negative predictive value of 76.5 (67.0-84.3) and a diagnostic odds ratio of 8.9 (CI95% 4.7-16.8).
Discussion and Conclusions

The potential role of IgA anti-MOMP assay in LGV screening

In a population of patients with a NAAT proven rectal Chlamydia infection, the IgA anti-MOMP assay detected LGV proctitis cases with a sensitivity, specificity, negative- and positive predictive value of all around 75% (table 3). These test characteristics make this assay not ideal for diagnostic purposes and it cannot replace a C. trachomatis biovar specific NAAT. However, for LGV population based surveys and in situation lacking biovar specific C. trachomatis NAATs’ the IgA anti-MOMP assay could serve as alternative screening marker.

The number of LGV cases among MSM is still increasing.[15,16] Recent reports of endemically acquired LGV among heterosexual patients in Spain and Portugal could herald transmission outside the initial core groups and needs close monitoring.[17,18] An IgA anti-MOMP assay could serve as a cost-effective marker to screen large populations on LGV.

For a correct NAAT based LGV diagnosis, it is important to obtain a specimen from suspected mucosal lesions under anoscopic vision. If these requirements cannot be met, a serological assay to confirm LGV could be an alternative. Moreover, a serology assay could be of additional value in the later stages of LGV when the pathogen possibly has become undetectable in the mucosal lining while the infection invaded into underlying connective tissue layers and lymphatics.

Other serologic assays to diagnose LGV

Before biovar specific NAAT became available, chlamydia cultivation was considered the gold standard for the confirmation LGV cases. Chlamydia cultivation is elaborate and lacks sensitivity. Therefore, serological assays like the Complement Fixation (CF) assay (a Chlamydia genus specific but not trachomatis specific assay) and the Micro Immuno-Fluorescence test (MIF, an IgG class Chlamydia species-specific serologic assay) were then used as an alternative method for LGV diagnostic purposes. [19] Nonetheless, false positive results with serological confirmed LGV cases due to non-L biovar chlamydia anal infections in MSM were already reported by Schachter.[20]

In the beginning of the recent LGV epidemic among MSM, we showed that the IgG anti-MOMP assay had a high positive predictive value for anal LGV.
infections in symptomatic patients but failed in asymptomatic cases.[21] The use of a Whole Immuno-Fluorescence (WIF) test based on crude LGV infected cells as antigen was proposed by Forrester et al., but this test is not standardised and not suitable for high throughput use.[22]

Anti-LPS assays performed worse compared to the anti-MOMP assays in our study. LPS based Chlamydia serology assays cross-react with other Chlamydia species like the widely prevalent *C. pneumonia* species. As a consequence, LPS based assays suffer from less specific results when used for *C. trachomatis* diagnostics.[23]

The IgA class anti-MOMP test performed better compared to the IgG based test to differentiate LGV from non-LGV infections (table 1), even in asymptomatic patients. IgA class serological assays are considered to reflect active infections whereas IgG class assays are a marker for both current and past infections. In a high risk population for STI with possible past infections this can lead to false positive results and loss of specificity, as reflected in table 1. Recently an IgA *Chlamydia* specific assay has been evaluated for diagnose anal LGV infections in a predominantly symptomatic population by van der Snoek et al.[24] A sum score of the IgA antibody response and the patients’ age was used as diagnostic criterion and older age correlated with anal LGV infections. Epidemiologic characteristics like age depend on the diseased population and can change over time. Therefore, combining a serological outcome with age has its limitations for diagnostic and screening purposes in other populations. Moreover, the diagnostic value of this assay in an asymptomatic population is unknown.[25]

**Asymptomatic LGV proctitis cases**

A considerable proportion of LGV cases in this study were asymptomatic at time of diagnosis. This is in contrast to recent reports from Great Britain where only 5% (3/61) asymptomatic cases were found in a retrospective survey.[26] Due to the retrospective data collection, recall bias could have caused over reporting of complaints experienced at the time of the clinic visit. This is supported by a prospective study performed at a large London clinic, were in 17% (6/35) asymptomatic LGV cases were found.[27] In the London study, anal swabs were collected without anoscopy in asymptomatic cases. This could have affected the sensitivity of the diagnostic tests and missed additional asymptomatic LGV cases.
In most studies on asymptomatic anal LGV infections, patient reported symptoms are used to define symptomatic cases.[26,27] This criterion is subjective but beneficial in situations lacking anoscopy. The presence of mucosal membrane abnormalities is an objective definition of symptomatic infections, but requires anoscopy.[5,6,21] We chose not to use this definition in the present study because previous investigations proved it lacks discriminative power from other anal infections commonly found in MSM, like gonorrhoeal and non-LGV chlamydia infections.[6,20] A third definition for symptomatic anal infections is based on the presence of leucos/hpf in anal smears. This criterion performs reasonably well as a predictor for anal LGV infections as we showed earlier.[5] Therefore we added this definition to our analysis, although a drawback for diagnostic purposes is the required anoscopy and laboratory routine.

One of the strong points of this study is the long follow-up period of patients with anal chlamydia infections; such data has not been reported previously. Although the IgA anti-MOMP reactivity dropped significantly 52 weeks after treatment, the COI titer remained well above the titer associated with LGV infections (figure 2). Thus, the assay seems not suitable to differentiate past effectively treated infections from successive re-infections, at least in the past year.

We conclude that the IgA anti-MOMP assay is a promising test to be used as an alternative screening tool for anal LGV infections, even for the detection of asymptomatic cases. Although biovar L specific NAAT are superior and remain a preferable diagnostic test for LGV infections, the IgA anti-MOMP assay could serve as a auxiliary test to exclude anal LGV proctitis in cases diagnosed with an anal chlamydia infection based on a routine (biovar non-specific) chlamydia NAAT test.

**Competing interests**

Potential conflicts of interest: None.

**Authors' contributions**

HDV: designed and supervised the study and wrote the manuscript
VS: performed part of the experiments and data analysis
SO: performed part of the experiments and data analysis
JP: performed part of the experiments and data analysis
RBG: advised on, and performed the statistical analysis and wrote the manuscript
AGCLS: performed part of the experiments
JSAF: designed and supervised the study
SAM: designed and supervised the study and wrote the manuscript

Acknowledgements

This study was financially supported with a grant of the Research and Development Fund of the Public Health Service of Amsterdam in 2004 and 2005. The assays used for this study were donated by medac, Hamburg, Germany. V.S. received a travel grant of medac, to present a poster at a conference.

The aims of this work is in part in line with the European EpiGenChlamydia Consortium which is supported by the European Commission within the Sixth Framework Programme through contract no. LSHG-CT-2007-037637. See www.EpiGenChlamydia.eu for more details about this Consortium.
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Figures

Figure 1  - Mean serology cut-off indices with 4 chlamydia specific serological assays, Amsterdam STI outpatient clinic.

The assays were performed on 42 patients with an anal LGV infection (solid squares) and 19 patients with an anal non-L biovar chlamydia infection (open squares). Except if indicated the patient groups differed significantly (p<0.01). MOMP = major outer membrane protein, LPS = lipopolysaccharide, n.s. = not significant, *absence of patient reported discharge, pain, itch, incomplete defecation or constipation. <10 leucos/hpf = less than 10 leucocytes per high power field in a Gram stained anal smear.

Figure 2  - Mean serology cut-off indices plotted one year after treatment with 4 chlamydia specific serological assays, Amsterdam STI outpatient clinic.

The assays were performed on 20 patients with an anal LGV infection (solid squares, black line and hatched area) and 19 patients with an anal non-L biovar chlamydia infection (open squares, gray line and gray shaded area). The x-axis is transformed logarithmic via x = log(t+1). MOMP = major outer membrane protein. LPS = lipopolysaccharide.
Figure 3 - Sensitivity and specificity plot with the IgA anti-MOMP serologic assay to differentiate anal LGV infections from non-LGV chlamydia anal infections in 203 men who have sex with men, Amsterdam STI outpatient clinic.

The assay was performed on 98 patients with an anal LGV infection and 105 patients with an anal non-LGV biovar chlamydia infection. Sensitivity (solid squares), specificity (open circles) and 95% confidence intervals (shaded area) are shown. The dotted lines indicate the cut off points (1.1, 2.0 and 3.0) described in table 3. MOMP = major outer membrane protein.

Tables

Table 1 - Test characteristics of 4 chlamydia specific serological assays to differentiate anal LGV infections from non-LGV chlamydia anal infections in 61 men who have sex with men, Amsterdam STI outpatient clinic.

The assays were performed on 42 patients with an anal LGV infection and 19 patients with an anal non-L biovar chlamydia infection. MOMP = major outer membrane protein, LPS = lipopolysaccharide, Ct+/LGV+ = anal LGV biovar specific chlamydia infection, Ct+/LGV- = anal non-LGV biovar chlamydia infection, CI95% = 95% confidence intervals.

Table 2 - Test characteristics of the IgA anti-MOMP assay to differentiate anal LGV infections from non-LGV chlamydia anal infections, in asymptomatic men who have sex with men, Amsterdam STI outpatient clinic.

The assay was performed on 38 men with no complaints (the absence of patient reported discharge, pain, itch, incomplete defecation or constipation) and 22 with <10 leucos/hpf (less than 10 leucocytes per high power field in a Gram stained anal smear). Ct+/LGV+ = anal LGV biovar specific chlamydia infection, Ct+/LGV- = anal non-LGV biovar chlamydia infection, MOMP = major outer membrane protein, CI95% = 95% confidence intervals.
Table 3 - Test characteristics of the IgA anti-MOMP assay to differentiate anal LGV infections from non-LGV chlamydia anal infections, in 203 men who have sex with men, Amsterdam STI outpatient clinic.

The assay was performed on 98 patients with an anal LGV infection (Ct+/LGV+) and 105 patients with an anal non-LGV biovar chlamydia infection (Ct+/LGV-), MOMP = major outer membrane protein, CI95% = 95% confidence intervals, <10 leucos/hpf = less than 10 leucocytes per high power field in a Gram stained anal smear.
Figure 2
Additional files provided with this submission:

Additional file 1: Table 1 IgA antiMOMP for LGV de Vries.pdf, 39K

Additional file 2: Table 2 IgA antiMOMP for LGV de Vries.pdf, 9K

Additional file 3: Table 3 IgA antiMOMP for LGV de Vries.pdf, 39K