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Evaluation of two commercial assays for the detection of lymphogranuloma venereum in rectal samples

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SHORT SUMMARY

In this study we find similar results between 2 commercial assays for detecting LGV in rectal samples

ABSTRACT

Background: The early identification of the *Chlamydia trachomatis* variants that cause lymphogranuloma venereum (LGV) is very important to establish an adequate antibiotic treatment. This identification should be made with molecular techniques that are easy to perform and accessible to most microbiology laboratories. The objective of this study was to evaluate two real-time PCR based assay (VIASURE *H. ducreyi* + *C. trachomatis* (LGV) Real Time PCR Detection Kit and the Allplex™ Genital ulcer Assay) for the detection of LGV in rectal samples.

Material and methods: Prospective study on positive rectal samples for *C. trachomatis*. All samples were processed in parallel by both tests. As a molecular reference method and to solve possible discrepancies between both assays, a PCR-based restriction fragment length polymorphism analysis (PCR-RFLP) analysis of the major outer membrane protein gene (omp1) was used.

Results: In total we detected 157 positive rectal samples for *C. trachomatis*, of which 36 were identified as LGV by PCR-RFLP. The positive percent agreement, negative percent agreement and overall percent agreement were 88,9%, 100%, and 97,3% respectively for the Allplex™ Genital ulcer assay and 91,6%, 100%, and 97,1% respectively for the VIASURE assay. In the direct comparison between the Seegene assay and the VIASURE assay, we obtained a kappa concordance index of 0.98 between both tests.

Conclusion: According to the results obtained, both tests could be used for the detection of LGV in rectal samples.

Keywords: Lymphogranuloma, diagnosis, VIASURE

INTRODUCTION

Lymphogranuloma venereum (LGV) is a sexually transmitted disease caused by invasive serovars L1, L2 or L3 of *C.trachomatis*. Classically, LGV has been characterized by the development of painless ulcers followed by painful inguinal lymphadenopathies and has been considered an endemic syndrome in developing countries. This perception began to change when in 2003 a first rectal LGV outbreak was described in Amsterdam between men who have sex with men (MSM)[1,2]. Since then, LGV has emerged in Europe and North America as a leading cause of proctitis and proctocolitis in MSM [3,4,5,6,7].

The recently published 2019 European guideline on the management of lymphogranuloma venereum[8] recommends that for the diagnosis of LGV, laboratories should follow a 2-step procedure. First, commercial nucleic acid amplification (NAAT) test should be used to detect *C. trachomatis* in suspected clinical samples, and then, if this detection were positive, a specific detection of the LGV should be done from the same specimen.

Numerous research teams have developed various molecular techniques to confirm LGV infection from clinical samples[9]. To our knowledge, there is still no commercial NAAT adequately evaluated for the detection of LGV in rectal samples.

The objective of our study was the evaluation of 2 newly developed assays for the detection of LGV in rectal samples, the VIASURE *H. ducreyi + C. trachomatis (LGV) Real Time PCR Detection Kit (*CERTEST BIOTEC, S.L Spain) and the Allplex™ Genital ulcer Assay (Seegene). The identification of LGV-associated biovars with the VIASURE assay is performed by the amplification of the *pmpH* gene using specific primers and a fluorescent-labelled probe. This assay has been validated only with urogenital samples. The Allplex ™ Genital ulcer Assay kit uses Seegene's proprietary MuDT ™ technology, which allows simultaneous amplification and detection

of Lymphogranuloma venereum (LGV) and 6 other pathogens that cause genital ulcers, herpes simplex virus type 1 (HSV-1), Herpes simplex virus type 2 (HSV-2), *H. ducreyi* (HD), Cytomegalovirus (CMV), *T. pallidum* (TP), and varicella-zoster virus (VZV). According to its insert, the kit is only validated for urogenital samples.

MATERIAL AND METHODS

Prospective study carried out in the microbiology laboratory of the University Hospital of Valme de Sevilla (Spain) during the period from October 2017 to February 2019. LGV detection was performed in parallel with both assays in all rectal samples where *C.trachomatis* DNA was detected. The samples were collected from patients treated at the sexually transmitted infection center in Seville (Spain). The study was approved by the Research Ethics Committee of the Hospital de Valme de Sevilla.

For *C.trachomatis* DNA detection, samples were collected with the Cobas PCR media (Roche Diagnostic) and processed with the Cobas CT/NG assay performed on the cobas®6800 System (Roche Diagnostic) following the manufacturer's instructions. Samples that were positive for *C.trachomatis* were frozen at -20ºC until being processed for LGV detection, no later than 7 days from the sample collection.

For processing with both assays (VIASURE and Seegene), the samples were thawed and 400 microliters of Cobas media were used for DNA extraction using the MagCore® Compact Automated Nucleic Acid Extractor (RBC Bioscience). The extracted DNA was dissolved at a final volume of 60 microliters. Five microliters of extracted DNA were used for processing. Each test was processed according to each manufacturer's instructions. Real-time PCR was performed using the CFX96^{TM} real-time PCR system (Bio Rad®Laboratories).

As a molecular reference method and to solve possible discrepancies between both tests, a PCR-based restriction fragment length polymorphism analysis of the major outer membrane protein gene (*omp1***)** was used**.** The following primers were used Fw ACAGATTCTCAAGGGCCAGTTC and Rv ACGCCTGAGATCTCCAAACTAGT (10). With this procedure, samples were assigned as being LGV-associated or non-LGV associated on the basis of their restriction digest profiles.

Data analysis

The assay performance of both assays was determined by calculating positive percent agreement (PPA), negative percent agreement (NPA) and overall percent agreement (OPA) values, with 95% confidence intervals taking the PCR-RFLP results as reference**.**

Concordance between both assays was analysed using Cohen's Kappa index so that with a value of K <0.20, the concordance is considered to be poor, K = 0.21-0.40 weak, k = 0.41- 0.60 moderate, K = 0.61-0.80 good and K = 0.81-1 very good.

RESULTS

During the study period, 1763 samples of rectal exudates were processed for the detection of *C.trachomatis* (1674 men and 89 women). Of these, 185 were positive (170 men and 15 women). A total of 157 samples (151 men and 6 women) were available for processing with both assays (19 were from symptomatic patients, 129 from asymptomatic patients and 9 from patients who had recent contact with patients with confirmed infection by *C.trachomatis*). Three samples were invalidated (internal control not amplified) in the Seegene assay and one with the VIASURE assay.

In total, PCR-RFLP analysis could be performed on 149 samples. Of them, 36 samples were classified as LGV biovars and 113 samples, were classified as non-LGV variants. All samples that were positive for LGV by analysis with PCR-RFLP came from men. None of the 6 samples

analyzed from women were positive for LGV. There were 8 patients with symptoms (proctitis o proctocolitis) and the other 28 were asymptomatic. Of the 36 positive samples by PCR-RFLP, the VIASURE assay was also positive in 33 and the Seegene assay in 32. Moreover, there were 3 samples that were negative with the Viasure assay and four samples with the Seegene assay. There were 3 invalid results with the Seegene test and 1 with the VIASURE test. All other PCR-RFLP samples were negative for LGV in both commercial assays (Table 1). The values of PPA, NPA and OPA, excluding invalid results were 88.9%, 100% and 97.26% respectively for the Seegene assay and 91.6%, 100%, and 97,9% respectively for the VIASURE assay (Table 2).

For the direct comparison between both the Seegene assay and the VIASURE assay, without considering the results of the PCR-RFLP, and excluding the samples with invalid results (2 with the Seegene assay and 1 with the VIASURE assay) we obtained 153 valid results. Of them, both assays were positive for LGV in 32 samples and negative in 120 samples resulting in a Kappa concordance index of 0.98. There was no sample that was positive by Seegene assay and negative by VIASURE assay. On the other hand, there was a positive sample by VIASURE assay that was negative by Seegene assay. This sample had a high cycle threshold (Ct) value (36.6) and was classified as LGV positive by PCR-RFLP. Three samples had invalid results (no internal control amplification) with Seegene assay. These 3 samples were repeated from the same DNA extract and in 2 of them the result was invalid again and in one it was negative for LGV. A sample was invalidated with the VIASURE assay (this sample was also invalidated with the Seegene assay). It was repeated with the same DNA extract and continued to be invalid. In all cases of invalid samples, the PCR-RFLP result was negative for LGV.

DISCUSSION

Early detection of L1-2-3 *C.trachomatis* variants is very important not only from an epidemiological point of view, but also for clinical and therapeutic aims, as it involves a longer

treatment with respect to infections caused by non-LGV variants (doxycycline 100 mg twice daily for 21 days, instead 1 week). LGV infection is asymptomatic in many patients, so it is necessary to be systematically screened on all patients with a *C. trachomatis* positive anorectal sample irrespective of symptoms, patients who had recent contact with confirmed LGV index cases and patients with symptoms suspected of having LGV [10,11].

The diagnostic algorithm for rectal LGV infection should begin with the detection of *C.trachomatis* using a nucleic acid amplification test (NAAT). Since FDA commercial available NAATs cannot distinguish *C.trachomatis* LGV from non-LGV biovars, if *C.trachomatis* is detected it is necessary to make a specific detection of LGV in the same specimen[8]. The objective of our study was to evaluate the assay performance of two tests, the Seegene assay and the VIASURE assay, to detect LGV in rectal samples since, at least in our medium; it is in this anatomical location where virtually all LGV infections occur[1-7]. The assay performance of both assays evaluated was good in terms of PPA NPA and OPA, taking the PCR-RFLP results as a reference. We did not find any false positive with any of the assays studied. On the other hand, only 3 samples (1.2% of the total samples studied) were falsely negative with both tests. A possible explanation for these 3 false negatives could be the presence of a variant of LGV not detected by any of the assays evaluated, but this option could not be confirmed since the PCR-RFLP test used as reference only distinguishes between LGV variants and non-LGV variants, but not among the different variants of the LGV (eg L1, L2, L2b, etc. ...). None of the tests evaluated gives information on the presence of non-LGV variants. Therefore another possibility to explain the 3 false negatives, is that in these samples, there would be a very low load of LGV and that it would not have been detected by either test evaluated (even if the internal control included in the kits were positive).

Moreover, all NAATs developed to detect LGV have been shown to have a lower sensitivity than the NAATs used in *C.trachomatis* screening[12,13]. It is possible that in our study there were samples

in which the LGV was not detected since the targets used (omp1 gene or pmpH gene) are present in a single copy, while in the Cobas CT/NG test used as screening a double target is used (a region on the CT cryptic plasmid and the ompA gene).

Regarding the comparison between both tests, the agreement was very good (K = 0.98). There was only one disagreement between them, which consisted of one sample that was negative with the Seegene assay and positive with the VIASURE assay. In this case, the Cycle threshold (Ct) value was very high (36.6), and the false negative of Seegene assay can be explained in a low bacterial load in the sample.

To our knowledge, there is no test marketed to exclusively detect LGV. Both evaluated assays have been designed in a multiplex format not only for the detection of LGV but also of other agents causing STDs. They have some differences in the kit format in which they are marketed. The VIASURE assay is shipped as freeze dried pellets, therefore, it has great reagent stability and a very broad expiration date. In contrast, in the Seegene kit, the reagents are frozen and the expiration date is shorter. As an advantage, this kit has greater versatility since it is designed for the detection of 6 other pathogens that cause genital ulcers not only LGV.

None of the tests are designed to distinguish between the different biovars of LGV. According to studies designed for this objective, the variant with the highest prevalence in our environment would be L2b [14-16], although the presence of other variants such as L2c has recently been reported which unexpectedly emerged in Europe [17]. We believe that this information is useful for epidemiological studies but not essential for the immediate management of patients.

In conclusion, both assays have adequate assay performance, were very easy to process, do not require any special infrastructure in the laboratory and could be used for the detection of LGV in rectal samples.

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