DIAGNOSTICS

# Confirming the *Chlamydia trachomatis* status of referred rectal specimens

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*Sex Transm Infect* 2007;**83**:327–329. doi: 10.1136/sti.2006.024620

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Accepted 3 April 2007
**Published Online First 2 May 2007**

**Objectives:** To assess the reliability of different laboratory methods for the detection of *Chlamydia trachomatis* in rectal specimens

**Methods:** 1782 rectal specimens confirmed as *C trachomatis* positive using a standard laboratory method, were forwarded to the Sexually Transmitted Bacteria Reference Laboratory (STBRL). All specimens were retested using a *C trachomatis* specific independent in-house real time polymerase chain reaction (PCR). If this test was negative, a second test (Artus Real-Art PCR Kit) was employed as a confirmation. A correlation between real time PCR results obtained at the reference centre (STBRL), and the method of *C trachomatis* detection used in the primary laboratory was undertaken.

**Results:** The percentage of specimens that could be confirmed as positive, compared with primary method of detection was as follows: *C trachomatis* culture 87.5%, strand displacement assay (SDA: Becton Dickinson) 93.4%, Cobas Amplicor (Roche) 89.2%, Aptima Combo Two assay (Genprobe) 83.3%, and enzyme immunoassays (EIA) 35.4%.

**Conclusions:** High rates of confirmation can be achieved using an independent real time PCR assay to examine rectal specimens which had initially tested *C trachomatis* positive using nucleic acid amplification tests and chlamydia tissue culture. This is not possible for specimens that had been screened using EIA tests, which reflects the low specificity of this test when used for rectal specimens. Laboratories currently using EIA based assays to test rectal specimens should review this approach.

The recent resurgence of lymphogranuloma venereum (LGV) in Western Europe has highlighted the need to test high risk patients for rectal chlamydial infection.[1] However, in the United Kingdom the testing of rectal specimens for *Chlamydia trachomatis* has been problematic for diagnostic laboratories because of the lack of a licensed nucleic acid amplification test (NAAT) platform for extragenital sites, a lack of culture facilities, and the continued and extensive use of enzyme immunoassay (EIA) methods.

In October 2004 the Health Protection Agency launched a LGV enhanced surveillance programme, which involved offering genotyping for *C trachomatis* from patients who were symptomatic. The positive *C trachomatis* status of all the specimens was confirmed upon receipt using a plasmid based in-house real time polymerase chain reaction (PCR) method which detects all serovars of *C trachomatis* before genotyping.[2] In the event a specimen was determined to be *C trachomatis* negative a further method—the Artus Real-Art PCR Kit—was employed to confirm its negative status. In order to ascertain the reproducibility of laboratory methods for the detection of *C trachomatis* in rectal sites, we examined the correlation between real time PCR results obtained at the reference laboratory, the Sexually Transmitted Bacteria Reference Laboratory (STBRL), and the methods used to determine the positive *C trachomatis* status of the specimen at the referring centre.

## METHODS

### Specimen inclusion criteria

Referral forms that accompanied all specimens forwarded to STBRL for LGV genotyping between October 2004 to September 2006 were examined. Criteria for inclusion in this analysis were that referral forms must state that a specimen was sourced from a rectal site and had tested positive for *C trachomatis* at the primary laboratory using a known and stated standard laboratory test.

### Confirming the *C trachomatis* status of rectal specimens

DNA extractions were performed on all referred specimens by one of three methods: (i) an automated extraction using the Corbett DNA X-Tractor with the Macherey-Nagel extraction for blood kit; (ii) a manual extractions using the Viral RNA Mini Kit (Qiagen); or (iii) MagNA Pure automated extraction (Roche). All extractions were performed according to the manufacturer's instructions.

### *C trachomatis* detection

The chlamydia status of all specimens was determined using a previously published method.[2] The PCR was run as a duplex containing primers targeted to a 88 bp region of the *C trachomatis* cryptic plasmid and primers targeted to the human RNase P gene which act as an internal control. All reactions were performed on a Corbett Rotorgene-3000.

In instances where specimens were determined to be *C trachomatis* negative a further method was used to ensure its negative status. the Artus Real-Art PCR Kit was performed on the Rotorgene, according to the manufacturer's instructions.

Instances where specimens were reported by STBRL as inhibited were the result of failure to generate a signal in either the *C trachomatis* or the internal control detection channels on the Corbett Rotorgene 3000 real time PCR machine. Specimens which were reported as equivocal by STBRL generated either (i) high *Ct* values (*Ct* >40 cycles), which is indicative of a low target load, or (ii) conflicting results between the two different real time PCR methods.

A comparison between real time PCR results at STBRL and the method of *C trachomatis* detection used in the primary laboratory was undertaken. The percentage of specimens that were confirmed as positive by STBRL was calculated.

**Abbreviations:** EIA, enzyme immunoassays; LGV, lymphogranuloma venereum; NAAT, nucleic acid amplification test; PCR, polymerase chain reaction; SDA, strand displacement assay; STBRL, Sexually Transmitted Bacteria Reference Laboratory

## RESULTS

A total of 1782 rectal specimens from 85 centres throughout the United Kingdom were confirmed as *C trachomatis* positive using a range of different laboratory platforms at the primary diagnostic centre. They were forwarded to STBLR where they were retested, using an inhouse plasmid based real time PCR. If this test was negative a second test (Artus Real-Art PCR Kit) was employed as a confirmation. When comparing the results generated at STBRL with the method used at the referring laboratory the percentage of specimens that could be confirmed as positive was as follows: *C trachomatis* culture 87.5%, strand displacement assay (SDA: Becton Dickinson) 93.4%, Cobas Amplicor (Roche) 89.2%, Aptima Combo Two assay (Genprobe) 83.3%, and enzyme immunoassays (EIA) 35.4% (table 1).

## DISCUSSION

The use of commercial *C trachomatis* platforms to examine rectal swabs for which they are unlicensed has provided diagnostic laboratories throughout the United Kingdom with ethical, legal, and financial dilemmas. In this study we were able to confirm 93.4% and 89.2% of specimens referred to STBRL, which had previously been reported as *C trachomatis* positive using the SDA and Cobas Amplicor assays, respectively. The results generated in this analysis indicate that despite their unlicensed status the SDA and Cobas Amplicor assays can confidently be used to examine rectal specimens for *C trachomatis*, as a substantial proportion of the positive results generated can be confirmed as positive using an assay with an independent target. It is important to acknowledge that specimens that could not be confirmed as positive, may have generated false negative results because of degradation of DNA during storage and transport. In some instance the specimens had been stored for long periods and at incorrect storage conditions before referral. Consequently, specimen integrity is often questionable and other issues such as low DNA loads, which could result in legitimate ''unconfirmed positive'' results, may also be responsible for some specimens being unconfirmed.[3] The data presented in this study compare favourably with other studies where genital swabs and urine specimens were retested using the same test in the same laboratory; 84%, 96.7%, and 98% of positives by SDA, PCR, and Aptima Combo Two could be confirmed on repeat testing.[3] However such comparisons should be interpreted with caution because of the unique specimens received by the STBRL in terms of both specimen site and *C trachomatis* prevalence.

When examining 24 specimens that had initially been tested by Aptima Combo Two at the primary laboratory only 83.3% could be confirmed. It is unfortunate that so few rectal specimens were available that had been initially tested with the Aptima Combo Two test but this is reflective of the current number of Genprobe platforms being used in diagnostic laboratories within the United Kingdom. It is generally widely accepted that not all chlamydia NAAT tests are of equal sensitivity and numerous studies have shown that the Aptima Combo Two platform is of superior sensitivity; consequently other NAATs platforms—such as our in-house real time PCR assay—may generate false negative results when attempting to confirm positive Aptima Combo Two results because of differences in analytical sensitivity.[4 5] This highlights the need for a larger scale study to be carried out to validate the Aptima Combo Two for the testing of extragenital specimens.

Rectal specimens, which had been determined to be *C trachomatis* positive using cell culture, were determined to have a 87.5% confirmatory rate. This was lower than expected and the result of the failure to confirm three culture positive rectal specimens. Given that two different real time PCR assays were used in this instance, this was unexpected given that the actual specimens referred were the cell culture supernatant fluid, rather than the primary rectal swab. It is unlikely that incorrect storage conditions could result in the complete DNA degradation in these samples and issues of PCR inhibition had been eliminated owing to the amplification of internal controls. It is possible that these three specimens represent three false positive *C trachomatis* cultures results, possibly because of the misclassification of cell artefacts as inclusions. This implies that high specificity of *C trachomatis* culture can only be maintained if inclusion bodies are correctly identified using a specific method; this often requires expensive reagents and skilled experienced laboratory personnel, and this is not always achievable even in the most experienced laboratory.

In contrast to the high confirmatory rate of NAAT based tests and culture to test rectal swabs, EIAs were found to perform very badly, with the real time PCR only being able to confirm 35.4% of specimens screened initially using EIA methods. The low confirmatory rate reflects the low specificity of EIA when testing rectal specimens, which has been described previously.[6] The data presented here further confirm that an EIA is an inappropriate test to use to examine a rectal specimen and that laboratories still using EIAs for testing should urgently find an alternative methodology.

The ability of different platforms to detect chlamydial infection in rectal sites is currently the subject of intense discussion. In this study we have presented data that show high confirmatory rates can be achieved using an independent real time PCR assay to examine rectal specimens that had initially tested *C trachomatis* positive using NAATs and chlamydia culture. Conversely, this was not the case for specimens that had been screened using EIA tests; this reflects the low specificity of this test when examining rectal

**Table 1** Comparison of reference confirmation and initial results for detection of *C trachomatis* in rectal specimens

| | Category of test used at local laboratory to determine positive status of rectal specimen | | | | |
|---|---|---|---|---|---|
| | | | NAATS: breakdown | | |
| Results of *C trachomatis* confirmatory real time PCR tests | EIA | Culture | SDA | Cobas Amplicor | Aptima Combo Two |
| Positive | 17 | 21 | 1144 | 411 | 20 |
| Negative | 25 | 3 | 56 | 39 | 3 |
| Inhibitory | 4 | 0 | 15 | 6 | 1 |
| Equivocal | 2 | 0 | 10 | 5 | 0 |
| % Confirmed positive (positive specimens/total number examined) | 35.4 | 87.5 | 93.4 | 89.2 | 83.3 |

EIA, enzyme immunoassay; NAATs, nucleic acid amplification tests; PCR, polymerase chain reaction; SDA, strand displacement assay.

specimens. It is hoped that these data will provide support to those primary diagnostic laboratories that are currently using NAATs to screen rectal specimens for *C trachomatis* despite their unlicensed status, and will encourage laboratories still screening rectal specimens with EIA based methods to move to a more appropriate technology.

## ACKNOWLEDGEMENTS

We would like to thank Ucheoma Ugoji for the testing of specimens. Contributors: All three authors contributed equally to study design. SA analysed the results and wrote the manuscript, and both CI and IM amended and approved the manuscript.

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