Pilot Study of COBAS PCR and Ligase Chain Reaction for Detection of Rectal Infections Due to Chlamydia trachomatis

Matthew R. Golden,1,2* Sabina G. Astete,1 Rosa Galvan,3 Aldo Lucchetti,3 Jorge Sanchez,3 Connie L. Celum,1 William L. H. Whittington,1 Walter E. Stamm,1 King K. Holmes,1 and Patricia A. Totten1

Division of Infectious Diseases and the Center for AIDS & STD, University of Washington,1 Seattle, and Public Health—Seattle and King County STD Program,2 Seattle, Washington, and Asociación Civil Impacta Salud y Educación, Lima, Peru3

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We tested rectal specimens from men who have sex with men for Chlamydia trachomatis by using COBAS PCR (Roche Diagnostics) and ligase chain reaction LCR (Abbott laboratories) and compared three PCR specimen-processing procedures. Chlamydiae were detected by one or more procedures in 22 of 186 specimens. All three PCR tests were positive for 17 specimens, all of which also tested positive by LCR.

Chlamydia trachomatis is a common cause of sexually acquired anorectal infections, particularly in men who have sex with men (MSM) (4, 6). Several health departments and the Centers for Disease Control and Prevention have recently issued guidelines advocating routine screening of MSM for rectal chlamydial infections by using culture (2, 3, 8). However, most laboratories do not perform chlamydial culture (1), and neither the Centers for Disease Control and Prevention nor the American Society for Microbiology recommends chlamydial culture (1). In several studies with men who have sex with men (MSM) (4, 6), PCR has been used for detection of Chlamydia trachomatis and LCR for detecting rectal infections with Chlamydia trachomatis and compared three different specimen-processing procedures for PCR testing.

As part of a protocol to screen men for participation in a study of MSM in Lima, Peru, during 1998, anorectal specimens were collected (J. L. Sanchez, W. L. H. Whittington, R. A. Zuckerman, R. L. Ashley, J. R. Lama, R. Galvan, J. Sanchez, K. Russell, and C. L. Celum, Conf. HIV Pathog. Treatment, abstr. 264, 2001). A Dacron swab was inserted into the anorectal canal, rotated, inoculated into 2SP (0.2 M sucrose, 0.02 M potassium phosphate buffer, and 0.001% phenol red [pH 7.5]) (5), and then stored initially at −20°C and then at −80°C until testing. Human subjects review committees at the University of Washington and Cayetano Heredia University approved the study protocol; participants provided written informed consent.

For PCR analyses, aliquots of each specimen were processed by three methods. For procedure 1, a 50-µl aliquot of each specimen was first diluted in lysis buffer and then was diluted in diluent as described in the Roche CT/NG sample treatment kit for specimens collected on swabs (Swab procedure; Roche Diagnostics Systems, Branchburg, N.J.). For procedure 2, a 125-µl aliquot of each specimen was centrifuged for 5 min at 12,500 × g and was treated as described in the Roche CT/NG sample treatment kit for urine (Urine procedure; Roche). For procedure 3, a 150-µl aliquot of specimen was treated with the MasterPure DNA Purification kit (Epicenter, Madison, Wis.) according to the manufacturer’s directions, with the exception that the DNA pellet precipitated in the last step was suspended in 30 µl of Tris-EDTA buffer (pH 8). Five microliters of purified DNA was then mixed with 7.5 µl of 2SP and equivalent amounts of Roche reagents. As a result of these purification procedures, the amounts of the original clinical specimen in the 100-µl PCRs were 12.5, 50, and 25 µl for procedures 1, 2, and 3, respectively. All specimens were tested for C. trachomatis according to the manufacturer’s instructions for the COBAS PCR (Roche).

For LCR testing, a 120-µl aliquot was centrifuged for 15 min at 13,000 × g, and the resulting pellet was suspended in 120 µl of urine resuspension buffer (Abbott Laboratories, Abbott Park, Ill.) to remove the inhibitory effects of phosphate in the 2SP collection medium. LCR testing was then performed on 100 µl of this treated specimen according to the manufacturer’s instructions (Abbott Laboratories). Since the LCR test does not contain internal controls to check for LCR inhibition, all specimens were diluted to a ratio of 1:6 to confirm initial results. This dilution was selected because previous experiments with dilutions of urines spiked with different concentrations of C. trachomatis resulted in no decreased detection of C. trachomatis by LCR at 1:6 dilution.

When sufficient clinical specimen remained, specimens producing discordant PCR and LCR results were purified by using procedure 3. Repeat LCR testing was done by using 5 µl of Epicenter purified DNA added to 100 µl of urine resuspension buffer (Abbott). Experiments with specimens producing concordant PCR and LCR results were repeated by using purified aliquots for all 15 specimens initially positive by LCR and all PCR specimen-processing procedures and a sample of 17 specimens that initially tested negative with both tests.

Among the 186 specimens, four were inhibited in PCRs, two
TABLE 1. Comparison of PCR by specimen-processing procedure and LCR for detection of C. trachomatis in rectal specimens

<table>
<thead>
<tr>
<th>No. of specimens</th>
<th>PCR results by specimen-processing procedurea</th>
<th>No. LCR positive by unpurified specimen</th>
<th>No. positive/no. tested by repeat testing with purified DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
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<td>15</td>
<td>+</td>
<td>+</td>
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<td>1</td>
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<td>I</td>
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</tr>
<tr>
<td>2</td>
<td>–</td>
<td>I</td>
<td>–</td>
</tr>
<tr>
<td>160</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

a 1, swab procedure; 2, urine procedure; 3, DNA purification procedure (see text for method details). I, inhibited; ND, not done due to insufficient specimen.

b Two specimens were not tested due to insufficient specimen availability.

each with procedures 1 and 2 (Table 1). Fifteen specimens (8.0%) tested positive and 160 (86%) tested negative by PCR with each of the three specimen-processing procedures and by initial LCR testing. Two additional specimens positive by PCR by all three specimen-processing procedures were LCR negative in the unpurified specimen and were LCR (and PCR) positive following DNA purification. Five specimens were PCR positive by only one of the processing procedures and were negative by LCR with unpurified specimen; two of these specimens became LCR positive following DNA purification. After DNA purification, all 15 specimens that initially tested LCR positive and PCR positive with all of the three specimen-processing procedures again tested PCR positive; 14 of these purified specimens retested LCR positive. All 17 initially negative specimens remained LCR and PCR negative after DNA purification (data not shown).

In summary, we found that agreement between PCR and LCR was high and that the three specimen treatment protocols gave similar PCR results. Only the third specimen-processing method, PCR after DNA purification, was completely free of inhibition. This method produced three positive results that were negative by PCR prior to DNA purification, two of which were confirmed by LCR. Nonetheless, among the 186 specimens tested, only two were inhibited by using the swab specimen-processing method, a surprising result given the minimal specimen processing performed. Initial PCR and LCR results agreed in 178 of 184 (98%) of these specimens, and 17 of the 18 PCR-positive specimens processed by procedure 1 were also LCR positive, either before or after DNA purification. Given the low frequency of inhibition, the high level of agreement between PCR and LCR on specimens processed by using the swab-processing procedure (procedure 1), and the relative ease and low cost of this procedure, we recommend the Roche swab-processing procedure when testing rectal specimens by PCR.

While our results suggest that PCR and LCR can be used to detect rectal chlamydial infections, the study was not designed to estimate the sensitivity or specificity of NAATs for detecting rectal chlamydial infections. Specimen storage conditions available in Lima (−20°C) did not permit us to perform reliable cultures for C. trachomatis, which might have served as a reference standard. Furthermore, specimens were collected in PCR media and PCR was performed after a single freeze/thaw cycle, while LCR was performed after a second such cycle. Thus, our procedures may have selectively decreased the sensitivity of LCR.

Despite these limitations, in this pilot study we have shown that PCR and LCR can be used to detect rectal chlamydial infections with C. trachomatis and that a commercially available specimen treatment regimen (Swab procedure; Roche), the least labor-intensive approach, appears promising for processing rectal specimens for PCR. Further research is needed to compare different NAATs and culture and to define the sensitivity and specificity of these different tests in detecting rectal chlamydial infections.

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REFERENCES