Journal of Clinical Microbiology, Jan. 2004, p. 239–241 Vol. 42, No. 1
0095-1137/04/$08.00+0 DOI: 10.1128/JCM.42.1.239–241.2004
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Validation of Roche COBAS Amplicor Assay for Detection of *Chlamydia trachomatis* in Rectal and Pharyngeal Specimens by an *omp1* PCR Assay

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Received 3 September 2003/Returned for modification 14 October 2003/Accepted 23 October 2003

Screening guidelines for men who have sex with men (MSM) recommend testing of extragenital sites (pharyngeal and rectal) for gonorrhoea and chlamydia. Testing of specimens from these sites is not validated by most commercial nucleic amplification tests, such as the COBAS Amplicor assay. To investigate the utility of the COBAS Amplicor assay for detection of *Chlamydia trachomatis* in extragenital specimens, this study developed and evaluated confirmatory tests using the *omp1* gene as an alternative target for amplification by PCR. Of anal and throat swabs collected from men in male-only saunas, 52 swabs that tested *C. trachomatis* positive by COBAS Amplicor and 30 swabs that tested as negative were included for confirmatory *omp1* PCR testing. A total of 49 (94%) COBAS Amplicor-positive samples were confirmed by the *omp1* PCR. A substantial proportion of specimens were confirmed by using a nested *omp1* PCR (27%). Not confirmed by any *omp1* PCR were three anal swabs (6%). It is most probable that these samples contained lower bacterial levels that were near or below the detection level of the *omp1* PCR assays. The findings of this study support the confident reporting of *C. trachomatis* detected by COBAS Amplicor in extragenital specimens and support the utility of this assay as a screening test for MSM.

Bacterial sexually transmitted infections (STIs) in men who have sex with men (MSM) are an important public health concern (9, 11). Their early detection and treatment may avoid transmission to sexual partners, serious sequelae, or STI-facilitated human immunodeficiency virus transmission (5, 6, 8). Screening for asymptomatic bacterial STIs is one method of control for such infections (7, 20), and guidelines have subsequently been developed for screening of MSM (1, 3, 4, 17, 19). These guidelines recommend routine screening of extragenital sites (pharyngeal and rectal) for *Neisseria gonorrhoeae* and *Chlamydia trachomatis* by using conventional tests, such as culture or nucleic acid amplification tests (NAATs).

NAATs are commonly used as screening tests for *N. gonorrhoeae* and *C. trachomatis*, but most commercial NAATs have not been validated for use with extragenital specimens. Some laboratories may have developed in-house NAATs for detection of *C. trachomatis* in extragenital specimens, such as *C. trachomatis* conjunctivitis (10). Preliminary data from commercial NAATs suggest that ligase chain reaction (LCR; Abbott Laboratories, North Chicago, Ill.) and the COBAS Amplicor PCR assay (Roche Diagnostics Systems Inc., Pleasanton, Calif.) are both sensitive and specific for *C. trachomatis* detection of extragenital specimens (12, 15, 24).

One method to investigate the validity of positive results with extragenital specimens from commercial NAATs is to conduct confirmatory testing by using an alternative specific target for amplification. For example, a PCR for the major outer membrane protein (MOMP) has been used for confirming chlamydia-positive genital specimens detected by commercial NAATs (18, 23). Because MOMP is coded for by the single-copy *omp1* gene, a PCR directed at *omp1* may be less sensitive than the COBAS Amplicor PCR or LCR that is directed at a multicopy plasmid target (16). An advantage of *omp1* PCR is its applicability to typing *C. trachomatis* in clinical specimens by sequencing the PCR product (2, 13, 16).

To investigate the utility of the COBAS Amplicor PCR assay as a screening test for *C. trachomatis* in MSM, this study conducted *omp1* PCR on COBAS Amplicor-positive rectal and pharyngeal specimens obtained from men attending male-only saunas.

MATERIALS AND METHODS

Participants and specimens. Specimens were collected from men who participated in a screening program in male-only saunas in Melbourne, Australia (14). All specimens from this program were tested for *C. trachomatis* by COBAS Amplicor PCR (14). A sample of 52 *C. trachomatis*-positive swabs were collected from 43 men (47 anal swabs and 5 throat swabs). Eight men testing positive had repeat positive anal swabs collected before treatment, and one man had *C. trachomatis* detected from both anal and throat swabs. The throat and anal swabs collected from 15 men testing *C. trachomatis* negative were also included in the analysis (15 anal swabs and 15 throat swabs).

DNA was isolated directly from the clinical samples by using the automated MagNA Pure LC (Roche Diagnostics), and the COBAS Amplicor PCR assay was performed as previously described (21). An additional PCR for amplification of β-globin sequence was conducted for detection of inhibition and sample adequacy (22).

***omp1* PCR amplification.** Three PCRs targeting the four *omp1* variable domains (VDs) were used (VD1-4, VD1/2, and VD3/4). PCR VD1-4 was used on all 52 swab samples, and PCRs VD1/2 and VD3/4 were used on samples that were negative by PCR VD1-4. Not all samples underwent all *omp1* PCRs, and the cumulative results of all three *omp1* PCRs were compared to the COBAS Amplicor PCR results.

PCR VD1-4 used the primers P1 (13) and OMP2 (13) to amplify an approximately 1.1-kb region of the *omp1* gene including all four VDs. PCR VD1/2 used

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TABLE 1. Comparative results of the COBAS Amplicor PCR assay and *omp1* PCRs for detection of *C. trachomatis*

Extragenital sample	*omp1* PCR result	No. of COBAS PCR samples[a]:		
		Positive	Negative	Total
Anal swab	Positive	44	0	44
	Negative	3	15	18
Throat swab	Positive	5	0	5
	Negative	0	15	15
Total		52	30	82

[a] Cospecificity = 100%; cosensitivity = 94%.

the primers P1 and CT6 (2) to amplify a region of *omp1* including VDs 1 and 2, and PCR VD2/3 used the primers CT6 (sense) and OMP2 to amplify a region including VDs 3 and 4. According to the *omp1* nucleotide sequence of *C. trachomatis* serovar strain J/UW36/Cx (GenBank accession no. AF063202), primer P1 is positioned at bases 1 to 22, primer OMP2 is at bases 1115 to 1136, and primer CT6 is at bases 655 to 679. The primary reason for conducting PCR VD1-4 followed by a second round of amplification with PCRs VD1/2 and VD3/4 was to sequence *omp1* amplicons, achieve sequence for all *omp1* VDs, and determine the corresponding *C. trachomatis* serovars of samples. (The sequencing results are currently being analyzed.)

PCR VD1-4 (13) was carried out with 20 μl of the DNA preparation and a 0.5 μM concentration of each primer in the PCR mixture (50-μl reaction volume) and using a decreased annealing temperature of 50°C in the PCR cycle. VD1/2 and VD3/4 were nested PCRs carried out as described for PCR VD1-4, using 2 μl of the VD1-4 product as target DNA and using an increased annealing temperature of 55°C in the PCR cycle. These nested PCRs were optimized using *C. trachomatis* reference strains and patient VD1-4 PCR-positive samples as positive controls and patient COBAS Amplicor *C. trachomatis*-negative samples as negative controls. Strict procedures avoiding specimen contamination and carryover were followed with nested PCRs. This included discarding PCR tube lids and replacement with new, sterile lids every time tubes were opened. Also, *C. trachomatis* reference strains with serotypes different from the predominant types in this study sample were used as controls, because any likely carryover would be indicated after sequencing of products.

Each time a PCR was conducted, the assay included one reaction mixture containing no DNA as negative control and one or two reference strains of *C. trachomatis* serovar B, D, E, F, G, K, or L2 as positive controls. Also, randomly selected COBAS Amplicor *C. trachomatis*-negative patient samples were used as negative controls. For nested PCRs VD1/2 and VD3/4, a positive VD1-4 PCR product was included as a positive control. PCR products were visualized by gel electrophoresis (5 μl of each PCR product on a 1.5% agarose gel containing ethidium bromide). The presence or absence of a band of the estimated product size determined the positive and negative *omp1* PCR results.

RESULTS

Of the 52 *C. trachomatis* PCR-positive samples by COBAS Amplicor, 35 (67%) were positive by *omp1* PCR utilizing VD1-4 primers. Of the 17 samples testing negative with PCR VD1-4, 14 (27%) were *omp1* positive on assay with nested PCRs VD1/2 and VD3/4. Overall, three anal swabs were not confirmed by any *omp1* PCR (6%). The results of *omp1* PCRs are summarized in Table 1. A total of 49 (94%; 95% confidence interval, 88 to 99%) COBAS Amplicor-positive samples were confirmed by the *omp1* PCRs. This represents confirmation of 38 of 41 (93%) men diagnosed with *C. trachomatis* by COBAS Amplicor. Table 1 shows the cospecificity (100%) and cosensitivity (94%) of *C. trachomatis* detection by *omp1* PCR.

DISCUSSION

In our study of extragenital samples (anal and throat swabs from males), 94% of COBAS Amplicor-positive samples were confirmed by testing for *omp1*. This supports both the confident reporting of *C. trachomatis* detected by COBAS Amplicor in extragenital specimens and the utility of this assay as a screening test for MSM.

A substantial proportion of extragenital specimens were confirmed positive for *C. trachomatis* by using a nested *omp1* PCR (14 samples [27%]) with only 3 anal swabs (6%) not confirmed by any *omp1* PCR. The multicopy plasmid target of the COBAS Amplicor assay offers high sensitivity, and a lower sensitivity with the *omp1* PCR was expected because *omp1* is a single-copy gene target. Other studies have also reported genital samples positive by a plasmid-directed PCR or LCR assay that could not be amplified by *omp1* PCR. Vincelette and colleagues (23) reported 12 (24%) endocervical swab specimens, 5 urethral swab specimens, and 3 (18%) urine specimens from women as false-positive COBAS Amplicor: i.e., not confirmed by *omp1* PCR (23). Pederson and colleagues (16) reported 53% of urine samples and 18% of vaginal flush samples that were positive by LCR (Abbott) could not be amplified by *omp1* PCR and have described reasons for the unsuccessful *omp1* PCR, including the low copy number of the target present in the sample (16).

The three COBAS Amplicor-positive and *omp1* PCR-negative anal samples all contained adequate amplifiable DNA and a lack of inhibitors, as shown by β-globin detection (14, 21, 22). It is most probable that these samples were not false positives, but rather contained bacterial levels that were below the detection level of the *omp1* PCR assay. In addition, one patient who had an *omp1*-negative anal sample also had *C. trachomatis* detected from a urine sample that was collected at the same time. This urine sample was COBAS Amplicor and *omp1* PCR *C. trachomatis* positive (data not shown) and supports the likelihood that the anal swab was *C. trachomatis* positive. To improve *omp1* detection in samples that may have DNA degraded from freeze-thawing, this study used primers targeting smaller regions of the *omp1* gene and nested PCR (PCRs VD1/2 and VD3/4). Also, to demonstrate the specificity of the *omp1* PCR methods used in this study, a selection of the *omp1* PCR products were sequenced. Approximately 80% of all PCR products had adequate concentrations of DNA for sequencing reactions. The results of sequencing are currently being analyzed and will be published separately (data not shown).

The calculated sensitivity and specificity of *C. trachomatis* PCRs (Table 1) demonstrate a high likelihood of *C. trachomatis* infection with a positive *omp1* PCR. Although the sensitivity was 93%, it does not rule out *C. trachomatis* infection in *omp1* PCR-negative cases, which may be due to low copy number. Until a larger-scale study is conducted, it is difficult to advocate that diagnostic laboratories implement routine *omp1* PCR testing to validate the COBAS Amplicor. Based on our results, there is a high likelihood of a positive COBAS PCR being a true positive. Laboratories may select to perform confirmatory assays such as *omp1* PCR; however, due to the lower sensitivity of *omp1*-directed PCR, some positives may not be confirmed. Nevertheless, this study demonstrates the utility of COBAS

Amplicor PCR for detection of *C. trachomatis* in anal and throat samples.

Because *C. trachomatis* is a potential risk factor for human immunodeficiency virus transmission, confident testing of sexually active MSM for *C. trachomatis* carriage at extragenital sites is essential. In many instances, NAATs for the detection of *C. trachomatis* are now the assays of choice for public health screening purposes. Until larger-scale studies are conducted for the validation of NAATs with extragenital specimens, the findings of this study support the confident reporting of *C. trachomatis* detected by COBAS Amplicor.

ACKNOWLEDGMENTS

This work was supported by funding from the Public Health Research Projects 2002-03 (Communicable Diseases), the Victorian Department of Human Services, Australia.

We gratefully acknowledge the research team involved in the collection of specimens used in this study (14).

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