Diagnosis of *Chlamydia trachomatis* Infections in Women

Urinary PCR Compared to Cervical Culture and PCR on Cervical Swabs in High Risk Females

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Diagnosis of *Chlamydia trachomatis* infections in women has traditionally depended on cell culture or enzyme linked immunosassay. Recently Polymerase Chain Reaction (PCR) has been shown to be more sensitive than these methods when performed on endocervical swabs. A total of 203 high risk females were enrolled in a comparative study of three methods for diagnosing *C. trachomatis* infections: McCoy cell culture and Amplicor\(^6\) PCR on endocervical swabs and urine. Thirty four had positive cultures, 38 positive PCR from cervix and 37 had positive PCR on urine specimens. When discrepancy occurred, the leftover Amplicor\(^6\) specimen was retested by Roche with Amplicor\(^6\) and a primer for the Major Outer Membrane Protein (MOMP) gene. None was false positive in cell culture or in urinary PCR but two were false positive in cervical PCR. In all three tests, 32 were positive. The sensitivity of culture was 97%, 92% in cervical PCR and 95% in urinary PCR. The specificity was 100% in both culture and urine PCR but 98% in cervical PCR. The results show that Amplicor\(^6\) PCR performed on female urine is more sensitive and as specific as cell culture.

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**Introduction**

*Chlamydia trachomatis* has become the most common and one of the most costly venereal diseases in the western world (1,2). Many patients carry the infection for a long time without any symptoms and can therefore disseminate the organisms unknowingly. Early detection of this infection is important in order to prevent complications like ectopic pregnancy, pelvic inflammatory disease and infertility. Culture of endocervical and urethral swab samples has been the gold standard in diagnosing Chlamydial infections. It is relatively easy to obtain an endocervical sample from women during a pelvic examination, but in some cases such an examination may be difficult or impossible. A number of rapid methods for detecting *Chlamydia trachomatis* have been developed but they are not as sensitive as culture (3–5). Recently the polymerase chain reaction (PCR) has been shown to be as sensitive and specific as culture when done on swabs from cervix and male urethra (6–8) and the same applies to another DNA amplification method, the ligase chain reaction (LCR) (9). PCR on male urine has been used routinely in our clinic for over a year with good results. A similar test on female urine would have a great advantage as screening would be considerably easier in many instances. In the following study a comparison was made between Amplicor\(^6\) PCR on urine in females, PCR on cervical specimens and cervical cell culture.

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**Patients**

The study was conducted in the Sexually Transmitted Disease (STD) clinic in Reykja-
vök, Iceland in November 1994 to January 1995. All women attending the clinic in a 70 day period who normally would be tested for *Chlamydia* were included. Pelvic examination was done in all cases. Signs like mucopurulent cervical discharge were recorded. Symptoms like inter menstrual bleeding, abnormal vaginal discharge or painful urination were also noted. All patients were asked about the number of sexual partners for the last three and six months and also if condoms were used. Patients receiving antibiotics two weeks prior to the visit to the STD clinic were excluded.

**Methods**

Three different methods were used to evaluate Amplicor® PCR for detecting *Chlamydia trachomatis* in female urine.

Two endocervical swabs were collected for *Chlamydia* cell culture and Amplicor® PCR (Roche) in an alternating sequence. After the examination 20–50 ml of first void urine were collected. The sample intended for culture was collected on a cotton swab (Medical wire Co) and put in 1.0 ml of 0.2 M sucrose phosphate buffer, antibiotics and 10% foetal calf serum and cooled with ice. One sample was collected with collection kits supplied by Roche. All samples were transported to the laboratory within four hours. Specimens for culture of *C. trachomatis* were agitated with glass beads and 0.6 ml of the buffer was added to two tubes with a monolayer of McCoy cells. The cells were centrifuged for one hour at 5000 g at 35°C. The supernatant was aspirated and replaced with maintenance media containing cycloheximide. The tubes were incubated at 35°C for 48–72 hours and the slide from one of the tubes was examined stained with Fluorescent Antibody (Syva MicroTrak FA). If the slide was conclusively positive or negative the slide from the second tube was stained. If the examination of the first tube was inconclusive the second was subcultured and the procedure repeated.

The Amplicor® assay was performed on a Perkin Elmer thermocycler supplied by Roche Molecular Systems. The test was performed according to manufacturer’s instructions. When discrepancy occurred, the leftover Amplicor® specimen was retested by Roche with Amplicor® and a primer for the Major Outer Membrane Protein (MOMP) gene. This applied when either one or both PCR samples were positive and culture negative, or if culture was positive and one or both PCR samples were negative.

**Results**

A total of 203 women were tested and enrolled in the study. The mean age was 21 and the median age was 20 years. The median and mean age of *Chlamydia* infected patients was 20. The age distribution of the patients is shown in the figure. The definition of infection was that either culture should be positive or
both PCR tests positive but in that case a confirmatory test with MOMP primers had to be positive. Percent infected was 19.2% but if *Chlamydia* culture was used as a definition of infection only 16.7% were positive. The results and calculations on the performance of the three different tests are shown in tables I and II. Fifteen of the 34 culture positive patients had no symptoms and 16 of 34 PCR cx/urine positive had no symptoms. Twenty three of those positive had white, purulent or transparent cervical discharge but 12 were normal on examination. Only one *Chlamydia* positive patient had always used a condom, five used it often 13 used it sometimes and 15 never. No difference in the use of condoms, was found between those positive in the PCR only and those positive in culture. Forty percent of those infected but only 28% of those not infected had two or more sexual partners during the three months prior to the study.

**Discussion**

Because collection of samples for the diagnosis of *Chlamydia* infections has often caused discomfort and sometimes humiliation for the female patients, new methods, where examination is not required, have definite advantages. Collecting a urine sample is in most instances considered acceptable to the patient. The PCR urinary diagnostics have been used for males in our clinic for over one year with very satisfying results and have been well accepted by our patients. It can be argued that omitting examination in the clinic may cause other diseases, like genital warts, to be missed. This is of some concern and examination of men and women is strongly recommended, whenever possible, regardless of which method is used for diagnosing Chlamydial infections.

**Table I. Results of the three different tests from 203 patients.**

<table>
<thead>
<tr>
<th></th>
<th>Positive</th>
<th>True positive</th>
<th>False positive</th>
<th>False negative</th>
<th>True negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture</td>
<td>34</td>
<td>34</td>
<td>0</td>
<td>5</td>
<td>164</td>
</tr>
<tr>
<td>PCR cx</td>
<td>38</td>
<td>36</td>
<td>2</td>
<td>3</td>
<td>162</td>
</tr>
<tr>
<td>PCR urine</td>
<td>37</td>
<td>37</td>
<td>0</td>
<td>2</td>
<td>164</td>
</tr>
<tr>
<td>All 3 tests</td>
<td>32</td>
<td>39</td>
<td>0</td>
<td>2</td>
<td>164</td>
</tr>
</tbody>
</table>

**Table II. Calculations on the performance of three different tests.**

<table>
<thead>
<tr>
<th></th>
<th>Culture</th>
<th>PCR cx swabs</th>
<th>PCR urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>87</td>
<td>92</td>
<td>95</td>
</tr>
<tr>
<td>Specificity</td>
<td>100</td>
<td>98</td>
<td>100</td>
</tr>
<tr>
<td>Predictive value of positive</td>
<td>100</td>
<td>95</td>
<td>100</td>
</tr>
<tr>
<td>Predictive value of negative</td>
<td>97</td>
<td>98</td>
<td>98</td>
</tr>
</tbody>
</table>

Amplicor® PCR has been shown to be more sensitive than the old gold standard, cell culture (7,8). It is difficult to resolve the issue of "false positive" Amplicor® PCR tests because of the lack of a standard sensitive enough. Using PCR for the MOMP gene is not quite satisfactory because it is slightly less sensitive than the Amplicor® PCR. The reason for this is that the Amplicor® PCR detects plasmid genes, of which there may be multiple copies in each *Chlamydia* cell. The MOMP gene, on the other hand, resides on the chromosome of which there is only one copy per cell. The two "false positive" tests from the cervix may therefore indeed have been true positives.

The results show that Amplicor® PCR performed on female urine is more sensitive and as specific as cell culture. The prevalence of isolated urethral asymptomatic *C. trachomatis* in the absence of cervical infection has been shown to be up to 24% (11,12). This could partly explain the higher sensitivity of urinary PCR. This urinary test can be used to test and screen asymptomatic females where other methods like cervical swabs for culture and PCR can be difficult to apply. Obviously, screening of asymptomatic populations like pupils of schools was difficult or impossible due to reluctance to undergo an examination where an urinary test is more acceptable. This opens new venues, in screening asymptomatic populations for *C. trachomatis*, that are now being explored (13).
REFERENCES


