

PCR TECHNIQUE DETECTION OF *CHLAMYDIA TRACHOMATIS* DNA BAND USING SILVER NITRATE STAINING

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ABSTRACT

Chlamydia trachomatis genital infection is one of the Sexually Transmitted Diseases that can cause cervicitis, salpingitis, ectopic pregnancy, infertility, chronic pelvic pain and pelvic inflammatory disease (PID) in women and epididymitis and non gonococcal urethritis (NGU) in men. The prevalence of this infection is 7.9% in women and 7.6% in men overall. The problem is that 70% of this infection in women and 50% in men are asymptomatic and can only be detected through accurate screening test. The nucleotide amplification using PCR technique could be used to amplify one DNA or RNA molecule of the microbe specific gene region that may increase the sensitivity and specificity to detect *Chlamydia trachomatis* in the "first-catch urine". The aim of this research was to study the accuracy (sensitivity and specificity) of the PCR with Silver Nitrate staining method in comparison to the commercial PCR (Amplicor, Roche) as a standard method. The result was no significant difference ($p < 0.05$) found in accuracy between PCR Silver Nitrate staining and PCR Amplicor; the sensitivity of PCR Silver Nitrate staining was 85.7% and the specificity was 100%, to detect *Chlamydia trachomatis* in the "first-catch urine".

Keywords: *Chlamydia trachomatis*, urine, PCR, silver nitrate, screening test

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INTRODUCTION

Chlamydia trachomatis genital infection is one of the Sexually Transmitted Diseases (STD). This infection is considered crucial because its rate of occurrence is relatively high compared to other diseases caused by infections that are recorded by the CDC (Center of Disease Control) in 2003. To this day, the standard method for detecting *Chlamydia trachomatis* in patients or in asymptomatic individuals is the culture on *McCoy cells* and immunofluorescence. However, besides the relatively low sensitivity range of 50-85% with 100% specificity, this test can only be done in a laboratory with expensive instruments and reagents and handled by experienced technicians; "cytologic examination" is also found to be less sensitive compared to culture method. The antigen detection method also shows very low sensitivity. The method of hybridization of nucleate acid with DFA (*Direct Fluorescent Antibody*) only reaches 70-95% sensitivity and 92-98% specificity, which is only reliable for symptomatic cases. As for the method of hybridization of nucleate acid using *DNA Amplification Assay*, there are 3 different formats: PCR (*Polimerase Chain Reaction*), LCR (*Ligase Chain Reaction*) and TMA (*Transcription Mediated Amplification*). Many studies indicate that these tests are more sensitive and specific than the culture method and

are more reliable in detecting both symptomatic and asymptomatic cases of Chlamydial genital infection by using first-catch urine specimen. This technique is not invasive so that it is easily acceptable by the diseased and community to detect Chlamydial genital infection early; but this technique is quite costly. This research aims to find a more efficient way to suppress the cost of this technique, that was by proving whether the PCR with Silver Nitrate staining method had comparable sensitivity and specificity with the PCR Amplicor (Roche) method as the standard. This research also investigated how effective was the diagnostic method using PCR with Silver Nitrate staining method compared to the PCR Amplicor (Roche) method.

Chlamydia trachomatis, included in the Chlamydiaceae family, is an intracellular obligate bacterium. Chlamydiae's cell wall is similar to Gram Negative bacteria, but it lacks the peptidoglycan layer and is composed of a relatively higher lipid component. Unlike other bacteria, Chlamydiae have a special life cycle. The infectious particles, rigid structures with envelopes with diameter of 0.3 μ m known as the "*Elementary Bodies*", will attach to the surface of a sensitive host cell. They start their own phagocytosis through a process called the phagosome. This organism can avoid fusion from a lysosome, thus keeping the cell from fagosome

sterization by lysosome activity. Therefore Chlamydiae is able to survive in the cell host (intracellular). Once this initial stadium is completed, “Elementary Bodies” will transform to a metabolically more active form, the “Reticulate Bodies”. “Reticulate Bodies” will double themselves through binary fission up to 18-24 hours after the infection, whereupon some “Reticulate Bodies” will transform back into the infectious “Elementary Bodies”. “Reticulate Bodies” and “Elementary Bodies” formed are still enclosed in phagosome, which can be seen microscopically as an “Inclusion” through iodine dye, Giemsa, or fluorescent antibody. The “Inclusion” from *Chlamydia trachomatis* contains a large amount of microorganisms. After 48-72 hours of infection, cells will burst and release infectious “Elementary Bodies”, which then will attack other cells. The target cells of *Chlamydia trachomatis* are the columnar epistole cells or squamocolumnar from a host. Looking at the life cycle of *Chlamydia trachomatis*, we can see that *Chlamydia trachomatis* causes a chronic infection and can cause scar on the infected area.

In women, this infection can cause cervicitis, salphingitis, ectopic pregnancy, infertility, chronic pelvic pain and pelvic inflammatory disease (PID). In men, this infection can cause epididymitis and non gonococcal urethritis (NGU), and is an important reservoir (transmission source) of this disease. The prevalence of this infection is 7.6% in men and 7.9% in women. The crucial problem is that there are more than 70% Chlamydia genital infections in women and 50% in men that are asymptomatic (without symptoms), so a screening test is needed. Screening test requires an accurate, fast, and easy method that does not cause pain to the diseased. An accurate detection method of Chlamydial infection is suitable to be used for screening test and for confirming infection diagnosis of a patient. At the end, with the right, accurate and quick diagnosis, a suitable therapy can be given to the diseased or asymptomatic individual to avoid further complication on the reproductive organ.

MATERIALS AND METHODS

This research involved laboratory observation. The samples tested were random samples from non-specific urethritic patients in the Department of Dermatology and Genital Polyclinic of Dr. Soetomo Hospital, Surabaya. The specimens are taken from first-catch urine, that was 10-30 ml urine samples that discharged by the patients at the first-catch urine after the external genital had been cleansed with liquid soap. In the first-catch urine there was microbial accumulation several hours prior to urination. Therefore, if *Chlamydia trachomatis* was present, it would accumulate in this

first-catch urine. Nucleic acid amplification would be able to increase detection sensitivity of *Chlamydia trachomatis* in the urine. Sensitivity can also be increased by labeling technique in the hybridization technique such as PCR Amplicor. PCR technique/method with Silver Nitrate staining was also expected to increase detection sensitivity by improving the clarity of DNA band observation. PCR result was 280 bp. Based on the aim of this research, analytical data were collected by descriptive statistics, negative predictive value and positive predictive value to test the comparability between PCR with Silver Nitrate staining with the PCR commercial kit method (Amplicor ROCHE).

RESULTS

Thirty samples of non-specific urethritic patients that were collected to detect the presence of *Chlamydia trachomatis* were given the same treatments, in which they were tested using PCR commercial kit (Amplicor/Roche) and PCR with Silver Nitrate staining. The result is shown in table 1. After being statistically analyzed using Mc. Nemar method, it was concluded that there was no significant difference between detection methods using PCR by commercial kit and the PCR by Silver Nitrate staining, with $p < 0.05$.

Table 1. The result of *Chlamydia trachomatis* detection with PCR commercial kit (Amplicor, Roche) and PRC with Silver Nitrate staining.

	Amplicor (standard method)		Total	P
	+	-		
Silver nitrate	12	0	12 (40%)	0.05
+	2	16	18 (60%)	
-				
Total	14 (46.6%)	16 (53.3%)	30	

Sensitivity of PCR Silver Nitrate staining method: $12/14 \times 100\% = 85.7\%$

Specificity of PCR Silver Nitrate staining method: $16/16 \times 100\% = 100\%$

Positive Predictive Value: $12/12 \times 100\% = 100\%$

Negative Predictive Value: $16/18 \times 100\% = 88.9\%$

Diagnosis Effectiveness: $(12+16) : 30 = 93.3\%$

DISCUSSION

Through this research it was found that 46.6% (14/30) Chlamydia infected patients were positively diagnosed

using PCR commercial kit (Amplicor, Roche) and 40% (12/30) using PCR Silver Nitrate staining. In a study in London, UK, the prevalence of genital infection by *Chlamydia trachomatis* was found to be 20% in young women, and 23% in young men. In her research, Carolyn M Black (1997) stated that 30-50% non-gonococcal urethritis cases in the United States were caused by *Chlamydia trachomatis*. This deviation might be caused by different sexual behaviors in developing and developed countries. However, it was possible that prevalence value increases as the country gets more developed and exposed to technology and information from various media.

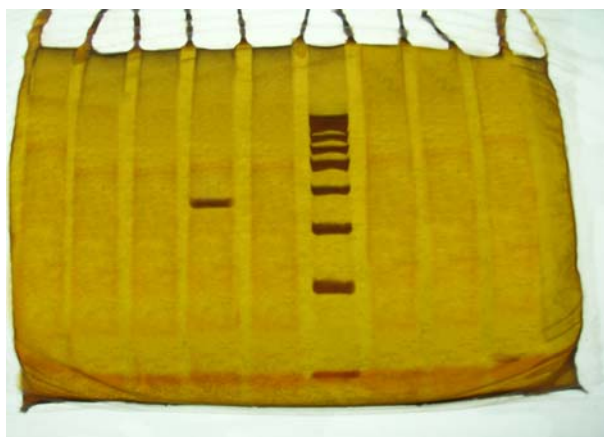


Figure 1. *Chlamydia trachomatis* detection result by PCR Silver Nitrate staining method.

The samples collected for this research were first-catch urines. The advantages were that they were not invasive or did not induce pain to the diseased, and the result that was obtained from an uncomplicated procedure was quite accurate. Caul and Herring (2001) stated that first-catch urines for men and women were adequate samples for “Nucleic Acid Amplification Test” using PCR method.

A method that is still used at a simple laboratory is Giesma staining from canalis cervicalis swab specimen. However, the sample collection is very inconvenient for patients with sexual diseases. Another disadvantage is that this method can only stain the “Inclusion Body” of Chlamydia, which contains glycogen. DNA amplification method recently is being developed because of its advantage. Only a little amount of DNA is required for the amplification to make the detection of *Chlamydia trachomatis* genital infection becomes more sensitive. This DNA can be well extracted from either the elementary bodies or reticulate bodies of *Chlamydia trachomatis* in a patient.

This research was looking for a specific, sensitive and accurate method by comparing DNA amplification method between PCR Silver Nitrate staining with PCR using commercial kit (Amplicor, Roche), which also can be used as a standard according to FDA. The result of this experiment showed that in samples from non-specific urethritis patients there was a specific band of *Chlamydia trachomatis* in agarose with Silver Nitrate staining. The sensitivity of PCR method using Amplicor (Roche) was obtained because a little target DNA could be amplified significantly. Another reason was the usage of “conserved” region and repeated region target DNA in plasmid DNA (Davis et al. 1994).

The sensitivity of PCR method using Amplicor (Roche) was obtained because the target DNA was a part of a specific region known as the “Cryptic plasmid” of *Chlamydia trachomatis* with primary CP24 and CP27 to determine DNA sequencing at 207 bp. Whereas, the sensitivity of PCR method with Silver Nitrate staining was obtained because it could detect a small amount of DNA from both conserved and repeated Plasmid DNA region. The specificity of this PCR method was a part of Major Outer Membrane Protein of *Chlamydia trachomatis* with primary T1 and T2 of 280 bp.

This research found that both methods seemed to have similar sensitivity and specificity. However, the advantage of PCR method using Amplicor (Roche) was that the last detection after amplification was followed by specific hybridization at the Cryptic plasmid of *Chlamydia trachomatis* and the result was read by using ELISA reader. But the positive predictive value showed 100% result, which indicated that the PCR with Silver Nitrate staining was comparative to or could replace PCR method with Amplicor Roche. By knowing the advantage of diagnostic method using DNA amplification, we can improve the accuracy (sensitivity and specificity) in detecting *Chlamydia trachomatis* genital infection so that a suitable treatment and medication can be determined immediately. The importance of this is that we can prevent further transmission of Chlamydia infection in an attempt to control genital infection by *Chlamydia trachomatis* whose negative effects on male and female reproduction organ have been known.

CONCLUSION

The sensitivity of PCR with Silver Nitrate staining method reaches 85.7% with sensitivity of 100%. The positive predictive value shows that PCR Silver Nitrate staining method is comparable to or can replace PCR Amplicor Roche method. It is expected that this DNA Amplification method can be used widely in STD

clinics and in infertility clinics because of the non-invasive specimen collection and the accuracy of the test for *Chlamydia trachomatis* genital infection early detection. These advantages lead to the determination of a suitable and immediate medical treatment thus prevents further negative effects on both male and female reproductive organs.

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