

# Nonculture Methods for Diagnosis of *Chlamydia trachomatis* Genital Infection

## Emphasis on the Newly Developed Ligase Chain Reaction

Julius Schachter

Schachter J

Læknablaðið 1995; 81: 526-7

### Introduction

The first non culture tests for diagnosis of *Chlamydia trachomatis* infection involved demonstration of inclusions in epithelial cell specimens. With the introduction of culture methods, it was recognised that this cytologic procedure is relatively insensitive. Isolation became accepted as the method of choice for diagnosing Chlamydial infection, but because of the obligate intracellular nature of Chlamydial growth, tissue culture systems were required and this restricted access to Chlamydial diagnostics as cell culture facilities were not widely available.

Soon after their introduction, antigen detection methods became very popular. They greatly improved access to *Chlamydia* diagnostics. The first procedure to be introduced was a direct fluorescent antibody (DFA) technique. It was particularly useful because it restricted the number of specimens that could be tested and required expensive microscopes and well trained microscopists.

It is generally considered that DFA is approximately 75-85% sensitive in detecting Chlamydial infection of the cervix and less sensitive with male urethral specimens. The comparison here is with isolation in cell culture as

the gold standard, and it must be recognised that cell culture is seldom more than 80-90% sensitive.

The other antigen detection tests, the enzyme immunoassays (EIAs) had at best a similar sensitivity to DFA, although some commercially available products were less sensitive. The advantages of EIA were that it could be batched and automated and thus allowed for broad based screening programs. The specificity of the original EIAs was 97-98% which precluded their use in screening in low prevalence populations. The introduction of confirmatory assays improved the specificity.

DNA probes are also available but are not appreciably more sensitive than DFA or EIA. The introduction of amplified DNA tests such as polymerase chain reaction (PCR) and ligase chain reaction (LCR) have promised to revolutionise *Chlamydia* diagnostics. These procedures, which typically target DNA sequences in the Chlamydial cryptic plasmid (present at seven to 10 copies per elementary body) are theoretically capable of detecting less than one Chlamydial particle whereas DFA and EIA will detect on the order of  $10^5$  or more. The amplified DNA product can be detected of a color product. Evaluations of LCR and PCR have required a reevaluation of the gold standard as some specimens can only be shown to be positive with other amplified DNA tests. These procedures are more sensitive than culture whereas antigen detection methods are less sensitive. Some workers have found the sensitivity of PCR to be reduced by presence of inhibitors - inhibitors are also found in LCR but seem to be less a problem.

From the Department of Laboratory Medicine at the University of California, San Francisco, California, U.S.A.

### The Ligase Chain Reaction

There have been several multi-center evaluations of ligase chain reaction in diagnosis of *Chlamydia trachomatis* genital infection in men and women. LCR results were compared to isolation in cell culture. Discrepant (tissue culture negative/LCR positive) test elementary bodies, and by use of alternate DNA probes that targeted the Chlamydial major outer membrane protein gene.

The LCR assay was found to have very few false positive results. Specificity was >99.5% for all types of specimens evaluated. This indicates that this test could be used in low prevalence populations and still have high positive predictive values. The sensitivity of the LCR assay with urethral swab specimens or first catch urine (FCU) specimens from men was 93%. Similar results were obtained whether the men were symptomatic or asymptomatic. Tissue culture sensitivity was 68%.

With cervical swabs the corresponding sensitivities were 94% for LCR and 65% for cell culture. There was greater variability across sites for cell culture sensitivity (52% to 92%) than for LCR sensitivity (87% to 98%). Thus, LCR offers a highly sensitive non culture

method for detecting Chlamydial infection of the cervix and the male urethra.

We also evaluated the LCR test for diagnosis of *Chlamydia trachomatis* infection using first catch urine specimens from women. When compared to cervical culture, the resolved LCR tests had a sensitivity of 94% and a specificity of 100%. Tissue culture isolation had a sensitivity of 65%.

LCR on FCU is a highly sensitive and specific method of diagnosing Chlamydial infection in women, as well as in men. It is more sensitive than TC and may well present public health authorities with a useful non invasive screening test for Chlamydial infection in asymptomatic women.

### REFERENCES

1. Chernesky MA, Lee H, Schachter J, et al. Diagnosis of *Chlamydia trachomatis* urethral infection in symptomatic and asymptomatic men by testing first-void urine in a ligase chain reaction assay. *J Infect Dis* 1994; 170: 1308-11.
2. Schachter J, Stamm WE, Quinn TC, et al. Ligase chain reaction to detect *Chlamydia trachomatis* infection of the cervix. *J Clin Microbiol* 1994; 32: 2540-3.
3. Lee HH, Chernesky MA, Schachter J, et al. Diagnosis of *Chlamydia trachomatis* genitourinary infection in women by ligase chain reaction assay of urine. *The Lancet* 1995; 345: 213-6.