

# Use of polymerase chain reaction on pooled cervical swabs to detect *Chlamydia trachomatis* infections in female sex workers in Singapore

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## ABSTRACT

**Introduction:** Infections caused by *Chlamydia trachomatis* (*C. trachomatis*) is one of the commonest sexually transmitted infections (STI). As there is currently no laboratory in Singapore that offers the chlamydia culture tests, alternative laboratory methods were developed and antigen detection methods such as enzyme immunoassays (EIA) proved to be even less sensitive than cell culture. This study was done to assess the accuracy and sensitivity of pooling five endocervical swabs collected for *C. trachomatis* testing by polymerase chain reaction (PCR) technology, as compared to the currently used EIA on individual swabs, in female sex workers who were seen at a STI clinic in Singapore.

**Methods:** A total of 1,182 endocervical swab specimens were analysed by EIA as well as in pools of five specimens using PCR. Any pool with a positive PCR result for *C. trachomatis* infections was subjected to repeat PCR testing of the five individual specimens in the pool.

**Results:** There were a total of 48 confirmed cases of *C. trachomatis* infection. EIA detected 19 positive samples for *C. trachomatis*, yielding a prevalence of 1.6 percent among the sex workers tested. Pooled PCR testing showed a higher prevalence rate of 4.1 percent, with 48 PCR positive samples. All cases that were EIA positive were also PCR positive. Individual runs on 200 random samples as well as on 220 individual samples from positive pooled results showed PCR inhibition rates ranging from 1.5 percent to 2.3 percent. However, the PCR inhibition rate was 0 percent with the use of pooling. Sensitivity of EIA was 39.6 percent, with 100 percent specificity. EIA tests had a false negative rate of 60.4 percent. PCR was found to be 100 percent sensitive and specific.

**Conclusion:** *C. trachomatis* infections among female sex workers attending the clinic were

found to be higher using PCR technology. Less sensitive methods such as EIA result in undertreatment of otherwise undetected cases. The pooling strategy using pool sizes of five specimens, with a disease prevalence of 4.1 percent is reliable and cost-effective, and has since been introduced in the current medical surveillance scheme for sex workers in the clinic.

**Keywords:** *Chlamydia trachomatis*, enzyme immunoassays, polymerase chain reaction

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## INTRODUCTION

Infections caused by *Chlamydia trachomatis* (*C. trachomatis*) are among the most common bacterial sexually transmitted infections (STI) in the world<sup>(1)</sup>, including Singapore<sup>(2)</sup>. It may result in substantial morbidity in young sexually active people. *C. trachomatis* causes a variety of clinical syndromes in males (including urethritis and epididymitis), females (including cervicitis, pelvic inflammatory disease, and tubal infertility), and newborns (including conjunctivitis, and pneumonia)<sup>(3)</sup>. It is the leading cause of tubal infertility in most developed countries<sup>(3)</sup>.

At the Department of STI Control Clinic (DSC) in Singapore, non-gonococcal urethritis (NGU) is the one of the commonest STI seen. One of the major obstacles to effective treatment control and prevention of infection by *C. trachomatis* is the lack of rapid, affordable and non-invasive sampling and diagnostic methods.

The traditional "gold standard" for detection of *C. trachomatis* infections had been cell culture, but this has several disadvantages, including its relatively low sensitivity rate of between 70% and 80%<sup>(4)</sup>, and the fact that it is a labour intensive and expensive test, requiring two to seven days to isolate the organism. Adding to these disadvantages are the requirements for special transport media to process the samples, and very stringent storage requirements, particularly in maintaining a cold chain in transport and storage.

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For these reasons, there is currently no laboratory in Singapore that offers the chlamydia culture tests.

Alternative tests using antigen detection methods, which include enzyme immunoassay (EIA), are now routinely used. However, many of these antigen detection methods are even less sensitive than cell cultures. Within the last several years, the use of molecular probes and DNA hybridisation assays has dramatically increased the sensitivity of detecting chlamydial infections. However, the use of nucleic acid amplification tests (NAAT) in Singapore has been minimal. The major obstacle to its widespread use has been the relatively high cost of the test. One possible method to reduce the costs of NAAT is to pool specimens for screening. If the pooled specimens are positive, individual specimens from that pool can be tested separately and the positive sample identified. On the other hand, if the pooled specimens are negative, no further testing is required.

The aims of this study were: to determine the accuracy in pooling five endocervical swabs collected for *C. trachomatis* testing by polymerase chain reaction (PCR) technology; and to determine the prevalence of *C. trachomatis* infections among commercial sex workers (CSWs) on medical surveillance at the DSC clinic in Singapore, using EIA and PCR methods.

## METHODS

Endocervical samples collected from 1,200 female CSWs attending the DSC clinic were tested using the Cobas AMPLICOR™ (Roche Diagnostics Corporation, Indianapolis, IN, USA) as well as the Abbott IMx SELECT™ (Abbott Laboratories, Chicago, IL, USA) diagnostic kit, which was the standard kit used at the DSC clinic at the time of the study. Samples for PCR were collected using a Dacron-tipped swab. This dry swab was stored at room temperatures and processed for PCR within 48 hours. Samples for EIA were also collected using Dacron-tipped swabs and processed within 48 hours. Specimens were stored at 4°C if they were not processed immediately. The sequence of specimen collection was rotated on an alternating basis such that each test had equal numbers of specimens that were collected first.

Pools of five cervical swabs each were created with aliquots of 100 µL from each of the samples taken for PCR analysis. Analysis was performed according to the manufacturer's instructions. In brief, the prepared specimens were transferred into amplification tubes containing primers for *C. trachomatis* nucleotides, the internal control and DNA polymerase. Amplification

was accomplished by the built-in thermocycler. The internal control of amplification in the Cobas AMPLICOR™ is a sequence of plasmid DNA with primer binding regions identical to those of the *C. trachomatis* target sequence. A unique probe-binding region differentiates the internal control from the target amplicon. The internal control is introduced into each amplification reaction and is co-amplified with the possible target DNA from the clinical specimen. After the amplification, the amplified nucleotide sequences for the internal control and *C. trachomatis* were automatically detected using colorimetric capture-hybridisation assays specific for *C. trachomatis*.

For EIA tests, the Abbott IMx SELECT™ diagnostic kit was used. This utilised a microparticle enzyme immunoassay for the quantitative detection of chlamydial antigen on the analyser. The IMx probe/electrode assembly dispenses microparticles to the sample well which contains the sample. The pre-extracted lipopolysaccharide (LPS) antigen of the sample binds to the microparticles forming a complex. An aliquot of the reaction mixture containing this complex is transferred to the glass fibre matrix of the reaction well, and the microparticle-LPS antigen complex binds irreversibly to the glass fibre matrix. Rabbit anti-chlamydial antibody is then dispensed into the matrix and binds with the microparticle-LPS complex, followed by the dispensing of biotinylated goat anti-rabbit antibody complex. Rabbit anti-biotin: alkaline phosphatase conjugate is dispensed onto the matrix and after washing to remove unbound materials, the substrate 4-methylumbelliferyl phosphate is added to the matrix and the fluorescent product is measured by the optical assembly.

## RESULTS

The test results are summarised in Table I. There were a total of 1,182 samples tested, with 48 cases of confirmed infections. The overall prevalence of *C. trachomatis* infections among the sex workers tested was 4.1%. Using EIA, 19 samples were detected to be positive for *C. trachomatis* infections. There were no false positive EIA results. Using EIA, a prevalence of 1.6% was detected, and there were another 29 cases that were not detected by EIA. The sensitivity of EIA was 39.6%, with a false negative rate of 60.4%. EIA was 100% specific, with a negative predictive value of 97.5%. Of the pooled samples using PCR, 44 positive pools were identified, and these 220 samples were then run individually using PCR again.

In addition to the 220 samples that were run individually from the positive pools, there were another

**Table 1. Summary of test results.**

Test	No. of patients in whom <i>C. trachomatis</i> was present (n=48)	No. of patients in whom <i>C. trachomatis</i> was absent (n=1,134)	Sensitivity (%)	Specificity (%)
<b>PCR</b>				
Positive	48	0	100	100
Negative	0	1,134		
<b>EIA</b>				
Positive	19	0	39.6	100
Negative	29	1,134		

Sensitivity = true positive test results / all patients with disease.

Specificity = true negative / all patients without disease.

200 other individual samples randomly picked to be run individually. Among the 220 individual runs from positive pooled specimens, there were five samples that were inhibitory. Among the 200 individual runs from negative pools, three cases were inhibitory. There were no inhibitory results from any of the pooled samples. Therefore, the PCR inhibition rate for pooled samples was 0%, and PCR inhibition rates ranged from 1.5% to 2.3% when run individually.

## DISCUSSION

*Chlamydia* are gram-negative, non-motile bacteria that exist as obligate intracellular parasites of eukaryotic cells due to their inability to synthesise ATP. The genus *Chlamydia* consists of three reported species: *C. trachomatis*, *C. psittaci*, and *C. pneumoniae*. *C. trachomatis* infections are now recognised as the leading cause of STI in the United States, where more than 4 million cases occur per year. *C. trachomatis* is the most frequent cause of NGU<sup>(5)</sup>. In Singapore, a previous study identified *C. trachomatis* as causing 46% of the cases of NGU seen in an STI Control clinic<sup>(6)</sup>.

Automated DNA amplification methods using ligase chain reaction (LCR) and PCR have proven to be highly sensitive, specific and superior to cells cultures and EIA for the detection of *C. trachomatis* infection in both men with NGU and asymptomatic women with high-risk behaviour. These NAATs can offer chlamydia control programmes a unique sensitive method for widespread population-based screening, with their quick turnaround time and high sensitivity and specificity. What has hampered the widespread utilisation of NAATs has been their high cost. One way to reduce the expense of employing NAAT is the pooling of specimens. There have been some previous studies investigating

the pooling approach for cervical scrapes. In a small study, Lisby et al showed that pooling in groups of 5 was 100% sensitive and reduced costs<sup>(7)</sup>. Kapala et al also showed recently that a pooling strategy could be utilised when performing LCR<sup>(8)</sup>.

Our findings that pooled specimens using PCR technology more than doubled the number of cases of *C. trachomatis* infections among asymptomatic sex workers when compared to EIA, confirming the findings of an earlier local study<sup>(6)</sup>. In that study, PCR had a sensitivity of 55.6% for endocervical specimens, compared with 22.2% for EIA, and 88.9% for urine specimens. The lower sensitivity for endocervical swabs in that study was due to the high rates of inhibitory specimens (12%), and compounded by the low numbers of patients tested (50). In this study, the sensitivity of PCR on pooled specimens utilising endocervical swabs was 100%, with 0% inhibitory specimens on pooled samples and an inhibitory rate of 1.5% to 2.3% when specimens were run individually. Pooling specimens thus provided the added advantage of reducing the number of inhibitory specimens without affecting the accuracy of the test. The presence of PCR inhibitors may also give false negative results. Interfering substances include the presence of excessive mucus in cervical sample and certain lubricants. Pooling the specimens diluted the effects of these potential inhibitory elements.

Organic and inorganic compounds that inhibit the amplification of nucleic acids by PCR are common contaminants in DNA samples from various origins. They can interfere with the reaction at several levels, leading to different degrees of attenuation and even complete inhibition of the reaction. A wide variety of PCR inhibitors have been reported, and they appear to be particularly abundant in complex samples such as animal fluids. Most of them, such as polysaccharides and haemoglobin, exhibit similar solubility to DNA. As a consequence, they are not completely removed during extraction protocols, remaining as contaminants in the final DNA preparation<sup>(9)</sup>. Although there were no cases of false positive results, it has been noted that bloody specimens may yield false positive reactions. Endocervical samples taken should thus be free of lubricants, with excessive mucus wiped off before the sample is taken and preferably as non-bloody as possible.

Among the 1,182 women tested, PCR detected 48 cases of *C. trachomatis* infections, compared to the 19 cases detected by EIA. Prevalence of *C. trachomatis* infections among the sex workers was 4.1% using pooled PCR, compared to a detection rate of only 1.6% using EIA. This would imply that using

EIA as the screening test in medical surveillance of the sex workers would potentially miss 60.4% of infections, leading to no treatment and the potential for complications and transmission to the clients of these sex workers. However, the overall prevalence of *C. trachomatis* infections among the sex workers tested was still relatively small. With the use of PCR on pooled specimens, the DSC clinic would be able to implement a more effective chlamydia control programme. The pooling strategy results in cost savings and in good dilution and inhibition characteristics.

At the time of writing, the clinic has since introduced routine PCR testing for *C. trachomatis* in sex workers. It should be noted, however, that the cost savings and pool sizes would be different depending on the prevalence of disease. For example, if the disease prevalence were 10%, then using pool sizes of five samples per pool would not be as cost-effective, as there would be more pools that would require individual runs. In the present low prevalence rate of 4%, pool sizes of five is cost effective without affecting accuracy. Further studies can be done in future using different pool sizes to determine the optimal pool size for maximum cost savings.

In an untreated patient, a positive PCR result signifies the presence of bacteria, and treatment should be initiated. However, due to the sensitivity of PCR, the test should not be repeated too soon as subsequent detection of chlamydia DNA does not imply infectivity, as even dead organisms are detected. A test of cure is not routinely done. Presently, at the DSC clinic, the monthly frequency

of screening for chlamydia using PCR for the medical surveillance scheme appears to be appropriate, as treated cases that were PCR positive became negative within a month. Larger studies on the optimal frequency of screening will be conducted in future.

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