The Impact of Switching to Polymerase Chain Reaction for the Diagnosis of Chlamydia trachomatis Infections in Women

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ABSTRACT

Background: We noted a marked increase in Chlamydia trachomatis (CT) infections in the Capital Health Region of NS coincident with substitution of a PCR for an enzyme immunoassay (EIA). We reviewed our experience to determine the cost of switching and the impact on the number of new infections diagnosed.

Methods: Information on the number of EIA and PCR tests performed on women was retrieved from an abstracted laboratory information database. We examined records of testing performed between April 1998 and December 2001. Prior to June 2001, all genital swabs were tested using the MicroTrak II Chlamydia EIA and confirmed by direct fluorescence examination. After July 2001, genital swabs were tested using the COBAS AMPLICOR® C. trachomatis test.

Results: During the study period, 62,288 EIA tests were performed on specimens submitted; 2,061 (3.33%) were positive. In the six months when testing was performed by the PCR method, 9,559 PCR tests were performed, 463 (4.84%) were positive; 46% increase. In the three years before PCR testing was implemented, an average of 1,626 specimens were submitted monthly. An average of 54 tests were positive (3.3%). The cost for each positive detected by PCR was $208 Cdn and $226 by EIA.

Conclusions: The switch to PCR for the diagnosis of CT produced a marked increase in the number of chlamydia infections diagnosed. The recent increase in the number of reported CT cases in Canada may be due in large part to more sensitive tests. Surprisingly, the cost of each positive test by PCR was $18 Cdn less than that of the EIA.

C hlamydia trachomatis is an important sexually transmitted disease. There is some evidence that an earlier decline in incidence has been replaced with a resurgence of infections. The decline had been attributed to a number of factors. There may be changing patterns of sexual activity among young adults, condom usage is more frequent, and more effective therapies are available.1,2 Probably one of the most important contributors to the decline in C. trachomatis infection was the increased availability of rapid and accurate diagnostic tests. Over the last two decades, testing has moved from tissue culture to direct immunofluorescence to enzyme immunoassay (EIA) and to molecular methods testing based on either the ligase or polymerase chain reactions (LCR and PCR).3 The sensitivity of these and other molecular based methods is significantly higher than that of previously employed methods.4-10

In the second half of 2001, we observed what appeared to be a significantly higher than normal number of cases of C. trachomatis infection in the Capital Health region of Nova Scotia. We wished to examine the impact of the switch on the proportion of specimens in which we detected C. trachomatis and to examine the cost implications.

MATERIALS AND METHOD

The Microbiology Laboratory of the Queen Elizabeth II Health Sciences Centre provides diagnostic microbiology services to the Capital Health region in the province of Nova Scotia. A small number of chlamydia EIA tests are also performed at the Izaak Walton Killam Health Centre; all other testing was performed in our laboratory. Information on the number of EIA and PCR tests performed on women was retrieved from an abstracted laboratory information service database. We examined records of testing performed between April 1, 1998 and December 31, 2001.

Chlamydia trachomatis testing

Prior to June 2001, all genital swabs submitted for diagnosis of chlamydial infection were tested using the MicroTrak, II
Chlamydia EIA (Behring Diagnostics Inc., Cuppertino, CA). Testing was performed on the Syva MicroTrak, II Autoreader (Behring Diagnostics Inc., Cuppertino, CA) in accordance with the manufacturer’s instructions. All specimens with a reactive or grey zone EIA were confirmed by direct fluorescence examination of the centrifuged sediment of the residual specimen using the MicroTrak, II DFA reagents supplied by the manufacturer. The presence of one or more elementary bodies in a direct fluorescence preparation was considered C. trachomatis antigen positive.

Following a switchover period between June and July 2001, specimens were tested almost exclusively using the COBAS AMPLICOR® Chlamydia trachomatis test (Roche Diagnostics, Hoffmann-LaRoche Ltd., Laval, Quebec) as described in the manufacturer’s package insert. Female urine testing is very rarely performed. Internal controls were used for female urethral but not cervical specimens. The Cobas Amplicor analyser automatically performed all of the amplification, hybridisation and detection steps. All results were interpreted according to manufacturer’s guidelines based on signal cut-off readings.

**Costing**

We calculated the cost of both EIA and PCR based upon reagent, other supplies and labour costs to the Queen Elizabeth II Health Sciences Centre at the time the switchover occurred. Included were the costs of confirmation testing and additional testing of grey zones and other problem specimens. The instruments on which testing was performed were acquired on a reagent rental agreement so that their cost was incorporated into the test cost. We did not include training time calculations.

**RESULTS**

During the study period, 62,288 EIA tests were performed on specimens submitted on females; 2,061 were positive (3.33%) (Figure 1 and Table I). In the six months when testing was performed almost exclusively by the PCR method, 9,559 PCR tests were performed; 463 (4.84%) were positive. This represented a 46% increase in the proportion of tests positive when compared with EIA. A very small number of PCR tests (62) were performed on female urine prior to the formal implementation of our PCR testing program for women. In the three years before PCR testing was implemented, an average of 1,626 specimens were submitted monthly. An average of 54 tests were positive (3.3%). Duplicate negative and positive tests were not removed from the analysis. The tests did not appear to be any significant changes to test ordering practices during the study period and the same clients used the laboratory. There were no significant differences in the number of EIAs performed monthly (mean 1,611 specimens, range, 1,373 to 1,715). The number of specimens during months when testing was done exclusively by PCR was 1,613, very close to the average in the preceding quarters.

The average cost to perform EIA testing (initial screening and subsequent confirmation and other testing) was $7.49 Cdn, the average cost of PCR was $10.10. The cost for each positive detected by PCR was $208 Cdn and $226 Cdn by EIA. The difference per positive test result was $18 Cdn.

**DISCUSSION**

The decision to switch to a PCR-based method from EIA does increase the cost of testing. Reagents are somewhat more expensive and there is an increase in the amount of technologists’ time required. At a time when financial resources available to labs are coming under increasing pressure, laboratories are often reluctant to make a switch to newer, more expensive technologies with only a perceived small increment in test sensitivity.

We were quite surprised by the magnitude of difference that we observed when we implemented the PCR testing of cervical specimens. Almost certainly, the increase relates to the increased sensitivity of the PCR method, although increases of smaller magnitude had been well recognized previously by other investigators. Formal comparison of PCR with EIAs had suggested that we might expect an up to 20% increase. However, Scoular et al. in a “real world” examination of the impact of LCR testing in Glasgow, demonstrated an increase in the detection rate from 4.8% to 7.8% when compared with an EIA. This represented a 62% increase.

It is unlikely that the observed increase in the proportion of tests that were positive reflects a change in physician practices since we simply replaced specimen collection kits for the EIA kits they were provided with. No changes in testing were suggested except that, for PCR testing, we asked that tests-of-cure not be performed for at least four weeks after treatment is administered. A statement to this effect is provided with each positive test that we
Infections might be diagnosed or treated at subsequently developed symptoms, some treated, if symptomatic at the time. If they many women would have spontaneous resolution of symptoms, others might be treated, if symptomatic at the time. If they subsequently developed symptoms, some infections might be diagnosed or treated at a later date. Untreated infections may have a number of consequences. It is estimated that Chlamydia trachomatis cervicitis increases the risk of HIV infection by 3-5 fold and may result in neonatal pneumonia and conjunctivitis. Others may develop complications of Chlamydia trachomatis infection with their attendant sequelae and cost. It is estimated that between 15 and 40% of chlamydia infections in women result in the development of pelvic inflammatory disease and that a number of these will subsequently become infertile. The cost of treating a case of pelvic inflammatory disease is at least $11,167 US. Many women who develop PID have further complications including chronic pelvic pain, ectopic pregnancy and infertility. Gorter and Gilly estimated the cost burden for chlamydia in females to be as high as $115 M in Canada in 1990. Many sexually active women seek testing before undertaking unprotected sexual activity and as a consequence of infections missed by EIA, secondary transmission is a significant concern. Any calculations of the cost benefits of screening with a more sensitive method such as PCR need to employ a dynamic model that considers the long-term impact of improved case detection on the incidence of the disease in subsequent years.

The incidence curve for Capital Health region of Nova Scotia will show a significant increase in the rate of chlamydia infections in women in 2001. We project a further increase for 2002 when testing for the whole year will be based upon PCR. It is important for public health officials to recognize that some of the apparent change in the incidence of chlamydia is artefactual but highlights the difficulty of diagnosing asymptomatic infections. This same phenomenon has likely been observed in many other geographic areas. Between 1997 and 2000, rate of Chlamydia trachomatis infection reported to Health Canada increased from 112.7 to 151.1 per 100,000 population. The impact of the introduction of new molecular diagnostics on the apparent incidence of sexually transmitted diseases needs to be better understood. A concurrent comparison of the two testing methods would have eliminated the possibility that the author’s observations were due to changing testing patterns or a change in the prevalence of CT infections in the community. However, since the dramatic increase in positivity was directly coincident with the switch to PCR, it is unlikely that such factors contributed to the differences we observed.

Clearly, the use of PCR for the diagnosis of Chlamydia trachomatis infections has advantages. In addition to the improved performance characteristics that others and we have found, the use of urine specimens or self-collected vaginal samples could encourage testing, especially in at-risk populations. Ultimately, the recognition of more infections should result in further decline in the incidence of this important disease in those regions where laboratory resources permit the use of newer technologies.

**REFERENCES**

5. Bauwens JE, Clark AM, Loefelholz MJ, Herman SA, Stamm WE. Diagnosis of Chlamydia trachomatis from female genital sites at the Queen Elizabeth II Health Sciences Centre Between April 1998 and December 2001
SWITCHING TO PCR FOR C. TRACHOMATIS


RÉSUMÉ

Contexte : Il y a eu une augmentation marquée des infections à Chlamydia trachomatis (CT) dans la région sanitaire de la capitale de la Nouvelle-Écosse, qui coïncidait avec l’utilisation de l’amplification par la polymérase (PCR) en remplacement des essais immunoenzymatiques (EIA). Nous avons fait le bilan de l’expérience afin de déterminer le coût du changement et ses incidences sur le nombre de nouveaux cas d’infection diagnostiqués.


Résultats : Durant la période à l’étude, 62 288 EIA ont été effectués sur les prélèvements soumis; 2 061 (3,33 %) étaient positifs. Au cours des six mois où l’on a eu recours à la méthode PCR, 9 559 essais ont été effectués, dont 463 (4,84 %) étaient positifs, soit une hausse de 46 %. Les trois années précédant la mise en œuvre des essais PCR, on soumettait en moyenne 1 626 prélèvements par mois, et 54 essais en moyenne étaient positifs (3,3 %). Le coût unitaire des essais PCR positifs était de 208 $CAN (contre 226 $ pour les EIA positifs).

Conclusions : L’adoption de la méthode PCR pour le diagnostic de la CT a entraîné une hausse marquée du nombre d’infections à Chlamydia diagnostiquées. La hausse récente du nombre de cas de CT signalés au Canada pourrait s’expliquer en grande partie par la sensibilité accrue des tests. Curieusement, le coût unitaire des essais PCR positifs était inférieur de 18 $CAN à celui des EIA.

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de soi pour les individus et les groupes, qui dans certains cas, pour survivre, ont dissimulé leur identité. L’appellation la plus récente, très lourdement influencée, bien sûr, par la Loi sur les Indiens, est celle de la Loi constitutionnelle de 1982 sur le Canada, où les peuples autochtones du Canada sont officiellement appelés Indiens, Inuits et Métis. Cette nouvelle définition « légale » ne favorise pas l’unité des peuples autochtones, mais plutôt le développement de tensions, certains groupes ayant accès à certains « droits » et « ressources » et d’autres non. Les effets destructeurs de la colonisation, notamment les différences socio-politiques entre groupes autochtones sur la question identitaire, empêchent les peuples autochtones d’agir collectivement pour assurer leur avenir au Canada.

Sur une note plus positive, la résurgence des identités culturelles s’accroit rapidement. Par exemple, dans le recensement de 1996, 60 000 Winnipegeois déclaraient des racines autochtones, mais seulement 45 000 se définissaient comme appartenant à la communauté autochtone. Les 15 000 restants sont peut-être des personnes qui se sont temporairement intégrées dans la société prédominante, mais n’ont pu maintenir un tel équilibre à long terme. La douleur psychologique peut s’étendre dans la société prédominante, mais n’ont jamais eu beaucoup de succès. D’autres font valoir que c’est l’examen consciencieux des connaissances sur la culture et de la façon dont celle-ci a été affectée par le contact soutenu entre les groupes qui permettra de concevoir des approches de la vie contemporaine ancrées dans la culture, et donc beaucoup plus susceptibles d’accroître la santé et le bien-être des Premières Nations, des Métis et des Inuits.