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.

La traduction du résumé se trouve à la fin de l'article.

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Chlamydia 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 (approximately 2,000 yearly). IWK testing was done primarily on children and women seeking gynecologic and obstetrical services at that hospital; 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 gray 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

Figure 1. Results of *Chlamydia trachomatis* testing from female genital sites at the Queen Elizabeth II Health Sciences Centre between April 1, 1998 and December 31, 2001

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. There 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.^{4,6,8-10} 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.¹¹ 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

TABLE 1

Summary of *Chlamydia trachomatis* Testing from Female Genital Sites at the Queen Elizabeth II Health Sciences Centre Between April 1998 and December 2001

	Apr-Jun 1998	Jul-Sep 1998	Oct-Dec 1998	Jan-Mar 1999	Apr-Jun 1999	Jul-Sep 1999	Oct-Dec 1999	Jan-Mar 2000	Apr-Jun 2000	Jul-Sep 2000	Oct-Dec 2000	Jan-Mar 2001	Apr-Jun 2001	Jul-Sep 2001	Oct-Dec 2001
Enzyme Immunoassay Testing															
Number of tests	4687	5143	5033	5090	4636	5009	4819	5081	4755	4928	4631	4800	3559	115	2
Number positive	151	147	146	168	149	170	164	187	169	177	156	167	103	7	0
Percent of EIA tests positive	2.96%	2.66%	2.72%	3.30%	3.21%	3.39%	3.40%	3.68%	3.55%	3.59%	3.36%	3.47%	2.89%		
PCR Testing															
Number of tests*	0	3	3	14	5	6	4	7	11	6	2	11	561	4583	4976
Number positive	0	0	0	0	0	1	2	0	1	0	0	0	30	208	255
Percent of PCR tests positive													5.35%	4.54%	5.12%
Total Number Tested	4687	5146	5036	5104	4641	5015	4823	5088	4766	4934	4633	4811	4120	4698	4978

* Prior to April 2001, occasional PCR tests were performed upon special request on urine specimens from women.

† Conversion period during which time both tests were offered

report. It is equally unlikely that change in the epidemiology of *C. trachomatis* occurred at the precise time of the switchover; in particular, we did not observe a change in the number of specimens submitted or the incidence of gonorrhoea in the same timeframe (Unreported data). A lower specificity might result in more false positives; however, numerous evaluations of the Cobas Amplicor PCR method suggests that, on average, fewer than 2% of results would be false positive.³

Because the incremental yield for PCR was proportionally higher than the incremental cost of PCR testing, PCR cost less than EIA per positive result. The prior cost of case detected by EIA was \$226 per case (\$7.49 x 100/3.33) before and \$208 after (\$10.10 x 100/4.84). To detect an additional case in our laboratory cost \$172.85 (\$10.10-\$7.49)/(4.84%-3.33%). This is much less than the £263 (pounds) per additional diagnosis when the LCR method was introduced into a Glasgow laboratory.¹¹ Costs of new molecular diagnostics such as LCR or PCR may vary considerably depending on the volume of testing, workflow patterns, labour costs, instrument use and whether other testing is bundled into purchase agreements. In the case of our laboratory, the very high test volume and bundled purchasing result in low test costs for PCR.

In addition to lower cost per positive test result, one can only speculate as to the financial or other impact of a false negative EIA test for *C. trachomatis*. Presumably, 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 *C. trachomatis* cervicitis increases the risk of HIV infection by 3-5 fold and may result in neonatal pneumonia and conjunctivitis.¹² Others may develop complications of *C. 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.¹³ The cost of treating a case of pelvic inflammatory disease is at least \$11,167 US.¹³ Many women who develop PID have further complications including chronic pelvic pain, ectopic pregnancy and infertility. Gorree and Gully estimated the cost burden for chlamydia in females to be as high as \$115 M in Canada in 1990.¹⁴ 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.¹⁵

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 *C. trachomatis* infection reported to Health Canada increased from 112.7 to 151.1 per 100,000 population.¹⁶

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 *C. 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.

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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.

Méthode : Les données sur le nombre d'essais EIA et PCR effectués sur des femmes proviennent d'une base de données de laboratoire résumées. Nous avons examiné les dossiers des essais effectués entre avril 1998 et décembre 2001. Avant juin 2001, tous les prélèvements génitaux étaient testés par EIA (MicroTrak II Chlamydia) et confirmés au moyen d'un examen direct par fluorescence. Après juillet 2001, les prélèvements génitaux ont subi le test de dépistage de la *C. trachomatis* COBAS AMPLICOR®.

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 différends 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'accroît rapidement. Par exemple, dans le recensement de 1996, 60 000 Winnipegois 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 sur plusieurs générations lorsque des individus ou des groupes sont forcés à renier un construit clé de leur humanité : la culture. Dans le recensement de 2001, 9 000 personnes de plus ont déclaré appartenir à la communauté métis de Winnipeg. Il est peu probable que cette hausse s'explique par la migration; on en déduira qu'il est plus socialement acceptable et admissible d'exister en tant que Métis dans la société d'aujourd'hui.

En conclusion, les importantes incidences culturelles, sociales, économiques et politiques de la colonisation ont fait en sorte que les peuples autochtones ont vécu dans des conditions de stress extrême pendant de nombreuses générations. Il n'est

pas étonnant que les « phénomènes du stress » et la « pathologie sociale et psychologique » s'y rattachant soient les produits de leur acculturation forcée. La compréhension de la pathogénie du phénomène du stress pourrait mener à l'élaboration d'approches ancrées dans la culture, contrairement à une tendance croissante et troublante qui consiste à employer la terminologie autochtone (et parfois ses cadres) tout en se contentant d'offrir les sempiternels programmes occidentaux qui n'ont jamais eu beaucoup de succès. D'aucuns 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.