National Collaborating Centre for Infectious Diseases
Centre de collaboration nationale des maladies infectieuses

Purple Paper

NAAT Testing for Gonorrhea and Chlamydia: A Review of Diagnostic Accuracy, Cost-Effectiveness, and Acceptability

The Canadian Agency for Drugs and Technologies in Health (CADTH) is a national body that provides Canada's federal, provincial and territorial health care decision-makers with credible, impartial advice and evidence-based information about the effectiveness and efficiency of drugs and other health technologies. The National Collaborating Centre for Infectious Diseases (NCCID) had recently requested CADTH to conduct a review on the diagnostic accuracy, cost-effectiveness and compliance with testing for gonorrhea and chlamydia. This issue of the *Purple Paper* is intended to be a companion for the full-length CADTH Technology Report, entitled "Urine Based Testing for Gonorrhea and Chlamydia: A Review of Diagnostic Accuracy, Cost-Effectiveness, and Compliance" [CADTH, 2009]. Here, we have condensed the CADTH Technology Report to highlight the conclusions and implications for public health practice and policy. Furthermore, we will provide additional information on the concept behind nucleic acid amplification tests (NAATs) and discuss other pertinent issues related to the interpretation of the CADTH Technology Report.

For the full-length CADTH Technology Report or the complete reference list, visit:

http://www.cadth.ca/media/pdf/L0124_Urine_Based_Testing_for_Gonorrhea_and_Chlamydia_final.pdf

Key Points

- NAATs are powerful molecular techniques for the screening and diagnosis of infectious microorganisms.
- Three commercially available NAATs are approved in Canada for the screening and diagnosis of chlamydia and gonorrhea.
- NAATs are, in most instances, highly sensitive and specific for chlamydia and gonorrhea testing on urine, cervical swab or urethral swab specimens. However, some studies show that factors within urine samples could hamper the performance of NAATs.
- Studies that directly measure the compliance with testing for chlamydia and gonorrhea are generally lacking, but several systematic reviews and observational studies report important factors that could enhance compliance.
- Cost-effectiveness and cost-savings of NAATs for screening for chlamydia and gonorrhea have been demonstrated in some mathematical modeling studies. However, there is a paucity of cost-effectiveness modeling studies that are based on Canadian data.
- No study regarding the stability of urine-based or swab-based specimens during storage or transport could be found.
- NAAT technologies can be adopted for other applications in public health.

What are NAATs?

Diagnostic tests for *Neisseria gonorrhoeae* and *Chlamydia trachomatis* have traditionally been based on laboratory cultures of the microorganisms, followed by a staining procedure to visualize under a microscope the actual bacteria (in the case of gonorrhea) or characteristic changes in indicator cells as a result of infection (in the case of chlamydia). Culture tests are generally highly specific, and in the case of *N. gonorrhoeae* also sensitive. However, culture tests, for chlamydia in particular, have a long turnaround time, and are highly labour intensive, technically complex, and difficult to standardize. Although culture tests for gonorrhea are technically less challenging and thus

do not require nearly the same level of expertise as chlamydial cultures, poor viability of *N. gonorrhoeae* during transport of the clinical specimens can significantly compromise the sensitivity of the culture test.

For these reasons, there has been a move among clinical microbiology laboratories to make greater use of molecular tests for the screening and diagnosis of infectious diseases. One such category of molecular tests is nucleic acid amplification tests (NAATs). As the name suggest, NAATs are designed to amplify nucleic acid sequences that are specific for the organism being detected. Detection by NAATs does not require viable organisms. Furthermore, NAATs are highly sensitive, requiring as little as a few copies of the DNA[a] or RNA from the target organism to produce a positive signal.

NAATs comprise several nucleic acid amplification methods, and many of them are a variation of the seminal "Polymerase Chain Reaction" (PCR) that has revolutionized every aspect of biological and medical sciences. At temperatures around 94°C, the two strands of a DNA molecule separate; when the temperature is lowered to around 55°C, the two strands again pair up. By exploiting this physical property of DNA, the core of the PCR technology relies on cycles of repeated heating and cooling for the enzymatic replication of the DNA. In each PCR cycle, newly-generated DNA duplex molecules are separated, with each of its two component strands then serving as a template for the next round of replication, therefore setting in motion a chain reaction in which the target DNA sequence is exponentially amplified [Mullis and Faloona, 1987; Lisby, 1999].

In addition to PCR, other examples of NAATs include transcription-mediated amplification (TMA), strand displacement amplification (SDA), ligase chain reaction (LCR), rolling circle amplification (RCA) and branched DNA signal amplification (bDNA) [Lisby, 2009; Gill and Ghaemi, 2008]. Many of these newer, second generation NAATs have abrogated the need of thermal cycling by including in the same reaction accessory enzymes that are capable of unwinding and separating the two DNA strands without heating. Currently, the three commercially available NAATs approved for detection of *C. trachomatis* and *N. gonorrhoeae* in Canada and the USA are based on the PCR (AMPLICOR®, Roche Molecular Systems, Inc), TMA (APTIMA®, Gen-Probe Incorporated) and SDA (ProbeTec™, Becton Dickinson and Company) platforms [CDC, 2002; Health Canada, 2009]. In Canada, the use of NAATs for the detection of chlamydia and gonorrhea is recommended for urine and swab (cervical and urethral) specimens [PHAC, 2008].

What is the comparative diagnostic accuracy of urine-based testing versus swab-based testing for gonorrhea and chlamydia?

Through a limited literature search, the CADTH Technology Report [2009] identified one meta-analysis on the sensitivity and specificity of three commercially available NAATs (PCR, TMA and SDA) for gonorrhea and chlamydia on urine samples (from both men and women), and cervical swab specimens or urethral swab specimens (from men only) [Cook et al., 2005]. Most of the 29 studies selected for the meta-analysis included a mixture of symptomatic and asymptomatic subjects.

For both chlamydia and gonorrhea testing in women, the pooled specificities of all 3 NAATs on either urine or cervical swab specimens were consistently high, ranging from 97.9%-99.6%[b]. However, the pooled sensitivities of the 3 assays on

[a] The genetic information on how to construct a cell is stored in the double-stranded DNA molecule. (Imagine a flexible ladder twisted like a ribbon, where the two sides of the ladder form the backbone of the DNA molecule and the rungs are symbols in the secret code. Like a ladder, the rungs hold the two complementary halves in place; however when the stability of the rungs is broken, the ladder can be split.) The genetic information is organized into discreet segments called genes. By specifying the sequence of amino acids within proteins, each gene dictates the function of individual proteins. When a gene is activated, its corresponding DNA segment is read and copied into structurally-related nucleic acid molecules, called RNA. Based on these RNA transcripts, the genetic code is subsequently translated into actual proteins. In multi-celled organisms, all cells in the body possess the identical set of genetic information – it is the array of active (i.e. expressed) genes that determines the function of the cell.

[b] Please note the percentages reported here for the specificity and sensitivity of each NAAT on urine or swab-based specimens are pooled summary estimates. For the corresponding 95% confidence intervals, please refer to the full-length CADTH Technology Report or to the original published article [Cook et al., 2005].

the 2 specimen types were more variable. While the pooled sensitivities of PCR and TMA for chlamydia testing on urine samples and those on cervical swab samples were similar (PCR: 83.3% vs. 85.5%; TMA: 92.5% vs. 96.7%), the pooled sensitivity of SDA on urine specimen was considerably lower compared to that obtained on cervical swab samples (SDA: 79.9% vs. 93.6%). The pooled sensitivities of the three NAATs for gonorrhea testing on urine versus cervical swab specimens were also variable (PCR: 55.6% vs. 94.2%; TMA: 91.3% vs 99.2%; SDA: 84.9% vs. 96.5%).

For chlamydia testing in men, the pooled specificities of PCR, TMA and SDA on either urine or urethral swab specimens were high, ranging from 93.8%-99.4%. The pooled sensitivities of the 3 NAATs on urine versus urethral swab specimens were: PCR, 84.0% vs. 87.5%; TMA, 87.7% vs. 95.9%; and, SDA, 93.1% vs. 92.4%. Performance studies of gonorrhea testing in men were only available on testing by PCR. The pooled specificity of PCR on urine and urethral swab samples were 99.7% and 99.0%, respectively; the pooled sensitivities on urine and urethral swab samples were 90.4% and 96.1%, respectively.

The results presented in this meta-analysis suggest that the 3 NAATs are, in most instances, highly sensitive and specific for chlamydia and gonorrhea testing on urine, cervical swab or urethral swab specimens. However, because only few studies on TMA and SDA were available to be included in this meta-analysis, and that estimation of pooled sensitivities and specificities was calculated based on a very small number of studies (range 1-4), these findings must be interpreted with caution.

The CADTH Technology Report also identified 9 additional observational studies on NAATs for chlamydia and gonorrhea, one of which was included in the above meta-analysis. These 9 studies were conducted in diverse clinic settings in the USA, the UK, Denmark, South Africa, Thailand and China, where the performance of NAATs was assessed on a variety of specimen types including urine, vaginal swabs, endocervical swabs and urethral swabs. With the exception of results from two studies, NAATs performed on urine specimens generally had lower sensitivity for detection of *C. trachomatis* and *N. gonorrhoeae* compared to the same tests performed on swab-based specimens. These results are in accord with the findings of the above meta-analysis.

What is the acceptability of urine-based testing versus swab-based testing for gonorrhea and chlamydia? What are the factors that affect acceptability?

The CADTH Technology Report identified two systematic reviews that could provide insights on the compliance (by means of acceptability) with urine-based versus swab-based testing for chlamydia. Instead of measuring compliance directly, the studies included in the two reviews used acceptability and acceptance of chlamydia testing [Marrazzo and Scholes, 2008], and views, attitudes and opinions about chlamydia screening, testing and diagnosis [Pavlin et al., 2006], as proxy indicators for compliance.

The first systematic review assessed the acceptability and acceptance (uptake) of urine testing for chlamydia among asymptomatic men, and included 3 categories of studies for analysis [Marrazzo and Scholes, 2008]:

1. Testing in established non-sexually transmitted disease (STD) clinic venues (urgent care clinics, freestanding clinics or health screening settings, correctional facilities, community centers) (2 studies)
2. Testing in home settings (6 studies)
3. Qualitative assessment of attitudes towards or experience with testing (3 studies).

When chlamydia testing was offered in established non-STD clinic settings (clinics, schools, and correctional facilities), median acceptability and uptake of testing by men is in the mid-60% range. However, acceptance rates could vary widely, dependent on a variety of factors such as venue and provider. Acceptance of home-based testing, including direct mailing of test kits, was lower. Men who declined testing generally reported low self-perception of risk for asymptomatic infection and inconvenience of providing test specimens as primary reasons. Given these findings, the authors suggested that a targeted approach to chlamydia testing among asymptomatic men in established community and clinic settings is most likely to yield higher acceptance rates than in home settings.

NCCID Comments:
This systematic review reported that median acceptability and uptake of testing for chlamydia by asymptomatic men in clinics, schools and correctional facilities was in the mid-60% range, although variability in acceptability existed. The latter is not surprising given the diversity and substantial differences among these settings. Caution is therefore needed when combining information from various clinic settings into a single analysis and when interpreting findings from such analytical approach. In addition to variability in uptake between venue types, the original published study noted that acceptability of testing also varied widely within venue types [Marrazzo et al., 2007].

In the second systematic review, Pavlin and colleagues [2006] assessed the views, attitudes and opinions of women about being screened, tested and diagnosed with *C. trachomatis*. Twenty-five eligible studies were included for analysis – 22 were conducted in the USA and UK and the remainder were from Holland, Sweden, Australia. Overall, the issues regarding chlamydia screening and diagnosis among women revolved around the need for knowledge and information, choice and support, and concerns about confidentiality, cost, fear, anxiety and stigma. Women were more likely to accept screening and testing for chlamydia if they understood chlamydia can cause asymptomatic infection and serious sequelae. A wide range of chlamydia testing options should be made available (urine tests, self-administered swabs, pelvic exams and clinician-collected swabs, home-testing and community-based testing), and tests should be free, easy and quick. Women felt that support for dealing with the implications of a chlamydia diagnosis was important, and chlamydia diagnoses should be normalized and destigmatized. They wanted assistance with partner notification, and assurance that their confidentiality was protected.

In addition to the two systematic reviews, the CADTH Technology Report also identified four observational studies examining the preference of women for the types of specimen collection method for chlamydia and gonorrhea testing. These studies were conducted in clinic settings in the USA, the Netherlands, UK and Canada. Results from these observational studies showed that self-collected urine or vaginal swab was preferred over cervical swab in a majority of women.

NCCID Comments:
The major limitation of these systematic reviews and observational studies is that the findings do not directly answer the question of compliance with chlamydia and gonorrhea testing originally posed to CADTH. This is probably due to a paucity of studies on the specific topic. Nonetheless, these studies may provide information on how testing can be made more acceptable to enhance compliance.

What is the cost-effectiveness of urine-based testing versus swab-based testing for gonorrhea and chlamydia?

Two mathematical modelling studies evaluating the cost-effectiveness of chlamydia and gonorrhea screening strategies in women were identified in the CADTH Technology Report.

The first modelling study determined the incremental cost-effectiveness of 3 chlamydia screening strategies in a STD clinic setting by comparing each to the reference PACE®2 test (Gen-Probe Incorporated) – a commercially available nucleic acid hybridization test[c] for the detection of *C. trachomatis* on endocervical swab specimens [Blake et al., 2008]. The 3 chlamydia screening strategies under scrutiny were:

1. A commercially available TMA test (APTIMA®, Gen-Probe Incorporated) on endocervical swab specimens
2. APTIMA® on self-obtained vaginal swab specimens
3. APTIMA®on urine samples.

Parameters on the prevalence of chlamydia, the proportion of asymptomatic infections, the proportion of infections that were treated, the proportion of women who required a Pap smear,

[c] Nucleic acid hybridization tests are based on the ability of complementary nucleic acid strands to specifically align and associate to form stable double-stranded complexes. A nucleic acid hybridization test uses a single-stranded DNA probe chemically linked to a fluorescing reporting label that is complementary to the nucleic acid of the target organism. A positive test is indicated by fluorescence detected in the nucleic acids prepared from a clinical specimen.

and the acceptability and sensitivities of the 3 chlamydia tests in question were derived from information of 324 women (92.6% black) who attended Baltimore STD clinics between April 5, 2004 and February 3, 2005.

The cost-effectiveness analyses were conducted from the public health care perspective and included only direct medical costs. The costs to process TMA tests were the same regardless of the specimen used; however, collection costs for urine or endocervical samples were higher than that for vaginal samples. The costs for speculum examination required in some patients were also considered in this analysis. The primary outcome measure was the number of cases of pelvic inflammatory disease (PID). Secondary outcome measures were infertility, ectopic pregnancy, and chronic pelvic pain. The projected time horizon was 10 years. Results from this study suggest that the use of self-obtained vaginal swab for the detection of *C. trachomatis* is both cost-effective and cost-savings in a STD clinic setting.

The second modelling study assessed the cost-effectiveness of gonorrhea screening in women aged 15-29 years, seeking care in urban emergency departments (EDs) using non-invasive or rapid point-or-care tests [Aledort et al., 2005]. Five *N. gonorrhoeae* detection methods were compared in terms of the net lifetime health consequences, costs, and cost-effectiveness of routine ED care (no screening for women without genitourinary symptoms) to gonorrhea screening:

1. Gram-stained smears of endocervical swab specimens
2. Urine-based NAATs
3. NAATs performed on endocervical swabs
4. Rapid immunochromatographic strip test (RIS)[d] performed on clinician-collected vaginal swabs
5. RIS on patient-collected vaginal swabs.

Screening women between 15 and 29 years with urine-based NAAT strategy was less costly and more effective than no screening and was therefore cost-saving. RIS using clinician-obtained vaginal swabs was the next most cost-effective strategy after the urine-based test. Screening with RIS on patient-obtained specimens, with Gram stain, and with NAATs on cervical specimens was less effective and cost more per person compared with the previous strategies. Results suggest that screening females aged 15 to 29 years for gonorrhea in some EDs with rapid, point-of-care tests, such as urine-based NAATs and RIS on clinician-obtained vaginal swabs, may be cost-effective and may provide cost-savings to society.

NCCID Comments:
Caution should be exercised when applying findings from these mathematical models. The generated cost-effectiveness estimates are highly dependent on the mathematical modeling methodology, design, assumptions and patient demographics on which the model is based. A more appropriate mathematical model would be based on the Canadian demographics, disease incidence and prevalence, and context. Until such studies are available, the mathematical modeling studies presented in the CADTH Technology Report should be viewed as a primer for further investigation or discussion.

What is the evidence regarding the stability of urine- or swab-based samples for gonorrhea and chlamydia testing during transport?

No study regarding the stability of urine-based or swab-based specimens during storage or transport could be found.

NCCID Comments:
In the absence of independent published studies on the optimal storage and transport conditions of urine- or swab-based specimens for NAATs, laboratory technologists rely on manufacturers' recommendations as their primary source of information. In general, both urine- and swab-based specimens are stable at room temperature, and may be transported to test sites at temperatures 2-30°C.

[d] Rapid immunochromatographic strip test (RIS) is based on the specific recognition and binding of the patient's antibodies to the target organism's antigens, which in turn are hybridized onto the test strip. A positive test is indicated by a colour change.

References

Aledort JE et al. The cost effectiveness of gonorrhea screening in urban emergency departments. *Sex Transm Dis*. 2005; 32:425-436.

Blake DR et al. Cost-effectiveness of screening strategies for *Chlamydia trachomatis* using cervical swabs, urine, and self-obtained vaginal swabs in a sexually transmitted disease clinic setting. *Sex Transm Dis*. 2008; 35:649-655.

Canadian Agency for Drugs and Technologies in Health (CADTH). Urine based testing for gonorrhea and chlamydia: a review of Diagnostic accuracy, cost-effectiveness, and compliance. 2009. http://www.cadth.ca/media/pdf/L0124_Urine_Based_Testing_for_Gonorrhea_and_Chlamydia_final.pdf [Assessed online June 1, 2010]

Centers for Disease Control and Prevention (CDC). Screening tests to detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections – 2002. *MMWR*. 2002; 51(No. RR-15):1-40.

Cook RL et al. Systematic review: noninvasive testing for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. *Ann Intern Med*. 2005; 142:914-925.

Gill P and Ghaemi A. Nucleic acid isothermal amplification technologies – a review. *Nucleosides Nucleotides Nucleic Acids*. 2008; 27:224-243.

Health Canada. Medical Devices Active License Listing. 2009. http://webprod.hc-sc.gc.ca/mdll-limh/prepareSearch-preparerRecherche.do?type=active&lang=eng [Accessed online June 6, 2010]

Lisby G. Application of nucleic acid amplification in clinical microbiology. *Mol Biotechnol*. 1999; 12:75-99.

Marrazzo JM et al. Acceptability of urine-based screening for *Chlamydia trachomatis* to asymptomatic young men and their providers. *Sex Transm Dis*. 2007; 34:147-153.

Marrazzo JM, Scholes D. Acceptability of urine-based screening for *Chlamydia trachomatis* in asymptomatic young men: a systematic review. *Sex Transm Dis*. 2008; 35(11Suppl):S28-S33.

Mullis KB, Faloona FA. Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. *Methods Enzymol.* 1987; 155:335-50.

Pavlin NL et al. Implementing chlamydia screening: what do women think? A systematic review of the literature. BMC Public Health. 2006; 6:221. doi:10.1186/1471-2458-6-221.

Public Health Agency of Canada (PHAC). *Canadian Guidelines on Sexually Transmitted Infections*. 2008 Edition. Ottawa, ON. Available online: http://www.phac-aspc.gc.ca/std-mts/sti-its/guide-lignesdir-eng.php

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