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Opportunities and pitfalls of molecular testing for detecting sexually transmitted pathogens

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Summary

In the last 20 years, nucleic acid amplification tests (NAATs) have gradually replaced traditional methods for the detection of sexually transmitted infections. NAAT technology comes with some considerable benefits for diagnosis, including increased sensitivity, rapid result turnaround and suitability for high throughput screening of asymptomatic individuals using more-readily available specimens. However, the transition to NAAT has not come without its problems. False-negative and false-positive results have been reported owing to various technical issues. Furthermore, increased reliance on NAATs for diagnosis have created the need to develop NAAT-based methods to inform treatment, being an area that presents its own set of challenges. In this review article, we explore NAAT-based detection of *Chlamydia trachomatis*, *Neisseria gonorrhoeae, Mycoplasma genitalium* and *Trichomonas vaginalis*. In doing so, we consider the benefits and limitations of NAAT-based technology and highlight areas where further research and development is in need.

Key words: Chlamydia, gonorrhoea, molecular, mycoplasma, NAAT, PCR, trichomonas.

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INTRODUCTION

The value of nucleic acid amplification techniques (NAAT) for detection and characterisation of sexually transmitted infections (STIs) cannot be disputed. For diagnosis, they are typically sensitive and specific, and can be used on a broad range of specimen types and do not require viable organism for detection; however, the latter may pose a problem for clinical interpretation. NAATs are now also being developed to inform treatment via the direct detection of mutations conferring antimicrobial resistance (AMR), as well as being used to determine treatment failure. Here we review the use of NAATs for the STIs *Chlamydia trachomatis*, *Neisseria gonorrhoeae, Mycoplasma genitalium* and *Trichomonas vaginalis*.

CHLAMYDIA TRACHOMATIS

Overview

Chlamydia is usually a silent infection and hence asymptomatic, or can present with non-specific symptoms. Asymptomatic *C. trachomatis* infections may go undetected and untreated, leading to serious sequelae and complications, particularly for pregnant women.[1] Neonates born to Chlamydia-infected mothers are at high risk of developing conjunctivitis and pneumonia. The disease also affects men. *Chlamydia trachomatis* has been associated with other complications such as reactive arthritis (formerly known as Reiter's syndrome).[2] Different serotypes of *C. trachomatis* are associated with different clinical presentations. Serovars A, B, Ba, and C cause trachoma eye infection,[3] which if not treated can lead to blindness;[4] serovars D, E, F, G, H, I, J, and K can cause urethritis, cervicitis, pelvic inflammatory disease (PID), infertility, ectopic pregnancy, epididymitis conjunctivitis in both neonates and adults, and neonatal pneumonia;[5–9] whilst serovars L1, L2 and L3 usually cause lymphogranuloma venereum (LGV) in tropical settings, but have been increasingly reported in cases of proctitis in men who have sex with men (primarily serovar L2b).[10]

Laboratory diagnosis of chlamydial infection

Conventional diagnostic assays for *C. trachomatis* include cell culture, enzyme-linked immunosorbent assays (ELISA) and direct fluorescent antibody test (DFA) techniques, but due to their laborious nature, insensitive detection, and prolonged result turnaround time, have now been replaced by NAATs for routine diagnosis. Current NAAT methodology (summarised in Table 1) utilises amplification of *C. trachomatis* nucleic acids for detection of *C. trachomatis* using real-time or digital polymerase chain reaction (PCR), transcription-mediated amplification (TMA), or strand displacement amplification (SDA). Many assays are now commercially available and these systems typically offer sensitive and specific detection of *C. trachomatis* (with specificity greater than 97% and sensitivity ranging between 86% and 100%[11]) combined with fully and partially automated instrumentation.

The use of NAATs has inherent limitations and requires vigilance in monitoring infection rates in order to identify potential problems in detection methodology. The fact that NAATs are targeted towards a particular nucleic acid sequence means that the sensitivity of the methods may be compromised where the sequence changes or is lost. In 2006, the Swedish Institute for Infectious Disease Control reported a 377 base-pair deletion in the *C. trachomatis* 7.5 kilobase cryptic plasmid, being a particular sequence used for the molecular detection of

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Table 1 Summary of common NAAT based methods for *Chlamydia trachomatis, Neisseria gonorrhoeae, Mycoplasma genitalium, Trichomonas vaginalis*

	Chlamydia trachomatis (CT)	*Neisseria gonorrhoeae* (NG)	*Mycoplasma genitalium* (MG)	*Trichomonas vaginalis* (TV)
Summary of diagnostic methods	Commercial tests include Roche Cobas 4800 CT/NG Test; Cepheid Xpert CT/NG Assay; Gen-Probe Aptima Combo 2; Aptima CT assay; BD ProbeTec ET CT/GC Amplified DNA; BD ProbeTec Q^x Amplified DNA; Abbott RealTime CT/NG assays. In-house PCR methods have been described and typically target the cryptic plasmid or MOMP genes.	Commercial NG tests include those listed for CT. In-house NG PCR assays are used in some settings, including confirmatory testing[40] and typically target the *porA, opa* or 16 S genes.	There are several commercial assays on the market, however none are yet FDA approved. Various in-house PCR assays have been described targeting the MgPa adhesion protein, 16 S rRNA and G3PDH genes.	Commercial tests include Gen-Probe Aptima TV and *BD* ProbeTec TV methods. In-house PCR methods have been described targeting the beta-tubulin and 18 S genes.[97]
Detection of resistance	Not available, no evidence of genetic mechanisms of resistance.	A PCR has been described that detects penicillinase-producing NG directly in clinical samples (PPNG-PCR[50]) implemented in WA. Similar methods predicting ciprofloxacin resistance have been described.[98,99]	Methods have been described to predict azithromycin susceptibility based on 23 S rRNA mutations.	Not available, but some evidence that mutation in the nitroreductase ntr4Tv and ntr6Tv genes is associated with nitroimidazoles resistance.[95,96]
Recognised problems	Sequence variation (e.g., in the cryptic plasmid) may affect sensitivity. Some commercial systems now use two targets to overcome this potential problem.	Cross reaction with commensal species leading to reduced specificity is a well-recognised problem, but later generation commercial methods are more specific.[100] Sequence variation affecting sensitivity has been reported for several in-house PCR targets.	Sensitivity issues may be observed because MG is typically a low organism load.	There have been questions over the specificity of certain TV target genes.[101–105]
Future directions	New methods for detection of mRNA will improve TOC testing.	New methods are needed to track NG AMR directly in clinical samples, particularly for ceftriaxone and azithromycin resistance.	New treatment options are required for MG, and routine and widespread testing is needed that includes monitoring of azithromycin resistance.	Further studies are required to understand the genetic basis of nitroimidazoles resistance.

AMR, antimicrobial resistance; NAAT, nucleic acid amplification testing; TOC, test of cure.

C. trachomatis in two established commercial assays.[12,13] This Swedish variant has since been detected in several countries.[14–16] Manufacturers have subsequently amended their assays to include different targets on the cryptic plasmid or otherwise included additional genomic targets to avoid false-negative results due to circulating mutant strains. Current commercial assays include the Roche Cobas 4800 CT/NG Test which targets cryptic plasmid DNA and a sequence of chromosomal DNA, Cepheid Xpert CT/NG Assay which targets a *C. trachomatis* chromosomal DNA sequence, Gen-Probe Aptima Combo 2 and Aptima CT assay which target the 23S rRNA and 16S rRNA genes respectively, and the BD ProbeTec ET CT/GC Amplified DNA, BD ProbeTec Q^x Amplified DNA and Abbott RealTime CT/NG assays that all target sequences within *C. trachomatis* cryptic plasmid DNA.

Competitive inhibition may be an additional issue causing false-negative results in multiplex CT/NG NAAT assays. This may be seen for co-infections where, for example, *N. gonorrhoeae* nucleic acids at high concentration monopolise the NAAT reaction, effectively preventing amplification and hence detection of *C. trachomatis* at lower concentration.[17] Such events are likely to be rare but laboratories should evaluate the ability of their multiplex assays to detect mixtures of *C. trachomatis* and *N. gonorrhoeae* nucleic acids by comparing results against individual not multiplexed *C. trachomatis* and *N. gonorrhoeae* NAAT methods.

A further limitation of NAAT technology is that the infrastructure requirements are typically such that they can only be performed in large clinical laboratories in metropolitan areas. This can cause problems for management of infections in remote settings as prolonged result turnaround times combined with high patient mobility may limit the ability to subsequently contact positive patients for treatment. Compared to other NAAT methods, the Cepheid Xpert CT/NG assay is relatively simple to perform, rapid and small in footprint and therefore has potential to be used as a point-of-care (POC) test. This POC potential of the Xpert CT/NG assay is currently being trialed for use in remote areas where healthcare access is limited.[18–20]

Urine and genital swab specimens are typically the only specimens which have been approved for detection of *C. trachomatis* using most commercial assays. Other specimens such as extra-genital specimens (eye, rectal and throat specimens) may also be tested using these commercial assays by laboratories that have performed in-house validation for such specimens. Although historically collection methods have been performed by the physician or pathology collector, studies