Journal of Molecular Diagnostics, Vol. 8, No. 1, February 2006

DOI: 10.2353/jmoldx.2006.050045

Review

Nucleic Acid Amplification Testing for *Neisseria gonorrhoeae*

An Ongoing Challenge

David M. Whiley,*† John W. Tapsall,‡ and Theo P. Sloots*†§

From the Clinical Virology Research Unit, Sir Albert Sakzewski Virus Research Centre, Royal Children's Hospital and Health Service District, Queensland; the Clinical Medical Virology Centre,† University of Queensland, Queensland; the Microbiology Department,‡ South Eastern Area Laboratory Services, Sydney, New South Wales; and the Microbiology Division,§ Queensland Health Pathology Service, Royal Brisbane and Women's Hospital Campus, Brisbane, Queensland, Australia*

Nucleic acid amplification tests (NAATs) for the detection of *Neisseria gonorrhoeae* became available in the early 1990s. Although offering several advantages over traditional detection methods, *N. gonorrhoeae* NAATs do have some limitations. These include cost, risk of carryover contamination, inhibition, and inability to provide antibiotic resistance data. In addition, there are sequence-related limitations that are unique to *N. gonorrhoeae* NAATs. In particular, false-positive results are a major consideration. These primarily stem from the frequent horizontal genetic exchange occurring within the *Neisseria* genus, leading to commensal *Neisseria* species acquiring *N. gonorrhoeae* genes. Furthermore, some *N. gonorrhoeae* subtypes may lack specific sequences targeted by a particular NAAT. Therefore, NAAT false-negative results because of sequence variation may occur in some gonococcal populations. Overall, the *N. gonorrhoeae* species continues to present a considerable challenge for molecular diagnostics. The need to evaluate *N. gonorrhoeae* NAATs before their use in any new patient population and to educate physicians on the limitations of these tests is emphasized in this review. ***(J Mol Diagn 2006, 8:3–15; DOI: 10.2353/jmoldx.2006.050045)***

Gonorrhea Epidemiology and Management

Neisseria gonorrhoeae is the etiological agent of the sexually transmitted disease (STD) gonorrhea, which globally causes an estimated 60 million new cases of gonococcal disease annually.[1] In 2003, it was second to *Chlamydia trachomatis* as the most reported notifiable sexually transmitted disease in the United States, with 335,104 cases of gonorrhea reported.[2] Infections with *N. gonorrhoeae* are primarily restricted to the mucus membranes of the endocervix, urethra, rectum, and pharynx. In females, gonorrhea is a major cause of pelvic inflammatory disease and may lead to tubal infertility, ectopic pregnancy, and chronic pelvic pain, whereas in males, it primarily causes urethritis. Importantly, these infections may often be asymptomatic, thereby contributing to further transmission and maintenance of the disease within populations.[1–3]

Control of gonorrhea, with a consequent reduction in morbidity due to its complications, is difficult and involves, among other factors, the need for complex social and behavioral change.[4] Laboratory contributions to the control of this disease include enhanced diagnosis and surveillance of antimicrobial resistance in the gonococcus to ensure that disease is both recognized and treated optimally.[4] However, the gonococcus is a readily transmissible, highly transformable, strictly human pathogen that is highly adapted to a particular biological niche where it adapts rapidly to host influences.[5,6] Notably, it has the capacity to alter its phenotypic and genotypic characteristics by numerous mechanisms, some of which are unique to the pathogenic *Neisseria*.[7] When coupled with its fastidious growth requirements, this capacity has led to difficulties in laboratory diagnosis and confirmation of this diagnosis by traditional culture-based methods and molecular-based approaches alike.

Supported by the Children's Hospital Foundation (grant no. 922–034), which is supported by the Woolworths Fresh Futures Campaign.

Accepted for publication July 28, 2005.

Address reprint requests to David Whiley, Clinical Virology Research Unit, Sir Albert Sakzewski Virus Research Centre, Royal Children's Hospital & Health Service District, Herston Road, Herston, Queensland, Australia 4029. E-mail: d.whiley@uq.edu.au.

An ideal diagnostic test for gonococcus is one in which sample collection is noninvasive and in which testing is cheap and can be performed simply and rapidly while the patient waits to obtain a result that is both sensitive and specific and that provides guidance regarding optimal treatment. Such a test does not exist, of course, despite concerted attempts for over a decade boosted by the added incentive of a substantial "reward" offered by the Rockefeller Foundation.[8] However, once developed and applied, testing that results in enhanced diagnosis can, if combined with appropriate treatment, effect significant reductions in disease incidence.[9] Thus, the applications for diagnostic tests for gonococci are many, and there is a need for improved laboratory tests that can be used both to screen for disease on a population basis and to establish an etiological diagnosis in the individual case.

Laboratory Diagnosis

Traditional Methods

Until the late 1980s, laboratory diagnosis of gonorrhea was limited to gram stain and bacterial isolation. The gram stain is a rapid tool and has comparable sensitivity to bacterial culture for symptomatic urethral gonorrhea in men. However, it is relatively insensitive for specimens collected from women and for specimens from extragenital sites where the specificity of gram stain may also be affected by the presence of commensal *Neisseria* species. Bacterial culture is generally regarded as sensitive and specific for the detection of gonorrhea, and to date, it remains the gold standard for definitive diagnosis. In addition to its relatively low cost, it is suitable for a broad range of specimen types and provides a viable organism for both antibiotic susceptibility testing and epidemiological investigation. Disadvantages of bacterial culture include the need to collect invasive specimens, which must be transported under appropriate conditions to maintain organism viability.[10,11] During the 1980s, an enzyme immunoassay (Gonozyme; Abbott Diagnostics, Abbott Park, IL) was also available but was withdrawn because of poor sensitivity.[12]

Nucleic Acid Tests

In the early 1990s, nucleic acid tests first became available for routine use. These include both nucleic acid hybridization assays and nucleic acid amplification tests (NAATs). The hybridization assays include the Gen-Probe PACE II (Gen-Probe, San Diego, CA)[13] and the Digene Hybrid Capture II assays (Digene Corp., Beltsville, MD).[14] These assays use a specific oligonucleotide probe to hybridize directly to *N. gonorrhoeae* nucleic acid present within a specimen. Reported sensitivity and specificity values of the hybridization assays showed that these may be below that of bacterial culture.[11,15,16]

To date, there have been four main commercial *N. gonorrhoeae* NAAT assays, including the Roche Cobas Amplicor (Roche Molecular Systems, Branchburg, NJ), the Gen-Probe APTIMA Combo 2 (AC2; Gen-Probe), the Becton Dickinson ProbeTec assay (Becton Dickinson, Sparks, MD), and the Abbott Ligase Chain Reaction (LCx) (Abbott Laboratories). All of these use multiplex NAAT assays, targeting both *C. trachomatis* and *N. gonorrhoeae*. In addition, each of these multiplex assays has used a unique *N. gonorrhoeae* gene target and amplification technology (Table 1). The Abbott LCx has previously been recalled because of manufacturing issues.[17] In addition to the commercial assays, numerous in-house *N. gonorrhoeae* NAAT assays have also been described. These have primarily used polymerase chain reaction (PCR), have targeted various *N. gonorrhoeae* genes, and for the most part, have not been multiplexed with other assays.

There are several advantages of *N. gonorrhoeae* NAATs. First, they offer improved sensitivity compared with bacterial culture. When compared with *N. gonorrhoeae* NAATs, gonoccocal culture ranges in sensitivity from 85 to 95% for acute infections and may fall as low as 50% for females with chronic infection.[11,18–20] The increased sensitivity of NAATs makes them particularly suitable for screening, enabling accurate diagnosis of both symptomatic and asymptomatic gonococcal infections, which is critical to control of the disease.[10] Second, specimens collected for NAAT assays do not require the

TABLE 1. Overview of Commercial *N. gonorrhoeae* NAATs

	Roche Amplicor	ProbeTec SDA	Abbott LCx	Gen-probe APTIMA
Gene target	Cytosine DNA methyltransferase gene	Multicopy pilin gene-inverting protein homologue	Opacity protein genes	16S ribosomal RNA gene
Amplification technology	PCR	SDA	LCR	TMA
Sensitivity	64.8 to 100%	84.9 to 100%	88.2 to 97.3%	91.3 to 98.5%
Specificity	93.9 to 100%	98.4 to 100%	98.5 to 100%	98.7 to 99.3%
Positive predictive value	31.3 to 100%	54.8 to 100%	59.3 to 100%	88.1 to 97.4%
Negative predictive value	99.5 to 100%	95.2 to 100%	98.5 to 100%	99.2 to 99.9%
Cross-reactivity with other *Neisseria* species	*N. cinerea, N. flavescens, N. lactamica, N. sicca, N. subflava*	*N. flavescens*, *N. lactamica*, *N. subflava*, *N. cinerea*	None identified	None identified
References	23, 49, 55, 56, 64, 72, 75, 88, 89, 104	23, 56, 57, 105	54, 56, 57, 76, 88, 89, 94–98	27, 76, 99