

Comparison of Two Nucleic Acid Amplification Tests to Culture for the Detection of Pharyngeal *Neisseria gonorrhoeae*

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INTRODUCTION

■ A recent laboratory advancement in the diagnoses of gonorrhea and chlamydia has been the introduction of nucleic acid amplification tests (NAAT). The enhanced sensitivity afforded by these methods (e.g. PCR, LCR, SDA, TMA)* allows for detection of lower numbers of these pathogens compared to the culture “gold standard” technique. Manufacturers of several STD amplification test kits have successfully received FDA clearance on their products for urine samples.

In San Francisco, men who have sex with men (MSM) have a comparatively high rate of pharyngeal gonorrhea. Due to the potential for transmission, screening for the presence of pharyngeal gonorrhea is part of the routine protocol at the San Francisco STD “City” Clinic. Because NAAT methods had been successfully introduced for testing genital specimens, and recognizing the potential advantages of an amplified test method for detecting gonorrhea from other anatomical sites, it was proposed to investigate the feasibility of DNA amplification tests for pharyngeal specimens. A previous evaluation comparing culture to the Ligase Chain Reaction (LCR) assay (Abbott Laboratories LCx) yielded favorable results [CID 2002; 34:173-6]. Therefore, a CLIA prompted validation study was undertaken comparing the BDProbeTec™ ET System (based on Strand Displacement Amplification) and Abbott’s LCx Probe System to culture.

* PCR = Polymerase Chain Reaction

LCR = Ligase Chain Reaction

SDA = Strand Displacement Amplification

TMA = Transcription Mediated Amplification

METHODS

The goal was to recruit a cohort of 200 MSM who had a history of fellatio in the preceding two weeks and no history of antibiotic use within the previous month. The evaluation was explained to eligible clients and verbal consent was obtained from the majority who met the qualifications. Three pharyngeal swabs taken between and posterior to the tonsillar pillars were collected from each study participant using the appropriate collection kits for the Abbott and BD tests and one swab was inoculated directly onto modified Thayer-Martin medium. Manufacturers’ instructions were followed using the Abbott LCR and BD SDA procedures. Culture was performed according to standard procedure with confirmatory testing on gram-negative diplococci isolated. A full set of specimens was collected and processed from a total of 177 participants.

RESULTS

Of the 177 participants 5.1 % were found to be gonorrhoea positive by culture, 9.6% by SDA (ProbeTec) and 10% by LCR (LCx). This is summarized in Table 1. Eight (8) participants tested positive for *N. gonorrhoeae* by all three methods and an additional five (5) participants by the DNA amplification methods only. Tables 2 and 3 compare each of the amplification methods to culture; Table 4 compares the combined amplification methods to culture test results. Use of amplification technology yielded an increase of 3% detection.

Of the 177 sets of specimens, there were 5 positive by the LCR method only, and 4 by SDA only. Because of the time limitation

on testing and scant amount of remaining sample none of the 5 LCR specimens and only 3 of the 4 SDA specimens could be repeated. In addition, there was no alternative testing method available to verify the presence of *N. gonorrhoeae* in these specimens.

Assuming all culture positive results (9) are true positives, as well as those where two out of three results were positive (5) then we have 14 true positive specimens from this cohort of 177. With these figures Sensitivity and Specificity was calculated for each method and these are shown in Table 5.

Table 1
Results of *N. gonorrhoeae* Detection by Culture, Ligase Chain Reaction (LCR) and Strand Displacement Amplification (SDA).

	POSITIVE	NEGATIVE	TOTAL
Culture	9 (5.1%)	168	177
LCR	18 (10%)	159	177
SDA	17 (9.6%)	160	177

Table 2
Comparison of the Ligase Chain Reaction (LCR) method to Culture

LCR	CULTURE RESULT		
	POSITIVE	NEGATIVE	TOTAL
Positive	8	10	18
Negative	1	158	159
Total	9	168	177

Table 3
Comparison of the Strand Displacement Amplification (SDA) Method to Culture

SDA	CULTURE RESULT		
	POSITIVE	NEGATIVE	TOTAL
Positive	8	9	17
Negative	1	159	160
Total	9	168	177

Table 4
Comparison of the Combined Amplification Methods to Culture

LCR & SDA	CULTURE RESULT		
	POSITIVE	NEGATIVE	TOTAL
Positive	8	5	13
Negative	1	163	164
Total	9	168	177

Table 5
Sensitivity and Specificity for Gonorrhea Testing

METHOD	SENSITIVITY	SPECIFICITY
Culture	9/14 (64.3%)	163/163 (100%)
LCR	13/14 (92.9%)	158/163 (96.9%)
SDA	13/14 (92.9%)	159/163 (97.5%)

DISCUSSION

■ These results have limited interpretation due to the small sample size; however, that the two amplification tests detected gonorrhea in 5 patients for whom the culture results were negative indicates a greater sensitivity of amplification methods over culture for pharyngeal specimens.

Because it is recognized that DNA amplification methods will detect non-viable organisms we could not rule out some NAAT positive/culture negative results were due to resolving infection even though recent antibiotic use was a disqualifier in the evaluation.

There is limited availability of other NAAT methods to help resolve discrepant results. A method for discrepancy resolution is needed to accurately determine the specificity of amplification tests in extra-genital sites.

The culture method indicated a high carriage rate (29%) of *N. meningitidis* in this cohort. Presence of this Neisseria species may have interfered with results by masking *N. gonorrhoeae* on culture plates, or possibly, cross-reacting with the amplified tests yielding false positive results.

Further studies are warranted to determine if there is a place for NAAT's in detecting *N. gonorrhoeae* in extra-genital sites.

