

# Correlation of Three Commercial Nucleic Acid Amplification Techniques and Conventional Assays for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in Female Endocervical Swab and Urine Specimens

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## ABSTRACT

■ Laboratory diagnoses of chlamydia and gonorrhea infections have evolved from conventional culture, long considered the gold standard, to nucleic acid amplification techniques (NAATs). The amplification approaches of ligase chain reaction (LCR), polymerase chain reaction (PCR), and strand displacement (SD) have all provided an increase in assay sensitivity. Enhanced sensitivity has led to first void urine as an acceptable alternative for specimen collection. In this study, paired endocervical swab and urine specimens were collected from 272 female subjects attending a local STD clinic. Specimens were used to correlate the detection rates of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (GC) utilizing NAATs as well as EIA, DFA, and conventional culture on artificial and living cell substrates. The LCx<sup>®</sup> LCR assay (Abbott Laboratories), Cobas Amplicor PCR assay (Roche Diagnostics), and BDProbeTec<sup>™</sup> ET SD assay (Becton Dickinson) were used to determine the detection rates of CT and GC from both urine and swab specimens; additionally, the detection rates of GC culture, CT cell culture, CT MicroTrak EIA (Wampole Laboratories), and CT MicroTrak DFA (Wampole Laboratories) established from endocervical swabs of the same subjects were included in the correlation. True positive subjects were defined as those in which two or more methods/specimens per subject generated positive results. LCR, PCR and SD detected 94%, 97% and 91%, respectively, of CT true positive subjects by testing endocervical swabs, whereas only 78%, 88% and 78%, respectively, were detected by testing the urine of the same subjects. The CT true positive detection rates of the endocervical swabs of these same subjects tested by cell culture, EIA, and DFA were 78%, 69% and 53%, respectively. Specimen adequacy for CT testing, as determined by DFA, indicated that 7.3% of the swabs collected were inadequate for evaluation due to improper specimen collection. All NAAT and non-NAAT methods used to detect GC in our study accurately identified the presence of gonorrhea in true positive subjects with the exception of urine tested by PCR.

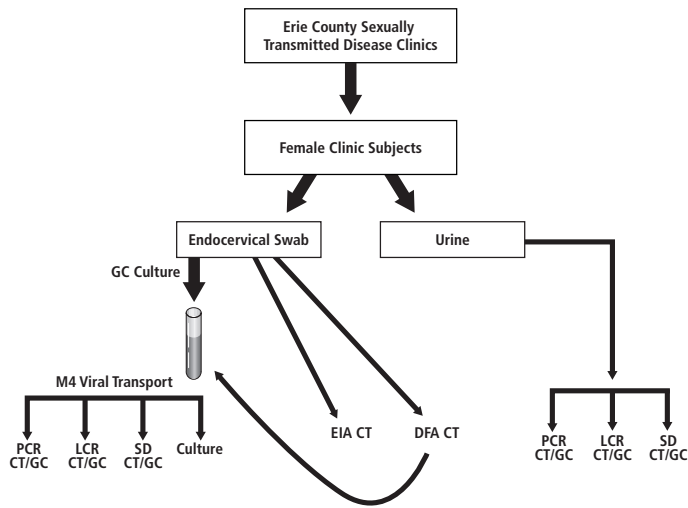
## INTRODUCTION

Nucleic acid amplification techniques (NAATs) that are currently available for the detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* are challenging the standard methods of culture, enzyme immunoassay (EIA), and direct fluorescent antibody (DFA). The increased sensitivity of the NAATs make it possible to use alternate specimens, specifically urine, as acceptable sources to test for the presence of these organisms. This study correlates the detection rates of various testing methods obtained from endocervical swab and urine samples. Paired specimens were collected from 272 female subjects attending a local STD clinic. All swab and urine specimens were tested for the presence of *C. trachomatis* and *N. gonorrhoeae* by ligase chain reaction (Abbott LCx), polymerase chain reaction (Roche PCR) and strand displacement (Becton Dickinson SD). Additionally, EIA (Wampole), DFA (Wampole) and cell culture (Kallestad) were used to detect *C. trachomatis*, and culture (JEMBEC plates) was used to detect *N. gonorrhoeae* from endocervical swabs. DFA also provided information on specimen adequacy for the swab specimens collected. True positives were defined in this study to be subjects whose specimens generated a positive result in at least two testing methods or specimen types.

The objectives of this study were to:

- Investigate the correlation among the detection rates of various testing methods used to detect *C. trachomatis* and *N. gonorrhoeae* in paired endocervical swab and urine specimens.
- Evaluate the use of a common viral transport medium (M4) for NAATs methodologies.
- Assess workflow patterns of the methodologies chosen for the study.

## METHODOLOGY



### METHODS 1. ENDOCERVICAL SWABS

Method	Target Organism
Ligase Chain Reaction (LCR) LCx Probe System (Abbott Laboratories)	<i>Chlamydia trachomatis</i> <i>Neisseria gonorrhoeae</i>
Polymerase Chain Reaction (PCR) Cobas Amplicor™ CT/NG Test (Roche Diagnostics)	<i>Chlamydia trachomatis</i> <i>Neisseria gonorrhoeae</i>
Strand Displacement (SD) BDProbe Tec™ ET Amplified DNA Assay (Becton Dickinson)	<i>Chlamydia trachomatis</i> <i>Neisseria gonorrhoeae</i>
Enzyme Immunoassay (EIA)/DFA Supplement Microtrak EIA/DFA (Wampole Laboratories)	<i>Chlamydia trachomatis</i>
Direct Fluorescent Antibodies Microtrak DFA (Wampole Laboratories)	<i>Chlamydia trachomatis</i>
Cell Culture McCoy Cells (BioWhittaker) Pathfinder Chlamydia Culture Confirmation System (Kallested Diagnostics)	<i>Chlamydia trachomatis</i>
Culture (JEMBEC plates)	<i>Neisseria gonorrhoeae</i>

### METHODS 2. URINES

Method	Target Organism
Ligase Chain Reaction (LCR) LCx Probe System (Abbott Laboratories)	<i>Chlamydia trachomatis</i> <i>Neisseria gonorrhoeae</i>
Polymerase Chain Reaction (PCR) Cobas Amplicor™ CT/NG Test * not currently FDA approved for <i>N. gonorrhoeae</i>	<i>Chlamydia trachomatis</i> <i>Neisseria gonorrhoeae</i> *
Strand Displacement (SD) BDProbe Tec™ ET Amplified DNA Assay (Becton Dickinson)	<i>Chlamydia trachomatis</i> <i>Neisseria gonorrhoeae</i>

## OBSERVATIONS

- The positivity rate (as defined by the study protocol) was 11.8% for *C. trachomatis* and 8.8 % for *N. gonorrhoeae* with a co-infection rate of 4.0% (see Table 1)
- Nucleic acid amplification techniques (NAATs) detected a greater number of *C. trachomatis* than non-amplification methods (see Table 2).
- Endocervical swabs tested for *C. trachomatis* by NAATs generated more positive results than the corresponding urine samples tested by NAATs (see Table 2).
- The detection rates of all methods and specimen types used to detect *N. gonorrhoeae* were similar with the exception of urine tested by PCR which is not currently FDA approved (see Graph 2).
- 85.3% and 95.6% of all subjects tested for *C. trachomatis* and *N. gonorrhoeae* respectively, demonstrated complete agreement among all of the testing methods and specimen types (see table 3).
- 40/272 (14.7%) and 12/272 (4.4%) of all subjects tested for *C. trachomatis* and *N. gonorrhoeae* respectively, demonstrated discordance among the results generated.
  - In 18 subjects, the DFA result was the only discrepant finding (due to improper specimen collection).
  - In 4 subjects, CT was detected in only one of the two specimen types (urine or endocervical swab).
  - In 3 subjects, CT was only detected in endocervical swab specimens by one methodology.
  - In 1 subject, the DFA demonstrated elementary bodies; albeit the quantity was insufficient to render a positive result.
  - In 5 subjects, CT was detected by at least 2 NAATs and was not detected by any non-NAATs.
  - Of the 12 subjects with discordant GC results, 7 true positive subjects demonstrated negative or grey zone urine results by PCR (inhibition).
  - In 3 subjects, GC was detected by PCR only.
  - Of the remaining discrepant subjects, no other trends were observed.
- 7.3% (19/260) of the endocervical swabs examined by DFA were unsatisfactory.

Table 1. True Positive Subjects

	True Positives	Positivity Rate
<i>Chlamydia trachomatis</i>	32 <sup>a</sup>	11.8%
<i>Neisseria gonorrhoeae</i>	24 <sup>a</sup>	8.8%

<sup>a</sup> 11 subjects were co-infected with *C. trachomatis* and *N. gonorrhoeae*

Table 2. Positive Results Generated by Each Methodology

<i>Chlamydia trachomatis</i>								
	Total Tested	True Positives	LCR	PCR	SD	Culture	EIA	DFA <sup>a</sup>
Endocervical Swab	272	32	30	31 (2 <sup>b</sup> )	29	25	22	18(1 <sup>c</sup> )
Urine	272	32	25	28	25	NA	NA	NA
<i>Neisseria gonorrhoeae</i>								
	Total Tested	True Positives	LCR	PCR	SD	Culture		
Endocervical Swab	272	24	24	23 (1 <sup>b</sup> )	24	24		
Urine	272	24	23	17 (2 <sup>b</sup> ) <sup>c</sup>	22	NA		

<sup>a</sup>200 of 272 DFA tests completed  
<sup>b</sup>False positives as defined by study protocol  
<sup>c</sup>Not currently FDA approved

Table 3. Overall Agreement of Test Results for Study Methods

	Total Subjects Tested	Positive Result Agreement	Negative Result Agreement	Discordant Results
<i>Chlamydia trachomatis</i>	272	14 (5.1%)	218 (80.2%)	40 (14.7%) <sup>a</sup>
<i>Neisseria gonorrhoeae</i>	272	16 (5.9%)	244 (89.7%)	12 (4.4%)

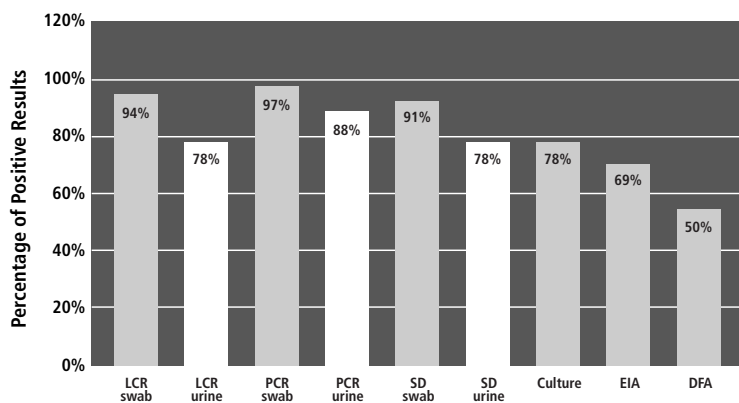
<sup>a</sup>The DFA result in 18 subjects was the only discrepant finding indicating improper specimen collection

Table 4. NAATs Workflow Characteristics

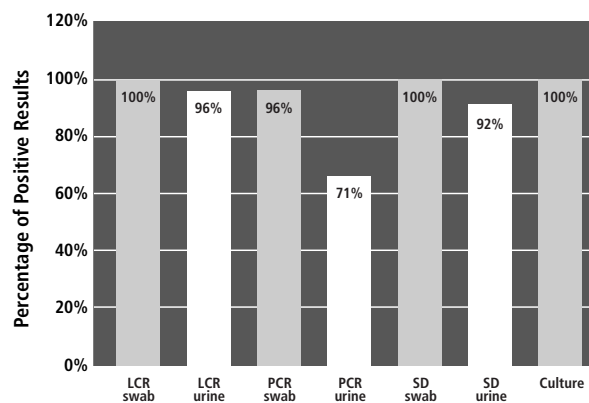
NAAT Method	Specimen Type	Specimens Tested per Batch <sup>a</sup>	Approximate Time to Complete Batch	Maintenance Time per Week
Abbott LCx	Swab	40	5.5 hours	1 hour
	Urine	40	6 hours	1 hour
Roche PCR	Swab	22	6 hours	15 minutes
	Urine	22	6.5 hours	15 minutes
Becton Dickinson SD	Swab	30	2.7 hours	5 minutes
	Urine	30	3.3 hours	5 minutes

<sup>a</sup> Separate detection of CT/GC (LCR), simultaneous detection of CT/CG (PCR and SD)

Graph 1. Percent of *Chlamydia trachomatis* True Positives Detected By various Methods



Graph 2. Percent of *Neisseria gonorrhoeae* True Positives Detected By various Methods



## CONCLUSIONS

- The positivity rate of *C. trachomatis* during the study period was impacted by the test methodology chosen and/or specimen submitted.
  - NAATs provided an advantage by yielding higher detection rates of *C. trachomatis* than culture, EIA and DFA for female endocervical swabs.
  - The ability to detect *C. trachomatis* in female subjects is enhanced by testing endocervical swabs as opposed to first void urine.
  - Urine specimens analyzed by NAATs had comparable positivity rates of *C. trachomatis* to female endocervical swabs tested by conventional non-amplified methodologies.
- NAATs did not provide a significant advantage over culture for detection of *N. gonorrhoeae*.
- M4 viral transport media is an acceptable collection/transport system for the detection of *C. trachomatis* and *N. gonorrhoeae* using LCR and SD methodologies based on FDA approval of M4 for PCR which showed comparable results.
- Overall, performance characteristics of the NAATs evaluated were similar, albeit, SD workflow characteristics demonstrated an advantage.

## SUMMARY STATEMENT

It is difficult to select the “best” NAAT method for laboratory diagnosis of CT and GC. That decision is best made considering the following:

- Sensitivity and specificity of each of the methods for each STD agent given the prevalence of the agent in the assessor’s area
- FDA approved claims for testing alternative (other than endocervical) specimens
- throughput characteristics
- general test utility
- and last, the bottom line cost per patient diagnosis of CT and GC

Finally, when applying the data from this study in any decision process, it needs to be noted that the findings are based upon one-half the affected population — females only. Clearly, however, the two products that merit consideration are the Becton Dickinson ProbeTec™ ET and Roche Amplicor systems.