



P-466 REPRODUCIBILITY OF POSITIVE GONORRHEA RESULTS USING A NUCLEIC ACID AMPLIFICATION TEST (NAAT) OVER A SIX YEAR PERIOD IN A STATE PUBLIC HEALTH LABORATORY.

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ABSTRACT

Objective: Nucleic acid amplification tests (NAAT) have become the standard for detection of Chlamydia and gonorrhea infections. These tests are known for their excellent performance, but concerns remain about specificity, especially in lower-prevalence settings where Positive Predictive Value (PPV) can be unacceptably low. Despite selectively screening for gonorrhea infections in Wisconsin Family Planning and STD clinics, the positivity rate is under 1% in many parts of the state. In order to increase the specificity and PPV, initially-positive gonorrhea results are retested before being reported. The goal of this study was to analyze the repeat-testing data gathered since early in 2001, in order to evaluate reproducibility and possibly adjust our retesting and reporting algorithm for gonorrhea (GC).
Methods: All specimens tested for gonorrhea on the BD Probe Tec instrument with "MOTA" ("method other than acceleration", a non-quantitative numeric value) scores over 2000, the manufacturer-specified cutoff for positive results, were retested to improve the PPV of positive results. The only exceptions were specimens with concurrent positive Chlamydia results; these were only retested if the initial GC MOTA was between 2000 and 20,000. Specimens repeating over 2000 MOTA were reported as positive; those with retest MOTA values under 2000 were reported as negative. Data including specimen identification numbers, submitting agency, dates of tests, reagent lot numbers, specimen type (urine or swab) and initial and repeat MOTA values were retained for each specimen. Analysis included stratification by specimen type and initial MOTA score and comparison of reproducibility across MOTA ranges and calendar year periods. Additional information was obtained on selected specimens in order to assess risk factors for GC infection.
Results: A total of 3887 specimens from February 2001 through February 2007 were included in the analysis. Several trends in the data were apparent. Overall reproducibility ranged from 78.2% (2001) to 95.3% (2006) and consistently improved over time. Reproducibility was higher in urine specimens, with a rate of 97.8% in 2006, than in swab specimens, with a 2006 rate of 89.5%. Swab specimens yielded a higher proportion of low-positive MOTA scores, with 12.6% of initially positive swab results falling in the 2000-10,000 range, as compared to urine specimens with only 2.6% in this range.
Conclusions: The reproducibility of initially-positive GC specimens using the BD Probe Tec was overall very good, especially in specimens with MOTA scores over 10,000. Clearly, specificity can be improved by retesting specimens in the low-positive range, with minimal loss of sensitivity. Non-repeating low-positive specimens were more likely to come from patients with risk criteria noted as "none" in the subset with this data available. However, quantification of the true impact on test performance (increased specificity versus decreased sensitivity) will require further study. Our retesting and reporting algorithm for positive GC specimens will change, partly as a result of this analysis.

BACKGROUND and OBJECTIVES

- The Wisconsin State Laboratory of Hygiene performs screening tests for Chlamydia and gonorrhea infections in support of the Region V Infertility Prevention Project and Wisconsin's own STD control efforts.
- WSLH has been using NAAT methods since 1997, starting with the Roche PCR test, and later the Abbott LCR assay. The entire screening program transitioned to the BD Probe Tec SDA in 2000/2001.
- WSLH tests over 60,000 specimens per year for Chlamydia, almost half also get a GC test. Rates have increased from 2001 to 2006. CT positivity went from 7.4% in 2001 to 8.2% 2006, GC from 2.5% to 4.5%.
- Because of the low positivity rate for GC in much of our testing population, positive GC results are retested before reporting, to increase the predictive value of the positive results. (GC positive specimens that are also positive for CT are not repeated unless in the equivocal range. Individuals testing positive for Chlamydia are at increased risk for GC as compared to Chlamydia-negative individuals, thus mitigating some of the concern about PPV. Timeliness of reporting is also a concern.)
- Six years of GC repeat-testing data was analyzed to determine whether repeat-testing and reporting algorithms could be improved.

METHODS

- Specimen Collection, handling and initial testing:** Urine (primarily male) and cervical swab specimens were collected, handled, processed and tested according to the manufacturer's product insert.
- Repeat GC Testing:** As a way to improve specificity and PPV, specimens with GC "MOTA" scores over 2000 were repeated on a subsequent run, either the same day or the following work day, and always within the manufacturers recommended timeframe and storage parameters.
- Result reporting:** Specimens with repeat results over 2000 MOTA were reported as positive; those with retest MOTA values under 2000 were reported as negative. (Supported by initial verification data in our lab.)
- Analysis included comparison of reproducibility by specimen type and initial MOTA score, and across MOTA ranges and calendar year periods.

RESULTS

Table 1. Gonorrhea Reproducibility, 2001-2006

	2001	2002	2003	2004	2005	2006
Reproducibility—All Specimens	78.2%	83.2%	91.8%	92.3%	94.3%	95.3%
# Urine specimens	311	420	298	338	443	535
Reproducibility— All Urine	86.8%	94.5%	94.6%	95.6%	96.6%	97.8%
# Urine <10K MOTA	37	21	25	30	22	14
Reproducibility Urine <10K	43.2%	59.1%	60.7%	63.3%	68.2%	57.1%
Reproducibility Urine >10K	92.7%	96.5%	98.1%	98.7%	98.1%	98.8%
# Swab specimens	424	388	178	168	155	229
Reproducibility – All Swab	71.9%	70.9%	87.1%	85.7%	87.7%	89.5%
# Swab <10K MOTA	87	119	18	22	24	29
Reproducibility Swab <10K	18.4%	19.3%	38.9%	27.3%	45.8%	41.4%
Reproducibility Swab >10K	85.8%	93.7%	92.5%	94.5%	95.4%	96.5%

Figure 1. Reproducibility of Positive GC Specimens

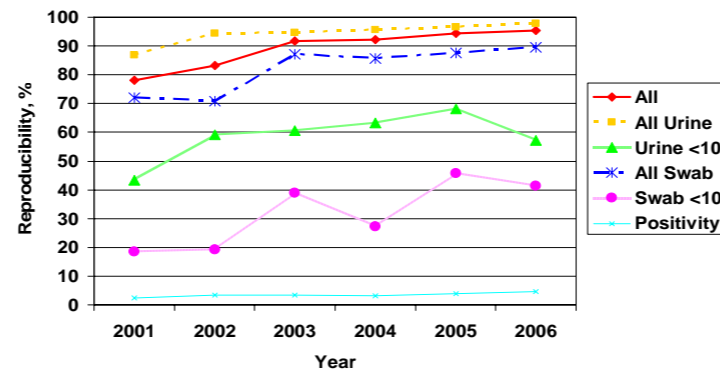
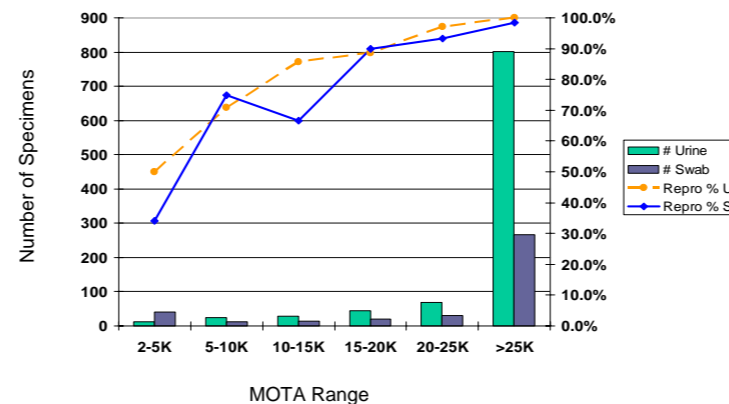


Figure 2. Reproducibility of Positive GC results by Initial MOTA Score (2005-06 Data.)



RESULTS/ DISCUSSION

A total of **3887** initially-positive GC specimens were included in the analysis; **2345 Urine** specimens, and **1542 Swabs**. Several variables were analyzed:

- Specimen Type:**
 - Reproducibility was higher in urine specimens than in swab specimens across all MOTA ranges and over time.
 - The proportion of urine specimens with initial MOTA values in the very low ranges was smaller than that for swabs. (See Fig. 2)
 - Positivity overall was higher in urine specimens (testing population.)
- MOTA Value:**
 - Reproducibility increased as initial MOTA values increased, and was lowest when the proportion of low-MOTA scores was highest. Looking at data from 2005/06, in specimens with MOTA scores under 5000, reproducibility was only 33.7%; in specimens over 15,000, reproducibility was 98.5%.
- Positivity:**
 - GC positivity overall was higher in 2006 (4.5%) than in 2001 (2.5%), due to efforts to reduce screening in low-prevalence areas. However, the largest increases in reproducibility were seen when GC positivity was steady, between 2001 and 2004.
- GC Risk Criteria Noted:**
 - Patient risk information was available for 45 specimens with initial MOTA values <10K in 2005. Of the 22 urines with this information, 15 repeated positive; 11 of these (73.3%) had a risk factor indicated. Of the 7 urines that did not repeat, only 4 (57.1%) had a risk factor listed. 9 of 23 swabs repeated positive; of these 7 (77.8%) indicated risk. Of the 14 swabs that did not repeat, 6 of 14 (42.9%) listed a risk factor.
- Over Time:**
 - Reproducibility increased each year for both urine and swab specimens, with the largest gains occurring in the earliest years of test use.

ADDITIONAL DATA: CT Reproducibility

Table 2. As part of another analysis, 145 positive CT specimens in the 2-10K MOTA range and 140 CT-negative specimens were repeated in duplicate.

Specimen Type	Initial MOTA	# Specimens	# Rpt. Neg/ Neg	# Rpt. Pos/ Neg	# Rpt. Pos/ Pos	% Reproducibility
Swabs	0-1999	94	94	0	0	100% (Neg.)
	All Pos.	94	26	16	52	72.3%
	2K-9999	45	15	11	19	66.7%
	10-20K	49	11	5	33	77.6%
Urine	0-1999	46	46	0	0	100% (Neg.)
	All Pos.	49	7	9	33	85.7%
	2K-9999	19	3	6	10	84.2%
	10-20K	30	4	3	23	86.7%

CONCLUSIONS

- Reproducibility of positive GC specimens over 10,000 MOTA is high enough that repeat testing may not be necessary or cost-efficient.
- Both results on repeat-discordant specimens should be reported to clinicians.
- Explanations for some of the trends noted, such as continued improvement over time, and differences in MOTA distribution between specimen types will require additional investigation.
- Additional investigation should be done to determine the impact on the actual **sensitivity** and **specificity** of the assay, and will include:
 - Further correlation with clinical risk data
 - Evaluation of the role of inhibitors, especially in swab specimens
 - Whether repeating low positives in duplicate can improve performance
 - Collection of additional data on Chlamydia result reproducibility