# VANISHING UNDIAGNOSED GONOCOCCAL INFECTIONS IN AN URBAN AMERICAN COMMUNITY

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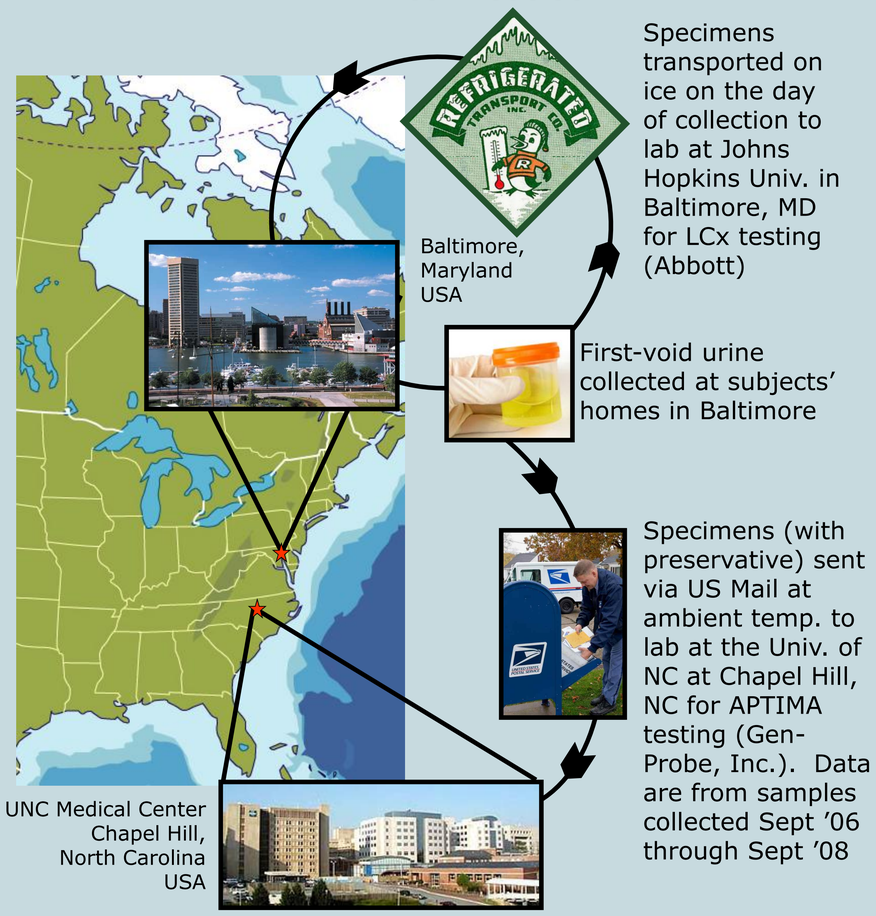
**Objective:** The Monitoring STIs Survey Program (MSSP) monitors trends in undiagnosed STIs among adolescents and young adults in an urban community in Maryland, US with historically high incidence of diagnosed STIs and high prevalence of undiagnosed STIs. We estimated the prevalence of undiagnosed gonococcal (GC) and chlamydial (CT) infections in 2006-2008 and compared these figures with data from a similar population survey conducted in the same community nearly a decade earlier (JAMA 2002 287:726).
**Methods:** From a probability sample of persons 15-35 years of age residing in community households with landline telephones, urine specimens were sent to the laboratory by mail with informed consent for STI testing. We used APTIMA Combo2 (Gen-Probe, Inc.) for GC/CT testing. Samples with initial positive results were retested and considered positive if both tests were positive. To assess effects of specimen collection and transport, negative urines were spiked with GC, mailed to the laboratory and tested. In the prior survey, urine collected at the household was kept chilled until laboratory testing by LCR (LCx, Abbott).
**Results:** Among 1712 urines tested, median volume was 60 mL (range, 10-100 mL). Samples were processed a median of 4 days after collection (range, 1-203 days). Under similar conditions, urines spiked with ≥ 250 cfu GC/mL (limit of detection for the assay) and mailed into the laboratory uniformly tested positive. Preliminary estimates show lower rates of undiagnosed GC and similar CT infection rates in the MSSP compared to the prior survey
**Conclusions:** The diagnostic test had expected analytical sensitivity, even with specimens that exceeded recommended limits for urine volume and storage time. Alternate explanations for the observed decline in prevalence of untreated GC in this urban US community (e.g. variation in specimen collection and testing, decline in population prevalence of treated and untreated infection) are explored.

## SPECIMEN COLLECTION, TRANSPORT AND TESTING METHODS

### 1997-1998 SURVEY




Specimens transported on ice on the day of collection to lab at Johns Hopkins Univ. in Baltimore, MD for LCx testing (Abbott)

First-void urine collected at subjects' homes in Baltimore

Specimens (with preservative) sent via US Mail at ambient temp. to lab at the Univ. of NC at Chapel Hill, NC for APTIMA testing (Gen-Probe, Inc.). Data are from samples collected Sept '06 through Sept '08

### 2006-2008 SURVEY

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**Table 1. Demographic and Social Characteristics of Survey Populations.**

| Characteristic | | Weighted[a] % for survey period '97-'98 (N = 579) | '06-'07 (N = 1248) | '07-'08 (N = 1033) |
|---|---|---|---|---|
| **Race** | White | 28.7 | 31.0 | 31.3 |
| | Black | 65.7 | 62.3 | 62.5 |
| | Other | 5.6 | 6.6 | 6.3 |
| **Gender** | Women | 52.0 | 52.1 | 52.2 |
| | Men | 48.0 | 47.9 | 47.8 |
| **Age** | 15-20 | 16.8 | 32.6 | 32.7 |
| | 21-25 | 23.9 | 23.3 | 23.0 |
| | 26-30 | 26.2 | 21.6 | 21.8 |
| | 31-35 | 33.2 | 22.5 | 22.5 |
| **Marital status** | Never married | 53.6 | 57.5 | 56.3 |
| | Married | 21.1 | 21.1 | 16.9 |
| | Not married, living with partner | 15.6 | 18.6 | 21.9 |
| | Widowed, divorced, separated | 9.6 | 2.9 | 5.0 |
| **Education** | < 8th grade | 6.1 | 3.8 | 3.7 |
| | Some high school | 16.0 | 24.1 | 24.9 |
| | High school graduate | 36.6 | 26.8 | 28.0 |
| | Trade/bus. sch./some college | 27.8 | 24.0 | 22.2 |
| | ≥ College graduate | 13.5 | 21.2 | 21.2 |

[a]Weighted to reflect variations in probabilities of sample selection from population.

**Table 2. Prevalence Estimates for STIs in Baltimore Population-Based Surveys 1997 – 2008.**

| Study Period[b] | Estimated[a] % Prevalence (SE or 95% CI) of infection with *N. gonorrhoeae* | *C. trachomatis* | *T. vaginalis* |
|---|---|---|---|
| 1997 – 1998[c] | 5.2 (SE, 1.4) | 3.0 (SE, 0.8) | not done |
| 2006 – 2007[d] | 0.1 (0.0, 0.7) | 4.6 (2.7, 6.4) | 6.3 (4.5, 8.1) |
| 2007 – 2008[d] | 0.1 (0.0, 0.6) | 3.3 (1.7, 4.9) | 6.2 (4.2, 8.3) |

[a]Weighted to reflect variations in probabilities of sample selection from population.
[b]For comparison, local health department rates of diagnosed GC and CT were 2.6% and 2.1%, respectively, in 1998 and 1.6% and 3.4% in 2007.
[c]Previous study population included persons 18-35 years of age.[1]
[d]MSSP study population includes persons 15-35 years of age.

**Table 3. MSSP urine specimen limitations.**

| Characteristic | | APTIMA STI Results[a] Positive (N = 136) | Negative (N = 1361) | *P* value[b] |
|---|---|---|---|---|
| Volume (mL) | median | 60 | 60 | 0.392 |
| | range | 10, 100 | 10, 100 | |
| % over 30 mL | | 91.9 | 94.2 | 0.375 |
| % reporting < 1h since previous urination | | 14.0 | 9.8 | 0.162 |
| Interval between collection and processing (days) | median | 5 | 4 | 0.022 |
| | range | 1, 203 | 1, 70 | |

[a]Positive = positive for GC, CT or TV; Negative = negative for GC, CT and TV.
[b]Z-test for proportions; Rank Sum Test for specimens volume and time to processing.

## SPECIMEN VALIDATION & QUALITY CONTROL FOR APTIMA TESTING

**Figure 1. APTIMA Combo 2 (AC2) detects *N. gonorrhoeae* (GC) below the published analytical sensitivity in urine spiked with whole GC and processed within 24h.** However, when the same samples were mailed into the laboratory and processed 10 days after collection, specimens containing ≤ 10 cfu/mL (red circles) were negative. Data plotted are mean RLU x 1000 ± sem from 6 samples at each concentration processed according to Gen-Probe instructions. $R^2$ = 0.999 for the nonlinear regression graph with data from freshly prepared urine specimens.

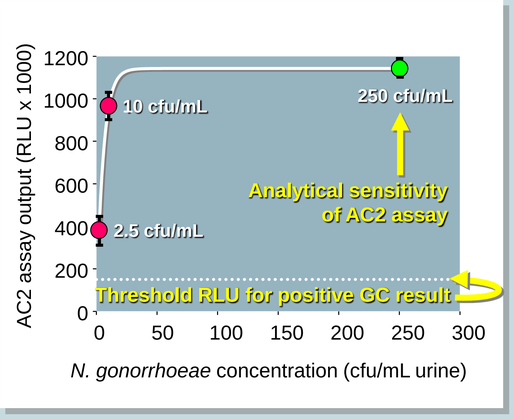


**Table 4. APTIMA repeat testing results.**

| Test Result | Number of specimens retested for GC Initial | GC Final | CT Initial | CT Final | TV Initial | TV Final |
|---|---|---|---|---|---|---|
| Pos | 2 | 2 | 47 | 47 | 96 | 97 |
| Neg | 123 | 124 | 78 | 79 | 33 | 45 |
| Eqv | 1 | 0 | 1 | 0 | 13 | NA |

Repeat testing was performed on all specimens with intial positive or equivocal results for GC, CT or TV. Pos, positive; Neg, negative; Eqv, equivocal; NA, not applicable. For TV testing, initial results between 10,000 and 30,000 RLU were considered equivocal. Initially equivocal specimens with repeat results ≥10,000 RLU were defined as TV positive; those with repeat results <10,000 were defined as TV negative.

**Self-collected vaginal swabs from women.** During the 3rd year of the MSSP, we are obtaining vaginal swabs in addition to urine from female respondents. To date, testing from 41 pairs of specimens suggests that urine is 60% sensitive for APTIMA STI testing compared to vaginal swabs. ***However, no additional cases of undiagnosed gonorrhea have been detected in vaginal swabs.***

## REFERENCES

1. Turner CF, Rogers SM, Miller HG, Miller WC, *et al.,* 2002 JAMA 287:726-733.
2. Isbey SF, Alcorn TM, Davis RH, *et al.* 1997 Genitourin Med. 73:378-382.

## CONCLUSIONS

- The majority of specimens in the MSSP exceed Gen-Probe limitations for urine volume, time since previous void and time to processing. ***Nevertheless APTIMA Combo 2 reliably detects ≥ 250 gonococci per mL urine under the conditions of this study.***

- Infections with fewer organisms would not be identified under these conditions. Organism burdens from men with gonococcal urethritis are on the order of ~$10^3$ cfu/mL urine[2], well above the limit of detection of APTIMA Combo 2. ***The concentration of gonococci in urine of asymptomatically infected individuals is not known.***

- Despite similar prevalence estimates for unrecognized chlamydial infection, ***undiagnosed gonorrhea is currently rare in Baltimore, MD and prevalence is over 98% lower than reported estimates for 1997-1998.*** *Reported* cases of gonorrhea have decreased by only 39% over the same period, suggesting that the earlier study may have overestimated the prevalence of unrecognized gonococcal infection.