Comparing First-Void Urine Specimens, Self-Collected Vaginal Swabs, and Endocervical Specimens To Detect Chlamydia trachomatis and Neisseria gonorrhoeae by a Nucleic Acid Amplification Test

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We set out to determine the prevalences of Chlamydia trachomatis and Neisseria gonorrhoeae by ligase chain reaction as well as to determine the prevalence of Trichomonas vaginalis by culture in a large and diverse national sample of non-health-care-seeking young women entering the military; we also sought to compare the abilities of three different techniques of collecting specimens (first-void urine, self-collected vaginal swab, and clinician-collected endocervical swab) to identify a positive specimen. A cross-sectional sample of young women was voluntarily recruited as a part of their routine entry pelvic examination visit, they completed a self-administered reproductive health questionnaire and provided first-void urine (used to detect C. trachomatis and N. gonorrhoeae) and self-collected vaginal swabs (used to detect C. trachomatis, N. gonorrhoeae, and T. vaginalis). The number of positive tests divided by the number of sexually active women screened by each sampling method determined the rates of prevalence. The rate of infection with any of the three sexually transmitted diseases (STDs) tested was 14.1%. The total positive rates for each STD (identified by ≥1 specimen) were the following: for C. trachomatis, 11.6%; N. gonorrhoeae, 2.4%; and T. vaginalis, 1.7%. The proportions of positives identified by specimen type were, for C. trachomatis and N. gonorrhoeae, respectively, endocervix, 65 and 40%; urine, 72 and 24%; and vagina, 81 and 72%. The proportions of positives when specimen results were combined were, for C. trachomatis and N. gonorrhoeae, respectively, cervix plus urine, 86 and 49%; cervix plus vagina, 91 and 93%; and vagina plus urine, 94 and 79%. We concluded that STDs were epidemic in this population. Self-collected vaginal swabs identified the highest number of positive test results among single specimens, with the combined cervix-vagina results identifying the highest number of positive results. Self-collected vaginal swab collections are a feasible alternative to cervical specimen collections in this population, and the use of multiple types of specimens increases the positive yield markedly.

Chlamydia trachomatis remains epidemic among sexually active young women (5). Health policy organization guidelines recommend annual chlamydia screening of sexually active adolescent and young adult females (1, 2, 29). Nucleic acid amplification technique (NAAT) applied to endocervical samples has proven to be a sensitive nonculture method to screen young women for C. trachomatis, with sensitivities ranging from 75 to 100% and with many reporting sensitivities greater than 90% (6, 7, 21, 22, 27, 30, 31). A further advance has been the application of the NAAT to first-void urine (FVU) samples to detect C. trachomatis with reported sensitivities of 50 to 95% (6, 7, 15, 22, 25, 27, 30, 31). This noninvasive form of specimen collection has been shown to be a cost-effective tool for chlamydial screening when compared to endocervical swabs because such collections do not require invasive pelvic examinations (24). Finally, the self-collected vaginal sample (e.g., swabs, tampons, and wash) has been a recent additional source for sexually transmitted disease (STD) specimens from young adult women with reported sensitivities by NAATs ranging from 75 to 100% for the detection of C. trachomatis (6–8, 12, 18, 19, 26, 28, 33), although this technique has not been approved by the U.S. Food and Drug Administration to date. Performances of the NAATs applied to Neisseria gonorrhoeae have been reported to be similar to those with C. trachomatis, with endocervical sample sensitivities of 89 to 97%, vaginal sample sensitivities of greater than 90%, and FVU sample sensitivities between 65 and 93% (4, 7, 8, 16, 30, 31).

It is difficult to compare the performance profiles of NAATs across studies, as they differ widely by the choice of STD test, specimen type, and the population studied. Comparative evaluations of different test systems and specimen sources, especially of self-collected specimens, are essential to the development of more consumer-friendly STD screening tests. However, there are few data published in which NAATs were used to compare three different anatomic specimens collected in parallel from the same female subject. In one such study, PCR was applied to identify C. trachomatis and N. gonorrhoeae from multiple specimen types (FVU, vaginal, and endocervical) during screening of 349 women from remote towns in Western Australia having gynecologic assessments (7) and found that the self-collected vaginal swab method identified

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more positive tests for both organisms than did samples from the other two anatomic sites. With this in mind, the present study reports the results of a ligase chain reaction assay for *C. trachomatis* and *N. gonorrhoeae* applied to FVU, self-collected vaginal, and clinician-collected endocervical specimens in a large cohort of young women in the United States upon their entry into the military.

**MATERIALS AND METHODS**

**Subjects and recruitment.** Young women entering 13 weeks of recruit training at the U.S. Marine Corps Recruiting Depot in Parris Island, S.C., were recruited to participate in “Focus—Fitness For Life,” a cognitive behavioral intervention designed to decrease STD acquisition and unintended pregnancy during their first year of enlistment. Participation was voluntary and required informed written consent in accordance with the requirements set by the military and university institutional review boards for human research. This study represents the baseline sociodemographic and biologic data. Between June 1999 and June 2000, 2,288 women voluntarily consented to participate in the study, and 2,157 (94%) women provided written consent.

**Procedures.** Participants underwent a routine pelvic examination with STD and Papanicolaou smear screening within 2 weeks of arrival. A self-report questionnaire and specimen collection (cervical, FVU, and two self-administered vaginal swab specimens) were carried out during this examination period.

**Questionnaire.** A self-report questionnaire about demographic characteristics, health and risk behaviors, and a reproductive clinical history (e.g., contraception, STD history, and pregnancy history, among other risk factors) was administered just prior to the routine pelvic examination.

**Specimen collection and processing.** Participants were instructed on the proper self-collection of the FVU (i.e., filling the first 20 ml in a marked cup) and vaginal specimens used to screen for *C. trachomatis, N. gonorrhoeae* (insert Dacron swab 2 in. [LCx STD kit], rotate around vagina three times), and *Trichomonas vaginalis* (repeat vaginal collection procedure with second swab [cotton]). Both swabs were immediately placed into a sterile screw-cap plastic specimen collection tube and immediately transported with the urine specimens to the clinic laboratory by a clinic assistant. The specimens were inoculated into their respective media within 5 min after the participant completed the self-administered vaginal specimen collection. After the urine and vaginal specimens were completed, the participants proceeded to their scheduled pelvic examination, during which the clinician first obtained the endocervical specimen according to the LCx protocol (for detection of *C. trachomatis* and *N. gonorrhoeae*) and then used a cytobrush to collect the Papanicolaou smear specimen.

Vaginal and urine specimens targeted for identification of *C. trachomatis* and *N. gonorrhoeae* were kept at 4°C immediately after collection and frozen at −70°C in the hospital freezer within 24 h of collection. They were shipped by overnight airlift to the author’s laboratory (J. Schachter) in batches, with the cold chain maintained by using specialized shipping containers containing dry ice. Endocervical specimens for *C. trachomatis* and *N. gonorrhoeae* were transported routinely, with the cold chain maintained, to the Naval Hospital laboratory for processing within 6 h of collection. Endocervical, vaginal, and FVU samples for *C. trachomatis* and *N. gonorrhoeae* were processed using LCx (4). In order to decrease costs and avoid unnecessary duplication, the endocervical swabs for *C. trachomatis* and *N. gonorrhoeae* were processed routinely by the Naval laboratory using the same LCx methodology as was used in the author’s laboratory (J. Schachter).

The second self-collected vaginal swab was tested for *T. vaginalis* using the Trichomonas In-Pouch TV (Biomed Diagnostics, San Jose, Calif.) according to the manufacturer’s instructions. These swabs were incubated in pouches at collection at 37°C and read for the presence of *T. vaginalis* at 2 and 5 days of inoculation by trained research assistants. Papanicolaou smears were prepared by the clinic’s clinician and sent to a local Nacy-approved hospital-based cytology laboratory.

Regarding the problem of specificity encountered with the LCx system in 2001, most of the LCx assays were completed before 1 February 2001 prior to problems encountered with the manufacturer’s specificity due to changes in components of the assay system. After that date, all positives (our final batch of specimens) were retested again before a positive result was reported, as required. If the repeat specimen-to-cutoff ratio was ≥1.0, it was read as a true positive; if the ratio was read as <1.0, it was considered a false positive and thus reported as a negative result. (Eleven positives out of 178 specimens in the final batch of specimens were found and confirmed positive on retesting during the last month of specimen processing, March 2001.)

**Data analysis.** This study compares the abilities of three different specimen types (endocervical, self-collected vaginal, and FVU) to identify a positive *C. trachomatis* and *N. gonorrhoeae* test. That is, any positive test by any collection method was considered the standard by which any single or combination of collection technique performances was measured.

**RESULTS**

Participants included 2,157 women (94% of those eligible), of which, 1,841 (85%) reported ever having been sexually active. Only data from sexually active women were included in the present study.

**Sociodemographic and reproductive health history.** This largely young (median age of 18 years, with 74% of women ranging from 17 to 19 years old), unmarried (92%), and ethnically diverse (43% racial or ethnic minority) cohort represents a “national” sample of young women (i.e., from all 50 states, Guam, Puerto Rico, and the U.S. Virgin Islands) who were not seeking health care services but who received a screening reproductive health assessment as part of their entry into the military. Most women did not report a history of a pregnancy (84% never pregnant), did report a history of sexual intercourse within the previous 3 months (85%), and reported having more than one lifetime partner (82%). Forty-four percent reported having ≥5 lifetime partners. At the last instance of intercourse, almost one-third used either no method or withdrawal only as a contraceptive technique, 20% used oral contraceptive pills, 7% used insertable (diaphragms or contraceptive jelly or foam) or injectable contraceptives, and 41% used condoms only as their contraceptives of choice (Table 1).

**Prevalences of *C. trachomatis*, *N. gonorrhoeae*, and *T. vaginalis*.** The rate of infection with any of the three STDs tested was 14.1%. The total positive rate for each STD (identified by ≥1 specimen) follows: for *C. trachomatis*, 11.6%; *N. gonorrhoeae*, 2.4%; and *T. vaginalis*, 1.7% (Table 2).

**Adequacy of the Papanicolaou smear.** As a measure of endocervical specimen adequacy for Papanicolaou specimen collection (presence of endocervical cells), we found that adequate cytology was achieved for >93% of cases.

**Performances of *C. trachomatis* and *N. gonorrhoeae* by specimen type.** All performance data are relative since the “gold standard” used here is based on the sum of “any positive” test from any specimen. (Specificities and predictive positive values based on our gold standard were 100%, and predictive negative values were 95 to 100% for both *C. trachomatis* and *N. gonorrhoeae* by any specimen type.) Positive results by specimen type are found in Table 2. The self-collected vaginal swab ranked highest in ability to detect a positive result among any of the three types of specimen collection for both *C. trachomatis* (81%) and *N. gonorrhoeae* (72%). For *C. trachomatis*, the urine specimen (72%) was the next-best single-specimen performer, followed by the endocervical specimen (64%). Combining the FVU specimen with the vaginal specimen boosted the detection rate of *C. trachomatis* to 94%, the highest detection rate of any single specimen or combination thereof. As expected, any combination of tests outperformed any single test.

The ability of the LCx to detect *N. gonorrhoeae* by specimen...
type was poorer in all single-specimen categories than the results for *C. trachomatis*. As stated earlier for *C. trachomatis*, the vaginal specimen had the highest detection rate of positives among the single-specimen evaluations (72%) and, if combined with the endocervical specimen, the positive detection rate was boosted to 93%. However, compared to vaginal samples, the endocervical and urine specimens performed particularly poorly for the detection of *N. gonorrhoeae*.

### DISCUSSION

**Overview.** This study represents a large cross-sectional sample of young women (*n* = 1,841) from every U.S. state and territory who were not seeking health care but who were screened for multiple STDs from multiple genitourinary specimens. The resultant study population was largely unmarried and young, as three-quarters of the participants were 17 to 19 years old. They were also at high risk for STD acquisition, as they were recruits at the time of entry into the military. It may also be that we had some false-negative results, as our self-administered swabs were transported dry to the laboratory before immediately being inoculated into the growth media, even though transport was done within 5 min of collection. Increasing loss of positivity for *T. vaginalis* over time as determined by repeated wet-mount examinations has been documented previously (14).

Finally, our overall rate for chlamydial infection, based on the sum of any positive result for any specimen, was 11.6%. Detection rates for chlamydia by specimen type follow: endocervix (7.5%), FVU (8.4%), and self-administered vaginal swab (9.6%). Our prevalence for chlamydia by FVU is similar to that reported for a young Army recruit population, the results for which were achieved by the use of ligase chain reaction applied to the FVU (9.2%) (9).

**Determining the best specimen type to detect positives during screening efforts.** Given the STD risk for these young women and the need to screen large numbers of young women in a short period of time (upon entry into the military), it is important to maximize the identification of these undetected STD infections while minimizing collection invasiveness, clinician and client time, and cost of collection and processing. In evaluating the individual specimen collection methods, the

<table>
<thead>
<tr>
<th>Specimen type(s)</th>
<th>% Prevalence of STDs caused by:</th>
<th>% of specimens positive for:</th>
</tr>
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<tbody>
<tr>
<td></td>
<td><em>C. trachomatis</em></td>
<td><em>N. gonorrhoeae</em></td>
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<td></td>
<td></td>
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<tr>
<td>Any positive specimen</td>
<td>11.6 (207/1,786)</td>
<td>2.4 (43/1,785)</td>
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<tr>
<td>Endocervix</td>
<td>7.5 (134/1,786)</td>
<td>1.0 (17/1,785)</td>
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<tr>
<td>Urine</td>
<td>8.4 (145/1,786)</td>
<td>0.6 (10/1,772)</td>
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<tr>
<td>Vagina</td>
<td>9.6 (167/1,746)</td>
<td>1.8 (31/1,744)</td>
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<tr>
<td>Endocervix and urine</td>
<td></td>
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<tr>
<td>Endocervix and vagina</td>
<td>91 (184/207)</td>
<td>93 (40/43)</td>
</tr>
<tr>
<td>Vagina and urine</td>
<td>94 (194/206)</td>
<td>79 (33/42)</td>
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</tbody>
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* Values in parentheses are numbers of women positive for an STD(s)/total number of women. Denominators may differ due to missing specimens.

* Referent is positive result with any specimen, so values in parentheses are numbers of positive specimens of given type/total number of positive specimens. Denominators may differ due to missing specimens.

* Self-collected vaginal swab.
self-collected vaginal swab was found to have the highest rate of detecting positive tests for both C. trachomatis and N. gonorrhoeae infection. These findings are similar to the few works available that compare the performances of all three collection specimens processed by NAATs, which showed that the vaginal specimen had the greatest ability to detect C. trachomatis and N. gonorrhoeae by LCx (4, 10) and by PCR (3, 7), with mixed results for the next-higher performer using LCx or PCR applied to urine and endocervical specimens.

Our endocervical specimens performed the most poorly of our three specimens for the positive detection of chlamydia. Such findings differ from several previously published studies (4, 10). It may be that we had a number of false-negative endocervical tests due to differences in specimen collection (13, 32), transport, and processing, as the endocervical specimens were processed in a different laboratory from that used for our vaginal and urine specimens. For example, it has been clearly shown that the cellular quality of the specimen collected has a direct impact on the ability to detect a true positive, whereby specimens deemed to have adequate cellularity were more likely to have a positive C. trachomatis result than those that had inadequate cells on smear (13, 32). Furthermore, the ability of the LCx to detect N. gonorrhoeae was unacceptably poor, especially with respect to the urine and endocervical specimens. It may be that our low prevalence for N. gonorrhoeae among this population of non-health-care-seeking asymptomatic young women had a negative impact on the ability of the LCx to yield an accurate result.

We also found an improvement in the identification of positive results when more than one method was used to screen. This finding was also shown using PCR on the same three specimen collection combinations in a population of women (7). The combination of the vaginal and urine specimens is of interest since those two methods represent self-collected specimens that require no additional clinician time to collect to enhance detection of positives. The combination of endocervical and vaginal sampling yielded a significant boost in detection for both C. trachomatis and N. gonorrhoeae (T. vaginalis was collected only by using the self-collected swab). Such findings may be translated into recommending that the clinician obtain a combined endocervical and vaginal sample on the same swab during a pelvic examination. This interpretation of the findings needs to be further evaluated.

Seeking the ideal STD screening specimen. Vaginal swabs proved to be the best single method at identifying positive results. They were also very readily accepted as a specimen source among these young women. STD screening by urine samples and vaginal swabs is much preferred by young women when compared to the traditional collections performed during routine pelvic examinations. In a study from our group of adolescent young women attending a teen clinic for a routine pelvic exam, a nonmedical research assistant instructed the young women regarding how to collect the FVU and self-administered vaginal swabs prior to their scheduled routine pelvic exam. After the visit, the young women were asked to rank their preference for specimen type should they need screening for STDs in the future. Not surprisingly, they ranked the urine first and the pelvic examination last, with the vaginal swab collection ranked immediately between the two (23). Vaginal specimens are an attractive alternative to FVU because vaginal swab specimens remain stable (i.e., they do not require immediate cold storage) enough to be shipped over a few days’ time to a remote laboratory. Vaginal specimens also require fewer steps to process than do urine samples in the laboratory. With both patient acceptance and ease of transport and processing, vaginal specimens might become the specimen of choice as work progresses toward the development of future home testing kits, for example. The vaginal specimen approaches the ideal screening specimen. It is noninvasive, it is not linked to a pelvic exam or to the requirement of a health professional or clinic for adequate collection, it is an easy and stable format for transport, including mail transport, and it offers ease of laboratory processing—among other attributes. Clearly, more attention is needed to evaluate specimen collection types, especially vaginal swabs, as we continue to develop and evaluate new STD test systems. In this same study of screening preferences of young sexually active women, 25% refused to participate in the collection of vaginal specimens giving such reasons as “lack of time,” not wanting to “touch myself,” and lack of trust in the new technique compared to a clinician’s collection performed at the routine pelvic examination (23). It must be noted that these young women were already sexually active at the time of the scheduled routine pelvic examination.

Summary. Young ethnically diverse young women entering the military from across the United States have very high rates of C. trachomatis infection. To ameliorate this continued epidemic of infection among our young women nationwide, it is necessary to increase screening for asymptomatic infection. Evaluating the performance of noninvasive collection of specimens such as urine and self-collected vaginal swabs furthers our knowledge of the ideal acceptable and sensitive screening specimen for the target population. This research emphasizes the importance of beginning to evaluate the efficacy of specific screening specimens and clinical algorithms for young women who are not symptomatic and are not seeking health care, especially within the STD-public health clinic setting, as performance profiles may differ. The final goal is to develop cost-effective screening tools that are acceptable, stable and accurate, and widely available to young women who are at risk for STD acquisition.

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