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Evaluation of Self-collected Vaginal Swab, First Void Urine and Endocervical Swab Specimens for the Detection of *Chlamydia Trachomatis and Neisseria Gonorrrhoeae* in Adolescent Females

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Abstract

OBJECTIVE—To assess the concordance of self-obtained vaginal swabs (SVS), first void urine samples (FVU) and provider-collected endocervical swabs (PES) for the detection of *chlamydia trachomatis* (CT) and *neisseria gonorrrhoeae* (NG) in adolescents.

METHODS—A total of 342 adolescent women and 1,080 baseline and semi-annual visits were analyzed. FVU, SVS and PES were collected at each biannual visit. All specimens were tested by BDProbeTec ET™ Amplified DNA Assay. Sensitivity, specificity, positive predictive value (PPV) negative predictive value (NPV) and Kappa Coefficient were calculated to evaluate the ability to identify possible infected cases using samples from three anatomic sites and the test agreement between any two of these three specimen types. Positive result from at least two of the three specimens collected from same subject at the same study visit was considered true positive.

RESULTS—The positivity rates for CT and NG were 26.6 and 11.7 per 100 women respectively. The sensitivities of SVS, FVU and PES for detecting CT were 97.3%, 89.2% and 90.1% respectively. For the detection of NG, the sensitivities of the three sampling methods were 100%, 88.6% and 95.5% respectively. The specificities were between 94.7% and 99.7% for both CT and NG. Kappa coefficients of CT test results were 0.89, 0.88 and 0.83 for specimen pairs SVS*PES, SVS*FVU and PES*FVU respectively. For the detection of NG, kappa coefficients were 0.91, 0.87 and 0.91 for those three pairs (all $P<0.0001$). Kappa > 0.75 is considered excellent agreement between specimens.

CONCLUSION—There were strong agreements among SVS, PES and FVU specimens on the detection of CT and NG infections in adolescent females using nucleic acid amplification test. SVS represented as high as or more sensitive an approach for detecting both CT and NG compared to PES. Although FVU was the least sensitive sampling method, it is also the least invasive method. Thus SVS and FVU may provide a reliable alternative to endocervical specimens for CT and NG screening.

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Keywords

chlamydia gonorrhea; DNA amplification test; reproductive tract infection screening; adolescent females

INTRODUCTION

Adolescent females have the highest rates of *chlamydia trachomatis* (CT) and *neisseria gonorrrhoeae* (NG) among all age groups in America (CDC Sexually Transmitted Diseases Surveillance 2005). Since most of the infected adolescent women are asymptomatic, successful detection of CT and NG infections relies on effective, convenient, non-invasive and low cost diagnostic method for broad screening. Several previous studies demonstrated that non-invasive alternatives to the traditional endocervical swab such as urine and vaginal samples to detect reproductive tract infections are required and well accepted among adolescents.[1–3]

Nucleic acid amplification test became available in the last decade for use in clinical microbiology laboratories and has been considered the most sensitive and promising test in identifying CT and NG organisms. The ability to detect CT and NG using urine and vaginal specimens without a pelvic examination is a key advantage of nucleic acid amplification test, and this ability facilitates screening females in situation other than traditional screening venues. However, given the current limited FDA approval for self-collected vaginal swabs (SVS) to health care settings only, it is necessary to demonstrate the equivalent performance of SVS to other specimen types in order to support the expanded CT and NG screening using SVS. This is especially important when screening high prevalence population.

In this study, we used BDProbeTec ET™ Amplified DNA Assay in detecting CT and NG infections. Although BDProbeTec ET™ has proved its performance both in men and women, most studies have been limited to adults or mixed age groups. The suitability of tests may vary by the population examined because of acceptability of testing, ability to properly obtain specimens, prevalence of infections and potential endogenous biological variables. The objective of this study was to assess the concordance of two self-collected sampling methods (urine sample and vaginal swab) and conventional provider-collected endocervical sampling (PES) for the detection of CT and NG in adolescent females using BDProbeTec ET™.

MATERIALS AND METHODS

Study population

Over a period of 5 years from 2001 to 2006, as part of a longitudinal study on hormonal contraceptive use, ectopy and sexually transmitted infection acquisition, a total of 350 adolescent females were recruited and followed up for the study. This study was carried out at an urban Adolescent Clinic in an academic institution. Healthy female adolescents were eligible to participate if they were 12–18 years old, sexually active and not currently pregnant or had not been pregnant in the last 3 months. With approval of the institutional review board, written informed consent was obtained from each enrolled adolescent female participant. Study participants completed a computer assisted sexual behavior questionnaire. A health care provider then completed a detailed medical history and conducted a clinical examination on the adolescent during every 6-month visit. First void urine (FVU), SVS and PES specimens were collected at baseline and semi-annual follow-up visits.

Specimen collection and storage

First void urine—At least one hour since last micturition and prior to swab collection, a urine specimen was collected from each subject into a sterile urine cup, after instructing the patients to collect only up to the 20 ml mark indicated with a black marker. At least 10 ml of urine was poured into a separate urine cup for BDProbeTec ET™ testing.

Self-collected vaginal swab—Prior to the pelvic examination, adolescents were briefly instructed to place a Dacron-tipped swab from the BDProbeTec ET™ collection devices 1 inch into the distal vagina, rotated 360° and handed back to the provider in the specimen container.

Endocervical swabs—Endocervical swab specimens were collected from each patient as part of the pelvic examination by the clinician using BDProbeTec ET™ manufacturer-provided collection devices according to package insert.

All specimens were stored in a refrigerator at 2 to 8°C prior to transfer to the main laboratory within 4 days of collection and processed according to the package insert instructions from the manufacturer.

Amplified DNA methods

The BDProbeTec ET™ (BD Biosciences, Sparks, MD) *C trachomatis* and *N gonorrhoeae* amplified DNA assay was used for the detection of DNA from CT and NG simultaneously. Swabs collected with the BDProbeTec ET™ specimen collection/transport kit and the portion of the urine sample were tested for *C. trachomatis* and *N. gonorrhoeae* according to the instructions in the manufacturer's package insert. Results were reported as positive, negative, or indeterminate. Indeterminate results occurred when the CT, NG, and separate amplification control were all negative, indicating inhibition of amplification.

Statistical Analysis

The detection of cervical, vaginal or urethral CT and NG infections by endocervical, vaginal and urinary sampling was evaluated. A positive result from at least two of the three specimens collected from the same subject at the same study visit was considered true positive. Sensitivity (= true positive/(true positive + false negative)), specificity (=true negative/(true negative + false positive)), positive predictive value (PPV=true positive/(true positive + false positive)) and negative predictive value (NPV=true negative/(true negative + false negative)) were calculated to examine the performance of FVU, SVS and PES in identifying possible infections. Indeterminate results falling into either true positive or true negative category were included in sensitivity, specificity, PPV and NPV calculations. The Kappa statistic (or Kappa coefficient) was calculated to evaluate the test agreement between any two of three specimen types. Excellent agreement was defined as a Kappa > 0.75.[4] We used SAS 9.1.2 (SAS Institute Inc., Cary, NC, USA) for the data analysis.

RESULTS

Among 350 adolescent females enrolled in the study, ninety-six percent were African American whose median age was 16 (range 12–18) years at their study entry visits. On average, the study participants initiated their sexual activity at 14 (range 10–18) years and had four sexual partners (Table 1).

Of 350 adolescent female subjects in the study, 342 participants and 1,080 baseline and semi-annual visits had BDProbeTec ET™ test results (including indeterminate results) available for CT and 1,079 visits for NG from all three of cervical, vaginal and urinary samples.

Among the 342 adolescents, the positivity rates for CT and NG were 26.6 and 11.7 per 100 women respectively. Notably, this was a high prevalence population. Of the 1,080 visits, 111 visits were tested positive for CT. For NG test, 44 out of 1,079 visits were positive based on our true positive standard. Unless mentioned specifically, the following analysis and discussion are based on subject visits.

Sensitivities, specificities, PPV, NPV and indeterminate results are shown in Table 2. Overall, self-collected vaginal specimen was the most sensitive sampling method, followed by endocervical specimen and urine sample in detection of CT and NG. The specificities and PPV of endocervical swabs in screening both the CT and NG infections were the best, followed by FVU and SVS. For NPV, the PES was again the most accurate sampling method for correctly classifying uninfected cases as negative. However, the SVS yielded the worst PPV for the detection of NG DNA among all three specimen types due to the 6 false positive results obtained by SVS. In addition, both SVS and FVU generated more false positives than PES and the majority of indeterminate results (96%). 'Indetermination' was reported by the lab indicating the BDProbeTec ET™ could not determine the status of a sample as whether positive or negative. In contrast to SVS and FVU, the PES produced the smallest number of false positive and indeterminate results for detecting both CT and NG. This could be the result of less contamination when collecting the endocervical specimen.

The agreement of test results from paired specimens between any two of PES, SVS and FVU and the detailed discrepancy data are showed in Table 3. It is evident that the overall agreements between specimens were great (from 97% to 99.3%). Nonetheless, there was a consistently better agreement among specimens from three sites for detecting NG than for CT. There were more discrepant results (n=32) for CT test between PES and FVU. For NG test, the most discrepant results (n=11) were seen between SVS and FVU.

The kappa coefficients were calculated to assess the agreement between paired specimens, as are indicated in Table 3. The kappa statistic was from 0.83 to 0.89 for the detection of *C. trachomatis* and from 0.87 to 0.91 for *N. gonorrhoeae*. A kappa greater than 0.75 demonstrates excellent agreement beyond chance, 0.40 to 0.75 represents intermediate to good agreement and below 0.40 expresses poor agreement[4]. Thus, kappa coefficients of 0.83 to 0.91 and all lower 95% CIs were above 0.75 in this study should be regarded as considerable strength of agreement between specimen pairs for detecting CT and NG infections.

DISCUSSION

The increased sensitivities and specificities of nucleic acid amplification techniques have led to the evaluation of less invasive specimen collection procedures for screening gonococcal and chlamydial urogenital infections. Despite their advantages, there are limitations of nucleic acid amplification tests. Of note, the strand displacement amplification test system used by BDProbeTec ET™ can be inhibited resulting in false negative results. False positive result could also be possible due to specimen contamination. Thus, we were relatively conservative in defining our internal measure of true positive in our analyses (In the clinical setting, however, we chose to consider even one positive out of three or any amount of specimens as an infection and administer treatment). Nonetheless, this study found strong agreement between self-collected vaginal swabs or urine specimens and provider collected endocervical swabs in the detection of both CT and NG infections. The performance of self-collected vaginal swabs was at least equivalent to PES or FVU.

A number of studies have reported the correlation among specimens from urine, vagina and endocervix in detecting CT and NG using various nucleic acid amplification tests. In two previous studies using PCR, the sensitivities are over 90 % for SVS and more than 70% for

FVU;[5] compared with cervical specimens, self-collected specimens have a sensitivity of 100% and a specificity of 93.4% for detection of *C trachomatis* infection.[6] In a BDProbeTec ET™ performance study, the sensitivities for CT by PES and FVU are 92.8% and 80.5%; the sensitivities for NG are 96.6% and 84.9%.[7] Compared to these previous studies, this study has shown similar performance of SVS and FVU.

Regarding the ability to identify CT and NG, our data has demonstrated that excellent performance can be achieved when noninvasive self-obtained vaginal swab specimens are used. Compared to PES, sampling the vagina by the patient represent similar or better sensitive approach for identification of both *C. trachomatis* and *N. gonorrhoeae* in young women. This finding is consistent with previous studies.[8,9] In addition to its promising ability to detect CT and NG infection, vaginal swab sampling method has proven to be preferred by women for screening CT and NG.[10–12]

Although the sensitivity of urine specimens may be suboptimal compared to SVS and PES, the FVU is the only alternative non-invasive sampling method approved by FDA for collection outside of health care settings. Thus, the FVU sampling methods, along with SVS, can serve as convenient substitutes especially when 1) they are properly choice to be used for large scale population screening or repeated sampling where the pelvic examination is not suitable due to the cost and time, 2) the cervical screening is refused or cannot be performed or 3) the providers or clinical facilities are not available.

Several potential limitations need to be mentioned. Since this study was to compare the abilities of sampling three anatomic sites to identify positive result(s) of CT or NG based on a single nucleic acid amplification test BDProbeTec ET™ on a one test per specimen basis, we had limited power to confirm infection status even using two positive results as our internal true positive standard. This standard may underestimate the number of 'infected patients' by disqualifying any single 'infected specimen' of three specimen types as compared to studies using multiple tests such as culture and/or various nucleic acid amplification tests. Therefore, misclassification could not be completely avoided. In addition, sensitivity of the BDProbeTec ET™ Amplified DNA Assay could be decreased if a specimen contained low number of target DNA of targeting pathogen(s), or due to amplification inhibitors. On the other hand, specimen contamination if strict quality control measures are not implemented or cross-reactivity with genes from related species (particularly for NG) could decrease specificity of the test. Thus, misclassification of a study subject's status in this study could be a result of our true positive definition, specimen collection, process and/or nucleic acid amplification test.[13,14] Another limitation was that indeterminate results by all three specimen types were not included in the kappa analysis, which might slightly influence the results of the kappa analysis. Finally, this was a high prevalence study population for both CT and NG. While the agreement and sensitivity was high in this context (high positivity rate population), the generalizability of these results to low prevalence and largely asymptomatic populations may still remain an issue, even when nucleic acid amplification tests are being used.

In conclusion, the agreement was excellent among SVS, PES and FVU in the detection of CT and NG by BDProbeTec ET™ in our study. Compared to PES and FVU, vaginal sampling performed by the women themselves was the most sensitive approach. The implications of our results are that since the performance of SVS was similar to that of the FDA approved PES and FVU sampling methods, SVS could be another noninvasive alternative in addition to FVU in screening CT and NG in adolescent females especially when prevalence is high. In addition, non-invasive SVS and FVU specimen collection will facilitate reproductive tract infection screening among at-risk youth eliminating the need for a clinical speculum examination. Finally, the use of SVS and FVU may be extended to broaden screening in non-clinical settings.

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Table 1
Descriptive Characteristics of study population

	Number of adolescent females	Percent (%)
Demographic characteristics		
Age (median = 16 years)		
12–14 years	52	14.9
15–16 years	146	41.7
17–18 years	152	43.4
Race/ethnicity (missing = 7)		
African American	329	95.9
Hispanic	2	0.6
Caucasian	3	0.9
Other	9	2.6
Current level of education completed (missing = 4)		
<9th grade	65	18.8
9th grade	57	16.5
10th grade	72	20.8
11th grade	61	17.6
12th grade	84	24.3
Technical/vocational	5	1.5
Some college	2	0.6
Socio-economic status		
Mother's final education (missing = 7)		
Less than high school	54	15.7
High school graduate	135	39.4
Technical/vocational	3	0.9
Some college	47	13.7
College degree	40	11.7
Gone to graduate school	2	0.6
Don't know	62	18.1
Father's final education (missing = 6)		
Less than high school	45	13.1
High school graduate	125	36.3
Technical/vocational	2	0.6
Some college	28	8.1
College degree	20	5.8
Gone to graduate school	4	1.2
Don't know	120	34.9
Sexual history		
Age at sexual debut (median = 14) (missing = 9)		
10–12 years	69	20.2
13–15 years	226	66.3
16–18	46	13.5
Lifetime number of sexual partners (median = 4) (missing = 9)		

	Number of adolescent females	Percent (%)
1	51	15.0
2–3	116	34.0
4–5	79	23.2
6–7	41	12.0
8–9 and more	54	15.8

Table 2

Performance of SVS, FVU and PES specimens in the detection of CT and NG infection

	True positive [a]	False positive	False negative	True negative	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Indetermination	
									True positive (%)[b]	True negative (%)[c]
SVS-CT	108	5	2	919	97.3	94.8	94.7	95.1	1 (0.9)	45 (4.6)
FVU-CT	99	5	12	926	89.2	95.6	95.2	94.9	0 (0.0)	38 (3.9)
PES-CT	100	4	11	961	90.1	99.2	96.2	98.5	0 (0.0)	4 (0.4)
SVS-NG	44	6	0	980	100	94.7	88.0	95.2	0 (0.0)	49 (4.7)
FVU-NG	39	1	4	996	88.6	96.2	95.1	96.0	1 (2.3)	38 (3.7)
PES-NG	42	0	2	1032	95.5	99.7	100	99.5	0 (0.0)	3 (0.3)

[a] as defined by positive results from at least two of SVS, FVU or PES specimens;

[b] indeterminate results fall into true positive category, percentage = number of indeterminate results/(number of indeterminate results + TP + FN);

[c] indeterminate results fall into true negative category, percentage = number of indeterminate results/(number of indeterminate results + TN + FP). Indeterminate results were included in the calculation of sensitivity, specificity, PPV and NPV.

Table 3

Agreement of CT and NG test results between any two of SVS, FVU and PES specimens

	Specimen Pairs	Overall agreement (%)[a]	Positive by both samples	Discrepancy		Kappa Coefficient[b]	95% CI
				(+/−)	(−/+)		
CT	SVS*PES	97.8	99	16	6	0.89	(0.84, 0.93)
	SVS*FVU	97.6	97	17	7	0.88	(0.83, 0.93)
	PES*FVU	97.0	89	16	16	0.83	(0.77, 0.89)
NG	SVS*PES	99.2	43	8	0	0.91	(0.85, 0.97)
	SVS*FVU	98.9	39	10	1	0.87	(0.80, 0.95)
	PES*FVU	99.3	37	3	4	0.91	(0.84, 0.97)

[a] agreement reading (including positive and negative results from both specimens) divided by all readings;

[b] Indeterminate results were not included in the kappa analysis, all p<0.0001.