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Multicenter Evaluation of the BDProbeTec ET System for Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in Urine Specimens, Female Endocervical Swabs, and Male Urethral Swabs

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The performance of the Becton Dickinson BDProbe Tec ET System *Chlamydia trachomatis* and *Neisseria gonorrhoeae* Amplified DNA Assays (BD Biosciences, Sparks, Md.) was evaluated in a multicenter study. Specimens were collected from 2,109 men and women, with or without symptoms, attending sexually transmitted disease, family planning, and obstetrics and gynecology clinics. Both swab and urine samples were collected, and the results obtained from 4,131 specimens were compared to those from culture and the LCx nucleic acid amplification test (Abbott Industries, Abbott Park, Ill.). PCR and cytospin of the culture transport medium with chlamydia direct fluorescent antibody staining were used to adjudicate chlamydia culture-negative results. Sensitivity and specificity were calculated both with and without use of the amplification control (AC), with little apparent difference in the results. Without the AC result, sensitivity for *C. trachomatis* and *N. gonorrhoeae* were 92.8 and 96.6%, respectively, for cervical swabs and 80.5 and 84.9% for urine from women. *C. trachomatis* and *N. gonorrhoeae* sensitivities were 92.5 and 98.5%, respectively, for male urethral swabs and 93.1 and 97.9% for urine from men. This amplified DNA system for simultaneous detection of chlamydial and gonococcal infections demonstrated superior sensitivity compared to chlamydia culture and has performance characteristics comparable to those of other commercially available nucleic acid-based assays for these organisms.

Chlamydia trachomatis and *Neisseria gonorrhoeae* infections are the most common bacterial sexually transmitted infections both in the United States and worldwide. The Centers for Disease Control and Prevention estimated that 2.5 to 3.3 million chlamydial infections and approximately one-fifth that number of gonococcal infections occurred in 1996 in the United States (2, 6). Individuals are often concomitantly infected with *C. trachomatis* and *N. gonorrhoeae* (20). Effective treatment exists for these infections, and the CDC Advisory Committee on HIV and STD Prevention strongly encourages active control programs targeting treatable sexually transmitted infections as a primary intervention in the human immunodeficiency virus epidemic (4). Untreated *C. trachomatis* and *N. gonorrhoeae* infections in women have both been linked to development of pelvic inflammatory disease (9, 12, 28, 29), ectopic pregnancy, and tubal factor infertility (23). Thus, detection and treatment of these infections deserves high priority.

Effective control programs have been instituted in several metropolitan areas in the United States and Canada (1, 13), as well as in several western European countries (19, 32), which have resulted in dramatic declines in the prevalence of both *C. trachomatis* and *N. gonorrhoeae* in targeted populations. However, such efforts are hampered by the difficulties inherent in detecting these infections. First, clinical management based on syndromic algorithms is often ineffective, as up to 70% of women and 50% of men with chlamydia do not demonstrate symptoms of disease (10, 21, 30). The insensitivity of clinical diagnosis has created a demand for laboratory testing for both *C. trachomatis* and *N. gonorrhoeae*. Second, identification of infected individuals is frequently hampered by the unavailability of sensitive culture systems. *N. gonorrhoeae* is a fastidious gram-negative bacterium which requires a CO_2-rich environment and specialized growth medium agar to be cultivated in a laboratory. *N. gonorrhoeae* culture can be performed in most laboratories associated with large health-care facilities, but samples collected at smaller clinics must be transported to centralized laboratories. During transportation of samples, maintenance of CO_2 concentration and an appropriate temperature (35 to 37°C) is crucial to the survival of organisms and therefore the sensitivity of the culture process. Therefore, although culture of *N. gonorrhoeae* can be highly sensitive (14), this type of testing may not be available in many settings. *C. trachomatis* is an obligate intracellular bacterium which can be cultivated only in tissue culture. Culture is performed only in highly specialized laboratories, and many factors, including sample collection, transportation conditions, cell lines, growth medium, and staining reagents, can have a dramatic impact on the sensitivity of *C. trachomatis* culture (10, 18, 26). Among

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laboratories that routinely perform tissue culture diagnosis of chlamydial infections, there is a high degree of variability in the sensitivity of the system in use (24, 31). Thus, even in clinical situations involving access to a testing facility, the sensitivity of the culture may be suboptimal for identification of infections, especially in asymptomatic populations. Finally, appropriate specimens for culture are endocervical swabs from women and urethral swabs from men. Screening of asymptomatic populations is facilitated by the use of minimally invasive or noninvasive specimens, which is not possible for culture of either *C. trachomatis* or *N. gonorrhoeae*.

In response to the shortcomings of *C. trachomatis* and *N. gonorrhoeae* culture as a means of wide-scale screening, assays have been developed which do not require viable organisms. The most recent of these, nucleic acid amplification tests (NAAT), target species-specific sequences of DNA for detection of infection. These assays are capable of detecting either or both organisms if present in a single patient specimen, and many of these assays can be performed on urine specimens, which are better suited for screening in asymptomatic populations. Here we describe a multicenter evaluation of a new NAAT which has been cleared by the Food and Drug Administration for identification of chlamydial and gonococcal infections. The BDProbeTec ET *Chlamydia trachomatis* and *Neisseria gonorrhoeae* Amplified DNA Assay (BDPT) utilizes a novel strand displacement amplification (SDA) method coupled with a fluorescent energy transfer (ET) measurement as the means of detection of amplified product. The BDPT is a rapid means of testing for *C. trachomatis* and *N. gonorrhoeae* simultaneously from swab or urine samples.

MATERIALS AND METHODS

Study population. Patients were enrolled at seven geographically diverse clinical sites that included both low- and high-prevalence populations. The clinical sites were The Cleveland Clinic Foundation (CCF), Indiana University (IU), Johns Hopkins University (JHU), University of California—San Francisco (UCSF), San Joaquin County Public Health Services (SJPHS), University of Alabama at Birmingham (UAB), and University of Maryland Medical System (UMMS). The sites included sexually transmitted disease (STD) clinics, obstetrics and gynecology clinics, family planning clinics, adolescent clinics, and emergency rooms. The majority of the patients were seen at STD or family planning clinics. This study was performed with the approval of each institution's institutional review board. Verbal or written informed consent was obtained from each enrolled patient. Patients who had taken antibiotics within the previous 21 days were excluded from the study.

Specimens. A urine specimen, followed by four endocervical swabs, was collected from female patients. The first swab was used to inoculate agar for *N. gonorrhoeae* culture. The remaining swabs were tested by *C. trachomatis* culture, BDPT, and LCx. The collection order of these three swabs was rotated throughout the study to minimize effects of sample variation. From males, two urethral swabs, followed by a urine specimen, were collected. The first swab was used to inoculate agar for *N. gonorrhoeae* culture and then was transported to the laboratory for the BDPTs. The second swab was used for *C. trachomatis* culture. Patients were instructed not to urinate during the hour prior to collection. Patients were then asked to provide 10 to 20 ml of urine; however, urine volumes of up to 90 ml were accepted. The urine collection cups were marked at 30 ml in order to facilitate compliance. All urine specimens were tested by both BDPT and LCx.

The specimens for the BDPT were stored at 2 to 30°C for up to 4 days prior to transportation and processing. For females, the CULTURETTE DIRECT Kit included two swabs. The large-tipped swab was used to clean the cervical os. The smaller swab was used to collect the sample for testing. The Mini-Tip CULTURETTE DIRECT was used to collect male specimens. Both male and female swabs were transported to the laboratory without transport medium. At the collection site, the urine was split and a Urine Processing Pouch, which contains resin and is intended to reduce the presence of inhibitors, was added to the collection cup (at least 10 ml of urine) for testing by BDPT. The remaining urine was stored and transported at a temperature between 2 and 8°C to the laboratory. Each laboratory's routine procedure was followed for the other specimen types and testing methods. Swab specimens for LCx were collected using the LCx sample collection kit.

Chlamydia cell culture. Chlamydia culture was performed using either 96-well microtiter plates or shell vials according to each laboratory's routine procedure (7, 31). Two sites performed a second passage on all cultures (UCSF and UAB). All sites used McCoy cells, with the exception of UMMS, which used Buffalo green monkey cells. Cells were evaluated for inclusions by immunofluorescent staining using monoclonal antibodies. Positive cultures were defined by finding at least one chlamydial inclusion in either the first or second passage.

N. gonorrhoeae culture. Specimens for *N. gonorrhoeae* culture were collected and immediately plated onto agar. All sites used modified Thayer Martin agar, except CCF, which used Martin Lewis medium. Cultures were examined after incubation at 37°C for 24 to 48 h in the presence of 5 to 10% CO_2. Gram-negative, oxidase-positive specimens were presumptively positive for *N. gonorrhoeae*. All presumptively positive colonies were confirmed using a biochemical method and at least one other method, either fluorescent antibody staining (7, 15), Gonogen, or GenProbe direct probe.

LCx. Swabs collected in the LCx specimen transport kit and a portion of the urine sample were tested for *C. trachomatis* and *N. gonorrhoeae* according to the instructions in the manufacturer's package insert.

BDPT. The BDPT system allows for the detection of DNA from *C. trachomatis* and *N. gonorrhoeae*. The amplification region for *C. trachomatis* is located in the chlamydial cryptic plasmid. Up to 10 plasmid copies can be present in each organism. The amplification region for *N. gonorrhoeae* is found in the chromosomal pilin gene-inverting protein homologue, which exists in multiple copies. A separate amplification control that contains a nucleic acid target sequence is available to confirm the absence of inhibitory substances in patient samples. The system is based on simultaneous SDA and detection of target DNA by the use of amplification primers along with a fluorescently labeled ET detector probe (16). Swab samples were eluted into 2 ml of sample diluent. Four milliliters of urine was centrifuged at 2,000 × *g* for 30 min and the resulting pellet was resuspended in 2 ml of sample diluent. All specimens in sample diluent were lysed by heating at 114°C for 30 min and allowed to cool to room temperature over at least 15 min. Processed samples were added to microwell strips to react with the SDA priming components. Samples remained in the priming wells at room temperature for at least 20 min and up to 6 h. Priming was completed by incubation at 72.5°C for exactly 10 min followed by transfer to amplification wells that had been preheated to 54°C. Plates were then sealed and immediately placed into the BDPT instrument. This thermally controlled fluorescent reader incubates the reaction mixture and monitors the real-time formation of amplification products. The system is capable of performing a total of 96 specimen and control reactions simultaneously. Results were reported through a computer-calculated algorithm as positive, negative, or indeterminate. Indeterminate results occurred when the *C. trachomatis, N. gonorrhoeae*, and amplification control (AC) were all negative, indicating inhibition of amplification. The assays are not multiplexed; *C. trachomatis* and *N. gonorrhoeae* assays are performed in separate reaction wells. A separate AC, designed to detect samples that may inhibit the SDA reaction, may be run on each sample. The purpose of the AC is to detect potentially inhibitory samples and to serve as an internal control to detect problems that could occur during sample processing and assay procedure steps (3, 17, 25). Samples with initial indeterminate results were repeated.

DFA staining. Direct fluorescent antibody (DFA) *C. trachomatis* staining was performed on specimens that were positive by either BDPT or LCx and negative by *C. trachomatis* culture. Slides were prepared by cytospin of 100 μl of the chlamydia culture transport medium (CTM) and stained with the Syva Micro Trak (Palo Alto, Calif.) *C. trachomatis* direct specimen reagent. Slides were considered positive for DFA staining if at least two elementary bodies were observed.

PCR. A third male swab was not collected for testing by LCx. Therefore, Amplicor PCR (Roche Diagnostic Corporation, Indianapolis, Ind.) was performed using the CTM remaining from the urethral *C. trachomatis* culture in cases where the BDPT or urine LCx was positive and the *C. trachomatis* culture was negative. This was intended to assist with adjudication of the infection status of men without incurring the discomfort of an additional swab. PCR was performed according to the instructions in the manufacturer's package insert.

Data analysis. Performance estimates for the BDPT were calculated compared to both culture and patient infection status. Female patients were defined as infected with *C. trachomatis* if (i) the endocervical culture was positive, (ii) the swab or the urine LCx result was positive and DFA staining was positive, or (iii) both the LCx swab and urine results were positive. Women were defined as

TABLE 1. *C. trachomatis* assay performance by specimen type

Specimen type	Patient status[a]	(*n*)	No. of culture-positive samples	BDPT vs culture		LCR vs culture		No. of patients with positive infection status[b]	BDPT vs patient infection status	
				% Sensitivity	% Specificity	% Sensitivity	% Specificity		% Sensitivity	% Specificity
Female										
Swabs	S	(599)	55	90.9	97.6	89.1	98.0	62	88.7	98.5
	A	(820)	47	100	96.1	100	97.2	63	96.8	97.9
	Subtotal	(1,419)	102	96.1	96.7	94.1	97.5	125	92.8	98.1
Urine	S	(574)	54	76.9	97.3	70.4	97.5	61	77.0	98.4
	A	(762)	46	91.3	96.9	82.6	97.5	62	83.9	98.3
	Subtotal	(1,336)	100	83.0	97.1	76.0	97.5	123	80.5	98.4
Male										
Swabs	S	(492)	96	95.8	89.9			125	93.6	95.9
	A	(186)	17	88.2	95.9			21	85.7	97.6
	Subtotal	(678)	113	94.7	91.7			146	92.5	96.4
Urine	S	(488)	95	95.8	86.5	92.6	89.1	124	94.4	92.6
	A	(187)	17	88.2	94.7	88.2	94.1	21	85.7	96.4
	Subtotal	(675)	112	94.6	89.0	92.0	90.6	145	93.1	93.8
Total		(4,108)	427	92.0	94.9	87.6	96.2	539	90.0	97.3

[a] S, symptomatic; A, asymptomatic.
[b] For females, a patient is considered infected if (i) culture is positive, (ii) LCR is positive in either swab or urine and DFA stain is positive, or (iii) LCR is positive in swab and urine. For males, a patient is considered infected if (i) culture is positive or (ii) LCR urine is positive and either DFA staining or PCR is positive.

having *N. gonorrhoeae* if (i) the endocervical culture was positive or (ii) both the swab and the urine LCx results were positive. Male patients were defined as infected with *C. trachomatis* if (i) the urethral culture was positive or (ii) the urine LCx result was positive and DFA staining or PCR of the CTM was positive. PCR of the CTM was used in lieu of an LCx swab but was performed only on culture-negative, LCx-positive samples. This testing was performed regardless of the BDPT result. Men were defined as infected with gonococci if the urethral culture was positive. At the time this trial was being performed, the only other amplified *N. gonorrhoeae* assay available for comparison was the LCx, and for males this was performed only on urine samples. Sensitivity, specificity, and positive and negative predictive values (PPV and NPV, respectively) were calculated by comparison to both culture results and patient infection status. Specimens with final BDPT results defined as indeterminate were not used in the calculations of performance estimates when AC data were included in the evaluation. In addition, performance estimates for the BDPT and LCx for female samples and male urines were compared using both the culture and infected patient standards. BDPT sensitivity and specificity were also calculated for patients coinfected with both *C. trachomatis* and *N. gonorrhoeae*.

RESULTS

A total of 4,131 specimens were collected from 2,109 patients. A total of 142 pregnant patients were enrolled. Twenty-two patient samples were excluded from the analysis of the *C. trachomatis* test's performance because of contaminated cell cultures and one specimen was excluded because a DFA staining result was missing. A total of 26 subjects' *N. gonorrhoeae* test results were excluded from the data analysis. Of these, 15 were excluded due to culture contamination and 11 were excluded due to failure to collect a swab for culture. Therefore, a total of 4,108 *C. trachomatis* and 4,105 *N. gonorrhoeae* specimens from 2,109 patients were used in the final data analysis. Paired specimens, from swabs and urine, were collected from 2,020 of the 2,109 patients. As expected, performance characteristics determined by comparison with *C. trachomatis* or *N. gonorrhoeae* culture consistently resulted in higher sensitivity and lower specificity than those calculated by comparison with the infected patient standard.

***C. trachomatis* detection from female specimens.** Of the 1,419 cervical swab specimens tested, 125 (8.8%) were identified as infected with *C. trachomatis* (Table 1). Culture of cervical swab samples identified 102 (81.6%) of the infected patients. Overall, the sensitivity of swabs using BDPT was similar to that seen using LCx (92.8 and 94.4%, respectively; $P > 0.05$). The specificity of BDPT was also similar to that of LCx (98.1 and 99.1%, respectively; $P > 0.05$). The ranges of prevalence, sensitivity, and specificity obtained for the two assays and culture at the different sites are shown in Table 2. The PPV for swab specimens at the various sites ranged from 75.1 to 100%. The NPV was greater than 97% at all sites. Only nine (0.6%) swab specimens were initially inhibitory and gave indeterminate results. All but one of the nine samples had satisfactory AC results following repeat testing. All of the nine specimens were from uninfected patients. Use of the AC with this specimen type had no effect on the calculated sensitivity and specificity of the BDPT.

Of the 1,336 female patients from whom urine was tested, 123 (9.2%) were identified as infected with *C. trachomatis* (Table 1). Cervical cultures from 100 (81.3%) of these patients were positive. Although the overall sensitivity with urine in the BDPT was lower than that with swabs (92.8 versus 80.5%; $P < 0.001$), it was comparable to the sensitivity of the LCx assay using urine (80.5 and 77.2%, respectively; $P > 0.05$). The specificity of the BDPT for urine specimens was equivalent to that of LCx (98.4 and 99.0%, respectively; $P > 0.05$). The performance of the BDPT and LCx assays using urine at each

TABLE 2. Performance of BDPT and LCx for detection of *C. trachomatis*, by clinical site

Specimen type	Clinical site	Prevalence (%)	No. of patients with positive infection status/*n*	BDPT vs patient infection status				LCR vs patient infection status				Culture vs patient infection status	
				% Sensitivity	% Specificity	% PPV	% NPV	% Sensitivity	% Specificity	% PPV	% NPV	% Sensitivity	% Specificity
Female													
Swabs	CCF	11.5	3/26	100	95.7	75.1	100	100	100	100	100	100	100
	IU	13.4	25/186	92.0	95.7	76.8	98.7	96.0	98.8	92.5	99.4	76.0	100
	JHU	9.0	10/111	70.0	99.0	87.4	97.1	60.0	99.0	85.6	96.2	80.0	100
	UCSF	5.3	7/133	100	100	100	100	100	100	100	100	100	100
	SJPHIS	4.4	22/498	95.5	99.2	84.6	99.8	100	99.4	88.5	100	59	100
	UAB	15.1	26/171	92.3	98.6	92.1	98.6	92.3	99.3	95.9	98.6	99.3	100
	UMMS	10.9	32/294	98.9	96.6	77.7	99.6	100	98.5	89.1	100	88	100
Urine	CCF	12.5	3/24	100	100	100	100	100	100	100	100		
	IU	13.5	25/185	72.0	97.5	81.8	95.7	68.0	98.1	84.8	95.2		
	JHU	9.0	10/111	50.0	100	100	95.3	40.0	100	100	94.4		
	UCSF	4.8	6/125	100	99.2	86.3	100	100	98.3	74.8	100		
	SJPHS	4.8	22/439	95.5	98.3	73.9	99.8	100	99.5	91	100		
	UAB	15.1	25/164	76.0	97.1	82.3	95.8	72.0	97.8	85.3	95.2		
	UMMS	11.6	32/275	84.4	98.4	87.4	98	78.1	99.2	92.8	97.2		
Male													
Swabs	IU	20.7	61/294	98.4	96.1	86.8	99.6					85.2	100
	JHU	19.8	39/197	89.7	98.1	92.1	97.5					84.6	100
	UCSF	9.1	1/11	100	100	100	100					100	100
	UAB	25.4	43/169	88.4	95.2	86.3	96					60.5	100
	UMMS	28.6	2/7	50.0	80.0	50	80					50.0	100
Urine	IU	20.7	61/295	98.4	94.0	81.1	98.6	93.4	97.0	89	98.3		
	JHU	19.4	38/196	89.5	93.7	77.4	97.4	92.1	96.2	85.4	98.1		
	UCSF	10.0	1/10	100	100	100	100	100	100	100	100		
	UAB	25.7	43/167	90.7	93.5	82.8	96.7	95.3	95.2	87.3	98.3		
	UMMS	28.6	2/7	50.0	80.0	50	80	100	80.0	66.7	100		

of the sites is shown in Table 2. The site-specific prevalence ranged from 4.8 to 15.1%. The PPV for urine in the BDPT ranged from 73.9 to 100%, and the NPV was consistently above 95%. Unlike the swab specimens, a substantial number of urine specimens failed to generate positive AC results. Of all urine samples, 161 (12.1%) were initially inhibitory: 7 (5.7%) samples from positive patients and 154 (12.7%) samples from negative patients had initial indeterminate results. Following repeat testing, 4 (57.1%) and 76 (49.4%) specimens from positive and negative patients, respectively, gave valid test results.

***C. trachomatis* detection from male specimens.** Of the 678 swab specimens tested, 146 (21.5%) were determined to be positive for *C. trachomatis* (Table 1). Culture identified 113 (77.4%) of the infected patients. Prevalence in the symptomatic population was significantly higher than in the asymptomatic group (25.4 and 11.3%, respectively; $P < 0.0001$). LCx was not performed on male swabs. The performance of the BDPT and LCx assays and culture at the different sites is shown in Table 2. The prevalence ranged from 9.1 to 28.6% at the six sites that enrolled male patients. The PPV at the study sites ranged from 50 to 100%. The site (UMMS) with unusually low performance values enrolled only seven males. If the two sites (UMMS and UCSF) with less than 20 enrollees are excluded from consideration due to the small number of patients, the PPV ranges from 86.3 to 92.1%. The NPV was 80% or higher at all sites. If the data from UMMS is not included, the NPV at the remaining sites was at least 96%. Only two swab specimens gave initially indeterminate results, and use of the AC had no effect on the sensitivity and specificity of the BDPT with this specimen type.

Urine was collected from 675 men, 145 (21.5%) of whom were identified as infected with *C. trachomatis* (Table 1). Urethral culture detected 112 (77.2%) of the infected patients. The prevalence ranged from 10.0 to 28.6% at the five participating sites (Table 2). The sensitivities of BDPT and LCx for urine specimens were similar (93.1% and 93.8%, respectively; $P < 0.05$). The specificity of BDPT with male urine (93.8%) was less than that of LCx (96.2%) ($P < 0.05$). The sensitivity and specificity of the two assays using male urine samples at each study site are shown in Table 2. The PPV ranged from 50 to 100%, and as with the swab results, if the sites with less than 20 enrollees are excluded, the PPV was 77.4 to 82.8%. The NPV was at least 80% when all sites were included and greater than 96% when the exclusion mentioned above was made during analysis. As with the female urine specimens, AC failure was seen more often in male urine samples than in swab specimens. Thirty-four (5.0%) were initially indeterminate, and all but 13 (38.2%) were resolved by repeat testing. None of the AC failures occurred in positive patient specimens. There was no change in the sensitivity and specificity of the BDPT results for male urine samples when the AC was included in the analysis.

***N. gonorrhoeae* detection from female specimens.** Swabs from 1,411 female patients were tested for *N. gonorrhoeae*, and 89 (6.3%) were identified as positive (Table 3). Culture identified 83 (93.2%) of the infected patients. Because of the excellent sensitivity of culture, the sensitivity of the BDPT was actually higher when compared to the patient-infected stan-

TABLE 3. *N. gonorrhoeae* assay performance by specimen type

Specimen type	Patient status[a]	(*n*)	No. of culture-positive samples	BDPT vs culture		LCR vs culture		No. of patients with positive infection status[b]	BDPT vs patient infection status	
				% Sensitivity	% Specificity	% Sensitivity	% Specificity		% Sensitivity	% Specificity
Female										
Swabs	S	(600)	48	95.8	98.7	95.8	0.3	51	96.1	99.3
	A	(811)	35	97.1	99.2	85.7	99.5	38	97.4	99.6
	Subtotal	(1,411)	83	96.4	99.0	91.6	99.4	89	96.6	99.5
Urine	S	(577)	46	84.8	99.2	87.0	98.9	49	83.7	99.6
	A	(754)	34	88.2	99.0	76.5	99.0	37	86.5	99.3
	Subtotal	(1,331)	80	86.3	99.1	82.5	99.0	86	84.9	99.4
Male										
Swabs	S	(496)	190	96.4	94.8			190	98.4	94.8
	A	(187)	4	100	99.5			4	100	99.5
	Subtotal	(683)	194	98.5	96.5			194	98.5	96.5
Urine	S	(492)	189	97.9	94.4	93.7	94.4	189	97.9	94.4
	A	(188)	4	100	100	100.0	98.9	4	100	100
	Subtotal	(680)	193	97.9	96.5	93.8	96.1	193	97.9	98.5
Total	Total	(4,105)	550	96.2	98.4	90.7	98.7	562	95.9	98.6

[a] S, symptomatic; A, asymptomatic.
[b] For females, a patient is considered infected (i) if culture is positive or (ii) if LCR is positive in swab and urine. For males, a patient is considered infected if culture is positive.

dard. The sensitivity of BDPT was equivalent to that of LCx (96.6 and 92.1%, respectively), as was the specificity (>99.5%) (both with *P* values of >0.05). The performance characteristics of the two assays and culture at the various sites are shown in Table 4. The prevalence of *N. gonorrhoeae* at the seven sites was 0 to 13.3%. The PPV for the different sites ranged from 75.2 to 100%, while the NPV was consistently greater than 98.5%. Only eight specimens had indeterminate results initially. Of these, seven were from uninfected patients and all but one were resolved by repeat testing. There was a minimal increase in sensitivity and no change in specificity when results were analyzed using AC results.

Urine was collected from 1,331 female patients, 86 (6.5%) of whom had *N. gonorrhoeae* (Table 3). Culture detected 80 (93.0%) of the infected patients while BDPT identified 83.7%. The performance of BDPT using urine samples was comparable to the performance of LCx using urine specimens. The specificity of the BDPT *N. gonorrhoeae* assay with urine was above 99%, equivalent to the specificity of LCx. The sensitivity and specificity of the BDPT for *N. gonorrhoeae* are shown in Table 3. The prevalence ranged from 0 to 13.2% among the study sites. The PPV of the BDPT was between 54.8 and 100%. The site with the PPV of 54.8% had a prevalence of 1.2%, the lowest of the study sites that identified *N. gonorrhoeae* in the study population. The NPV ranged from 95.3 to 100%. The performance of all assays by site is shown in Table 4. A total of 174 (13.1%) specimens had initial indeterminate results. One of these specimens was from an infected patient and 79 (45.4%) were from symptomatic patients. Following repeat testing, 88 (50.6%) of the samples were amplified successfully, including the sample from the positive patient. Since the AC failures were predominately from negative patients, using the AC had no effect on the sensitivity or specificity of the assay.

***N. gonorrhoeae* detection from male specimens.** Swab specimens for *N. gonorrhoeae* culture were collected from 683 men, of whom 72.6% were symptomatic and 194 (28.4%) were culture positive (Table 3). Since culture was used as the sole definition of infected patients, it was arbitrarily assumed to have 100% sensitivity. Interestingly, 190 (97.9%) of the positive patients were symptomatic. The remaining 4 infections were identified from among the 188 asymptomatic men enrolled. The sensitivity and specificity of the BDPT were 98.5 and 96.5%, respectively. In order to avoid collection of an extra urethral swab from male subjects, LCx was not performed on swabs from males, so no comparison was possible. Performance characteristics described by site are shown in Table 4. The prevalence ranged from 0 to 42.9% at the five sites that enrolled male subjects. The PPV in these high-prevalence populations ranged from 81.8 to 100%, and the NPV was always above 98%. AC failure occurred in two (0.3%) of the swab specimens. Neither was from a positive patient and both were resolved with repeat testing. Use of the AC had no effect on the performance of the BDPT for *N. gonorrhoeae*.

Urine was obtained from 680 men; 72.4% were symptomatic and 193 (28.4%) were positive (Table 3). Of the positive patients, 189 (97.9%) were symptomatic. The sensitivity of BDPT was high (97.9%) and comparable to the sensitivity of LCx (93.8%) ($P < 0.05$). The specificity with male urine was also similar to that of LCx (96.5 and 96.1%, respectively). The performance of BDPT and LCx using male urine at the various sites is shown in Table 4. The prevalence of *N. gonorrhoeae* ranged from 0 to 42.9% at the five sites. The PPV obtained

TABLE 4. Performance of BDPT and LCx for detection of *N. gonorrhoeae*, by clinical site

Specimen type	Clinical site	Prevalence (%)	No. of patients with positive infection status/*n*	BDPT vs patient infection status				LCR vs patient infection status				Culture vs patient infection status	
				% Sensitivity	% Specificity	% PPV	% NPV	% Sensitivity	% Specificity	% PPV	% NPV	% Sensitivity	% Specificity
Female													
Swabs	CCF	NA[a]	0/26	NA	100	NA	100	NA	100	NA	100	NA	100
	IU	12.3	23/187	100	100	100	100	95.7	100	100	99.4	95.7	100
	JHU	13.3	15/113	93.3	99.0	93.5	99	93.3	100	100	99	80.0	100
	UCSF	3.0	4/132	100	100	100	100	100	100	100	100	100	100
	SJPHS	1.2	6/486	100	99.6	75.2	100	66.7	100	100	99.6	100.0	100
	UAB	11.7	20/171	90.0	98.0	85.6	98.7	95.0	98.7	90.6	99.3	100	100
	UMMS	7.1	21/296	100	99.6	95	100	90.5	100	100	99.3	90.5	100
Urine	CCF	NA	0/24	NA	100	NA	100	NA	95.8	NA	100		
	IU	12.4	23/186	91.3	99.4	95.6	98.8	69.6	99.4	94.3	95.9		
	JHU	13.2	15/113	66.7	100	100	95.2	93	100	100	99		
	UCSF	3.2	4/124	100	100	100	100	100	100	100	100		
	SJPHS	1.2	5/430	80.0	99.3	54.8	99.8	60.0	99.5	59.3	99.5		
	UAB	12.2	20/164	90.0	100	100	98.6	90.0	97.9	85.6	98.6		
	UMMS	6.6	19/290	84.2	98.9	84.4	98.9	89.5	100	100	99.3		
Male													
Swabs	IU	28.6	84/294	98.8	99.0	97.5	99.5					100	100
	JHU	26.7	54/202	100	91.9	81.8	100					100	100
	UCSF	NA	0/11	NA	100	NA	100					NA	100
	UAB	31.4	53/169	98.2	97.4	94.4	98.3					100	100
	UMMS	42.9	3/7	100	100	100	100					100	100
Urine	IU	28.5	84/295	95.2	98.6	98.4	98.1	94.0	99.0	97.4	97.6		
	JHU	26.9	54/201	100	92.5	83.1	100	96.3	91.8	81.2	98.5		
	UCSF	NA	0/10	NA	100	NA	90	NA	100	NA	100		
	UAB	31.1	52/167	100	97.4	94.6	100	90.4	95.7	90.5	95.7		
	UMMS	42.9	3/7	100	100	100	100	100	100	100	100		

[a] NA, not available.

using urine ranged from 83.1 to 100%, and the NPV was greater than 98% at each of the study centers. Forty-two urine specimens had initially indeterminate results. None of these were from positive patients and 24 (57.1%) were resolved by repeat testing. The AC assay had no impact on the performance of the BDPT for *N. gonorrhoeae*.

Performance of BDPT on samples from coinfected patients. A total of 73 patients, 30 female and 43 male, were infected with both *C. trachomatis* and *N. gonorrhoeae* (Table 5). BDPT on the female swab samples from coinfected patients performed slightly better than on urine samples, with swab *C. trachomatis* and *N. gonorrhoeae* sensitivities of 93.3 and 100%, respectively, compared to urine sensitivities of 83.3 and 86.7% for *C. trachomatis* and *N. gonorrhoeae*, respectively. However, the performance of BDPT on urine in female patients with coinfections was marginally better for both *C. trachomatis* and *N. gonorrhoeae* than on urine from patients in whom a single infection was identified. BDPT on urine from male patients performed slightly better than on swab specimens, with *C. trachomatis* sensitivities of 93.0 and 86.0%, respectively, and *N. gonorrhoeae* sensitivities of 97.7 and 95.3% for urine and swabs, respectively. The sensitivity for *C. trachomatis* and *N. gonorrhoeae* was not markedly different in patients with a single infection compared to coinfected patients.

Performance of BDPT on samples from pregnant patients. Separate performance characteristics were calculated for specimens collected for the 142 pregnant females enrolled in the study. *C. trachomatis* sensitivity and specificity compared to patient infection status for female swabs were 94.4% (17 of 18) and 98.4% (122 of 124), respectively, and for urines were 83.3% (15 of 18) and 100% (120 of 120), respectively. For *N. gonorrhoeae*, sensitivity and specificity compared to patient infection status for female swabs were 100% (2 of 2) and 98.6% (137 of 139), respectively, and for urines were 100% (2 of 2) and 98.5% (133 of 135), respectively.

Effect of sample characteristics on performance of BDPT. Most of the bloody specimens were from female endocervical swabs. Of the 1,419 endocervical swabs collected, 101 were classified as grossly bloody and 242 as moderately bloody. No statistical differences in sensitivity or specificity were found for either the *C. trachomatis* ($P = 0.20$ and 0.65, respectively) or *N. gonorrhoeae* assay ($P = 0.28$ and 0.71, respectively). In addition, the effect of urine volume on assay performance was analyzed separately for male and female urine specimens. Decreased sensitivity in larger volumes for male or female specimens was not observed for either the *C. trachomatis* or *N. gonorrhoeae* assay. Some variability in sensitivity estimates was observed; however, a trend toward lower sensitivity was not observed.

DISCUSSION

In today's laboratory environment, there is a conflict between cost containment and the introduction of new technology, such as molecular amplification methods for STD testing. While amplification testing offers superior performance that is

TABLE 5. BDPT sensitivity in patients infected with *C. trachomatis* or *N. gonorrhoeae* only compared with coinfected patients

Specimen type	Patient status[a]	No. of coinfected patients	% *C. trachomatis* sensitivity		% *N. gonorrhoeae* sensitivity	
			C. trachomatis only patients	Coinfected patients	*N. gonorrhoeae*-only patients	Coinfected patients
Female						
Swabs	S	14	89.6	85.7	94.6	100.0
	A	16	95.7	100.0	95.5	100.0
	Subtotal	30	92.6	93.3	94.9	100.0
Urine	S	14	76.5	78.6	82.9	85.7
	A	16	82.6	87.5	85.7	87.5
	Subtotal	30	79.6	83.3	84.0	86.7
Male						
Swabs	S	43	97.6	86.0	99.3	95.3
	A	0	85.7	[b]	100.0	
	Subtotal	43	95.2	86.0	99.4	95.3
Urine	S	43	95.1	93.0	98.0	97.7
	A	0	85.7		100.0	
	Subtotal	43	93.1	93.0	98.0	97.7
Total		146	90.4	89.0	96.1	95.2

[a] S, symptomatic; A, asymptomatic.
[b] NA, not available.

cost-effective in many cases (9, 10, 29), there are also technical challenges associated with the optimal performance of these assays. Many are still laborious and require technical expertise. Amplification technology also allows the use of less-invasive samples such as urine for STD testing.

The BDPT addresses many of the problems inherent with amplification testing, using previously available NAAT while maintaining the advantages of high sensitivity and specificity. The system offers higher throughput than other available NAAT and there is no need for separate work areas or unidirectional workflow. The assay also provides an optional AC, the ability to obtain results for both *C. trachomatis* and *N. gonorrhoeae* from a single urine or swab sample, medium-free transport for swabs, and room temperature storage of reagents and samples. The total assay time ranges from 3 to 4 h, depending on the specimen type and quantity. Swab specimens are stable for up to 6 days at 2 to 27°C and urine specimens are stable for up to 2 days at 2 to 27°C or 6 days at 2 to 8°C.

As presented previously, the BDPT performance is comparable to that of other available commercial amplification methods, and these consistently perform better than *C. trachomatis* culture (27). Because of the higher sensitivity of amplified assays compared to culture, use of culture results as the only standard artificially increases the sensitivity and decreases the specificity calculated for the NAAT. Therefore, the performance characteristics of BDPT were compared to those calculated for LCx. Comparison of male swab *N. gonorrhoeae* results was not possible, since an additional swab specifically for LCx was not collected. Therefore, the results presented here may be an underestimation of the specificity and an overestimation of the sensitivity of this assay for *N. gonorrhoeae* in male specimens.

A potential limitation of this analysis was the use of a single assay from two body sites as a defining criterion for infected patients. The use of a single assay to define infected patients (e.g., positive LCx results from both the swab and urine sample of a culture-negative patient) may lead to incorrect classification of a small number of patients. Theoretically, an LCx positive result from both the cervix and urine of a culture-negative patient may not indicate an infection but merely a reproducible false-positive result. However, based on the previously established performance of LCx (5, 14, 20), the probability of two independent samples both giving false-positive results should be minimal. While specimen processing and handling errors may result in inaccurate results, no systematic cross-reactivity with common urogenital flora has been demonstrated for the LCx assay. Therefore, the design of this study attempted to avoid the inherent problems associated with evaluation of an assay whose performance exceeds that of the existing standard (e.g., culture) by running two LCx assays for all female samples and a urine LCx for all males enrolled. At the time this study was performed, no other NAAT was commercially available for both *C. trachomatis* and *N. gonorrhoeae* testing. The use of an alternate amplification assay to define infected patients would have been difficult and expensive, with little likely benefit.

Sensitivity and specificity for male urine samples and male swabs were equivalent in the aggregate data. However, as previously reported for all other FDA-approved NAAT (5), a lower sensitivity was observed for female urine samples than for endocervical swabs for both *C. trachomatis* and *N. gonorrhoeae* assays. In the populations studied, female urine samples were more likely to produce indeterminate results than their paired swab specimens, indicating the more inhibitory nature

of urine specimens. The data indicate that a very low level of indeterminate results, i.e., inhibition (<1.0%), is observed for swab specimens. However, indeterminate rates for female urine specimens were higher (12% initially and 6% after repeat testing).

High indeterminate rates were observed at several of the clinical sites during the studies. (Three of the seven sites had initial indeterminate rates twice as high as the other sites [16 versus 8%]). The clinical sites were instructed to decant forcefully (i.e., decant spun urine with a flick of the wrist to make sure excess urine was removed). This resulted in a lowering of the number of indeterminate results from these sites. Testing of indeterminate results performed at BD Biosciences after study completion demonstrated that proper processing could drastically reduce the number of indeterminate results. Out of 42 urine samples tested, only 3 yielded indeterminate results after more rigorous residual urine removal. This procedure dramatically reduced the number of final indeterminate samples. The difference between sites is probably best explained by variations in technique.

Differences were also observed among the sensitivities of all diagnostic methods between the sites. The sensitivity varied by specimen type as well as by assay type. The range of sensitivity observed for both *C. trachomatis* and *N. gonorrhoeae* testing was generally smaller for the BDPT than for LCx. Interlaboratory differences in technique may also account for the reduced sensitivity of LCx seen in this study compared to the figures reported in the literature. Other investigators have questioned the reproducibility of both PCR and ligase chain reaction, indicating that variability of results also occurs within a single laboratory (8, 22). Variability in the performance of kit-based assays emphasizes the need for both formal training in assay protocols and equipment maintenance and strict adherence to package insert instructions when performing these assays.

In conclusion, the BDPT ET *C. trachomatis* and *N. gonorrhoeae* assays proved to be sensitive and specific in the populations studied. The small number of asymptomatic males enrolled in this study limits the conclusions that may be drawn concerning the performance of this assay in such populations. Investigators agreed that the BDPT was easy to use, required low maintenance, and had high throughput. The closed-system design and assay workflow were seen as advantageous. The ability of the assay to be performed on samples collected without transport medium and transported at room temperature provides a significant advantage over currently available NAAT.

As public health initiatives expand into large-scale screening programs aimed at asymptomatic or low-prevalence populations, it will be crucial to be able to interpret the results of assays which lend themselves to noninvasively collected samples. Therefore, further studies in low-prevalence and asymptomatic populations are needed to evaluate the performance of this assay, as well as other commercially available NAAT, in those situations. Additional studies of the performance of the BDPT, focusing on asymptomatic males, are currently under way.

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REFERENCES

1. **Addiss, D. G., M. L. Vaughn, D. Ludka, J. Pfister, and J. P. Davis.** 1993. Decreased prevalence of *Chlamydia trachomatis* infection associated with a selective screening program in family planning clinics in Wisconsin. Sex. Transm. Dis. **20:**28–35.
2. **American Social Health Association.** 1998. Sexually transmitted diseases in America: how many cases and at what cost? Kaiser Family Foundation, Menlo Park, Calif.
3. **Black, C. M.** 1998. "But Doctor, I'm celibate" the potential for, sources, and implications of false-positive (and -negative) results of tests for *Chlamydia trachomatis*. Clin. Microbiol. Newsl. **20:**120–124.
4. **Centers for Disease Control and Prevention.** 1998. HIV prevention through early detection and treatment of other sexually transmitted diseases—United States. Morb. Mortal. Wkly. Rep. **47**(RR-12)**:**1– 24.
5. **Ferrero, D. V., H. N. Meyers, and D. E. Schultz.** 1998. Performance of the Gen-Probe amplified *Chlamydia trachomatis* assay in detecting *Chlamydia trachomatis* in endocervical and urine specimens from women and urethral and urine specimens from men attending sexually transmitted disease and family planning clinics. J. Clin. Microbiol. **36:**3230–3233.
6. **Goessens, W. H. F., J. W. Mouton, W. I. van der Meijden, S. Deelen, T. H. van Rijsoort-Vos, Lemmens-den N. Toom, H. A. Verbrugh, and R. P. Verkooyen.** 1997. Comparison of three commercially available amplification assays, AMP CT, LCx, and COBAS AMPLICOR, for detection of *Chlamydia trachomatis* in first-void urine. J. Clin. Microbiol. **35:**2628–2633.
7. **Groscoe, S. L., A. A. Zaidi, S. J. DeLisle, W. C. Levine, and M. E. St. Louis.** 1999. Estimated incidence and prevalence of genital *Chlamydia trachomatis* infections in the United States, 1996. Sex. Transm. Dis. **26:**339–344.
8. **Grownowski, A. M., S. Copper, D. Baorto, and P. R. Murray.** 2000. Reproducibility problems with the Abbott Laboratories LCx assay for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. J. Clin. Microbiol. **38:**2416–2418.
9. **Howell, M. R., J. C. Gaydos, K. T. McKee, Jr., T. C. Quinn, and C. A. Gaydos.** 1999. Control of *Chlamydia trachomatis* infections in female army recruits: cost-effective screening and treatment in training cohorts to prevent pelvic inflammatory disease. Sex. Transm. Dis. **26:**519–526.
10. **Jones, C. A., R. C. Knaup, M. Hayes, and B. P. Stoner.** 2000. Urine screening for gonococcal and chlamydial infections at community-based organizations in a high-morbidity area. Sex. Transm. Dis. **27:**146–151.
11. **Jones, R. B., B. Van Der Pol, and B. P. Katz.** 1989. Effect of differences in specimen processing and passage technique on recovery of *Chlamydia trachomatis*. J. Clin. Microbiol. **27:**894–898.
12. **Kamwendo, F., L. Forslin, L. Bodin, and D. Danielsson.** 1998. Programmes to reduce pelvic inflammatory disease—the Swedish experience. Lancet **351**(S3)**:**25–28.
13. **Katz, B. P., M. J Blythe, B. Van Der Pol, and R. B. Jones.** 1996. Declining prevalence of chlamydial infection among adolescent girls. Sex. Transm. Dis. **23:**226–229.
14. **Koumans, E. H., R. E. Johnson, J. S. Knapp, and M. E. St. Louis.** 1998. Laboratory testing for *Neisseria gonorrhoeae* by recently introduced nonculture tests: a performance review with clinical and public health considerations. Clin. Infect. Dis. **27:**1186–1193.
15. **Laughon, B. E., J. M. Ehret, T. T. Tanino, B. Van Der Pol, H. H. Hansfield, R. B. Jones, F. N. Judson, and E. W. Hook, III.** 1987. Fluorescent monoclonal antibody for confirmation of *Neisseria gonorrhoeae* cultures. J. Clin. Microbiol. **25:**2388–2390.
16. **Little, M. C., J. Andrews, R. Moore, S. Bustos, L. Jones, C. Embres, G. Durmowicz, J. Harris, D. Berger, K. Yanson, C. Rostkowski, D. Yursis, J. Price, T. Fort, A. Walters, M. Collis, O. Llorin, J. Wood, F. Failing, C. O'Keffe, B. Scrivens, B. Pope, T. Hansen, K. Marino, K. Williams, et al.** 1999. Strand displacement amplification and homogeneous real-time detection incorporated in a second-generation DNA probe system, BDProbe TecET. Clin. Chem. **45:**777–784.
17. **Mahony, J., S. Chong, D. Jang, K. Luinstra, M. Faught, D. Dalby, J. Sellors, and M. A. Chernesky.** 1998. Urine specimens from pregnant and nonpregnant women inhibitory to amplification of *Chlamydia trachomatis* nucleic acid by PCR, ligase chain reaction and transcription mediated amplification: identification of urinary substances associated with inhibition and removal of inhibitory activity. J. Clin. Microbiol. **36:**3122–3126.
18. **Mahony, J. B., and M. A. Chernesky.** 1985. Effect of swab type and storage temperature on the isolation of *Chlamydia trachomatis* from clinical specimens. J. Clin. Microbiol. **22:**865–867.
19. **Meyer, I., V. Goulet, V. Massari, and A. Lepoutre-Toulemon.** 1994. Surveillance of sexually transmitted diseases in France: recent trends and incidence. Genitourin. Med. **70:**15–21.
20. **Morré, S. A., I. G. M. Van Valkengoed, R. M. Moes, A. J. P. Boeke, C. J. L. M. Meijer, and A. J. C. Van den Brule.** 1999. Determination of *Chlamydia trachomatis* prevalence in an asymptomatic screening population: performance of the LCx and COBAS Amplicor tests with urine specimens. J. Clin. Microbiol. **37:**3092–3096.
21. **Mosure, D., S. Berman, D. Fine, S. Delisle, W. Cates, Jr., and J. R. Boring III.** 1997. Genital chlamydia infections in sexually active female adolescents: do we really need to screen everyone? J. Adolesc. Health **20:**6–13.

22. **Mulcahy, G. M., E. A. Albanese, and B. L. Bachl.** 1998. Reproducibility of the Roche Amplicor polymerase chain reaction assay for detection of infection by *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in endocervical specimens. Clin. Chem. **44:**1575–1578.
23. **Paavonen, J., and W. Eggert-Kruse.** 1999. *Chlamydia trachomatis*: impact on human reproduction. Hum. Reprod. Update **5:**433–437.
24. **Pate, M. S., and E. W. Hook III.** 1995. Laboratory to laboratory variation in *Chlamydia trachomatis* culture practices. Sex. Transm. Dis. **22:**322–326.
25. **Rosenstrauss, M., Z. Wang, S.-Y. Chang, D. DeBonneville, and J. P. Spadoro.** 1998. An internal control for routine diagnostic PCR: design, properties and effect on clinical performance. J. Clin. Microbiol. **36:**191–197.
26. **Schachter, J., and P. B. Wyrick.** 1994. Culture and isolation of *Chlamydia trachomatis*. Methods Enzymol. **236:**377–390.
27. **Schachter, J.** 1997. DFA, EIA, PCR, LCR and other technologies: what tests should be used for diagnosis of chlamydial infections? Immunol. Investig. **26:** 157–161.
28. **Scholes, D., A. Stegachis, F. E. Heidrich, H. Andrilla, K. K. Holmes and W. E. Stamm.** 1996. Prevention of pelvic inflammatory disease by screening for cervical chlamydial infection. N. Engl. J. Med. **334:**1362–1366.
29. **Shafer, M. A., R. H. Pantell, and J. Schachter.** 1999. Is the routine pelvic examination needed with the advent of urine-based screening for sexually transmitted diseases? Arch. Pediatr. Adolesc. Med. **153:**119–125.
30. **Simms, I., M. Catchpole, R. Brugha, P. Rogers, H. Mallinson, and A. Nicoll.** 1997. Epidemiology of genital *Chlamydia trachomatis* infections in England and Wales. Genitourin. Med. **73:**122–126.
31. **Van Der Pol, B., T. C. Quinn, C. A. Gaydos, K. Crotchfelt, J. Schachter, J. Moncada, D., Jungkind D. H. Martin, B. Turner, C. Peyton, and R. B. Jones.** 2000. Multicenter evaluation of the Amplicor and automated Cobas Amplicor CT/NG tests for the detection of *Chlamydia trachomatis*. J. Clin. Microbiol. **38:**1105–1112.
32. **Walckiers D., P. Piot, A. Stroobant, J. Van der Veken, and E. Declercq.** 1991. Declining trends in some sexually transmitted diseases in Belgium between 1983 and 1989. Genitourin. Med. **67:**374–377.