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Reproducibility of Positive Test Results in the BDProbeTec ET System for Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*

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Nucleic acid amplification tests such as the BDProbeTec ET (BDPT) system are more prone to reproducibility problems than are antigen detection tests and culture. A repeat testing algorithm for all samples with method other than acceleration (MOTA) scores greater than or equal to the cutoff value (2,000) was developed for the BDPT system and applied in a clinical laboratory setting. All positive samples were retested, and if the result of the second test was below the cutoff value, a third test was performed to resolve the discrepancy. Overall, 11 (5.3%) of 207 samples initially positive for *Chlamydia trachomatis* and 11 (10.7%) of 103 samples initially positive for *Neisseria gonorrhoeae* were not confirmed by repeat testing of the original sample. Poor reproducibility was associated with low-positive MOTA scores (2,000 to 9,999) for both analytes. Only 21 (80.8%) of 26 low-positive samples in the *C. trachomatis* test and 4 (33.3%) of 12 low-positive samples in the *N. gonorrhoeae* test retested as positive. The reproducibility of both tests with samples with initial MOTA scores of ≥10,000 increased to 96.7%. The data suggest that retesting of low-positive samples is warranted and could reduce the number of potentially false-positive test results.

Nucleic acid amplification tests (NAATs) offer several advantages over culture and other methods for the detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in clinical specimens. These advantages include increased sensitivity, high throughput, no requirement for viable organisms, and the use of urine as an alternative to more difficult-to-obtain specimens. Disadvantages of NAATs include high cost, false-negative results due to the presence of amplification inhibitors in specimens, and false-positive results due to specimen cross-contamination.

The BDProbeTec ET (BDPT) system (Becton Dickinson and Company, Franklin Lakes, N.J.) uses strand displacement amplification and fluorescent resonance energy transfer probes to simultaneously amplify and detect the DNAs of *C. trachomatis* and *N. gonorrhoeae*. The amplification targets are DNA sequences in the cryptic plasmid of *C. trachomatis* and in the pilin gene-inverting protein homologue of *N. gonorrhoeae* (4). A recent multicenter evaluation of the BDPT system demonstrated that it has sensitivity superior to that of chlamydia culture and performance characteristics similar to those of other commercially available NAATs for these organisms (7). According to a recent College of American Pathologists survey (2003 HC6-A), the BDPT system was the most common NAAT used by participants for detection of *C. trachomatis* and *N. gonorrhoeae*.

NAATs are more prone than other tests to false-positive results due to specimen cross-contamination. False-positive test results are particularly problematic in low-prevalence patient populations, in which the impact on the positive predictive value of the tests is greatest. However, regardless of the population or health care setting, false-positive results for *C. trachomatis* and *N. gonorrhoeae* can have adverse medical, social, and psychological impacts on patients.

The Centers for Disease Control and Prevention has recently issued guidelines for the selection, use, and interpretation of screening tests to detect *C. trachomatis* and *N. gonorrhoeae* infections (2). These guidelines suggest several approaches by which to detect false-positive test results. The approaches include (i) testing of a second specimen with a different test that uses a different target, antigen, or phenotype and a different format; (ii) testing of the original specimen with a different test that uses a different target, antigen, or phenotype and a different format; (iii) repetition of the original test of the original specimen with a blocking antibody or a competitive probe; and (iv) repetition of the original test of the original specimen. However, only the last approach is practical in most clinical laboratories using NAATs because of different sample collection devices and requirements for the various tests, lack of confirmatory tests, and logistical problems in obtaining second samples from patients.

Problems with reproducibility of positive test results have been documented with the LCx (Abbott Laboratories, Abbott Park, Ill.) and AMPLICOR (Roche Diagnostics Corp., Indianapolis, Ind.) assays for detection of *C. trachomatis* and *N. gonorrhoeae* (1, 3, 6). The reproducibility of the BDPT system in a clinical laboratory setting has not been reported. We developed a repeat testing algorithm in order to document the reproducibility of positive BDPT system test results.

All samples were tested once for both *C. trachomatis* and *N. gonorrhoeae* according to the manufacturer's instructions, and samples with method other than acceleration (MOTA) scores of greater than or equal to 2,000 (cutoff value) for either *C. trachomatis* or *N. gonorrhoeae* were retested for both organisms in the next run by use of the same sample. The MOTA score is a metric used to assess the magnitude of the signal generated as a result of the reaction. The magnitude of the MOTA score is not indicative of the level of the organism in the specimen,

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TABLE 1. Reproducibility of BDPT positive test results by initial MOTA score

Test result	No. (%; 95% CI[a]) of tests with initial MOTA scores of:	
	2,000–9,999	>10,000
C. trachomatis		
Repeat positive[b]	21 (80.8; 65.7–95.9)	175 (96.7; 94.1–100)
Repeat negative[c]	5 (19.2; 0–45.9)	6 (3.3; 0.7–5.9)
N. gonorrhoeae		
Repeat positive	4 (33.3; 6.6–60)	88 (96.7; 93–100)
Repeat negative	8 (66.7; 40–93.4)	3 (3.3; 0–7)

[a] CI, confidence interval.
[b] Either two out of two or two out of three tests with MOTA scores of ≥2,000.
[c] One out of three tests with a MOTA score of ≥2,000.

since the amount of target is only one of many factors that influence the MOTA score.

If the MOTA score of the second test was above the cutoff, then the sample was considered positive and no further testing was performed. If the MOTA score of the second test was below the cutoff, then a third test was performed with the original sample in the next run. Samples with MOTA scores below the cutoff in the third test were considered negative, and those with MOTA scores above the cutoff were considered positive. Samples were stored at 2 to 8°C for up to 4 days before testing was completed. Samples stored for more than 6 h between repeat tests were vortexed and reheated before testing, as recommended by the manufacturer.

The laboratory provides service to internal medicine, obstetrics and gynecology, student heath clinics, and emergency departments at two hospitals. The prevalence of patients with test results positive for *C. trachomatis* or *N. gonorrhoeae* during the study period was 8.1 or 3.9%, respectively. Specimens were collected and transported to the laboratory in accordance with the manufacturer's instructions.

Testing was performed in a molecular diagnostic laboratory by six experienced medical technologists using multiple lots of reagents from 29 July 2002 to 5 March 2003. The amplification control provided by the manufacturer was included in tests from swab specimens until 1 February 2003 and in all urine tests. All positive and negative controls gave the expected values for all of the runs included in the data analysis. One run in which an obvious break in technique occurred, as indicated by failed negative controls, was excluded.

On the basis of the initial MOTA scores of greater than or equal to 2,000, *C. trachomatis* DNA was detected in 156 swab and 51 urine samples. The range of MOTA scores for the 207 positive samples was 2,118 to 40,642, with a median of 17,723. The reproducibility of the positive test results by the initial MOTA score category is shown in Table 1. Only 80.8% of the samples with initial MOTA scores between 2,000 and 9,999 were positive when retested. The reproducibility of results for samples with initial scores of greater than or equal to 10,000 was 96.7%. All of the 38 samples with scores greater than or equal to 27,000 were reproducibly positive.

The initial positive results for *C. trachomatis* were confirmed for 194 samples (93.7%) with the first retest. The MOTA scores of the 13 samples that required a third test to resolve a discordance between the results of the first two tests are shown in Table 2. The initial MOTA scores of these samples ranged from 2,118 to 26,882, and only two were resolved as positives by our algorithm. Discordant results were found with all specimen types, occurred throughout the study period, and were not associated with any one operator (Table 2).

On the basis of initial MOTA scores of greater than or equal to 2,000, *N. gonorrhoeae* DNA was detected in 73 swab and 30 urine samples. The range of the MOTA scores of the 103 positive samples was 2,235 to 51,699, with a median of 27,986. The reproducibility of the positive test results by initial MOTA score category for the *N. gonorrhoeae* test is shown in Table 1. Only 33.3% of the samples with MOTA scores between 2,000 and 9,999 were positive when retested. The reproducibility of results from samples with MOTA scores greater than or equal to 10,000 was 96.7%. All of the 69 samples with initial scores greater than or equal to 25,000 retested as positive.

The initial positive results for *N. gonorrhoeae* were confirmed for 90 samples (87.4%) with the first retest. The MOTA scores of the 13 samples that required a third test to resolve a discordance between the results of the first two tests are shown in Table 3. The initial MOTA scores of these samples ranged from 2,235 to 23,946, and only two were resolved as positives.

TABLE 2. Discordant-result analysis of samples positive in the BDPT *C. trachomatis* test

Sample no.	Sample type (gender[a])	Run 1		MOTA score			Interpretation[b]
		Date	Operator	Run 1	Run 2	Run 3	
2715	Urine (F)	9/9/02	A	5,046	68	68	Negative
1388	Swab (F)	10/11/02	B	2,721	1,423	3,034	Positive
474	Swab (M)	10/30/02	A	21,157	0	37	Negative
2442	Swab (F)	11/6/02	A	26,882	92	276	Negative
3070	Swab (F)	11/6/02	A	6,995	216	0	Negative
3001	Swab (F)	12/13/02	C	26,848	0	152	Negative
2583	Urine (F)	12/18/02	D	18,370	10	1,270	Negative
529	Urine (M)	12/18/03	D	18,029	211	0	Negative
3036	Swab (F)	1/15/03	D	25,159	0	0	Negative
3088	Swab (F)	1/29/03	E	9,208	0	33	Negative
3148	Swab (F)	2/3/03	B	8,781	5	42	Negative
2872	Swab (F)	2/21/03	A	2,118	0	1	Negative
2702	Swab (F)	3/3/03	B	2,554	0	36,159	Positive

[a] M, male; F, female.
[b] Positive, two of three tests with MOTA scores of ≥2,000; negative, two of three tests with MOTA scores of <2,000.

TABLE 3. Discordant-result analysis of samples positive in the BDPT *N. gonorrhoeae* test

Sample no.	Sample type (gender[a])	Run 1		MOTA score			Interpretation[b]
		Date	Operator	Run 1	Run 2	Run 3	
1144	Swab (F)	8/2/02	A	8,546	1,249	166	Negative
1513	Swab (F)	8/19/02	A	7,715	1,885	904	Negative
2661	Urine (F)	10/4/02	D	3,742	280	320	Negative
3070	Swab (F)	11/6/02	A	16,943	461	390	Negative
2588	Swab (M)	11/8/02	D	4,046	294	9,288	Positive
2696	Swab (M)	12/20/02	C	5,007	398	278	Negative
2907	Swab (F)	12/20/02	C	8,360	342	122	Negative
2967	Swab (F)	1/13/03	C	2,863	343	316	Negative
1126	Swab (F)	2/3/03	B	3,295	556	14,868	Positive
2830	Swab (F)	2/5/03	B	3,412	188	288	Negative
2832	Swab (F)	2/5/03	B	23,946	580	229	Negative
445	Swab (F)	2/21/03	A	2,235	561	1,023	Negative
439	Swab (F)	2/24/03	B	20,795	288	178	Negative

[a] M, male; F, female.
[b] Positive, two of three tests with MOTA scores of ≥2,000; and negative, two of three tests with MOTA scores of <2,000.

One sample, no. 3070, gave discordant results in both the *N. gonorrhoeae* and *C. trachomatis* tests. As noted for the *C. trachomatis* test, discordant results were not associated with specimen type, run date, or operator (Table 3).

The manufacturer suggests that supplemental testing may be useful for verifying the presence of *C. trachomatis* or *N. gonorrhoeae* DNA in low-positive samples (MOTA scores of 2,000 to 9,999) but provides no direction to laboratories in how to resolve these test results. In theory, testing of a second sample with a different test that uses a different target would be the best approach but is not practical in most settings.

The clinical performance data for BDPT system tests given in the package insert showed that 28.9% of the low-positive test results for *C. trachomatis* and 42.3% of the low-positive test results for *N. gonorrhoeae* were false positives when arbitrated by culture, direct fluorescent-antibody testing, and another NAAT. Although we did not use alternative test methods in our algorithm, the nonrepeat rates we found for low positives were similar to the false-positive rates reported for these tests by the manufacturer. The false-positive rates reported by the manufacturer, together with the poor reproducibility of low-positive test results described here, support the establishment of a gray zone for these tests and supplemental testing of samples with test results in the gray zone. Our data suggest that the gray zone should be 2,000 to 9,999 for both tests.

Reproducibility problems are not unique to the BDPT system. Similar problems have been reported for the other NAATs (3, 5, 6). The sources of the poor reproducibility of low positives in the BDPT system are not clear. Lack of reproducibility was not associated with any one operator, as five of the six technologists performing the tests experienced similar problems (Tables 2 and 3). Degradation of target DNA and generation of amplification inhibitors were considered since some positive lysates were stored for as long as 4 days before testing was completed. We found no relationship between how long a lysate was stored and the reproducibility of the positive results; in fact, some of the MOTA scores were higher in the retests. In addition, the MOTA scores of the amplification control in those samples with poorly reproducible results remained consistent and robust in the retests, eliminating inhibition as an explanation (data not shown). More likely explanations include random events associated with primer, target, and enzyme interactions leading to signal generation in the absence of target DNA, sporadic target DNA carryover during sample transfer from tubes to microwells or between microwells during the procedure, and samples with organism concentrations near the limits of detection of the tests.

Sample cross-contamination is always a concern with NAATs and probably accounts for some of the nonreproducible results in our study. However, it should be stressed that none of the negative controls showed evidence of this problem, and there was no temporal clustering of the nonreproducible test results. These parameters are used in many laboratories to recognize sample cross-contamination problems. Replicate testing has been shown to increase the sensitivity of other nucleic acid amplification tests, particularly for samples with small amounts of target present. This has been attributed to sampling variability. Although our study was not specifically designed to evaluate the effect of replicate testing on the sensitivity of the BDPT system tests, we found that 2 (1%) of the 196 samples repeatedly positive for *C. trachomatis* and 2 (2.2%) of the 92 samples repeatedly positive for *N. gonorrhoeae* did not test positive on the first repeat. In addition, we performed replicate testing of the 92 samples that were initially negative for *C. trachomatis* and the 196 samples that were initially negative for *N. gonorrhoeae* because of positive results for the other organism. In each test, only one additional positive sample was detected. These results suggest that testing of all samples in duplicate would yield only modest increases in the sensitivities of these tests.

In summary, our data support the establishment of a gray zone for the BDPT system tests for *C. trachomatis* and *N. gonorrhoeae* and confirmation of low positives by retesting. The algorithm proposed here is a practical way for laboratories to limit the number of potentially false-positive test results without adding substantial costs to screening programs for these important pathogens.

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