

## Direct Comparison of the BD ProbeTec ET System with In-House LightCycler PCR Assays for Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* from Clinical Specimens

Michael G. Koenig,\* Sandra L. Kosha, Brandon L. Doty, and David G. Heath

Landstuhl Regional Medical Center, Landstuhl, Germany

Received 19 April 2004/Returned for modification 13 June 2004/Accepted 3 August 2004

The commercial BD ProbeTec ET (BDPT) system for the detection of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* from clinical specimens was compared with our in-house LightCycler real-time PCR (LC-PCR) assays. Specimens initially positive by the BDPT system were retested by our LC-PCR assays. Our results for *C. trachomatis* testing indicate a 91.2% agreement when the results of 114 clinical specimens, initially positive by BDPT over a wide range of method-other-than-acceleration (MOTA) scores, were retested by our LC-PCR assay. The agreement between the two systems improved to 96% when only MOTA scores of >30,000 were retested by the LC-PCR assay. The overall agreement between the two systems for *Neisseria gonorrhoeae* detection from 155 clinical specimens was only 77.4%, with agreement particularly low (24.1%) for MOTA scores ranging from 2,000 to 19,999. Repeat testing of specimens with the BDPT only closely correlated with that seen by others demonstrating that reproducibility of the BDPT system for specimens initially within the MOTA score range from 2,000 to 9,999 is problematic, especially for *Neisseria gonorrhoeae* testing. With our study, we proposed an algorithm for *C. trachomatis* and *N. gonorrhoeae* testing which involves screening with the BDPT system followed by selective use of our in-house LC-PCR assays.

The arrival of nucleic acid amplification tests for the detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* DNA from clinical specimens provided an opportunity to improve testing for laboratories receiving specimens where organism viability was an issue and high throughput was important. However, nucleic acid amplification tests, because of their greater sensitivity compared to conventional culture techniques, have the disadvantage of producing a greater number of false positive results (2). This is especially problematic in patient populations which are at low prevalence for *C. trachomatis* or *N. gonorrhoeae* because the effect of false positives on the positive predictive value is more pronounced at lower prevalence rates.

Our laboratory began with the BD ProbeTec ET (BDPT) (Becton Dickinson and Company, Franklin Lakes, N.J.) more than 3 years ago because of its high sensitivity, lower cost, and high-throughput capability. The BDPT can amplify DNA from both *C. trachomatis* and *N. gonorrhoeae* in separate wells, and plates can optionally contain wells which monitor inhibition of amplification for each specimen. The BDPT assay functions by means of strand displacement amplification with amplified DNA being detected by means of fluorescent energy transfer probes (5). After switching to nucleic acid amplification testing, we soon realized the potential for reporting a higher number of false positives compared to prior conventional testing based on our analyses of the BDPT package insert. The BDPT assay produces a method-other-than-acceleration (MOTA) score for each specimen with lower MOTA scores (2,000 to 9,999) being more prone to contain false positives (supported

with discrepant analysis), for both *C. trachomatis* and *N. gonorrhoeae* testing, compared to specimens producing higher MOTA scores.

We therefore incorporated a testing algorithm which involved repeat testing when initial results gave a MOTA score between 2,000 and 9,999. Those specimens negative on repeat testing were deemed indeterminate, and another specimen was requested. However, the recent results of others (2) and our own internal examination revealed that for lower MOTA score ranges, the reproducibility of the BDPT assay is problematic with the possibility that, even with repeat testing with only the BDPT assay, false positive results could still be reported. A recent publication stated that when testing low prevalence populations, all positive tests should be considered presumptive and additional testing should be considered (1). The same publication also suggested approaches to additional testing and included repeat testing of the original specimen “with a different test that uses a different target, antigen, or phenotype and a different format” as one of four possibilities.

Therefore, the purpose of our study was to see how we could best utilize an in-house LightCycler PCR (LC-PCR) assay for both *C. trachomatis* and *N. gonorrhoeae* testing as a means of improving our BDPT assay screening results. Our study compared the BDPT assay directly with our LC-PCR assay over a wide range of MOTA scores for both *C. trachomatis* and *N. gonorrhoeae* tests. With this study, we devised a new algorithm for both *C. trachomatis* and *N. gonorrhoeae* testing which incorporates the use of our LC-PCR assays.

### MATERIALS AND METHODS

**Becton Dickinson ProbeTec ET (BDPT).** Specimen processing and BDPT performance followed the manufacturer’s guidelines (Becton Dickinson and Company). For a description of the BDPT assay, the reader should refer to earlier publications (5, 9).

\* Corresponding author. Mailing address: Landstuhl Regional Medical Center, Auf Dem Kirchberg, Attn: Virology, Gebaeude 3738, Zimmer 204, 66849 Landstuhl, Germany. Phone: 49 6371-86-7809. Fax: 49 6371-86-6390. E-mail: michael.koenig@ind.amedd.army.mil.

**Patient population.** Our specimens were collected from U.S. Army, U.S. Air Force, and U.S. Navy active-duty personnel and their dependents. During the period from January to September 2003, 21,981 specimens were tested, and, of these, 18,376 specimens were endocervical, 2,589 were urethral, and 523 were urine. The high number of endocervical specimens is the result of a required annual screen. Our positive prevalence rate during this period was 7% for *C. trachomatis* and 1.6% for *N. gonorrhoeae*, with 0.3% of specimens positive for both *C. trachomatis* and *N. gonorrhoeae*. The positive BDPT *N. gonorrhoeae* specimens used in this study were collected from April to September 2003 and the BDPT-positive *C. trachomatis* specimens were collected in August and September 2003. Only Becton Dickinson-approved specimen sources with Becton Dickinson-specific collection kits were tested.

**DNA purification.** DNA for the LightCycler PCR and relative sensitivity studies was extracted with the High Pure viral nucleic acid kit (Roche Diagnostics GmbH) according to the manufacturer's instructions. DNA was eluted with 50  $\mu$ l of elution buffer and stored at  $-20^{\circ}\text{C}$ .

**LC-PCR.** Our in-house PCR assays were performed with the LightCycler (Roche Diagnostics, Mannheim, Germany). LC-PCR for *N. gonorrhoeae* was performed as described by Whiley et al. (10) with the HO1 and HO2 primer set and the NG-LC1 and NG-LC2 probes to amplify a portion of the *ccpB* gene present on a cryptic plasmid (4, 10). Briefly, premade reaction mixtures were obtained from the LightCycler FastStart DNA master hybridization probes kit (Roche Diagnostics). Capillaries were loaded with a reaction mixture consisting of 2  $\mu$ l of 10-fold-concentrated kit master reagent (reagent 1), 0.35 pmol (4 mM) of  $\text{MgCl}_2$  (reagent 2), 2.0 pmol of primer HO1, 8.0 pmol of primer HO2, 4.0 pmol each of probes NG-LC1 and NG-LC2, and 5  $\mu$ l of target DNA. All final reaction mixes were made up to 20  $\mu$ l with sterile PCR-grade water (reagent 3). Each final reaction mix was subjected to an initial 10 min of incubation at  $95^{\circ}\text{C}$ , followed by 55 cycles consisting of denaturation at  $95^{\circ}\text{C}$  for 10 s, annealing at  $55^{\circ}\text{C}$  for 10 s, and extension at  $72^{\circ}\text{C}$  for 20 s.

For *C. trachomatis* detection by LC-PCR, we adapted the primers described earlier (6) to the SYBR Green LC-PCR format. The total reaction volume per capillary was 20  $\mu$ l. Briefly each capillary was loaded with 2  $\mu$ l of LightCycler FastStart reaction/enzyme mix SYBR Green 10-fold concentrated (Roche Diagnostics), 1.6  $\mu$ l of 25 mM  $\text{MgCl}_2$  (3 mM), 4.0 pmol (0.2  $\mu\text{M}$ ) of each primer, 5  $\mu$ l of purified DNA (or water as negative control) and diluted to 20  $\mu$ l with molecular grade water. Initially the FastStart enzymes were activated by heating at  $95^{\circ}\text{C}$  for 10 min. This denaturation was followed by 40 amplification cycles starting with a denaturation at  $95^{\circ}\text{C}$  for 10 s, annealing at  $55^{\circ}\text{C}$  for 10 s, and elongation at  $72^{\circ}\text{C}$  for 25 s, followed by a single measurement. All transition rates were at  $20^{\circ}\text{C}$  per s. The amplification was followed by the melting curve. After a 2-s denaturation at  $95^{\circ}\text{C}$  and hybridization at  $55^{\circ}\text{C}$  for 10 s, the temperature was raised to  $95^{\circ}\text{C}$  with a transition rate of  $0.2^{\circ}\text{C}$  per s. Only those specimens that gave a clearly distinguishable peak from the primer-dimer (negative control) were considered positive. The above parameters for the *C. trachomatis* LC-PCR were considered optimal after performing this assay under different  $\text{MgCl}_2$  concentrations (from 1 to 5 mM) and different annealing temperatures (50 to  $60^{\circ}\text{C}$  in  $2.5^{\circ}\text{C}$  increments).

**DNA precipitation.** Because the volume of specimen used by the LightCycler is normally 5  $\mu$ l, we added a DNA precipitation step for all patient specimens positive by BDPT but negative by LC-PCR as a means of equalizing the amount of patient specimen used by both assays. We then retested the precipitated specimens by LC-PCR. For each precipitation, 200  $\mu$ l of the specimen was added to an extraction column (see DNA purification) and eluted with 50  $\mu$ l of elution buffer. For each LightCycler capillary tube, 2.5  $\mu$ l of a 2.5 M sodium acetate solution (pH 5.8) was added to 25  $\mu$ l of the purified DNA solution. A 10-fold volume of 100% molecular grade ice-cold ethanol was then added and placed for 30 min at  $-80^{\circ}\text{C}$ . The DNA was centrifuged for 1 h at  $16,000 \times g$  in a tabletop centrifuge. The precipitates were washed once with ice-cold 70% ethanol and then air dried. The pellets were resuspended in 15  $\mu$ l of an  $\text{MgCl}_2$  solution (3 mM for *C. trachomatis* and 4 mM for *N. gonorrhoeae*) and SYBR Green Fast Start Mastermix, and primers were added for *C. trachomatis* testing and Hybridization FastStart Mastermix and primers and probes for *N. gonorrhoeae* testing. The final volume for both *C. trachomatis* and *N. gonorrhoeae* testing was 20  $\mu$ l.

**Comparison of relative sensitivities between the BDPT strand displacement amplification and LC-PCR assays.** To compare the relative sensitivity of the LightCycler with the BDPT strand displacement amplification, we made positive control DNA from frozen stock cultures of *C. trachomatis* and *N. gonorrhoeae* (ATCC 19424). The *C. trachomatis* used was obtained from a College of American Pathologists Survey VR1-18 (2002) after identification from growth in McCoy cells. *C. trachomatis* cultures were grown in McCoy cells, and when more than 75% of cells were positive for growth of *C. trachomatis* by immunofluorescence, cells were harvested and placed in 200- $\mu$ l aliquots for DNA purification by

extraction with the High Pure viral nucleic acid kit (Roche Diagnostics) according to the manufacturer's instructions. DNA was eluted with 50  $\mu$ l of elution buffer and stored at  $-20^{\circ}\text{C}$ .

For *N. gonorrhoeae*, two to three colonies were resuspended in 500  $\mu$ l of molecular grade water and boiled for 15 min; 200- $\mu$ l aliquots were subjected to DNA purification as for *C. trachomatis*. Tenfold dilutions of purified *C. trachomatis* or *N. gonorrhoeae* DNA were made in molecular grade water, and 5  $\mu$ l of each 10-fold dilution was used for LC-PCR. For BDPT, 20  $\mu$ l of each 10-fold dilution was diluted to 400  $\mu$ l with BDPT diluent and 100  $\mu$ l was used per test. In so doing, the relative concentrations of DNA used for both the LC-PCR and the BDPT were equivalent. The detection limit was defined as the highest dilution giving a positive result.

To compare the sensitivities of the BDPT assays with the LC-PCR assays from clinical specimens, 10 patient specimens of both endocervical and urethral origin and negative by the BDPT assay were pooled. This pool was then subjected to BDPT assays for both *N. gonorrhoeae* and *C. trachomatis* and again found to be negative (MOTA score < 2000). The specimen pool was used to create separate serial dilutions for both *N. gonorrhoeae* DNA and *C. trachomatis* DNA to  $10^{-9}$  of the originally spiked pool. The dilution series spiked with *N. gonorrhoeae* DNA was subjected to both the BDPT and LC-PCR assays for *N. gonorrhoeae* detection, while the dilution series spiked with *C. trachomatis* DNA was subjected to both the BDPT and LC-PCR assays for *C. trachomatis* detection.

## RESULTS

***C. trachomatis* BDPT repeat testing and comparison with LC-PCR.** With known *C. trachomatis* DNA, the melting point of the *C. trachomatis* amplicons in the SYBR green LC-PCR was determined to be  $82^{\circ}\text{C}$  ( $\pm 1^{\circ}\text{C}$ ) and was clearly distinguishable from the melting point of the primer-dimers (data not shown). Therefore, specimens that showed a distinguishable peak at  $82^{\circ}\text{C}$  ( $\pm 1^{\circ}\text{C}$ ) were considered positive. Those PCRs that had no distinguishable peak near or at  $82^{\circ}\text{C}$  ( $\pm 1^{\circ}\text{C}$ ) were considered negative. We then compared our LC-PCR assay with the *C. trachomatis* DNA dilution series we tested previously with the BDPT and found both systems to have the same relative sensitivity when DNA dilutions were performed in molecular grade water. To compare the relative sensitivity of the LC-PCR with BDPT assay from clinical specimens, actual pooled patient specimens were used to create a dilution series. Our results indicate that, for the pooled patient specimen dilution series, the LC-PCR assay was able to detect *C. trachomatis* DNA at the  $10^{-7}$  dilution while the BDPT assay was able to detect DNA at the  $10^{-6}$  dilution. Hence, in our hands, the LC-PCR was 10-fold more sensitive in detecting *C. trachomatis* DNA from a pooled clinical specimen than was the BDPT assay (data not shown).

Next, we randomly selected 114 BDPT *C. trachomatis*-positive specimens. Of these 114 specimens, 11 were found to have a MOTA score of between 2,000 and 9,999 and were retested by the BDPT assay (Table 1). Three of the 11 initially positive specimens were negative upon retesting, and only three retested again within the MOTA range of 2,000 to 9,999. The remaining five of these initially positive specimens gave repeat results with MOTA scores ranging from 10,000 to  $>30,000$ .

To compare the BDPT assay directly against the LC-PCR, we retested the original 114 BDPT initially *C. trachomatis*-positive specimens with our LC-PCR assay (Table 2). Prior to precipitation of DNA for LC-PCR analysis, 97 (85%) repeated positive and 14 (12.2%) turned out to be negative by LC-PCR. When these 14 negative specimens were subjected to precipitation and centrifugation and submitted to another round of LC-PCR testing, the comparison between the BDPT assay and the LC-PCR became even more impressive. All BDPT-re-

TABLE 1. BDPT repeat testing results for 11 *C. trachomatis*-positive specimens with an initial MOTA score of between 2,000 and 9,999

Second MOTA score	No. of specimens with initial MOTA score of:	
	2,000–4,999	5,000–9,999
<2,000	3	0
2,000–4,999	1	1
5,000–9,999	0	1
10,000–14,999	0	1
15,000–19,999	1	0
20,000–24,999	1	0
25,000–29,999	0	0
>30,000	0	2

tested specimens with an initial MOTA score between 2,000 and 9,999 correlated exactly with the LC-PCR assay (after precipitation) results. Further, in the high MOTA score range of >30,000, there was a 96% (79 of 82 specimens) agreement between the two systems. Overall, when precipitation is used for the LC-PCR assay, there was a 91.2% agreement between the BDPT assay and the LC-PCR assay for detection of *C. trachomatis* DNA and an 85% agreement when precipitation is not used for the LC-PCR assay. Three of the 114 specimens were initially positive by the BDPT assay but retested as negative by both the BDPT and the LC-PCR assay (before and after the precipitation step). The precipitation step was used so that relative concentrations of DNA tested from patient specimens would be equivalent for the LC-PCR assay compared to the larger sampling volume used by the BDPT assay.

***N. gonorrhoeae* BDPT repeat testing and comparison with LC-PCR.** To compare the relative sensitivity of the BDPT assay to the LC-PCR assay, we tested serial dilutions of DNA purified from a lysate of an *N. gonorrhoeae* stock culture. The LC-PCR could detect 1 log dilution more than the BDPT assay (data not shown) and therefore, its relative sensitivity is approximately 10-fold higher than that of the BDPT when dilutions were made in molecular grade water. Results of testing for relative sensitivity when a pooled clinical specimen is used to create the dilution series showed that the LC-PCR assay was able to detect *N. gonorrhoeae* DNA at a dilution of 10<sup>-9</sup> while the BDPT assay was able to detect *N. gonorrhoeae* DNA at only the 10<sup>-7</sup> dilution. Hence, in a pooled clinical specimen, our LC-PCR assay was 100-fold more sensitive for detection of *N. gonorrhoeae* than was the BDPT assay (data not shown).

Next, we randomly selected 155 BDPT *N. gonorrhoeae* initially positive specimens with 13 of these specimens having an

TABLE 3. BDPT repeat testing of 13 *N. gonorrhoeae*-positive specimens with an initial MOTA score of between 2,000 and 9,999

Second MOTA score	No. of positive specimens with initial MOTA score of:	
	2,000–4,999	5,000–9,999
<2,000	8	0
2,000–4,999	1	0
20,000–24,999	1	0
25,000–29,999	2	0
>30,000	0	1

initial MOTA score of between 2,000 and 9,999. On retesting these 13 specimens, we found eight to retest below a MOTA score of 2,000 while five specimens ranged in MOTA scores between 2,000 and > 30,000 (Table 3). Of the 155 BDPT initially positive specimens, 120 repeated positive on the LC-PCR and 35 were negative (Table 4). As with *C. trachomatis*, we were able to precipitate 32 of the 35 discrepant results between the BDPT assay and the LC-PCR. However, unlike that shown for our *C. trachomatis* results, precipitating negative specimens and submitting them to another round of LC-PCR testing did little to alter the initial results (only 2 of 32 initially negative LC-PCR results became positive after precipitation and retesting).

Overall, we saw a high percentage of discrepant results between the BDPT and the LC-PCR *N. gonorrhoeae* assays (35 of 155, or 22.5%). It is clear from our analysis that for BDPT *N. gonorrhoeae* testing, discrepant results with the LC-PCR assay occur throughout the different MOTA ranges but are more concentrated in the MOTA range from 2,000 to 30,000 (only 64% agreement in this range). Of particular note is that only 5 of 13 positive specimens in the MOTA score range of 2,000 to 9,999 repeat positive by the BDPT assay, and only one specimen in this range was positive by our LC-PCR assay. Agreement between the BDPT assay and our LC-PCR assay was also poor in the MOTA score range of 10,000 to 19,999. Here 16 specimens were positive by the BDPT assay but only six of these specimens were also positive by the LC-PCR assay (Table 4).

**DISCUSSION**

Our laboratory has used the BDPT assay for more than 3 years, and based on the analysis of the BDPT assay package insert, we decided almost immediately to institute retesting of all specimens with an initial MOTA score range between 2,000

TABLE 2. Results comparing 114 BDPT *C. trachomatis*-positive specimens by MOTA score with LC-PCR assay results

Test	No. of samples positive at MOTA score of:					Total
	<2000	2,000–9,999	10,000–19,999	20,000–29,999	≥30,000	
BDPT	3 <sup>a</sup>	8 <sup>a</sup>	8	13	82	114
LC-PCR positive	0	7	4	10	76	97
LC-PCR precipitation positive <sup>b</sup>	0	8	6	11	79	104
% Agreement <sup>c</sup>	100	100	75	85	96	91.2

<sup>a</sup> Tested twice with the BDPT assay with the first assay giving results between 2,000 and 9,999.

<sup>b</sup> Total number of LC-PCR-positive specimens (initial LC-PCR positives plus positives after precipitation of initially negative LC-PCR specimens)

<sup>c</sup> Agreement between the BDPT- and LC-PCR-positive specimens (initially positive and those positive after precipitation).

TABLE 4. Results comparing BDPT *N. gonorrhoeae*-positive specimens by MOTA score with LightCycler assay results

Test	No. of samples positive at MOTA score of:				Total
	2000–9,999	10,000–19,999	20,000–29,999	≥30,000	
BDPT	13 <sup>a</sup>	16	58	68	155
BDPT retest	5	NT <sup>b</sup>	NT	NT	ND <sup>c</sup>
LC-PCR positive	1	6	49	64	120
% agreement <sup>d</sup>	7.7	37.5	84.5	94	77.4

<sup>a</sup> See Table 3 for results of these specimens when subjected to retesting with the BDPT assay.

<sup>b</sup> NT, not tested.

<sup>c</sup> ND, not determined.

<sup>d</sup> Agreement comparing the initial BDPT results with the LC-PCR assay.

and 10,000. Those specimens that repeated with a MOTA score range of equal to or greater than 2,000 were reported as positive, while those specimens which repeated with a MOTA score below 2,000 were reported as indeterminate. This algorithm was applied for both *C. trachomatis* and *N. gonorrhoeae* testing. Indeterminate results, for us, are somewhat problematic because health care providers are often confused as to what indeterminate results mean. In addition, for indeterminate results we request an additional specimen which can also be problematic because it is often difficult to locate individuals for a second specimen as we test for all armed services throughout the European theater.

Therefore, our purpose, for designing an in-house LC-PCR assay, was as an additional, confirmatory test enabling us to lessen the number of or eliminate indeterminate test results. Another reason for adding confirmatory testing comes from a recent publication highlighting the lack of reproducibility of the BDPT assay for both *C. trachomatis* and *N. gonorrhoeae* testing (2). If shown to have at least equal sensitivity, a second nucleic acid amplification test assay, used judiciously and sparingly, would enable us to be more confident of our results and still allow cost containment. The confidence would come from the knowledge that our in-house LC-PCR assay is amplifying a separate and distinct DNA target for both *C. trachomatis* and *N. gonorrhoeae* DNA than of the BDPT assay. A false positive result by both assays, even at the low MOTA range for our BDPT assay, would seem highly unlikely.

To begin our study, we compared the relative sensitivity of the BDPT assay with our in-house LC-PCR assay by isolating DNA from control organisms and testing a series of dilutions on both systems. With this method, our LC-PCR assay was equivalent to the BDPT assay for *C. trachomatis* DNA but more sensitive, by a single 10-fold dilution, for *N. gonorrhoeae* DNA (data not shown). Next, we performed the LC-PCR assay on 114 BDPT-positive specimens, covering a large range of MOTA scores, for *C. trachomatis*. Eleven of these specimens initially tested positive by the BDPT assay with MOTA scores ranging from 2,000 to 9,999. Only 72.7% (8 of 11) were positive by BDPT on retesting giving similar results to that shown earlier (80.8%) (2) and the BDPT package insert (71.2%) when retesting initially *C. trachomatis*-positive specimens in the MOTA score range of 2,000 to 9,999. Three of these 11 specimens that were initially positive but retested with a MOTA score of <2000 were also negative by LC-PCR. We saw only a 50% agreement between the BDPT and the LC-PCR in the MOTA range of 10,000 to 19,999 but this improved to 75% when *C. trachomatis* DNA was precipitated prior to the LC-

PCR assay. Similarly, in the MOTA range between 20,000 and 29,999 the agreement between the BDPT and the LC-PCR (with precipitation) improved to 85% and further improves to 96% when MOTA scores are equal to or greater than 30,000. We decided to precipitate patient specimens so that the relative amounts of DNA tested between the BDPT assay and the LC-PCR assay would be equivalent after the precipitation procedure.

Our analysis comparing the BDPT assay with the LC-PCR assay to detect the presence of *N. gonorrhoeae* DNA differed significantly from our comparative results for *C. trachomatis* detection. In contrast to BDPT *C. trachomatis* testing, in the MOTA score range of 2,000 to 9,999, only 5 of 13 specimens repeated positive by the BDPT *N. gonorrhoeae* assay (38.4%) which correlates well with earlier reproducibility results of 33% (2). Our in-house LC-PCR was able to detect *N. gonorrhoeae* DNA in only one of the originally 13 positive specimens. Our results for positive specimens in the MOTA score ranges from 10,000 to 19,999 were also lower than expected because we were able to achieve only a 37.5% agreement between the BDPT assay and our in-house LC-PCR assay (6 out of 16 specimens were positive by both assays). We saw significant improvement in the correlation of results between the two systems when the MOTA score ranges increased to between 20,000 and 29,999. Here we saw an 84.5% agreement which improved to 94% when MOTA scores of ≥30,000 were examined.

One argument that could explain our results is that *N. gonorrhoeae* DNA is lost during the purification process for our in-house LC-PCR assay. This would explain why we had a much greater correlation at the higher MOTA score range where greater DNA concentration is likely. Specimens with a small amount of initial *N. gonorrhoeae* DNA may be undetectable in our *N. gonorrhoeae* assay after our purification process. Our result, in which we analyzed a pool of 10 human specimens with spiked *N. gonorrhoeae* DNA, does not support this hypothesis, however, since our in-house LC-PCR assay was 100-fold more sensitive than the BDPT assay and showed that relatively minor amounts of DNA survive our purification process for the LC-PCR assay. An alternative explanation is that the primers used to generate the amplicons in our LC-PCR assay for *N. gonorrhoeae* hybridize to a cryptic plasmid which may not be present in all *N. gonorrhoeae* clinical isolates, as opposed to the BDPT assay, which detects the presence of *N. gonorrhoeae* genomic DNA. However, one study indicated that the cryptic plasmid known to contain the *ccpB* gene is present in 96% of *N. gonorrhoeae* isolates taken from clinical speci-

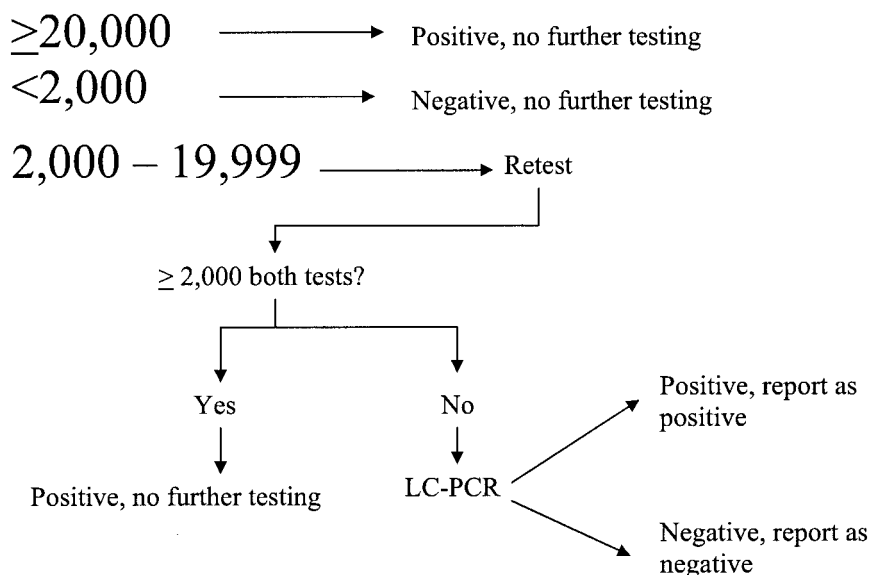


FIG. 1. Testing algorithm for the BDPT and LC-PCR assays for both *C. trachomatis* and *N. gonorrhoeae*. Numbers refer to MOTA scores.

mens (4) and that the *ccpB* gene can also be found chromosomally (3, 7).

Yet another consideration is that the BDPT assay itself lacks good reproducibility at the lower MOTA score range (2). Hence, a specimen which is initially positive with the BDPT assay at the MOTA score range between 2,000 and 10,000 may represent a false positive and hence the lack of correlation with our in-house LC-PCR assay for *N. gonorrhoeae*. Indeed, it would be highly useful to include probit analyses on the BDPT *C. trachomatis* and *N. gonorrhoeae* assays so that end users could judge these assays for reproducibility at different initial MOTA scores. Such probit analyses would be similar to that conducted by Smieja, et al (8) for in-house *C. trachomatis* PCR assays.

For *N. gonorrhoeae* testing results in the lower MOTA score range from 2,000 to 30,000, our data did not correlate well with data listed in the BDPT package insert. They list 6 of 24 specimens as false positives (25% false positives) in the 2,000 to 10,000 MOTA score range, while our results show only a 38.4% reproducibility (5 of 13 specimens repeated as positive) based on retesting by BDPT. We had only a 37.5% agreement between the BDPT and our in-house LC-PCR at the MOTA score range of 10,000 to 19,999 while the BDPT analysis shows a possible false positive rate of only 10.8% for this MOTA score range. However, the BDPT analysis shown in the package insert also includes results of culturing which we did not include in our analysis.

Our comparative results, between the BDPT assay and the LC-PCR for both *C. trachomatis* and *N. gonorrhoeae* testing, suggest that we repeat the BDPT assay on all initially positive specimens in the MOTA score range of 2,000 to 29,999 since above 30,000 our LC-PCR assay and the BDPT assay are in strong agreement. However, the results of Culler et al. (2) show that the BDPT assay is very reproducible at MOTA scores above 10,000 (they do not break it down further between 10,000 and 19,999 and equal to and greater than 20,000) while BDPT assay package insert results suggest that the

BDPT assay is very reproducible at MOTA scores at or above 20,000. Therefore, our algorithm (Fig. 1) would be similar to our earlier testing algorithm except that we would expand our repeat range from 2,000 to 9,999 MOTA score to between 2,000 and 19,999 with repeat positives (i.e.,  $\geq 2000$  MOTA score on both BDPT assays) reported as positive. Those specimens that are negative (i.e.,  $< 2,000$  MOTA score) on the second BDPT assay would be considered indeterminate and would automatically be subjected to the LC-PCR assay to include the precipitation step. Those specimens positive on the third analysis by LC-PCR would be reported as positive and those that are negative would be reported negative. No indeterminate results would be reported under this algorithm.

Use of the LC-PCR on specimens only after two rounds of BDPT testing (initially positive followed by a negative) would substantially reduce the number of LC-PCR assays performed compared to performing the LC-PCR assay on all specimens with a MOTA score between 2,000 and 19,999. Indeed, our analysis shows that over a typical 2-month period, we tested 5,577 specimens for both *C. trachomatis* and *N. gonorrhoeae*. For *C. trachomatis*, 403 specimens (7.2%) were positive with 148 specimens yielding a positive result with a MOTA score between 2,000 and 19,999 (36.7% of all positives). Of these 148 specimens, 102 specimens had a MOTA score between 10,000 and 19,999 and 46 had a MOTA score between 2,000 and 9,999.

To construct a cost analysis, we estimate for *C. trachomatis*, based on the false positive rates proposed by the BDPT assay package insert, that approximately 15% of the specimens between 10,000 and 19,999 would likely be false positives on retesting with the BDPT assay and 30% would be negative on retesting with an initial MOTA score between 2,000 and 9,999. This would make a total of 29 *C. trachomatis* indeterminate specimens which would be subjected to our LC-PCR assay with our proposed testing algorithm during a typical 2-month period. At approximately US\$6 per LC-PCR assay, this would add an additional US\$174 to our testing costs, not counting

labor. A similar analysis for *N. gonorrhoeae* indicates that only 32 specimens had a MOTA score between 2,000 and 19,999 during the 2-month period analyzed because our rate of positives for *N. gonorrhoeae* is typically less than 2% for our testing population. Therefore, the cost of retesting for *N. gonorrhoeae* would be relatively minor.

An argument could be made that all initial retesting should be done by LC-PCR, but our analysis indicates that while the cost per test for both the BDPT assay and the LC-PCR were approximately the same at our location, the number of samples that can be completed per day with the BDPT assay is approximately 550 and approximately 200 with the LC-PCR. Thus, when factoring in the cost of labor, the BDPT assay is a much cheaper assay to perform.

Our results and the results of Culler et al. (2) show that initial testing results for *N. gonorrhoeae* giving MOTA scores between 2,000 and 9,999 should not be reported as positive without further testing because of the lack of reproducibility.

The goal of our study was to see if our in-house LC-PCR could be useful in confirming both *C. trachomatis* and *N. gonorrhoeae* detection by the BDPT assay at MOTA score ranges where reproducibility or the percentage of false positives is high by BDPT's own study data. Our results indicate that in-house LC-PCR confirmatory testing is useful for resolving indeterminate results for *C. trachomatis* testing and for confirming results for *N. gonorrhoeae* testing in the MOTA score range where the BDPT assay's reproducibility is poor.

A vast majority of our specimens were taken from asymptomatic female patients as part of a yearly screen. Hence, reporting a false positive result as a positive patient result could potentially have a devastating social impact. An examination of our current prevalence rates indicates that, within our population, *C. trachomatis* is present in approximately 7% of our testing population while *N. gonorrhoeae* is present in less than 2% of our population. Therefore, we would expect the positive predictive value for our *N. gonorrhoeae* testing to be

far less than that of our *C. trachomatis* testing. Confirmatory testing is therefore especially critical for *N. gonorrhoeae* when with the BDPT assay giving MOTA scores of less than 10,000 but should also be used as confirmatory testing for *C. trachomatis* as outlined in our testing algorithm.

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