

# A Long Term Evaluation of the BDProbeTec™ ET System for the Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in a High Volume Clinical Laboratory

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## ABSTRACT

We tested 34,212 first void urine specimens using the BDProbeTec™ ET system for the detection of *C. trachomatis* (CT) and *N. gonorrhoeae* (GC) during the months of June 2000 to May 2001 in Saskatchewan. This included 26,119 female and 8,093 male patient specimens. All specimens were processed according to the manufacturer's instructions and tested within 72 hours of collection. Amplification controls were included in all specimens for detection of assay inhibition. The daily workload is about 150–180 specimens, with three results per specimen using two ProbeTec instruments. The positivity rate for CT in males and females was 13.08% and 7.69% and for GC was 2.98% and 1.21% respectively. The inhibition rate for CT and GC in males was 0.82% and 0.84% respectively; and in females was 1.12% and 1.08% respectively. The two instruments were able to handle the volume within the eight-hour shift and without any major breakdowns during the twelve-month evaluation. This study demonstrated that the ProbeTec instrument is applicable for use in a high volume setting and that the inhibition rate for both assays in both sexes is below 1.2%. The low inhibition rate results in few repeat tests or specimen recollections.

## INTRODUCTION

The BDProbeTec ET system is a semi-automated system using strand displacement amplification technology that simultaneously amplifies and detects *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (GC) DNA. An amplification control is also included to monitor assay inhibition. This assay has been evaluated in clinical trials and comparison studies have shown it to be highly sensitive and specific. The current study evaluated the long term performance of the BDProbeTec ET system in detecting *C. trachomatis* and *N. gonorrhoeae* in male and female urine specimens, calculated its ability to process large volumes of specimens, determined the inhibition rate in our patient population and evaluated the reproducibility of the gonorrhoeae assay by repeat testing all GC positive results and by performing our own in-house PCR assay.

## MATERIALS AND METHODS

### Patient Population

Between June 2000 to May 2001, 8,093 male and 26,119 female first void urine specimens collected from three sexually transmitted disease clinics and other family physicians' offices were tested for both *C. trachomatis* and *N. gonorrhoeae* using the BDProbeTec ET system. All urine specimens were collected and transported to the Provincial Laboratory within 24 to 48 hours after collection. All urine specimens were processed according to the manufacturer's instructions. The amplification control was tested with each specimen assay to monitor inhibition.

### BDProbeTec ET System Assays

The manufacturer's protocol was strictly followed. Briefly, a urine processing pouch was added to at least 20 ml of each first catch urine and left at room temperature for a minimum of 2 hours before proceeding. A 4-ml aliquot of the treated urine was then transferred to a sample tube and centrifuged at 2000xg for 30 minutes at room temperature. The supernatant was then decanted, and the pellet was resuspended in 2 ml of diluent and then vortexed for 60 seconds. Positive and negative controls were processed according to the manufacturer's instructions. The tubes were then incubated at 114°C for 30 minutes in a lysing heater and allowed to cool at least 15 minutes at room temperature. Priming and amplification microwells for chlamydia, gonorrhoeae and the amplification control were placed in appropriate plates. One hundred and fifty microliters of lysed specimen and controls were then transferred into each of the corresponding columns of the priming microwells and incubated at room temperature for a minimum of 20 minutes. After incubation, the priming microwell plate was placed into

the priming heater (72.5°C). The amplification microwell plate was placed into the warming heater (54°), and both were incubated for 10 minutes. At the end of this incubation period, 100 µl from each column of the priming microwell plate was transferred to the corresponding column of the amplification microwell plate. The amplification well plate was then sealed and immediately transferred to the instrument for testing.

### Repeat Testing of GC on the BDProbeTec ET System and In-house PCR Testing

Positive GC Samples were repeat tested with BDProbeTec and with our in-house PCR assay (PCR). Re-testing was performed from the processed urine sample. For purpose of reporting results we used the following algorithm to interpret data.

#### GC Results Interpretation

Initial ProbeTec Result	Repeat ProbeTec Result	PCR Result	Interpretation
+	+	+	Positive
+	+	-	Indeterminate
+	-	-	Negative
+	-	+	Indeterminate

### BDProbeTec ET System Reliability

During the 12 months of evaluation, the system was monitored for major breakdowns and other system problems to assess the reliability of the system. A major breakdown was defined as an instrument failure which required a BD instrument technician to come to the laboratory to repair the instrument in order to resume operation.

## RESULTS

■ Inhibition rates by month for CT and GC are shown in Table 1 for female urine and Table 2 for the male urine. The indeterminate column under gonorrhoeae shows the number of initial gonorrhoeae positives that were processed according to the above algorithm.

*C. trachomatis* was detected in 7.69% of the female urine specimens and in 13.09% of the male urine specimens for an overall positivity rate of 8.97%. *N. gonorrhoeae* was detected in 1.21% of the female urine specimens and in 2.98% of the male urine specimens for an overall positivity rate of 1.63%. In Figure 1, the % positive results for both CT and GC results by month are shown.

Non-specific inhibition rates for *C. trachomatis* and *N. gonorrhoeae* were 0.82% and 0.84% respectively for urine from males and 1.12% and 1.07% for urine from females. In

Figure 2, the inhibitory rates for both the CT and GC assays by month are shown.

The overall number of GC positive samples, which did not repeat positive in both the BDProbeTec ET system and PCR during the study, was 133/34212 or 0.4%. Of the 133 samples, 26 repeated positive in the BDProbeTec ET system but were negative in the PCR test. This may be due to lower sensitivity with our in-house PCR assay. One specimen was positive by PCR but not in the BDProbeTec ET system repeat test. This sample may have had a low target load that resulted in the BDProbeTec ET system negative result on the repeat test.

During the twelve months of high volume testing, there were no major breakdowns of the instruments. The system was shown to be capable of performing high throughput testing.

Table 1. Monthly breakdown of assay performance in female urine specimens

Month	Chlamydia			Gonorrhoeae				Total
	Negative	Positive	Inhibition	Negative	Positive	Inhibition	Indeterminate	
June	2005	195	34	2158	37	31	16	2242
July	1692	117	34	1804	8	32	2	1846
August	1974	175	42	2133	22	39	1	2195
September	1974	186	22	2125	41	19	1	2186
October	2055	176	29	2218	28	22	1	2269
November	2048	182	39	2207	26	41	0	2274
December	1745	139	21	1862	24	20	0	1906
January	2122	173	17	2257	39	15	1	2312
February	1914	185	16	2069	25	21	0	2115
March	2289	166	19	2431	22	21	0	2474
April	1744	142	6	1866	20	5	1	1892
May	2221	173	14	2370	23	15	0	2408
TOTALS	23783	2009	293	25500	315	281	23	26119

Table 2. Monthly breakdown of assay performance in male urine specimens

Month	Chlamydia			Gonorrhoeae				Total
	Negative	Positive	Inhibition	Negative	Positive	Inhibition	Indeterminate	
June	522	85	3	592	16	3	1	612
July	468	78	18	529	19	18	1	567
August	571	85	12	640	18	11	0	669
September	487	74	8	545	20	7	0	572
October	590	92	1	658	26	1	0	685
November	590	94	9	662	22	9	0	693
December	492	80	3	552	18	5	0	575
Jan-01	653	98	0	725	25	0	1	751
Feb.	648	88	7	723	12	8	0	743
Mar.	731	89	2	800	20	2	0	822
Apr.	556	83	1	621	17	1	1	640
May	649	113	2	733	28	3	0	764
Totals	6957	1059	66	7780	241	68	4	8093

Figure 1. Assay Positivity Rate by Month

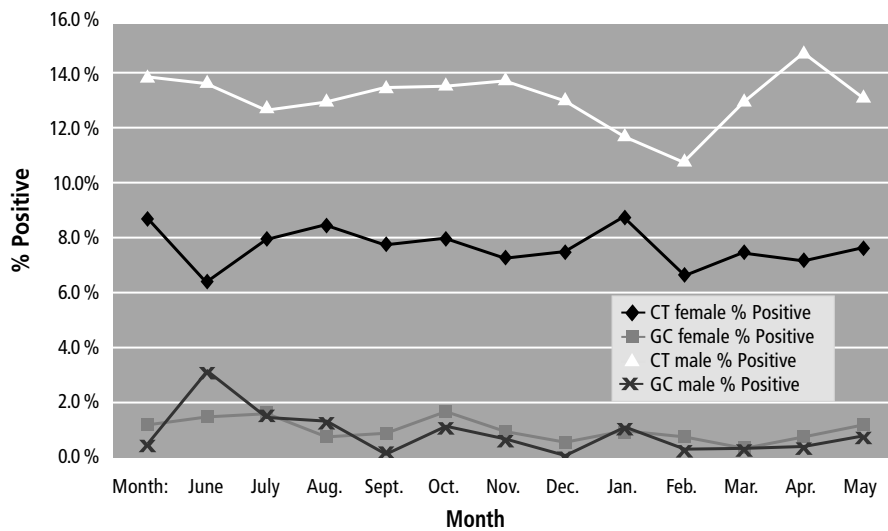
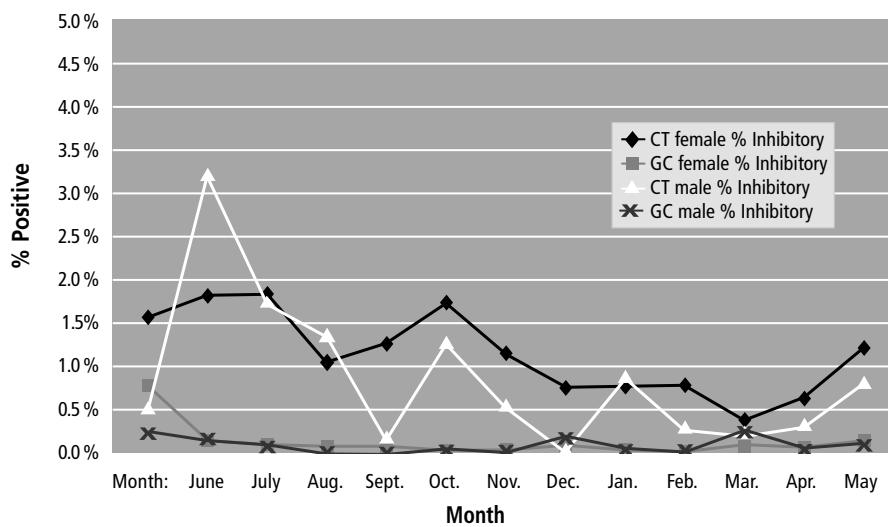


Figure 2. BDProbeTec ET System Inhibition Rates by Month



### CONCLUSION

- The positivity rate determined in this study was similar to rates found in prior years using amplified assays for CT and GC detection.
- The low inhibition rate of <1.2% for both sexes combined resulted in few repeat tests or specimen recollections.
- The higher indeterminate rate (initial positives not confirmed by both repeat test and in-house PCR assay) of the GC assay in June 2000 may reflect the learning curve to perform the assays. After July 2000, the number of indeterminate specimens has dropped to one or less each month showing the assay to be highly reproducible.
- The BDProbeTec ET system was utilized over the twelve months of this study without down time and was capable of handling large volumes of urine specimens. One instrument is able to process 150 specimens with three results per specimen (chlamydia, gonorrhoeae, and amplification control) within a single 8-hour shift. The high throughput capacity of the BDProbeTec ET system combined with high sensitivity and specificity of the assays allowed us to achieve accurate results with excellent efficiency.