

# In-House Verification of the BDProbeTec™ (BDP) *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) Amplified Assay using Abbott LCx (LCX) STD Swab Specimen Collection System

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## ABSTRACT

Changing methodologies for STD testing is complicated by the need to replace collection devices in offices and clinics. This study looks at the use of the LCx collection system (our current method) with the BDP assay (the proposed new method). To verify the performance of the new method, we first compared the two methods using their respective collective devices. Paired endocervical specimens were randomly collected from patients presenting to Montgomery County STD Clinic. True positives were defined as specimens positive by both methods. For the paired specimens analyzed (n=297), prevalence of CT and GC was 10.1% and 7.1% respectively. After discrepant analysis the sensitivity/specificity for both CT and NG using BDP was 100%/100%.

Two processing techniques were used for BDP testing of routine (non-STD clinic) urethral or endocervical laboratory specimens submitted in the LCx collection device. In the first technique, 150 µl of untreated LCx sample was added directly to the BDP sample diluent. In the second technique, 200µl of untreated LCx was added to 1 mL PBS, centrifuged, and the liquid portion decanted. Two ml of BDP sample diluent was added to the drained pellet. Further processing of the specimens was done according to the manufacturer's specifications. A specimen was considered a true positive if it was found to be positive by LCx on initial and repeat testing.

Using the first technique (N=389), the prevalence of CT and NG was 4.6% and 3.3%. Sensitivity/specificity for CT was 77.8% and 99.7%, and for NG was 100% and 99.7%. After discrepant analysis (repeat testing), adjusted BDP results were as follows: sensitivity/specificity for CT was 94.4% and 100%, and for NG was 100% and 100%.

Using the second technique (N=330), the prevalence of CT and NG was 6.6 and 1.5%. Sensitivity/specificity for CT was 86.4% and 99.7%, and for NG was 100% and 99.7%. After discrepant analysis (repeat testing), adjusted BDP results were as follows: sensitivity/specificity for CT was 100% and 100%, and for NG was 100% and 100%.

Based on this data, both methods have equivalent sensitivity and specificity when specimens are collected as recommended by the manufacturer. Use of the LCx specimens processed directly into the BDP diluent does not offer adequate sensitivity for CT unless all testing is done in duplicate. Use of the centrifuged technique offers an acceptable alternative for detection of CT although some true positive specimens will be missed. Both processing methods offered adequate sensitivity and specificity for NG.

## PURPOSE

The purpose of this study was to evaluate different techniques for using the Abbott LCx Collection device for testing with the BDProbeTec for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*.

## METHODS

### SAMPLE PROCESSING AND TESTING

#### Part 1 – Method Performance Verification (Paired Endocervical Samples)

Paired endocervical specimens (N=297) were collected in random order from patients presenting to the Montgomery County STD Clinic. Specimens were transported to the laboratory and tested directly or stored at -70°C until tested. Both the LCx and the BDP assays were performed according to the package insert provided by the manufacturer.

#### Part 2 – BDP using LCx Collection Device (Direct Method)

Using endocervical or urethral specimens (N=389) submitted to our laboratory for routine testing, 150 µL of the untreated LCx sample was removed and added directly to 2 mL of BDP Swab Sample Diluent. The LCx specimens were tested directly and positives were repeated on the day of testing (as per our standard protocol). The BDP specimens were tested directly or stored frozen at -70°C. After testing, BDP specimens were stored frozen at -70°C until repeat testing was performed as necessary.

#### Part 3 – BDP using LCx Collection Device (Centrifugation Method)

Using endocervical or urethral specimens (N=330) submitted to our laboratory for routine testing, 200 µL of the untreated LCx sample was removed and added directly to 1 mL of sterile PBS. The samples were then centrifuged for 30 minutes at 2000x g. The supernatant was decanted and 2 mL of BDP Swab Sample Diluent was added to resuspend pellet. The LCx specimens were tested directly after removal of 200 µL and positives were repeated on the day of testing (as per our standard protocol). The BDP specimens were tested directly or stored frozen at -70°C. After testing, BDP specimens were stored frozen at -70°C until repeat testing was performed as necessary.

### DATA INTERPRETATION

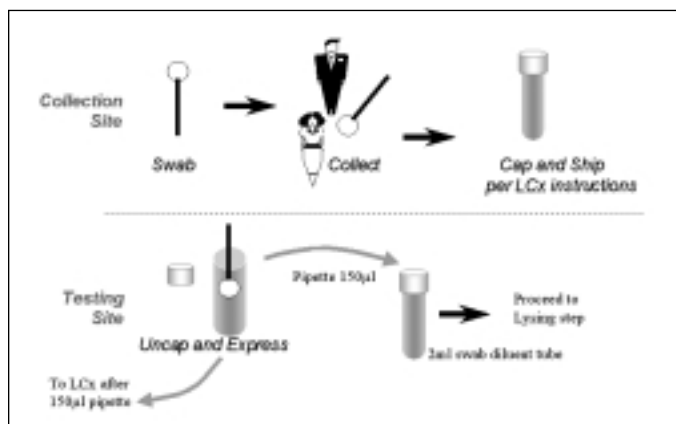
#### Part 1 – Method Performance Verification

Results of the LCx and BDP methods were compared and discrepancies were resolved by repeat testing of both samples in the pair. A true positive was defined as a specimen which tested positive twice using either BDP or LCx.

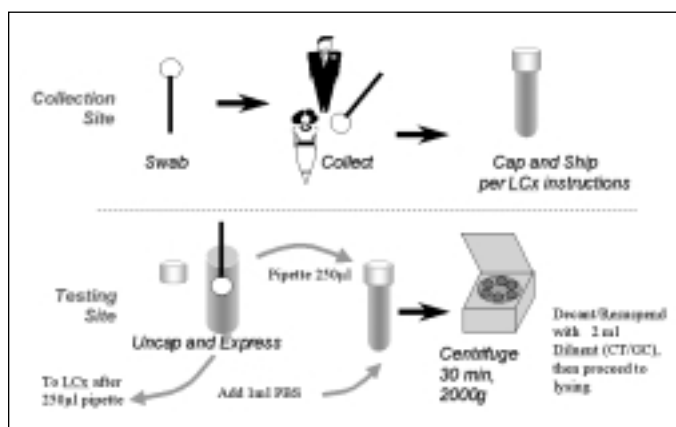
#### Parts 2 and 3 – BDP using LCx Collection Device

True positives were defined as specimens which tested positive by LCx on initial and repeat testing. Discordant analysis: Testing was repeated for BDP and LCx. If the second BDP result agreed with LCx, it was considered concordant.

### DIRECT METHOD



### CENTRIFUGATION METHOD



## RESULTS

### Part 1: Initial Method Performance Verification

*Chlamydia*  
(With Repeat Testing of Positives)

	LCx Pos	LCx Neg
BDP Pos	30	0
BDP Neg	0	267

*Gonorrhea*  
(With Repeat Testing of Positives)

	LCx Pos	LCx Neg
BDP Pos	21	0
BDP Neg	0	275

LCx CT and NG Sens/Spec/PPV/NPV = 100/100/100/100%

BDP CT and NG Sens/Spec/PPV/NPV = 100/100/100/100%

### Discrepant Analysis

*Chlamydia*

	BDP		LCx*		Interpretation	Final Result
	1st	Repeat	1st	Repeat		
1	neg	neg	Pos 1.35	neg	1 <sup>st</sup> LCx result-FP	True Neg
2	Pos 3,387	neg	neg	QNS	1 <sup>st</sup> BDP result-FP	True Neg
3	neg	neg	Pos 1.06	neg	1 <sup>st</sup> LCx result-FP	True Neg

\* Per Abbott, during study period, various lots of LCx CT reagents were prone to false positive reactions.

*Gonorrhea*

	BDP		LCx		Interpretation	Final Result
	1st	Repeat	1st	Repeat		
1	Pos 38,676	Pos 25,298	neg	Pos 5.15	1 <sup>st</sup> LCx result-FN	True Pos
2	neg	neg	Pos 1.29	neg	1 <sup>st</sup> LCx result-FP	True Neg

**CONCLUSION:** Following our standard laboratory protocol for testing (which does routinely include repeat testing of all positive specimens), the BDP correlated 100% with LCx results.

### Part 2: BDP using LCx Collection Device (Direct Method)

*Chlamydia*  
(Before Discrepant Analysis)

	LCx Pos	LCx Neg
BDP Pos	14	1
BDP Neg	4	370

*Gonorrhea*  
(Before Discrepant Analysis)

	LCx Pos	LCx Neg
BDP Pos	13	2
BDP Neg	0	374

BDP CT Sens/Spec/PPV/NPV = 77.8/99.7/93.0/98.9%

BDP NG Sens/Spec/PPV/NPV = 100/99.5/88.7/100%

### Discrepant Analysis

*Chlamydia*

	BDP		LCx		Interpretation	Final Result
	1st	Repeat	1st	Repeat		
1	Pos 17,513	neg	neg	QNS	1 <sup>st</sup> BDP result-FP	True Neg
2	neg	Pos 34,640	Pos 2.17	Pos 3.70	1 <sup>st</sup> BDP result-FN	True Pos
3	neg	Pos 34,222	Pos 1.03	Pos 3.89	1 <sup>st</sup> BDP result-FN	True Pos
4	neg	Pos 4,199	Pos 1.59	Pos 1.68	1 <sup>st</sup> BDP result-FP	True Pos
5	EQV	neg	Pos 1.89	Pos 2.72	BDP result-FN	Discrepant False Neg

*Gonorrhea*

	BDP		LCx		Interpretation	Final Result
	1st	Repeat	1st	Repeat		
1	Pos 29,037	Pos 23,443	Neg 0.53	QNS	Probable LCx FN	True Pos
2	Pos 30,031	neg	Neg 0.04	QNS	Probable BDP FP	Discrepant False Pos

### Adjusted Results

*Chlamydia*

	LCx Pos	LCx Neg
BDP Pos	17	0
BDP Neg	1	371

*Gonorrhea*

	LCx Pos	LCx Neg
BDP Pos	13	1
BDP Neg	0	375

BDP CT Sens/Spec/PPV/NPV = 94.4/100/100/100%

BDP NG Sens/Spec/PPV/NPV = 100/100/92.9/100%

**CONCLUSION:** Use of the LCx specimens processed directly into the BDP diluent does not offer adequate sensitivity for CT unless all testing is done in duplicate. The direct method did offer adequate sensitivity and specificity for NG.

Part 3: BDP using LCx Collection Device (Centrifugation Method)

**DISCUSSION**

*Chlamydia*  
(Before Discrepant Analysis)

	LCx Pos	LCx Neg
<b>BDP Pos</b>	19	1
<b>BDP Neg</b>	3	312

*Gonorrhea*  
(Before Discrepant Analysis)

	LCx Pos	LCx Neg
<b>BDP Pos</b>	5	1
<b>BDP Neg</b>	0	329

BDP CT Sens/Spec/PPV/NPV = 86.4 /99.7/95.0/99.0%  
BDP NG Sens/Spec/PPV/NPV = 100/99.7/83.3/100%

Direct comparison of LCx versus BDP for detection of CT and NG demonstrated similar performance characteristics for both tests. The sensitivity, specificity, and predictive values for both tests were 100% after discrepant analysis. The LCx CT assay had two false positive results on initial testing. This is attributable to problems with false positive CT results with the reagents provided by the manufacturer. These were detected by repeat testing of the positive samples which is standard protocol in our laboratory.

The direct method for processing LCx specimens for testing with BDP did not give adequate sensitivity with CT for routine use. The dilution of the original LCx specimen (150 µL into 2 mL) may have put it below the level of detection for the BDP CT assay or alternately may have caused inhibition due to substances in the LCx collection media. The freeze thawing of processed specimens may have removed inhibitors so that repeat testing was positive by BDP. Repeat testing of all samples would not be practical for large volumes of samples. In situations where the number of LCx collection devices received by the laboratory is small, testing duplicates may be a viable option until conversion of all collection devices is possible.

The centrifugation method for processing LCx specimens for testing with BDP gave adequate sensitivity with CT for routine use. The use of all of the LCx specimen (versus the 200 µL used here) would probably improve sensitivity further.

Both the direct and centrifuged methods for processing LCx samples gave adequate sensitivity and specificity for detection of GC when tested with BDP.

Discrepant Analysis

*Chlamydia*

	BDP		LCx		Interpretation	Final Result
	1st	Repeat	1st	Repeat		
1	neg	neg	Pos 1.33	Pos 2.19	BDP result-FN	Discrepant False Neg
2	neg	neg	Pos 1.11	Pos 1.33	BDP result-FN	Discrepant False Neg
3	neg	Pos 35,632	Pos 3.30	Pos 3.32	1 <sup>st</sup> BDP result-FN	True Pos
4	Pos 10,537	neg	neg	neg	1 <sup>st</sup> BDP result-FP	True Neg

*Gonorrhea*

	BDP		LCx		Interpretation	Final Result
	1st	Repeat	1st	Repeat		
1	Pos 2,698	neg	neg	neg	1 <sup>st</sup> BDP result-FP	True Neg

Adjusted Results

*Chlamydia*

	LCx Pos	LCx Neg
<b>BDP Pos</b>	20	0
<b>BDP Neg</b>	2	313

*Gonorrhea*

	LCx Pos	LCx Neg
<b>BDP Pos</b>	5	0
<b>BDP Neg</b>	0	330

BDP CT Sens/Spec/PPV/NPV = 94.4/100/100/100%  
BDP NG Sens/Spec/PPV/NPV = 100/100/100/100%

**CONCLUSION:** Use of the centrifuged technique offers an acceptable alternative for detection of CT although some true positive specimens will be missed. The direct method did offer adequate sensitivity and specificity for NG.

**SUMMARY**

- Following our standard laboratory protocol for testing (which does routinely include repeat testing of all positive specimens), the BDP correlated 100% with LCx results.
- Use of the LCx specimens processed directly into the BDP diluent does not offer adequate sensitivity for CT unless all testing is done in duplicate. The direct method did offer adequate sensitivity and specificity for NG without the need for repeat testing of specimens.
- Use of the centrifuged technique offers an acceptable alternative for detection of CT although some true positive specimens will be missed. The centrifuged method did offer adequate sensitivity and specificity for NG without the need for repeat testing of all specimens.
- The use of LCx specimen collection devices for testing with the BDP is an acceptable alternative to sending specimens to a reference laboratory for LCx testing. The centrifugation method using 200 µL of LCx sample is the better of the two methods we examined.