

Analytical Reproducibility of the BD Viper™ System with XTR™ Technology (extracted mode) for the Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* When Using the BD ProbeTec™ CT/GC Q^x Amplified DNA Assays

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ABSTRACT

OBJECTIVE: The objective of this study was to determine the reproducibility of qualitative test results obtained with a sixteen panel member panel of simulated *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (GC) positive and negative urine and swab specimens using the BD Viper™ System with XTR™ Technology (BD Viper System with XTR) and BD ProbeTec™ CTQ and GCQ Amplified DNA Assays. The study included analysis of within run and between run reproducibility in addition to instrument to instrument reproducibility across three Viper instruments. The target levels tested were designed to span above and below the analytical limits of detection (LODs) of the CTQ and GCQ assays.

METHODS: Three clinical sites were each provided five identical reproducibility panels, each consisting of 90 simulated urine and swab samples. The 90 panel members were prepared using BD ProbeTec CT/GC Q^x Swab Diluent (Q^x Swab Diluent) and were either left unspiked (0x) or spiked with known quantities of CT serovar H and/or GC strain ATCC 19424 at 2x or 5x the specified analytical LOD for each analyte. Samples that were not spiked with one or both organisms were considered to be negative for the respective analyte(s). Additionally, simulated swab panels contained a clean endocervical swab. Panels were run once a day at each facility over five consecutive days. A second series of 90 panel members was prepared in Q^x Swab Diluent at target levels of 1:10 (0.1x) and 1:100 (0.01x) of the specified analytical LOD for each organism. The levels for these panel members were selected to fall within the dynamic range of the analytical LOD curves of the assays. Samples in each panel were randomized and blinded to the user prior to testing.

VALIDATION: The study generated a total of 2250 results for each assay. For the CTQ assay, the percentage correct for the panel members at 0x, 2x, and 5x the analytical LOD is 99.6% (538/540), 100.0% (540/540), and 100.0% (270/270) respectively, across days and sites. For the second panel members, the percent positive at 0.1x the LOD was 67.7% (303/450) across days and sites, and the percent positive for the panel members at 0.01x the LOD was 10.4% (47/450) across days and sites. For the GCQ assay, the percentage correct for the panel members at 0x, 2x, and

5x the LOD is 99.2% (536/540), 100.0% (540/540), and 100.0% (270/270) respectively, across days and sites. For the second panel members, the percent positive for the panel members at 0.1x the LOD was 91.8% (413/450) across days and sites, and the percent positive for panel members at 0.01x the LOD was 26.7% (120/450) across days and sites. There was no statistical difference in performance between sites.

CONCLUSIONS: For the CT/GC negative simulated urine and swab samples and those spiked with organisms at either 2x or 5x the specified analytical LODs of the CTQ and GCQ assays, there was >99% agreement with the expected results. As expected, lower levels of agreement were obtained with samples spiked below the analytical LODs of the two assays.

INTRODUCTION

Nucleic acid amplification tests (NAATs) have become the standard practice in the detection of sexually transmitted diseases. BD has developed two novel assays for the detection of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (GC) DNA from swab and urine specimens with the BD Viper™ System with XTR™ Technology (extracted mode). Walk-away automation is achieved using the BD Viper XTR in conjunction with nucleic acid extraction using FOX technology and real-time fluorescent detection using the BD ProbeTec™ CT Q^x and GC Q^x Amplified DNA Assays. Sample tubes are sealed with a pierceable cap (P-cap), which streamlines workflow.

Here we describe the reproducibility of qualitative test results both within and between testing sites and BD Viper XTR instrument runs, for detection from simulated urine and swab specimens containing known concentrations of CT and GC organisms. Target levels were selected in order to span a range above and below the specified analytical limits of detection (LOD) for each assay.

METHODS

Preparation of panels

Panel 1: Each of three testing sites was provided with five identical reproducibility panels, each of which consisted of 90 samples (Table 1). Each of the 90 panel members was prepared using Swab Diluent for the BD ProbeTec CT/GC Q^x Amplified DNA Assays (Q^x Swab Diluent) and enumerated stocks of CT serovar H elementary bodies (EB) and/or cells of GC strain ATCC 19424. Spike levels corresponded to either 0, 2X or 5X the specified analytical LOD for each analyte in Q^x Swab Diluent. Unspiked specimens or those containing only CT elementary bodies were considered as negative controls for the GC Q^x Assay. Likewise, unspiked specimens or those containing only GC cells were considered to be negative controls for the CT Q^x Assay.

Each simulated endocervical/urethral swab panel member contained 2 mL of spiked or unspiked diluent together with a Q^x endocervical swab collection device broken off into the tube.

Each simulated urine/vaginal specimens consisted of 2 mL of spiked or unspiked diluent with no swab present. All samples were capped with P-caps and stored at -20°C±10°C until assayed.

Samples in each panel were randomized and blinded to the user prior to testing. On each day of testing a complete set of panel members (1-10 in Table 1) was thawed and tested twice, for a total of 18 results per day per testing site for each member.

Panel 2: A second series of 90 panel members was prepared in Q^x Swab Diluent at target levels of 0.1X and 0.01X the specified analytical LODs for each assay (Table 2). The target levels for these panel members were selected to fall within the dynamic range of the analytical LOD curves of the assays. One operator tested a complete series of panel members each day for five consecutive days, across three BD Viper XTR instruments.

Table 1: Panel 1 – Summary of Reproducibility Panel Members

Panel Member	Panel Member Replicates	<i>C. trachomatis</i> CT Serovar H (EB/mL)	<i>N. gonorrhoeae</i> GC ATCC 19424 (cells/mL)	Simulated Sample Type
1	9	0	0	Urine / Vaginal
2	9	30 (Low)*	0	Urine / Vaginal
3	9	0	100 (Low)	Urine / Vaginal
4	9	30 (Low)	250 (High)	Urine / Vaginal
5	9	75 (High)	100 (Low)	Urine / Vaginal
6	9	0	0	Endocervical Swab
7	9	30 (Low)	0	Endocervical Swab
8	9	0	100 (Low)	Endocervical Swab
9	9	30 (Low)	250 (High)	Endocervical Swab
10	9	75 (High)	100 (Low)	Endocervical Swab

*"Low" and "High" are relative to the specified analytical LOD for each of the Viper XTR CT Q^x and GC Q^x Assays (15 CT EB/mL and 50 GC cells/mL, respectively)
Low = 2X LOD; High = 5X LOD.

Table 2: Panel 2 – Summary of Reproducibility Panel Members

Panel Member	Spike Level		Simulated Sample Type
	<i>C. trachomatis</i> CT Serovar H (EB/mL)	<i>N. gonorrhoeae</i> GC ATCC 19424 (cells/mL)	
1	1.5*	5	Urine / Vaginal
2	0.15	0.5	Urine / Vaginal
3	1.5	5	Endocervical Swab
4	0.15	0.5	Endocervical Swab

*Spike levels were 0.1X or 0.01X the specified analytical LODs for the respective assays (1.5 CT EB/mL and 5 GC cells/mL or 0.15 CT EB/mL and 0.5 GC cells/mL, respectively).

METHODS CONTINUED

Figure 1: BD Viper XTR Workflow

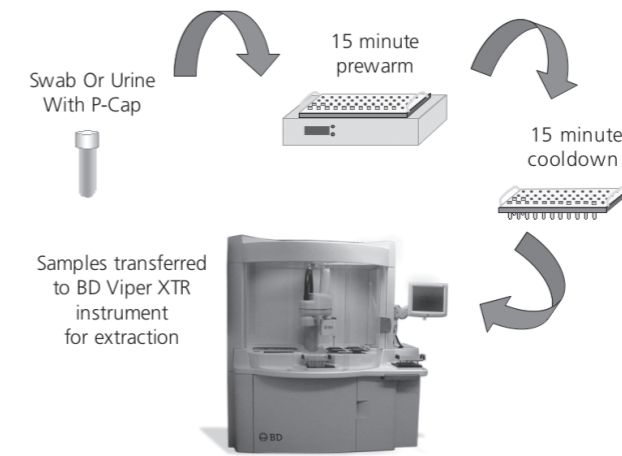
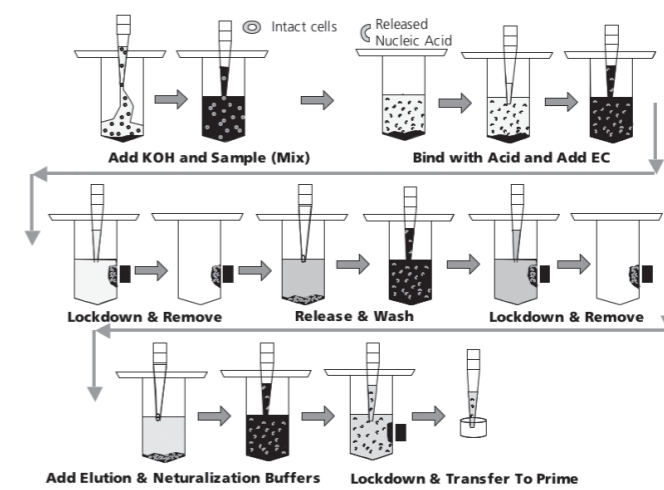


Figure 2: Automated Extraction Technology



DATA ANALYSIS

Results of the BD ProbeTec CT/GC Q^x Amplified DNA Assays results were analyzed using a novel algorithm based on the maximum relative fluorescence (MaxRFU) observed during the course of the amplification reaction.

RESULTS

Table 3: CT Q^x Assay – Endocervical/Urethral Swab and Urine/Vaginal Sample Reproducibility: Percent Correct Shown by Site and Combined Across Testing Sites for Panel 1

Specimen	Panel (CT/GC)	Testing Site						Total / Results Panel					
		1		2		3		PASS					
		PASS		PASS		PASS		Yes		No			
		Yes	%	Yes	%	No	%	Yes	%	No	%		
Endocervical / Urethral Swab	(0/0)	45	100.0	43	95.6	2	4.4	45	100.0	133	98.5	2	1.5
	(0/100)	45	100.0	45	100.0	0	0	45	100.0	135	100.0	0	0
	(30/0)	45	100.0	45	100.0	0	0	45	100.0	135	100.0	0	0
	(30/250)	45	100.0	45	100.0	0	0	45	100.0	135	100.0	0	0
	(75/100)	45	100.0	45	100.0	0	0	45	100.0	135	100.0	0	0
	Total	225	100.0	223	99.1	2	0.9	225	100.0	673	99.7	2	0.3
Urine / Vaginal	(0/0)	45	100.0	45	100.0	0	0	45	100.0	135	100.0	0	0
	(0/100)	45	100.0	45	100.0	0	0	45	100.0	135	100.0	0	0
	(30/0)	45	100.0	45	100.0	0	0	45	100.0	135	100.0	0	0
	(30/250)	45	100.0	45	100.0	0	0	45	100.0	135	100.0	0	0
	(75/100)	45	100.0	45	100.0	0	0	45	100.0	135	100.0	0	0
	Total	225	100.0	225	100.0	0	0	225	100.0	675	100.0	0	0

Table 4: CT Q^x Assay – Descriptive Statistics for Reproducibility Panel 1

Specimen	CT EM/mL	GC Cells/mL	% Correct	95% CI	Max RFU Mean	Within Run		Between Run Within Testing Site		Between Testing Site	
						SD	%CV	SD	%CV	SD	%CV
Endocervical / Urethral Swab	0	0	98.5% (133/135)	(94.8%, 99.8%)	29.93	233.03	778.54	0.00	0.00	33.93	113.35
	30	0	100.0% (135/135)	(97.3%, 100.0%)	2011.23	114.05	5.67	0.00	0.00	14.78	0.74
	0	100	100.0% (135/135)	(97.3%, 100.0%)	1.36	6.00	442.70	1.04	76.93	0.00	0.00
	30	250	100.0% (135/135)	(97.3%, 100.0%)	1991.91	118.02	5.93	17.60	0.88	10.42	0.52
	75	100	100.0% (135/135)	(97.3%, 100.0%)	1954.80	169.35	8.66	0.00	0.00	0.00	0.00
Urine / Vaginal	0	0	100.0% (135/135)	(97.3%, 100.0%)	0.93	5.02	542.38	0.00	0.00	0.00	0.00
	30	0	100.0% (135/135)	(97.3%, 100.0%)	1999.78	131.75	6.59	34.19	1.71	0.00	0.00
	0	100	100.0% (135/135)	(97.3%, 100.0%)	0.76	3.36	442.40	0.00	0.00	0.00	0.00
	30	250	100.0% (135/135)	(97.3%, 100.0%)	1995.17	125.77	6.30	33.10	1.66	52.94	2.65
	75	100	100.0% (135/135)	(97.3%, 100.0%)	2014.38	109.48	5.43	0.00	0.00	0.00	0.00

Table 5: CT Q^x Assay – Descriptive Statistics for Reproducibility Panel 2

Specimen	Dilution	% Positive	95% CI (Positive)	Max RFU Mean (Positive)	% Negative	95% CI (Negative)	Max RFU Mean (Negative)
Endocervical/ Urethral Swab	1/10	70.2 (158/225)	(63.8, 76.1)	1794.2	29.8 (67/225)	(23.9, 36.2)	2.6
Endocervical/ Urethral Swab	1/100	10.2 (23/225)	(6.6, 14.9)	1643.8	89.8 (202/225)	(85.1, 93.4)	1.6
Urine/Vaginal	1/10	64.4 (145/225)	(57.8, 70.7)	1733.9	35.6 (80/225)	(29.3, 42.2)	4.6
Urine/Vaginal	1/100	10.7 (24/225)	(7.0, 15.5)	1666.6	89.3 (201/225)	(84.5, 93.0)	2.4

RESULTS CONTINUED

Table 6: GC Q^x Assay – Endocervical/Urethral Swab and Urine/Vaginal Sample Reproducibility: Percent Correct Shown by Testing Site and Combined Across Testing Sites for Panel 1

Specimen	Panel (CT/GC)	Testing Site						Total / Results Panel					
		1		2		3		PASS					
		PASS		PASS		PASS		Yes		No			
		Yes	%	Yes	%	No	%	Yes	%	No	%		
Endocervical / Urethral Swab	(0/0)	45	100.0	44	97.8	1	2.2	45	100.0	134	99.3	1	0.7
	(0/100)	45	100.0	45	100.0	0	0	45	100.0	135	100.0	0	0
	(30/0)	45	100.0	43	95.6	2	4.4	45	100.0	133	98.5	2	1.5
	(30/250)	45	100.0	45	100.0	0	0	45	100.0	135	100.0	0	0
	(75/100)	45	100.0	45	100.0	0	0	45	100.0	135	100.0	0	0
	Total	225	100.0	222	98.7	3	1.3	225	100.0	672	99.6	3	0.4
Urine / Vaginal	(0/0)	45	100.0	44	97.8	1	2.2	45	100.0	134	99.3	1	0.7
	(0/100)	45	100.0	45	100.0	0	0	45	100.0	135	100.0	0	0
	(30/0)	45	100.0	45	100.0	0	0	45	100.0	135	100.0	0	0
	(30/250)	45	100.0	45	100.0	0	0	45	100.0	135	100.0	0	0
	(75/100)	45	100.0	45	100.0	0	0	45	100.0	135	100.0	0	0
	Total	225	100.0	224	99.6	1	0.4	225	100.0	674	99.9	1	0.1

Table 7: GC Q^x Assay – Descriptive Statistics for Reproducibility Panel 1

Specimen	CT EM/mL	GC Cells/mL	% Correct	95% CI	Max RFU Mean	Within Run		Between Run Within Testing Site		Between Testing Site	
						SD	%CV	SD	%CV	SD	%CV
Endocervical / Urethral Swab	0	0	99.3% (134/135)	(95.9%, 100.0%)	13.8	151.28	1096.26	0	0	0.59	4.3
	30	0	98.5% (133/135)	(94.8%, 99.8%)	28.1	220.68	785.27	0	0	33.81	120.32
	0	100	100.0% (135/135)	(97.3%, 100.0%)	1859.52	94.07	5.06	0	0	19.19	1.03
	30	250	100.0% (135/135)	(97.3%, 100.0%)	1847.26	117.65	6.37	0	0	25.88	1.4
	75	100	100.0% (135/135)	(97.3%, 100.0%)	1855.93	119.39	6.43	0	0	42.18	2.27
Urine / Vaginal	0	0	99.3% (134/135)	(95.9%, 100.0%)	15.75	162.35	1031.1	0	0	0	0
	30	0	100.0% (135/135)	(97.3%, 100.0%)	1.06	3.13	295.78	0.74	69.73	0.51	48.25
	0	100	100.0% (135/135)	(97.3%, 100.0%)	1898.96	86.08	4.53	22.84	1.2	0	0
	30	250	100.0% (135/135)	(97.3%, 100.0%)	1884.21	93.95	4.99	13.77	0.73	0	0
	75	100	100.0% (135/135)	(97.3%, 100.0%)	1867.2	87.69	4.7	0	0	19.24	1.03

Table 8: GC Q^x Assay – Descriptive Statistics for Reproducibility Panel 2

Specimen	Dilution	% Positive	95% CI (Positive)	Max RFU Mean (Positive)	% Negative	95% CI (Negative)	Max RFU Mean (Negative)
Endocervical/ Urethral Swab	1/10	92.9 (209/225)	(88.7, 95.9)	1324.6	7.1 (16/225)	(4.1, 11.3)	41.4
Endocervical/ Urethral Swab	1/100	30.7 (69/225)	(24.7, 37.1)	835.9	69.3 (156/225)	(62.9, 75.3)	7.2
Urine/Vaginal	1/10	90.7 (204/225)	(86.1, 94.1)	1165.9	9.3 (21/225)	(5.9, 13.9)	34.2
Urine/Vaginal	1/100	22.7 (51/225)	(17.4, 28.7)	872.7	77.3 (174/225)	(71.3, 82.6)	7.8

RESULTS CONTINUED

For Panel 1, >99 % agreement was observed with expected results for both the CT Q^x and GC Q^x Assays across days and testing sites. The CT Q^x Assay results for Panel 1 are presented in Tables 3 and 4. The GC Q^x Assay results for Panel 1 are presented in Tables 6 and 7.

For Panel 2, positive results were obtained with a high proportion of specimens even though the target levels were below the specified analytical LODs for the assays. The CT Q^x and GC Q^x Assay results are presented in Tables 5 and 8. (Note: No pre-determined acceptance criteria were assigned to test results for the 0.1x and 0.01x panel members).

A summary of results for both reproducibility panels is provided in Table 9.

Table 9: Summary of Panel 1 and 2 Results

Target Level (X LOD)	N	Number (%) Correct v's Expected Result*	
		CT Q ^x	GC Q ^x
0*	540	538 (99.6)	536 (99.2)
0.01X**	450	47 (10.4)	120 (26.7)
0.1X**	450	303 (67.7)	413 (91.8)
2X	540	540 (100)	540 (100)
5X	270	270 (100)	270 (100)

*For target negative samples, negative results were expected for both CT Q^x and GC Q^x assays. Positive results for either or both the assays were expected for all other target levels.

**Spike levels are below the specified analytical LODs for each assay.

CONCLUSIONS

BD has demonstrated that the BD Viper™ System with XTR™ Technology yields a high degree of reproducibility for positive and negative results within and between instruments. The BD ProbeTec™ CT Q^x and GC Q^x Amplified DNA Assays each yielded 100% agreement with expected results for spike levels of 2X and 5X their respective specified analytical LODs, and >99% agreement for negative specimens. A high proportion of positive results were also obtained at spike levels below the specified analytical LOD for each assay, indicating the robustness of the system at low target levels.

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