

Compatibility of Accurun[®] and Amplichek[™] Positive Controls with the BD ProbeTec[™] ET CT/GC Amplified DNA Assays

A.E. SALLOOM AND B.T. DELLONE

BD Diagnostics • 7 Loveton Circle • Sparks, MD 21152, USA

REVISED ABSTRACT

Today, nucleic acid amplification technology is widely accepted as the standard of care for diagnosis of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (GC) infections. An integral aspect associated with the implementation of molecular diagnostic assays is the development of appropriately rigorous methods for quality control within the testing laboratory. Under the Clinical Laboratories Improvement Amendments (CLIA) program (42 CFR 493 - Final Rule)¹, each laboratory that introduces an unmodified, FDA-cleared or -approved system must demonstrate, among other requirements, accuracy and precision that are similar to the performance specifications established by the manufacturer, prior to reporting patient test results. Due to the low prevalence of many analytes and a lack of independently confirmed positive specimens, this frequently presents a significant challenge and necessitates the use of seeded samples and/or commercially available mock specimens to demonstrate the adequacy of assay performance.

Here we describe a series of experiments that were performed to evaluate the compatibility of Boston Biomedica ACCURUN[®] 341 and Bio-Rad Amplichek[™] CT/GC DNA Swab and Urine Positive Controls with the BD ProbeTec[™] ET CT/GC/AC Amplified DNA Assays and determine their applicability as an independent means by which to monitor accuracy and precision.

To mimic processing and testing of expressed urogenital swab specimens, 100 μ L aliquots of the ACCURUN 341 and Amplichek Swab Controls were diluted in 2 mL of BD ProbeTec ET CT/GC Assay Sample Diluent, heated at 114°C for 30 min, cooled and then assayed.

To mimic the processing of urine, 4 mL volumes of Phosphate Buffered Saline/Bovine Serum Albumin (PBS/BSA) were used as mock specimens. Each volume of PBS/BSA was spiked with 100 μ L bacterial suspension from the ACCURUN 341 or Amplichek Urine Controls and then processed by centrifugation and decanting according to the standard BD ProbeTec ET CT/GC/AC procedure. Each centrifuged pellet was resuspended in 2 mL Sample Diluent, heated at 114°C for 30 min, cooled and assayed.

The data presented here demonstrate the compatibility of the ACCURUN 341 and Amplichek Swab and Urine Controls with the BD ProbeTec ET CT/GC/AC Amplified DNA Assays. For swab specimens, equivalent performance was observed for CT and GC with both types of control. A lower proportion of positive results were observed using the CT ACCURUN 341 controls in the mock urine processing protocol than with the Amplichek Urine Positive Controls, although this difference was not statistically significant ($p = 0.090$).

The availability of these controls provides an independent means by which to monitor the performance of the BD ProbeTec ET CT/GC/AC Assays in accordance with CLIA guidelines, although it is important to follow the appropriate manufacturer's instructions when evaluating procedures associated with alternative specimen types.

INTRODUCTION

Infections caused by *C. trachomatis* (CT) and *N. gonorrhoeae* (GC) are the most common sexually transmitted bacterial diseases in the United States and worldwide. An estimated 2.8 million Americans are infected with CT each year. In 2003, 877,478 CT infections were reported to the Centers for Disease Control and Prevention (CDC) from 50 states and the District of Columbia with an incidence in women of 466.9 cases per 100,000. GC infections also occur in approximately 700,000 people in the United States each year. In 2003, 335,104 cases of GC were reported to CDC with an incidence of 116.2 per 100,000 persons.²

Nucleic acid amplification technology is now widely accepted as the standard of care for diagnosis of CT and GC infections. Integral to the implementation of molecular diagnostic assays is the development of appropriately rigorous methods for quality control within the testing laboratory. Under the Clinical Laboratories Improvement Amendments (CLIA) program,¹ each laboratory that introduces an unmodified, FDA-cleared or -approved system must demonstrate, among other requirements, accuracy and precision that are similar to the performance specifications established by the manufacturer, prior to reporting patient test results. Due to the low prevalence of many analytes and a lack of independently confirmed positive specimens, this frequently presents a significant challenge and necessitates the use of seeded samples and/or commercially available mock specimens to demonstrate the adequacy of assay performance.

Here we describe a series of experiments to evaluate the compatibility of Boston Biomedica ACCURUN[®] 341 and Bio-Rad Amplichek[™] CT/GC DNA Swab and Urine Positive Controls with the BD ProbeTec[™] ET CT/GC/AC Amplified DNA Assays, and determine their applicability as an independent means by which to monitor the performance of the BD ProbeTec ET CT/GC/AC assays in accordance with CLIA guidelines.

¹CLIA Regulations 42 CFR 493.1253

²<http://www.cdc.gov/std>

MATERIALS AND METHODS

MATERIALS:

Boston Biomedica (BBI) ACCURUN 341 Series 300 *Chlamydia trachomatis/Neisseria gonorrhoeae* DNA Positive Control P/N A341-5025

Bio-Rad Amplichek Swab CT/GC Control, Amplified Positive P/N 133

Bio-Rad Amplichek Urine CT/GC Control, Positive P/N 136X

Sigma® Phosphate Buffered Saline with BSA (PBS/BSA), pH 7.4 P/N P-3688

BD PBS/BSA buffer, pH 7.6

BD ProbeTec ET CT/GC Sample Diluent

BD ProbeTec ET CT/GC Positive Control

BD ProbeTec ET CT/GC Negative Control

METHODS:

Two lots each of Bio-Rad Amplichek Swab CT/GC Control, Bio-Rad Amplichek Urine CT/GC Control, and Boston Biomedica (BBI) ACCURUN 341 Series 300 *Chlamydia trachomatis/Neisseria gonorrhoeae* DNA Positive Control were used for all testing described below. BD ProbeTec ET CT/GC positive and negative controls were rehydrated with CT/GC sample diluent and used as run controls throughout. The BD ProbeTec ET sample process and assay workflows are depicted in Figures 1 & 2. The MOTA algorithm was used to determine positive, negative, or indeterminate results (Figure 3).

Swabs: Bio-Rad Amplichek CT/GC Positive DNA Swab Controls and BBI ACCURUN CT/GC DNA Positive Controls were prepared and processed according to procedures outlined in the manufacturer's product inserts (Figure 1).

Urine: Bio-Rad Amplichek CT/GC Positive DNA Urine Controls were processed according to procedures outlined in the manufacturer's product insert (treating and analyzing 4 mL as a patient specimen) (Figure 1). In order to verify that the target levels used in these controls would provide robust performance, serial dilutions were prepared in BD PBS/BSA buffer in order to determine if a smaller volume of control could be used. In each case, PBS/BSA was spiked with either 2 mL, 1 mL, 500 µL, 250 µL or 100 µL bacterial suspension for a total volume of 4 mL (Figure 4). Based on this analysis, all subsequent experiments with Amplichek urine controls were performed by spiking 100 µL into 4 mL of BD and Sigma PBS/BSA.

Although the manufacturer does not provide a protocol for use of their controls with the BD ProbeTec ET urine processing procedure, BBI ACCURUN CT/GC DNA Positive Controls were tested by spiking 100 µL of control into 4 mL of BD and Sigma PBS/BSA buffer. Once controls were spiked into PBS/BSA, they were processed as outlined in Figure 1. Processed specimens were assayed according to the normal BD ProbeTec ET CT/GC/AC testing procedure (Figure 2).

Figure 1. BD ProbeTec ET Sample Process Workflow for Swab and Urine Samples

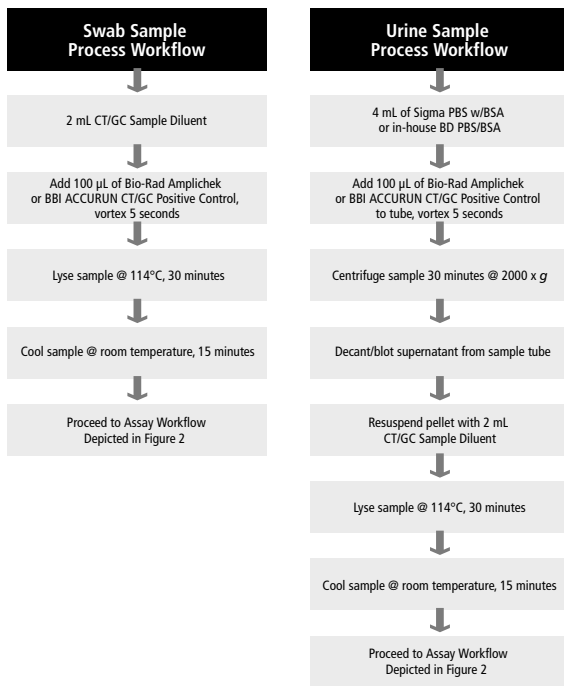


Figure 2. BD ProbeTec ET Assay Workflow

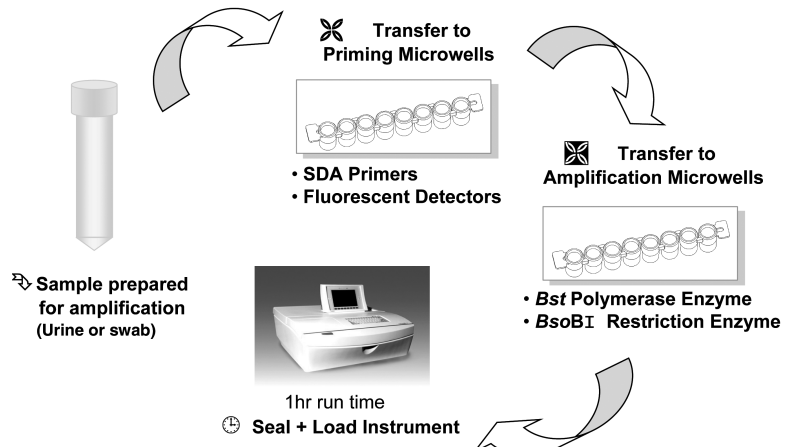
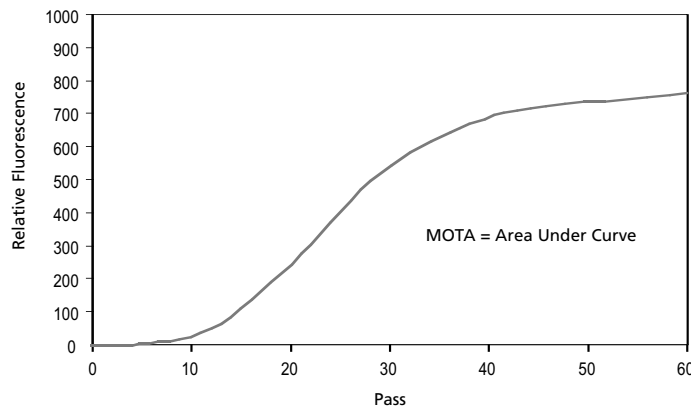


Figure 3. BD ProbeTec ET System MOTA Algorithm



- Metric other than Acceleration (MOTA)
- Defined as the area under the fluorescent kinetic curve

MOTA Score		
CT or GC	AC	Result
>2000	Any	Positive
<2000	>1000	Negative
<2000	<1000	Indeterminate

All data collected during swab testing events are summarized in reference Table 1 and Figure 5. All data collected during urine testing events are summarized in reference Table 2 and Figure 6. There were no indeterminate AC results in any assay run.

Table 1. Summary of CT/GC/AC Swab Data
Proportion Positive by Assay (n=60#)

	CT	GC	AC
Bio-Rad Amplichek	100%*	100%	100%
BBI ACCURUN	97%*	100%	100%

*p=0.150
#60 replicates from multiple processed tubes

CONCLUSION:

No statistical difference in performance between Bio-Rad and BBI controls for CT/GC/AC assays (p value > 0.05).

RESULTS

Table 2. Summary of CT/GC/AC Urine Data
Proportion Positive by Assay and Source of PBS/BSA (n=60#)

	CT		GC		AC	
	Sigma	BD	Sigma	BD	Sigma	BD
Bio-Rad Amplichek	98%*	97%**	100%	100%	100%	100%
BBI ACCURUN	92%*	90%**	100%	100%	100%	100%

* p=0.090
** p=0.140
#60 replicates from multiple processed tubes with Sigma PBS/BSA and 60 replicates from multiple processed tubes with BD PBS/BSA

CONCLUSIONS:

No statistical difference in performance between Bio-Rad and BBI controls for CT/GC/AC assays (p values > 0.05).

Differences observed with the CT results according to source of PBS/BSA were not statistically significant (Bio-Rad Sigma vs. BD p= 0.558; BBI Sigma vs. BD p=0.752).

Figure 4. Titration of Bio-Rad Amplichek CT/GC Positive Urine Controls

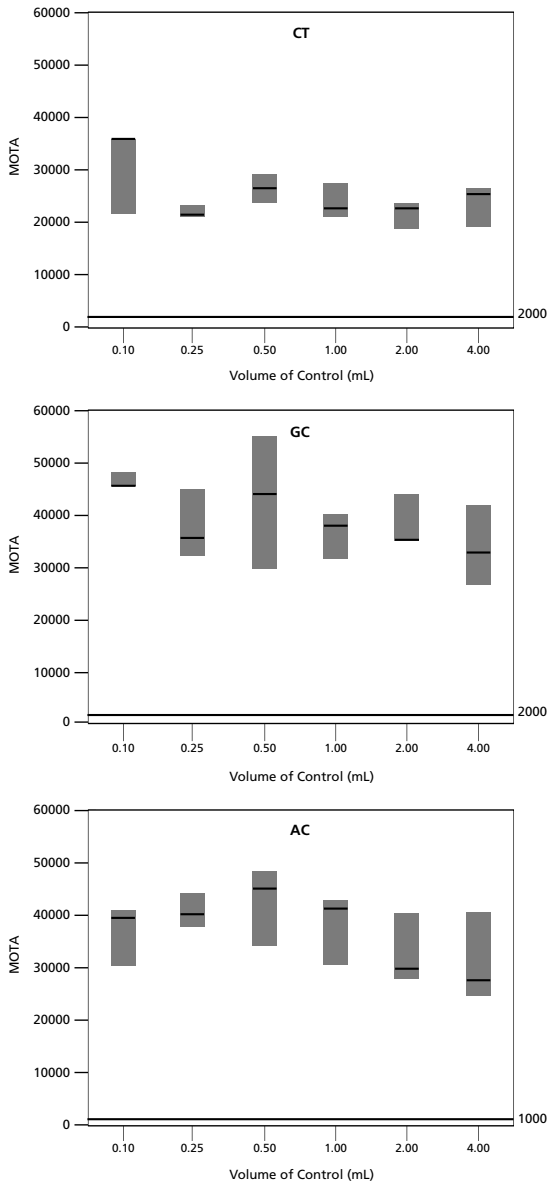


Figure 5. Performance Comparison of CT/GC/AC Swab Process

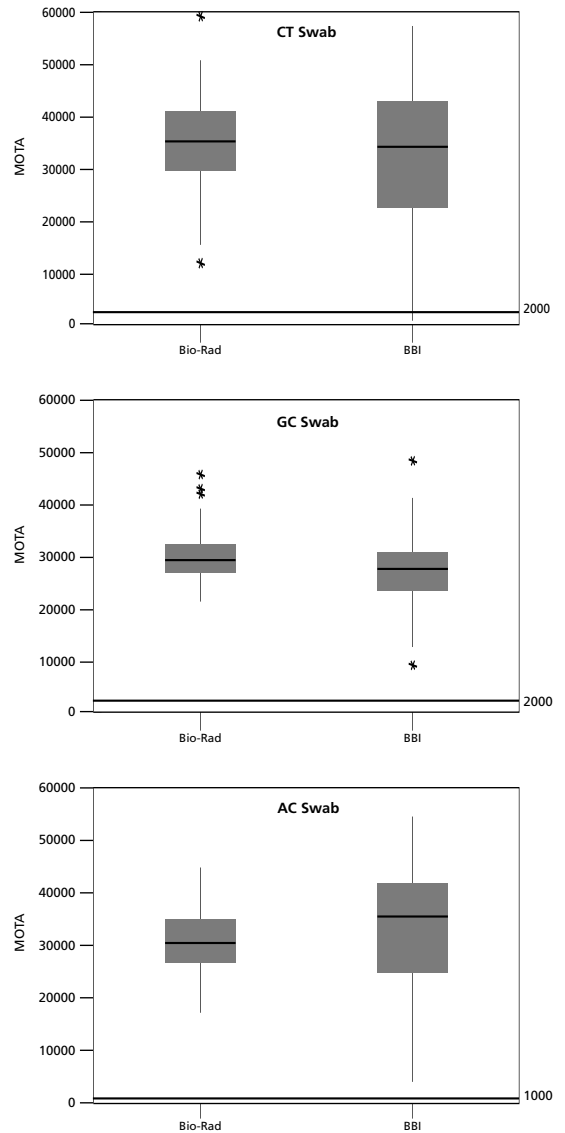
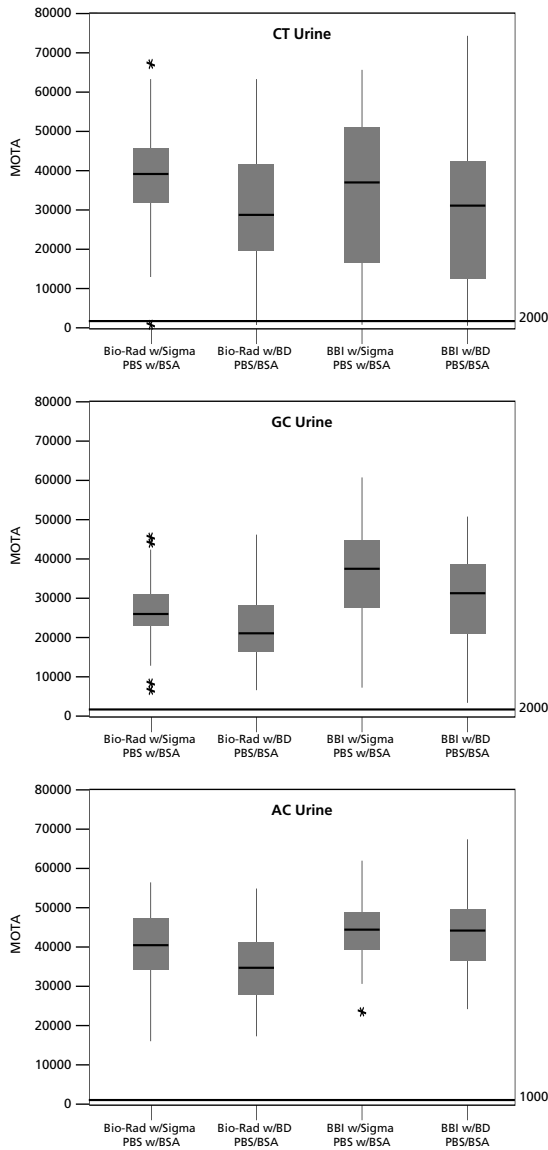


Figure 6. Performance Comparison of CT/GC/AC Urine Process



CONCLUSIONS

- The data presented here demonstrate the compatibility of the Boston Biomedica (BBI) ACCURUN 341 CT/GC DNA Positive Controls and Bio-Rad Amplichek CT/GC DNA Swab and Urine Positive Controls with the BD ProbeTec ET CT/GC/AC Amplified DNA Assays.
- With the swab processing protocol, similar performance was observed with both manufacturers' controls across the CT, GC and AC assays.
- BBI does not provide a specific protocol for the use of ACCURUN 341 CT/GC DNA Positive Controls with the BD ProbeTec ET urine protocol. The false negative CT results obtained with Amplichek and ACCURUN in the urine processing protocol might reflect the absolute level of target DNA present, or the inability to recover the target upon centrifugation due to lysis of the organisms in the matrix in which the controls are prepared.
- Nevertheless, with appropriate adherence to the manufacturer's instructions for use, the commercial availability of standardized Bio-Rad Amplichek and BBI ACCURUN controls provides an independent and convenient means by which to monitor the performance of the BD ProbeTec ET CT/GC/AC assays in accordance with CLIA guidelines.